INSTITUT FÜR ERNÄHRUNGS- UND LEBENSMITTELWISSENSCHAFTEN
DONALD STUDIENZENTRUM
am Forschungsinstitut für Kinderernährung, Dortmund

Aspects of carbohydrate quality and their relevance for risk markers of type 2 diabetes and related health outcomes

INAUGURAL – DISSERTATION

zur

Erlangung des Grades

Doktor der Ernährungs- und Haushaltswissenschaft

(Dr. oec. troph.)

der

Landwirtschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt im Februar 2014

von

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Tag der mündlichen Prüfung: 15.09.2014
Erscheinungsjahr: 2014

ACKNOWLEDGEMENTS

During my time as a PhD student, many people supported me for which I am deeply thankful. I would like to mention some of them in particular:

First of all, I am very thankful to Prof. Dr. Thomas Remer for his support and his willingness to always answer and discuss my questions. His insights and views were always very instructive and particularly enriched this thesis regarding physiological aspects.

Furthermore, I would like to thank Dr. Sarah Egert and Prof. Dr. Ute Nöthlings for their immediate agreement to co-supervise this thesis and their valuable comments on my manuscript.

This thesis would not exist without Dr. Anette Buyken, and I am deeply grateful to her. Dear Anette, you not only got me enthusiastic about the GI, but also about epidemiology. Thank you for providing me with all your knowledge, your helpful advice, and for always encouraging me, particularly during setbacks. Also, it is due to you, your relentless support and your relations, that I had the chance to work with the Australian data and that I am now spending a research fellowship at the University of Sydney.

Special thanks go to Dr. Christian Herder, Head Inflammation Workgroup at the German Diabetes Center, for his support in preparing three of the publications included in this thesis as well as overseeing the measurements of inflammatory markers in the laboratory of his group. Furthermore, I would like to thank both Prof. Dr. Michael Roden, Director of the Institute for Clinical Diabetology, German Diabetes Center in Düsseldorf, and Prof. Dr. Stefan Wudy, Head Pediatric Endocrinology & Diabetology Center of Child and Adolescent Medicine, Giessen, for additional measurements of risk markers of type 2 diabetes in their laboratories.

As a part of my thesis I was given the opportunity to analyze data from the Blue Mountains Eye Study, conducted at the Centre for Vision Research, Westmead Millennium Institute, University of Sydney, Australia. I am very thankful to Prof. Dr. Paul Mitchell for giving me this opportunity. Furthermore, I am particularly grateful to Prof. Dr. Jennie Brand-Miller, School of Molecular Bioscience and Boden Institute of Obesity, Nutrition, Exercise and Eating Disorders, University of Sydney, for supporting me and more importantly, giving me the invaluable chance to work within her research group in Sydney.

Also, I like to thank all other co-authors, who helped to improve this thesis by providing their constructive criticism and suggestions.

Especially, I would like to thank Gesa for her comments and help on this thesis – regarding both the content and format. Working together with you was a great pleasure from the beginning on and I miss stopping by your office, discussing problems, assigning numerous GI and FII values together or jumping for joy if a paper got accepted.

All the other PhD-students and colleagues at the Research Institute of Child Nutrition also made my time as a PhD student enjoyable. Particularly, I would like to thank my “roommate” Katharina for her ideas and advice, and for sharing our nice little working day habits. Also, the “corner office” with all its changing inhabitants has always been a wonderful second “home” for me.

In addition, my thanks go to the DONALD participants and their families as well as the BMES participants, without whom this unique data would not exist.

Last but not least I would like to express my heartfelt thanks to my parents, my sister and Moritz for their unconditional support, for reminding me that work is not always the most important thing in life and for giving me the precious feeling of always being there for me. Moritz, also thank you so much for proof-reading my thesis, helping me with the figures, and always restocking my chocolate supply – particularly in the last few weeks.

PUBLICATIONS

This thesis aimed to examine aspects of carbohydrate quality and their relevance for risk markers of type 2 diabetes mellitus and related health outcomes. It resulted in the following:

Scientific papers

- Joslowski G, **Goletzke J**, Cheng G, Günther ALB, Bao J, Brand-Miller JC, Buyken AE. Prospective associations of dietary insulin index, glycemic index, and glycemic load during puberty with body composition in young adulthood. International Journal of Obesity (London) (2012) 36, 1463-1471. doi: 10.1038/ijo.2011.241
- **Goletzke J**, Herder C, Joslowski G, Bolzenius K, Remer T, Wudy SA, Rathmann W, Roden M, Buyken AE. A habitually higher dietary glycemic index during puberty is prospectively related to increased risk markers of type 2 diabetes in young adulthood. Diabetes Care (2013) Jul; 36(7):1870-6. doi: 10.2337/dc12-2063
- **Goletzke J**, Buyken AE, Gopinath B, Rochtchina E, Barclay AW, Cheng G, Brand-Miller JC, Mitchell P. Carbohydrate quality is not associated with markers of hepatic fat accumulation over 5 years in an older population. British Journal of Nutrition (2013) Jan 23:1-8. doi:10.1017/S0007114512005867
- Buyken AE, **Goletzke J,** Joslowski G, Felbick A, Cheng G, Herder C, Brand-Miller J. The role of carbohydrate quality in chronic low-grade inflammation – a systematic review on observational and intervention studies. American Journal of Clinical Nutrition (in press)
- **Goletzke J**, Buyken AE, Joslowski G, Bolzenius K, Remer T, Carstensen M, Egert S, Nöthlings U, Rathmann W, Roden M, Herder C. Prospective association between carbohydrate nutrition during puberty and markers of chronic low-grade inflammation in younger adulthood (under revision)

Oral presentations

- Joslowski G, **Goletzke J**, Cheng G, Bao J, Brand-Miller JC, Buyken AE. Prospective associations between dietary insulin index during puberty and body composition in young adulthood.
    - International Journal of Obesity Supplements (2011) 1: S16
    - Proceedings of the German Nutrition Society (2011) 15: 10
- Buyken AE, Joslowski G, **Goletzke J**, Cheng G, Bao J, Brand-Miller JC. Prospective associations between dietary insulin index, glycemic index and glycemic load during

puberty and body composition in young adulthood. German Epidemiologic Society Meeting Abstract (2011) 6:64

- **Goletzke J**, Buyken AE, Joslowski G, Bolzenius K, Remer T, Carstensen M, Egert S, Nöthlings U, Rathmann W, Roden M, Herder C. Prospective association between carbohydrate nutrition during puberty and markers of chronic low-grade inflammation in younger adulthood. German Epidemiologic Society Meeting Abstract (2013) 78

Posters

- Buyken AE, Joslowski G, **Goletzke J**, Cheng G, Bao J, Brand-Miller JC. Prospective associations between dietary insulin index, glycemic index and glycemic load during puberty and body composition in young adulthood. Diabetologia 2011; 54 (Suppl.1): S542
- **Goletzke J**, Herder C, Joslowski G, Bolzenius K, Remer T, Wudy SA, Rathmann SA, Roden M, Buyken AE. A habitually higher dietary glycemic index during puberty is prospectively related to increased risk markers of type 2 diabetes in young adulthood.
    - Diabetologia 2012; 55 (Suppl.1): S137
    - Proceedings of the German Nutrition Society (2013) 18: 68
- Buyken AE, **Goletzke J**, Joslowski G, Felbick A, Cheng G, Herder C, Brand-Miller J. The role of carbohydrate quality in chronic low-grade inflammation – a systematic review on observational and intervention studies. Proceedings of the German Nutrition Society (2013) 18: 107
- **Goletzke J**, Buyken AE, Joslowski G, Felbick A, Cheng G, Herder C, Brand-Miller J. The role of carbohydrate quality in chronic low-grade inflammation – a systematic review on observational and intervention studies. Obesity Facts 2013; 6 (Suppl.1): S222

Articles in national journals

- **Goletzke J**, Buyken AE. Mit Ballaststoffen vorbeugen. UGB-Forum (4/12)
- **Goletzke J**, Buyken AE, Kohlenhydatqualität in der Pubertät und Risiko für Diabetes mellitus Typ 2 im jungen Erwachesnenalter – DONALD News.
    - Pädiatrische Praxis 5/2013
    - Ernährungsumschau 4/2013

SUMMARY

Aspects of carbohydrate quality and their relevance for risk markers of type 2 diabetes mellitus and related health outcomes

Concern has been raised that the commonly advocated low-fat, high-carbohydrate diet might be actually detrimental for the growing number of people with impaired IR since it favors postprandial rises in glucose and insulin, which are associated with an increased risk of type 2 diabetes mellitus (T2D). Successful prevention strategies to fight the increasing prevalence rates of obesity, T2D and related chronic diseases are urgently needed. Since insulin-resistant individuals are particularly prone to glycemic excursions, this might also extend to puberty, a period characterized by physiological IR. A further age group, which to date has not been addressed, are elderly people, who represent a growing proportion of our population and for whom specialized prevention strategies might be necessary.

Therefore, the **overall aim** of the present thesis was to investigate the relevance of different aspects of carbohydrate quality for selected risk markers of T2D. In this regard, prospective associations between puberty and young adulthood as well as 5-year longitudinal relations in older age were examined.

Major data source was the DOrtmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study, which includes data on dietary intake, anthropometry, and health from birth until adulthood. Moreover, data from the Blue Mountains Eye Study (BMES) was used, where information on nutritional status and markers of liver function was repeatedly collected from an older Australian cohort. Additionally, a systematic literature search was conducted on the association between carbohydrate quality and chronic low-grade inflammation in adults.

Four analyses (Study I, II, III, and V) and one systematic review (Study IV) were performed. **Study I**, including 262 participants of the DONALD Study, showed that a higher habitual dietary insulin index, but not a higher glycemic index (GI), during puberty was related to a higher percentage body fat in young adulthood. **Study II** revealed that a habitually higher dietary GI during puberty was the only aspect of carbohydrate nutrition which was consistently related to the analyzed T2D risk markers i.e. homeostasis model assessment IR (HOMA-IR), alanine-aminotransferase (ALT), and gamma-glutamyltransferase (GGT) in a subsample of the DONALD Study (n=226 and n=214, respectively). In **Study III**, again based on data from the DONALD Study (n=205), a higher habitual pubertal intake of carbohydrates from higher GI food sources and a lower intake of whole grains was associated with higher levels of the pro-inflammatory cytokine interleukin-6 in younger adulthood. In this regard,

Study IV showed that the observational evidence in adults is less consistent for a beneficial role of a lower GI or GL compared to dietary fiber/whole grain. However, there is less consistent evidence from intervention studies for anti-inflammatory benefits of higher fiber or whole grain diets than there is for low-GI/GL diets (60 studies were included in the systematic review). Benefits of higher fiber and whole grain intakes suggested by observational studies may hence reflect confounding. Finally, in **Study V**, including 866 older people from the BMES, no longitudinal relation was observed between the different aspects of carbohydrate quality and liver enzymes and serum lipids.

In conclusion, our results suggest a particular relevance of postprandial glycemic – and also insulinemic – excursions during puberty for risk markers of T2D during adulthood. Overall, efforts to improve carbohydrate quality should not focus solely on a high whole grain intake, but needs to be complemented by an advice for a preferred selection of low-GI foods.

ZUSAMMENFASSUNG

Aspekte der Kohlenhydratqualität und ihre Relevanz in Bezug auf Risikomarker für Typ 2 Diabetes und assoziierte Erkrankungen

Zunehmend werden Bedenken laut, dass die derzeitige Empfehlung, sich fettarm und kohlenhydratreich zu ernähren, ungünstig für die steigende Zahl an Menschen mit gestörter Insulinresistenz ist, da sie zu postprandialen Blutglukose- und Insulinanstiegen führt, welche wiederum mit einem erhöhtem Risiko für Typ 2 Diabetes mellitus (T2D) verbunden sind. Daher sind vor dem Hintergrund der steigenden Prävalenz von Übergewicht, T2D und weiteren chronischen Erkrankungen erfolgreiche Präventionskonzepte dringend notwendig. Da insbesondere insulinresistente Personen sehr empfindlich auf Blutzuckeranstiege reagieren, könnte dies auch auf die Phase der Pubertät zutreffen, die durch eine physiologische Insulinresistenz gekennzeichnet ist. Eine weitere, bisher kaum berücksichtigte Altersgruppe sind ältere Menschen, die einen immer größeren Anteil in unserer Gesellschaft ausmachen, und für die möglicherweise speziell zugeschnittene Präventionskonzepte erforderlich sind.

Das **übergeordnete Ziel** dieser Arbeit war, die Relevanz verschiedener Aspekte der Kohlenhydratzufuhr für Risikomarker von T2D zu untersuchen. Von Interesse waren hierbei prospektive Assoziationen zwischen der Pubertät und dem Erwachsenenalter sowie 5-Jahres-Veränderungen bei älteren Personen.

Daten für diese Untersuchungen lieferte die DOrtmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study, in der Informationen zur Ernährung, Anthropometrie und dem Gesundheitsstatus von der Geburt bis ins Erwachsenenalter erhoben werden. Außerdem wurden Daten aus der Blue Mountains Eye Study (BMES) herangezogen, in der wiederholt Informationen zur Ernährung und zu Markern der Leberfunktion in einer älteren australischen Kohorte erfasst wurden. Zusätzlich wurde eine systematische Literaturrecherche zum Zusammenhang zwischen der Kohlenhydratqualität und chronisch geringgradiger Entzündungsneigung im Erwachsenenalter durchgeführt.

Vier Auswertungen (Studie I, II, III, V) und eine systematische Literaturrecherche wurden durchgeführt (Studie IV). **Studie I**, in der 262 Probanden aus der DONALD Studie eingeschlossen wurden, zeigte, dass ein gewohnheitsmäßig höherer Insulin Index, jedoch nicht ein höherer glykämischer Index (GI), in der Pubertät mit einem höheren Körperfettanteil im jungen Erwachsenenalter assoziiert war. In **Studie II** war ein habituell höherer GI in der Pubertät der einzige Aspekt der Kohlenhydratqualität, der in einer Untergruppe der DONALD Studie (n=226 bzw. n=214) konsistent mit den untersuchten T2D Risikomarkern

(Homeostasis model assessment IR (HOMA-IR), Alanin-Aminotransferase (ALT) und Gamma-Glutamyltransferase (GGT)) zusammenhing. **Studie III** basierte ebenfalls auf Daten der DONALD Studie (n=205) und konnte zeigen, dass eine gewohnheitsmäßig hohe Zufuhr von Kohlenhydraten aus Lebensmitteln mit einem höheren GI sowie eine niedrigere Aufnahme von Vollkorn während der Pubertät mit höheren Werten des proinflammatorischen Cytokins Interleukin 6 assoziiert war. In diesem Zusammenhang wurde aus **Studie IV** ersichtlich, dass die vorhandene Evidenz aus Beobachtungsstudien weniger eindeutig für den günstigen Einfluss eines niedrigen GI ist als für die Ballaststoff- und Vollkornzufuhr. Im Gegensatz dazu ist die Evidenz aus Interventionstudien weniger konsistent, dass eine ballaststoff- und vollkornreichen Kost verglichen mit einer Kost mit niedrigem GI/GL anti-inflammatorische Effekte hat (60 Studien wurden im systhematischen Review eingeschlossen). Günstige Effekte eines hohen Ballaststoff- und Vollkornverzehrs aus Beobachtungsstudien lassen Confounding vermuten. Schließlich deutete **Studie V,** basierend auf Daten von 866 Probanden aus der BMES, darauf hin, dass kein longitudinaler Zusammenhang zwischen den verschiedenen Aspekten der Kohlenhydratqualität und den Leberenzymen oder Serumlipiden besteht.

Zusammenfassend lässt sich festhalten, dass unsere Ergebnisse auf eine besondere Relevanz von postprandialen Blutglukose-, sowie Insulinanstiegen während der Pubertät für verschiedene Risikomarker von T2D im jungen Erwachsenenalter hinweisen. Insgesamt sollten Bemühungen, die Kohlenhydratqualität zu steigern, sich nicht ausschließlich auf Vollkornprodukte fokussieren, sondern um den Hinweis für eine bevorzugte Auswahl von Lebensmitteln mit einem niedrigen GI erweitert werden.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

%BF	Percentage body fat
%En	Percentage of total energy intake
95% CI	95% confidence interval
ALT	Alanine aminotransferase
AUC	Area under the curve
BIA	Bioelectrical impedance analysis
BMES	Blue Mountains Eye Study
BMI	Body mass index
CHD	Coronary heart disease
CVD	Cardiovascular disease
DEGS1	German Health Interview and Examination Survey for Adults
DEXA	Dual-energy X-ray absorptiometry
DGAC	Dietary Guidelines Advisory Committee
DGE	German Nutrition Society (Deutsche Gesellschaft für Ernährung)
DiOGenes	Diet, Obesity and Genes
DONALD	Dortmund Nutritional and Anthropometric Longitudinally Designed
EFSA	European Food and Safety Authority
EPIC	European Prospective Investigation into Cancer and Nutrition
ER	Endoplasmatic reticulum
EsKiMo	Ernährungsstudie als KiGGS Modul
FAO	Food and Agriculture Organization
FFA	Free fatty acids
FFQ	Food frequency questionnaire
FFM, FFMI	Fat mass index, fat-free mass index
FII	Food insulin index
GDM	Gestational diabetes mellitus
GGT	Gamma glutamyltransferase
GI	Glycemic index
GL	Glycemic load
HbA1c	Glycosylated haemoglobin
HOMA	Homeostasis model assessment
hsCRP	high-sensitivity C-reactive protein
IASO	International Association for the Study of Obesity
IDF	International Diabetes Federation

IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IL	Interleukin
IOTF	International Obesity Task Force
IQR	Interquartile range
IR	Insulin resistence
KiGGS	German Health Interview and Examination Survey for Children and Adolescents (Kinder- und Jugendgesundheitssurvey)
KORA	Kooperative Gesundheitsforschung in der Region Augsburg
LEBTAB	In-house nutrient database (Lebensmitteltabelle)
MRI	Magnetic resonance imaging
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic Steatohepatitis
NFκB	Nuclear factor κB
NHANES	National Health and Nutrition Examination Survey
OA	Original Article
OGTT	Oral glucose tolerance test
PAI-1	Plasmingen activator inhibitor type 1
PREVIEW	PREVention of diabetes through lifestyle Intervention and population studies in Europe and around the World
RESIST	Researching Effective Strategies to Improve Insulin Sensitivity in Children and Teenagers
SD, SDS	Standard deviation, standard deviation score
SE	Standard error
SEM	Standard error of the mean
T1D, T2D	Type 1 diabetes mellitus, Type 2 diabetes mellitus
TG	Triglyceride
TNF-α	Tumor necrosis factor α
US	United States
WHO	World Health Organization

1. INTRODUCTION

Obesity and type 2 diabetes mellitus (T2D) can be considered today's main public health burdens worldwide – and their prevalence rates are expected to increase even further in the upcoming years [1, 2]. Thus, successful prevention strategies to fight these increasing rates are urgently needed. Besides physical activity, dietary approaches are considered to be an important part in obesity and diabetes prevention. However, despite the plethora of available studies investigating the effect of different diets on the risk of obesity, T2D, and related health outcomes, an agreement has not yet been reached.

Not least due to the findings of two large intervention studies at the beginning of this century, showing that a high-fiber, low-fat diet as part of a lifestyle modification including physical activity can prevent the progression to manifest T2D in individuals with impaired glucose tolerance [3, 4], this diet composition is generally advocated. However, one decade later, concern has emerged that a low-fat high-carbohydrate diet might be actually harmful for the increasing number of persons with insulin resistance (IR), since it induces postprandial glucose and insulin excursions, thereby increasing the risk to develop T2D [5-7].

Furthermore, the focus has changed from solely looking at the carbohydrate proportion to also taking into account its quality. Indeed, most nutritional recommendations entail the advice to consume a diet characterized by a high fiber and whole grain intake [8-11]. Moreover, it has been suggested that all successful dietary approaches for T2D prevention alternatively proposed to the common low-fat diet share a unifying mechanism: They induce less glycemic and insulinemic excursions, and hence reduce the strain on beta cells [5]. In this regard, interest in the concepts of the dietary glycemic index (GI) and dietary glycemic load (GL) has increased. These concepts estimate the relative and absolute glycemic responses to consumed carbohydrates, respectively [12, 13]. Recently, changes in glucose and insulin homeostasis, while being closely associated with T2D pathogenesis, have also been linked to obesity, low-grade inflammation and hepatic steatosis [14]. These conditions are tightly linked: While obesity is considered to be an initial factor in disease development, chronic low-grade inflammation is a shared condition both in obesity and diabetes [15]. Moreover, hepatic IR often parallels systemic IR and results in hepatic fat accumulation [16]. This coexistence and more importantly linkage of these diseases, i.e. T2D, obesity, low-grade inflammation and hepatic steatosis, makes it evident to address them together and gives the chance to reveal potential underlying mechanisms by which dietary aspects impact on these health outcomes.

Although being relevant for all age groups, associations between carbohydrate nutrition and diabetes risk markers have been extensively investigated especially in middle-aged

populations. However, other periods in life might also be relevant: As insulin resistant individuals are particularly prone to postprandial rises in glucose and insulin [17, 18], this might also extend to puberty, a period characterized by physiological IR [19]. The rising awareness that prevention should start early in life underlines the importance of investigating the role of carbohydrate nutrition during this phase. Finally, progress in health care systems implies that people are getting older [2]. Hence, preventive strategies tailored to the special needs and adapted to metabolic alterations related to aging are becoming indispensable.

Taken together, prospective data on the relevance of carbohydrate quality on the risk of T2D and related health outcomes can be regarded insufficient – particularly in pediatric and elderly populations. Moreover, attention should also be paid to the potential importance of nutrition during critical periods such as puberty. Hence, one main aim of this thesis was to investigate the relevance of carbohydrate quality during puberty for different risk markers related to T2D and related health outcomes in younger adulthood: First, the association with adult body composition will be examined, as obesity frequently precedes T2D (*Aim 1*). Second, the relevance for different risk markers of T2D will be assessed (IR and hepatic steatosis markers) (*Aim 2*), to then look at the association with inflammatory markers, the main mechanism discussed linking carbohydrate intake to T2D risk (*Aim 3, 1st research question*). The DOrtmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study represents an ideal data basis to investigate these prospective associations between dietary factors and later risk markers. Additional to these analyses in sub-samples of the DONALD Study, two further research questions will be addressed: In a more systematic approach, evidence on the relatively new research field of low-grade inflammation will be assembled for different aspects of carbohydrate quality in both observational and intervention studies (A*im 3, 2nd research question*). Finally, to also consider carbohydrate quality in an elderly population, data from the Australian Blue Mountains Eye Study (BMES) will be used, offering the possibility to examine the longitudinal association between carbohydrate nutrition and hepatic steatosis markers – a further condition closely linked to T2D and highly prevalent in older age groups (*Aim 4*).

Outline of this thesis

This thesis builds on data from two observational studies and additionally comprises a systematic review. In the *Background* section (Chapter 2), the different aspects of carbohydrate quality will be defined and the epidemiology of T2D will be summarized along with its related disease outcomes, namely obesity, low-grade inflammation and hepatic steatosis. Moreover, available evidence linking carbohydrate quality and these health outcomes will be presented – both from a mechanistic and an epidemiological point of view. Based on this overview, four *Research Aims* (Chapter 3) will be formulated and addressed subsequently. In Chapter 4, the *General Methodology* of the DONALD Study, BMES, and the conducted systematic review on which this thesis is based will be described. The research aims will be addressed in five O*riginal Articles*, which will be summarized in Chapter 5. The result of these publications will be brought into a wider context in the *General Discussion* section (Chapter 6) and will be finally summarized in Chapter 7 (*Summary and Perspectives*). In this chapter, ideas for future research will also be suggested. This thesis is cumulative and does not include detailed descriptions of the performed statistical analyses or the obtained results. The information on the analytical approaches, detailed presentations of the results and discussions of specific findings can be found in the original articles (OA) (Appendices 1-5).

2. THEORETICAL BACKGROUND

2.1 Aspects of carbohydrate quality

Carbohydrates represent a diverse group with different chemical structures and physiological properties [20]. According to the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Consultation on carbohydrates in human nutrition, which took place in Rome 1997, the primary classification of carbohydrates should be based on chemistry; that is, by molecular size, degree of polymerization, the type of linkage and character of individual monomers. This classification results in three main groups: (1) sugars, which comprise mono- and disaccharides as well as polyols, (2) oligosaccharides, the so called short chain carbohydrates like malto-oligosaccharides, and (3) polysaccharides, which can be further divided into starch and non-starch polysaccharides [21]. However, within the 2006 scientific update the FAO/WHO acknowledged that, when classifying carbohydrates solely on chemical characteristics, nutritional implications cannot be drawn directly, since each of the three main groups has various and overlapping physiological effects. Moreover, during this update the recommended intake of 55% to 75% of total daily energy from carbohydrates was questioned and it was emphasized that the nature of dietary carbohydrates appears to be more important than the total intake [22]. Likewise, the German Nutrition Society (DGE) concluded in the evidence based dietary guidelines for "Carbohydrate Intake and Prevention of Nutrition-Related Disease" published in 2011, that for primary prevention purposes the quality of carbohydrate nutrition is more important than its quantity [23].

To date, numerous classifications based on different physiological properties exist, including terms such as available and unavailable carbohydrates, simple and complex carbohydrates or glycemic and non-glycemic carbohydrates, which are more or less suitable for translation into health benefits [20, 24]. This thesis focuses on the terms dietary fiber, whole grain, added sugar, dietary glycemic index (GI), glycemic load (GL), and dietary insulin index, insulin load to describe different aspects of carbohydrate quality, which are subsequently explained in detail.

2.1.1 Dietary fiber and whole grain

In 2006, the FAO/WHO scientific update on carbohydrates proposed to define **dietary fiber** as intrinsic plant cell wall polysaccharides reflecting the naturally occurring polysaccharides in vegetables, fruits and whole grains, for which health benefits are clearly established [22]. However, according to the FAO/WHO Codex Alimentarius definition from 2008, three

different categories of dietary fiber exist, all of which cannot be hydrolyzed by endogenous enzymes in the small human intestine: Besides naturally occurring fiber in the plant cell wall, according to this definition, also extracted and synthetic carbohydrate polymers, for which physiological health benefits can be verified by scientific evidence, are considered as dietary fiber [25]. This definition was further specified in the Ninth Vahouny Fiber Symposium, organized by the International Life Sciences Institutes from Europe and America, where it was agreed upon that non-digestible carbohydrates with a degree of polymerization of more than three are also considered as dietary fiber [26]. These two approaches to define dietary fiber – the so-called plant-rich diet and the indigestibility approach – are still subject to debate. An argument against the extension of the term dietary fiber to include extracted and synthetic carbohydrate polymers, as proposed by the indigestibility approach, is the insufficient evidence for these polymers, whose effects are diverse and often not directly associated with health benefits [24]. Furthermore, concern has been raised that this would lead to the misinterpretation that an insufficient fiber intake can be compensated with extracted and synthetic carbohydrate polymers. By contrast, the plant-rich diet approach is in accordance with dietary recommendations to increase dietary fiber consumption in the form of fruits, vegetables and whole grains. Furthermore, the plant cell walls are nutrient dense with respect to vitamins, minerals, and phytochemicals, all of which are associated with beneficial health outcomes [24].

As mentioned above, besides fruits and vegetables, **whole grains** are a main source of dietary fiber and a high consumption is commonly recommended. However, the existing definitions and meanings of the term ‘whole grain’ are as diverse as those existing for dietary fiber [20]. The Whole Grain Label Statement of the United States (US) Food and Drug Administration (FDA) defined whole grains as: “Cereal Grains that consist of the intact, ground, cracked or flaked caryopsis, whose principal anatomical components – the starchy endosperm, germ and bran – are present in the same relative proportions as they exist in the intact caryopsis.”[27]. In scientific studies though, a less strict definition is often applied [28], e.g. considering foods with added bran but no endosperm or germ as whole grains [29]. In 2008, the Whole Grain Task Force stated that it “supports the use of the term whole grain for products of milling operations that divide the grain into germ, bran, and endosperm, but then recombine the party into their original proportions before the flour leaves the mill” [30]. However, recombined whole grain flours rarely comprise the single ingredients in the same proportion as in the intact caryopsis [31]. Hence the Whole Grain Task Force proposed a less strict addition to the US FDA term such as “… as they exist in the intact caryopsis to the extent feasible by the best modern milling technology” [30]. With regards to possible health benefits, it would furthermore be useful to distinguish between intact whole grains (e.g.

kernels) and those physically disrupted (e.g. whole grain flour), as the latter one exerts a completely different postprandial glycemic response compared to intact kernels [31]. Further discordance exists regarding the definition of a whole grain food. According to the US FDA it is defined as “a product containing >51% whole grain by weight per reference amount customarily consumed per day” [32]. Other suggested cut-offs for whole grain foods are >10% or >25% of the content as whole grain, allowing a wider range of foods to be considered and hence reducing the possibility of underestimating the overall whole grain intake [20].

2.1.2 Added sugar

Total sugar comprises all mono- and disaccharides present in foods except polyols. However, as several foods, like fruits, vegetables or milk, are naturally high in sugars and their consumption is not related to adverse health outcomes, the need for a further differentiation became evident [24]. That led to suggestions of numerous different terms to describe sugar intake, among them free sugars, added sugars, extrinsic and intrinsic sugars and non-milk extrinsic sugars. One common problem of these terms is that they are lacking a coherent definition and may hence cause confusion for the consumer. Moreover, these terms relate to food attributes not associated with the sugars per se but the food matrix [20]. According to the Institute of Medicine in the US, added sugars are defined as sugars and syrups added to the food or beverage during processing or preparation [33]. While the voluminous texture of fruits and vegetables normally prevents against an overconsumption of sugars from these sources, added sugars are more likely to be consumed in high proportions and are therefore more closely related to a high energy intake [24]. Hence, under a public health perspective, the differentiation between naturally occurring and added sugars could be useful. Moreover, a common approach in epidemiological studies is to further differentiate between different sources in the diet. Particularly, sugars from sugar-sweetened beverages are often investigated separately, since the consumption of these beverages has been linked to T2D risk [34].

2.1.3 Dietary Glycemic Index and Glycemic Load

The concept of the **GI** was introduced by Jenkins et al. because it became evident that the chemical composition of carbohydrate containing foods cannot predict their postprandial blood glucose responses [35]. Before that, the overall assumption persisted that mono- and disaccharides – the so-called simple sugars – induce high blood glucose increases, whereas polysaccharides – the complex carbohydrates – were related to comparably lower increases [36].

The GI of a food is defined as the 2-hour incremental area under the curve (AUC) of glucose response following the intake of 50 g of available carbohydrates from a test food as compared to the 2-hour AUC of glucose response induced by the same amount of carbohydrates from ingested glucose (reference food) [35]. In their initial studies, Jenkins et al. could also show that there was no association between the GI and the sugar or fiber content of a food [35]. Hence, many products rich in dietary fiber have a high GI [37]. Glycemic responses of common foods are illustrated in **Figure 1**. However, it has to be noted, that most European potato varieties and preparation methods are associated with lower glycemic responses compared to those common in the US and Australia [38].

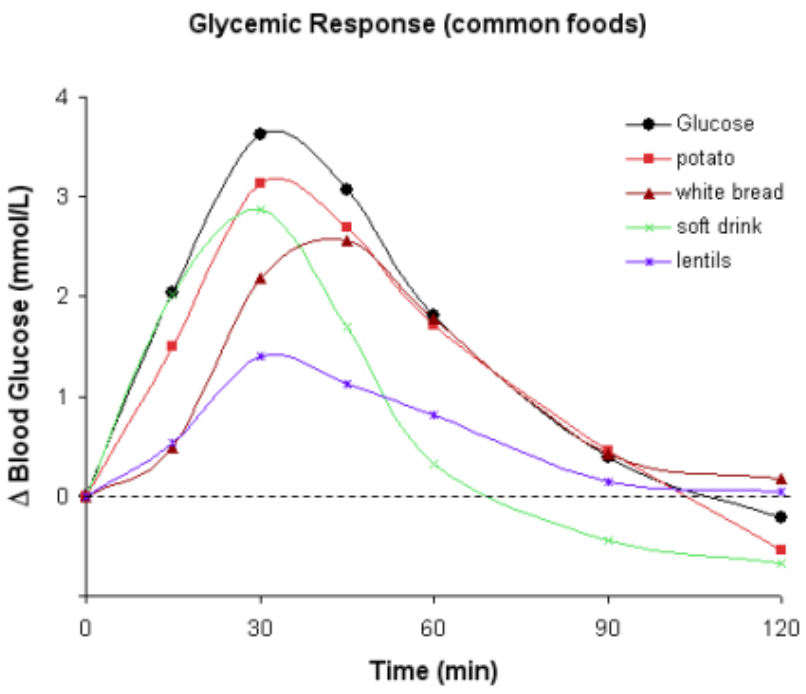

Figure 1**:** The glycemic response to 50g carbohydrate portions of common foods in relation to the reference food glucose (GI=100), from [39].

As there are numerous factors influencing the glycemic response of a food, the dietary GI cannot be predicted and thus needs to be measured. Factors affecting the postprandial blood glucose curve are for instance the degree of processing (the glycemic response is higher when the gross matrix structure is homogenized) or the amylase and amilopectin content (amylopectin has a greater effect on glucose rises) [40]. The measurement of the GI follows a standardized procedure where the capillary blood glucose levels are observed in at least 10 healthy subjects up to two hours after ingestion of 25g or 50g available carbohydrates from the test food (depending on the carbohydrate content of the food, since the amount should be edible in 5 to 15 minutes with the addition of 250 ml water) as well as the reference food glucose [40]. The mean of the individual ratios of the two measured AUC are then multiplied by 100 to derive the foods GI value. Over the years, GI values for a wide

range of carbohydrate containing foods have been compiled [37, 41], which can be grouped into low-GI (GI ≤ 55), moderate-GI (GI > 55 to ≤ 70) and high-GI foods (GI > 70) [37].

To also take the carbohydrate content of a food into account, which affects the absolute glucose response together with the GI, the concept of the **GL** was introduced by Salmeron et al. [42, 43]. The GL represents the amount of carbohydrates adjusted for its glycemic potency and is defined as the amount of available carbohydrates multiplied by their respective GI.

In epidemiological studies, not the GI or GL values of a single food are of interest but the dietary GI and GL to estimate the overall glycemic effect of the diet. For this purpose, GI assignments need to be made for every carbohydrate containing food recorded by the study participants. However, dietary assessment methods vary in their precision: Mean GI values are assigned to food groups in food frequency questionnaires (FFQ), while in the case of weighed dietary records a more precise assignment can be made. The carbohydrate content (in grams) of each food consumed is then multiplied by the food's GI to obtain its GL. The sum of these GL values for each subject divided by 100 corresponds to the total daily GL. The overall dietary GI is obtained by dividing the total daily GL by the total daily carbohydrate intake multiplied by 100.

2.1.4 Dietary Insulin Index and Insulin Load

The blood glucose response after consumption of a food or meal is not always proportional to the insulin response. Yet, the insulinogenic effect of foods is commonly estimated by its GL which takes both carbohydrate content and GI into account [44]. Indeed, according to a recent Australian study, the dietary GL was shown to be the best predictor of postprandial insulin response in 121 test foods and 13 meals [45], but it only yields an indirect estimate. However, protein – especially when consumed together with carbohydrates, also leads to increases in insulin responses while at the same time reducing glycemia [46]. Furthermore, fat consumed with carbohydrates decreases postprandial glycemia but not insulinemia [47, 48]. As GI measurement cannot be conducted in foods with little or no carbohydrate content, the GI and thus the GL are not available as indicators for insulin responses to these foods.

In contrast, the concept of the **food insulin index (FII)** provides a classification of all foods according to their postprandial insulin response [44] and is defined as the 2-hour insulinemic response (AUC) following the intake of 1000 kJ of a food (2000 kJ for meals) relative to the 2-hour insulinemic response to glucose i.e. the reference food (FII = 100). Since the measure of comparison for the FII is energy as opposed to carbohydrate for the GI, also foods with no or only little carbohydrate content can be considered [49]. To date, 121 FII values, measured

at the Human Nutrition Unit School of Molecular and Microbial Bioscience at the University of Sydney, Australia in groups of 10 individuals, are published [45].

Similar to the dietary GI and GL, the overall dietary **insulin index** and dietary **insulin load** of the diet can be estimated based on the FII, which needs to be assigned to all foods primarily. For this assignment, the principal consideration is the dietary GL when matching a carbohydrate-rich food with a published FII, as it best predicts the FII [45, 49]. For foods low in carbohydrates, the protein content is additionally considered. The average dietary insulin load can be calculated by summing the product of FII, energy content and consumption frequency over all recorded food items [50]. The average dietary insulin index can then be calculated by dividing the insulin load by total energy intake [50]. While both insulin index and insulin load resemble the dietary insulin demand they still have slightly different interpretation: The dietary insulin index is more a qualitative measure, which ranks foods according to their postprandial insulin response, whereas the dietary insulin load gives an insight of the quantity of the insulin demand of the diet.

2.2 Risk markers of type 2 diabetes mellitus and related health outcomes

T2D represents a growing public health problem with both incidence and prevalence rates as well as related comorbidities increasing rapidly – also in younger age groups [2, 51]. Knowledge on different risk markers, indicating an increased risk before a disease manifests, and moreover, insight into the interaction between IR or T2D and closely linked conditions such as obesity, chronic low-grade inflammation and hepatic steatosis is pivotal to better elaborate preventive approaches. Furthermore, it might be necessary to consider special circumstances in different stages of life such as physiological occurring IR during puberty or age related metabolical changes in older age groups [19, 52].

2.2.1 Type 2 diabetes mellitus

Definition and diagnosis

The term diabetes mellitus comprises heterogenic metabolic disturbances, all resulting in an hyperglycemic condition and sharing the underlying cause of either an impaired insulin secretion due to malfunction of the pancreatic beta cells, an impaired insulin effect on target cells or both [53]. There are three main types of diabetes: T1D, T2D, and gestational diabetes mellitus (GDM). Other specific types of diabetes mellitus also exist, such as maturity-onset diabetes of the young. However, their proportion is very small. The focus of this thesis is on T2D, formerly also known as non-insulin dependent diabetes or adult-onset diabetes, which accounts for 90-95% of all diabetes cases. While an absolute deficiency of

insulin secretion exists in T1D, T2D is caused by a resistance to insulin action accompanied by an inadequate compensatory insulin secretion [54].

Diagnosis of diabetes is traditionally based on fasting plasma blood glucose values either from single measurements or from the results of a 75g oral glucose tolerance test (OGTT) (see **Box 1**). HbA1c (glycosylated haemoglobin), while being already frequently used to monitor blood glucose control, was introduced as a further diagnostic criterion firstly in 2009, when an International expert committee came to the conclusion to recommend the use of HbA1c in diabetes diagnosis. The reasons for this were on the one hand the international standardized method for measuring HbA1c and on the other hand the growing evidence from epidemiological studies that the specifity of a diabetes diagnosis is sufficient for HbA1c values ≥ 6.5% [54].

Further criteria exist to define those patients who do not yet have a manifest diabetes but neither glucose levels considered normal and healthy – summarized under the term "intermediate hyperglycemia" (see **Box 1**).

Box 1: Diagnosic criteria for diabetes mellitus/intermediate hyperglycemia according to IDF/WHO [1]:

Diagnostic test: oral glucose tolerance test (OGTT)

Diabetes mellitus:

- Fasting plasma glucose ≥ 126 mg/dl (≥ 7.0 mmol/L) **or**
- 2-h plasma glucose* ≥ 200 mg/dl (≥11.1 mmol/L)

Impaired fasting glucose (IFG):

- Fasting plasma glucose 110 mg/dl to 125 mg/dl (6.1 - 6.9 mmol/L) **and (if measured)**
- 2-h plasma glucose* <140 mg/dl (<7.8 mmol/L)

Impaired glucose tolerance (IGT):

- Fasting glucose <126 mg/dl (<7.0 mmol/L) **and**
- 2-h plasma glucose* ≥140 mg/dl to <200 mg/dl (≥7.8 - <11.1 mmol/L)

* if 2-h plasma glucose is not measured, status is uncertain as diabetes or IGT cannot be excluded

However, a large number of individuals suffering from T2D or intermediate hyperglycemia remain undiagnosed and are themselves unaware of their health condition as hyperglycemia develops gradually with the absence of classical symptoms at earlier stages [54].

Several direct, indirect and surrogate measures exist to assess insulin sensitivity/resistance and beta cell function in humans: While the "hyperinsulinemic euglycemic glucose clamp technique," a direct measure of insulin sensitivity developed in 1979 [55] is still considered

the gold standard [56], this method can be regarded time-consuming, labor intensive, and expensive. A widely used indirect test to assess glucose tolerance in clinical practice is the oral glucose tolerance test (OGTT), which however does not provide information on insulin sensitivity/resistance per se [57]. Homeostasis model assessment (HOMA), introduced by Matthews et al. in 1985 [58], is a simple surrogate index derived from fasting steady-state glucose and insulin concentrations, which has been widely used especially in large-scale epidemiological studies to estimate both IR (HOMA-IR) and beta cell secretion (HOMA-β) [56, 57]. Other indices and risk markers exist displaying different advantages and limitations and therefore the decision which marker to use needs to be based on the underlying study conditions [57]. The fact that T2D is often paralleled by hepatic steatosis, led to the recognition of the liver enzymes alanine aminotransferase (ALT) and gamma glutamyltransferase (GGT) – commonly used as surrogate parameters for hepatic fat content – as risk markers for T2D [59-63]. The link between systemic and hepatic IR will be described in more detail in Chapter 2.2.4.

Epidemiology

According to the 2013 update of the 6th edition of the International Diabetes Federation (IDF) Diabetes Atlas, worldwide 382 million people between 20 and 79 years of age live with diabetes, which implies a prevalence of 8.3% [64]. Of interest, the IDF estimates that globally half of those who have diabetes are unaware of their health status – 85% of these live in low- and middle-income countries. The proportion of undiagnosed diabetes cases is estimated by using representative population-based studies which report the percentage of these cases [2]. Furthermore, worldwide, 6.9% of the adult population (316 million people), are estimated to have IGT [64]. In Europe, 56.3 million people are estimated to have diabetes, of which 20.1 million are undiagnosed, and further 60.6 million adults are estimated to have IGT. The diabetes prevalence in Europe amounts to 6.8%. Worldwide, this is the second lowest prevalence following Africa – however, over one-quarter of the global diabetes health care costs are spent in this continent [64]. For Germany, data from the "German Health Interview and Examination Survey for Adults" (DEGS1) conducted between 2008 and 2011 indicated a prevalence of 7.2% among adults aged between 18 and 79 years with an additional 2.1% of undiagnosed cases [65]. Compared to data from the German National Health Interview and Examination Survey 1998 the diabetes prevalence increased by 38% [65]. According to IDF data, 1.6 million people suffer from diabetes in Australia – besides Germany another country under study in this thesis – implying a prevalence of 7.8% [64]. Of note, Australia has one of the highest prevalence of abnormal glucose tolerance among developed countries [66]. IDF figures furthermore show that the global number of diabetes cases is expected to rise up to 552 million – implying a prevalence of 9.9% – by 2030. In Europe and Australia, the

prognostic prevalence for 2030 is 7.1% and 9.3%, respectively [2]. Notably, this global increase in diabetes cases is inversely related to income status and is expected to parallel the increasing adult population [2]. Besides giving estimates for diabetes prevalence, the Global Burden of Disease project additionally published estimates of mean glucose levels. Using a complex, multi-level approach, they came up with a global age-standardized mean fasting plasma glucose of 5.50 mmol/L for men and 5.42 mmol/L for women, implicating a rise of 0.07 mmol/L and 0.09 mmol/L per decade, respectively [67].

Compared to adults, it is more difficult to give overall T2D prevalence numbers for children and adolescents. A recent systematic review, which aimed to summarize available global incidence and prevalence rates for children and adolescents, came to the conclusion that there is a substantial variation among countries, age categories and ethnic groups. This variation in incidence and prevalence data is largely caused by population characteristics and methodical dissimilarities. According to this review, worldwide diabetes incidence rates in children and adolescents ranged from 0 to 330 per 100,000 person years and the observed prevalence ranged from 0% to 5.3% among included studies [51]. A recent publication based on data from the National Health and Nutrition Examination Survey (NHANES) (1999 to 2010) showed a prevalence of 0.48% for T1D and of 0.36% for T2D among 12 to 19 year old US adolescents. Undiagnosed T2D accounted for 34% of the T2D cases [68]. With a total diabetes prevalence of 0.84% (T1D and T2D together) this data suggests an increase compared to data from NHANES III (1988 to 1994), when a total prevalence of 0.41% was estimated. Even if the data has to be evaluated with caution because of the low precision of diabetes estimates [68], the proportion of T2D cases of the combined diabetes prevalence appeared to increase from 31% to 43% comparing data from NHANES III with the recent survey [68, 69]. Furthermore, the consideration of early disturbances in carbohydrate metabolism is equally important, as this increases the risk for later T2D: Alarmingly, a population based US study identified 15.7% of the adolescents to have IR as being defined by fasting insulin levels [70]. For Germany, a prevalence of 0.14% for T1D and T2D was observed in the German Health Interview and Examination Survey for Children and Adolescents (KiGGS), analyzing data from 2003 to 2006 of children and adolescents up to the age of 17 [71]. In a cross-sectional German survey it was noted that 2.5% of the included 721 school-leaving students (mean age: 15.5 years) had impaired fasting glucose and impaired glucose tolerance or T2D [72]. Similarly, data from the diabetes registry of Baden-Württemberg indicated a prevalence of 2.3 per 100.000 among children and adolescents aged 0 to 20 years [73]. Hence, compared to the US, T2D prevalence rates are much lower in Germany, but nonetheless need to be taken serious in terms of public health interventions.

Risk factors

There are several risk factors which are associated with T2D, including family history of diabetes, overweight, unhealthy diet, physical inactivity, increasing age, high blood pressure, ethnicity, IGT, or the previous development of GDM. According to IDF and the Global Burden of Disease project, the increase in diabetes prevalence is mostly caused by a rising incidence due to demographic changes like aging of the population, by increases in risk factors such as obesity and sedentary lifestyle being observed more frequently, and by a rising lifespan of patients with diabetes due to better health care options [2]. According to the Robert-Koch Institute which conducted the German surveys, aging of the population accounts for 14% of the increase seen in Germany over the last 13 years. With data from the DEGS1, it could also be shown that the diabetes prevalence is increasing with age, body mass index (BMI) group, and a lower socioeconomic status [65].

Consequences

If not treated at an early stage, long-term damage, dysfunction, and failure of different organs due to chronic hyperglycemia may occur in patients with diabetes. These complications include retinopathy with potential loss of vision, nephropathy causing renal failure, peripheral neuropathy being associated with foot ulcers or amputations, and autonomic neuropathy leading to gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Moreover, diabetes is associated with an increased risk for development of hypertension or abnormalities in the lipid metabolism as well as cardiovascular disease (CVD). Of note, recent reports suggest, that T2D among adolescents – the so-called early onset T2D – requires particular attention, as it is more difficult to control compared to the later-onset form and also displays a more aggressive disease phenotype, i.e. a more rapid decrease in beta cell function [74-76].

Data from the IDF Diabetes Atlas, stating that 5.1 million people died (accounting for 8.4% of the global all-cause mortality among adults) and health care costs of 548 billion USD arose in 2013 due to diabetes, underlines the great public health impact of diabetes and its associated comorbidities [64].

Insulin resistance

An impaired insulin secretion and/or IR represent the underlying pathology of T2D. Both conditions are closely linked. However, to fully understand the linkage between T2D and pathophysiological relevant phenotypes such as obesity, chronic low-grade inflammation and hepatic fat accumulation, an understanding of the metabolic effects of IR, also on a molecular level, is essential.

Insulin is an anabolic hormone, secreted by the beta cells of the Langerhans islets in the pancreas, which is essential for tissue development, growth, and glucose homeostasis.

Insulin secretion is increased in response to enhanced circulating levels of glucose, amino acids, and to a small degree also of free fatty acids. The main targets of insulin can be considered adipose tissue, muscle cells and central organs such as the liver. For maintenance of normoglycemia, insulin stimulates glucose uptake in the skeletal muscle and adipose tissue, glycogen storage in the muscle and liver, and inhibits gluconeogenesis in the liver and kidney as well as glycogenolysis in the liver. Furthermore, insulin enhances triglyceride (TG) synthesis in the liver and adipose tissue and inhibits lipolysis. Regarding protein metabolism, protein synthesis is promoted and its degradation suppressed in muscle and liver cells [77, 78] (**Figure 2**).

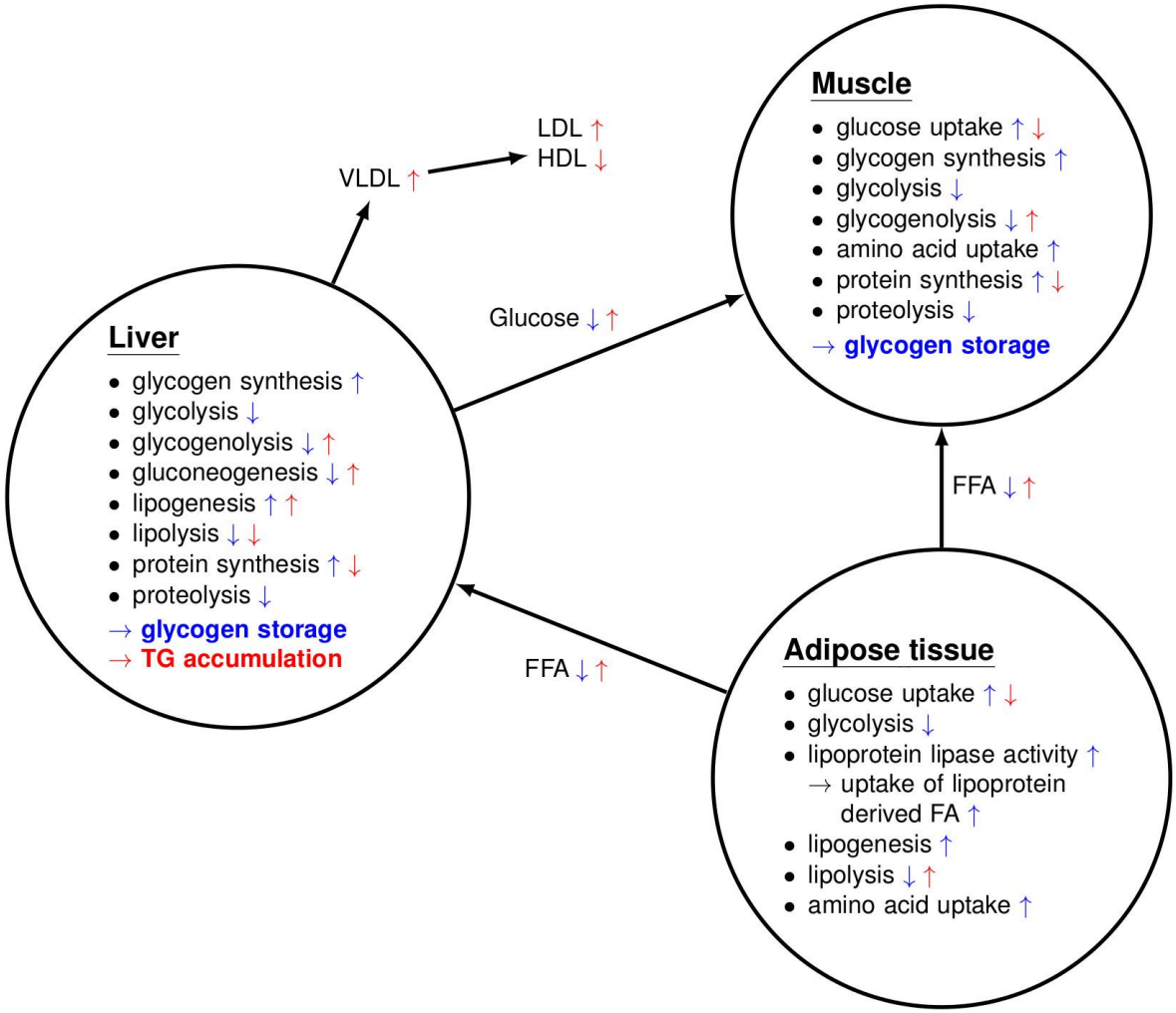

Figure 2: Physiological and impaired insulin actions and their main metabolic consequences (adapted from [79, 80]).
Blue arrows/font refer to the healthy state and red arrows/font to the insulin resistant.
Abbreviations: FFA, free fatty acids; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein

Under conditions of IR, these diverse signaling pathways are disturbed, leading to systemic hyperglycemia, which in turn increases the strain on beta cells [78]. The insulin resistant

state is moreover closely linked to further metabolic perturbations such as hyperlipidemia [79] (**Figure 2**).

On a molecular level, insulin affects responsive cells by binding to its receptor on the cell surface, which leads to downstream signaling events. The insulin receptor is a transmembrane $\alpha_2\beta_2$heterodimer, whose α subunits are extracellular and entail insulin binding elements, while the linked β units interfuse the plasma membrane. The receptor belongs to the tyrosine kinase receptors. Under physiological, insulin-sensitive conditions, insulin binding to α subunits causes the β subunits to phosphorylate themselves, which activates the catalytic activity of the receptor. The activated intrinsic kinase then phosphorylates several proximal substrates such as members of the insulin receptor substrate family, leading to the activation of two major signaling pathways: First, the Ras-mitogen-activated Protein Kinase (MAP-Kinase) pathway, leading to the completion of functions related to cell growth and gene expression and second, the Phosphatidylinositol-3-Kinase (PI3K)-AKT/protein kinase B (PKB) pathway, which modulates most metabolic functions of insulin such as glucose transport and synthesis of glycogen, proteins, and lipids [78, 81, 82].

These physiological insulin signaling pathways can be disturbed through e.g. alterations in insulin receptor expression, ligand binding, phosphorylation, kinase activity, or impaired proximal signaling, hence causing IR [78]. A variety of counter-regulatory pathways exists which inhibit insulin-signaling, leading to diminished insulin action in target tissues. There are two main pathways for inhibition of insulin-signaling, involving the cJun N-termial kinase (JNK) and the inhibitor of nuclear factor κB (NFκB) kinase β (IKKβ) [15]. These two kinases induce IR through different mechanisms: JNK phosphorylates serine residues on IRS-1, being thereby counter-regularly to the physiological tyrosin kinase cascade. IKKβ induces IR through transcriptional activation of NFκB, which in turn promotes the expression of numerous target genes whose products, among them are different cytokines, transcription factors and surface proteins, induce IR [14].

The described disturbances, leading to counter-regulatory molecular pathways, can be induced in different conditions and by various signals such as inflammation, excess circulating saturated fatty acids, necrotic residues (cell-extrinsic), as well as mitochondrial dysfunction, oxidative stress, or membrane/endoplasmatic reticulum (ER) stress (cell-intrinsic) caused by either hyperglycemia or hyperlipidemia [15]. The particular role of fatty acids and inflammatory markers in IR will be described in chapters 2.2.2 and 2.2.3, respectively.

2.2.2 Obesity

Obesity is considered a major risk factor for IR and T2D – according to WHO estimates, 44% of the diabetes burden is attributable to overweight and obesity [83]. Epidemiological evidence suggests a strong association between obesity, particularly abdominal adiposity, and an elevated risk for T2D as well as CVD [84-87]. Overweight and obesity are defined as abnormal or excessive body fat accumulation that may have detrimental health effects [83]. Different indices exist to assess overweight and obesity: the body mass index (BMI) can be considered the most common, although crude indicator among adults and is defined as weight/height^2 (kg/m^2). It classifies an individual as overweight if having a BMI >25 kg/m^2 and as obese if the BMI is >30kg/m^2 [83]. However, it has been criticized that the BMI does not distinguish between weight associated with muscle or fat mass [88-90] nor does it allow a relation between BMI and body fat, as this varies according to build and proportion [83]. Thus, additional consideration of age, sex, ethnicity, physical activity, and body fat distribution is necessary for a correct use and interpretation of BMI values. Compared to the gold-standard method of computer tomography as well as the equally accurate method magnetic resonance imaging (MRI), both waist circumference or the waist-to-hip circumference ratio provide reasonable validity to assess abdominal adiposity [91, 92]. Besides BMI as a surrogate marker of body fatness, a distinction between fat mass and fat-free mass is often suggested – either using percentage body fat (%BF), fat mass index (FMI) (%BF / height^2), or fat-free mass index (FFMI) ([weight - weight · %BF] / height^2) [90, 93-95]. It is not clear what the gold standard method for measurement of %BF is, as even the best methods used are indirect [92]. Historically, densitometry has served as a standard, but due to its impracticability in epidemiological studies, it has often been replaced by dual energy x-ray absorptiometry (DEXA). DEXA can be considered a practicable method, providing reproducible measurements of the different body components, i.e. fat mass, fat-free mass, and bone-mineral mass. However, the x-ray and scanning unit is expensive and both trained radiology personnel as well as software is needed for proper usage [92]. In contrast, skinfold measurements are low in costs, which is one reason why this technique is probably the most widely used method in epidemiological studies. However, it is essential to have good trained personnel to achieve valid measurements. While it provides a direct measure of body fat, it has to be noted that not all fat is accessible to the calipers, e.g. intraabdominal fat [92]. To define excess body fat, %BF reference values have been published [96, 97].

According to the WHO, since 1980, obesity prevalence has nearly doubled worldwide [83]. Global estimates from the International Association for the Study of Obesity (IASO) and the International Obesity Task Force (IOTF) state that 2010 approximately one billion people were overweight and 475 million people were obese [98]. For Europe, IASO/IOTF estimate

that 60% of the adult population (260 million) were overweight or obese in 2010 [98]. According to DEGS1, 53.0% of women and 67.1% of men over the age of 18 years are overweight and 23.9%, and 23.3%, respectively, are obese. Thereby, prevalence increases with age group [99]. For Australia, the Australian Health Survey 2011-2012 revealed that 63.4% of Australians aged 18 years and older were overweight or obese, with a prevalence of 75% among those aged 65 to 74 years [100].

For children and adolescents, classification of overweight and obesity is done by using sex- and age-specific BMI percentiles as the BMI varies during childhood growth [101]. These percentiles are available on a national and international basis. In Germany, reference curves from Kromeyer-Hauschild, which are based on data from 17 regional studies conducted between 1985 and 1999 [102], and the newer percentiles from the KiGGS study conducted between 2003 and 2006 [103], exist. Compared to Kromeyer-Hauschild, the KiGGS data already includes 50% more overweight children, reflecting the more recent BMI distribution [104]. Hence, the Kromeyer-Hauschild percentiles should be used for classification of overweight and obesity, which is defined as a BMI above the 90^{th} and 97^{th} percentile, respectively [102]. For an international comparison, the IOTF has developed an international standard, which provides age- and sex-specific cut-offs corresponding to an adult BMI of 25 and 30 kg/m^2 at 18 years of age and is based on six nationally representative surveys from Brazil, Great Britain, Hong Kong, the Netherlands, Singapore, and the US [105]. To adjust for changes occurring with normal growth, BMI z-scores, also called standard deviation scores (SDS), are often calculated using population based BMI reference data. These scores express in units of standard deviations (SD) how far away a child's BMI lies from the mean BMI value for sex and age [106]. For definition of excess body fat in children and adolescents, reference percentile curves were developed with data from 1985 British Caucasian children aged 5 to 18 years, where a %BF above the 85^{th} and 95^{th} percentile is defined as overfat and obese, respectively [107].

Regarding prevalence data for children, IASO/IOFT estimate that worldwide up to 200 million school aged children are overweight with 40-50 million of them being obese, and that in Europe 20% of all school aged children (12 million) are overweight or obese [98]. For Germany, data from the KiGGS study, using the definition for overweight and obesity by Kromeyer-Hauschild [102], revealed that 15% and 6% of all children aged three to 17 years were overweight or obese, respectively. Regarding adolescents, 15.4%, 18.4%, and 17.1% of the seven to ten, eleven to 13, and 14 to 17 year olds, respectively, were overweight or obese. Comparing this prevalence to the reference population from the 1980ies and 1990ies, overweight and obesity in the youth has risen by 50% in Germany, with the highest increases among the 14 to 17 year olds [104].

The underlying pathology of obesity is either an enlargement of fat cells or an increase in their number [108]. However, the former condition is associated with metabolic dysregulation [109]: Large adipocytes secrete more fatty acids and additionally different types of cytokines, chemokines and hormone-like factors – the so-called adipokines – which are able to modulate IR as well as inflammatory, thrombotic, and coagulation systems [84, 108]. Elevated levels of free fatty acids contribute essentially to IR by affecting the primary targets of insulin: In the liver, production of glucose and triglycerides and secretion of very low density lipoproteins (VLDL) is increased. In the muscle, excess free fatty acids reduce insulin sensitivity through the inhibition of insulin-stimulated glucose uptake. The resulting increase in circulating levels of glucose and to some extent free fatty acids induces an elevated insulin secretion from the pancreatic beta cells, which in turn leads to hyperinsulinemia. (see **Figure 2** in Chapter 2.2.1). Regarding the role of insulin in lipid metabolism, it is of interest that adipose tissue lipolysis is the most sensitive pathway with regard to its action. This implies that under the condition of IR, more free fatty acids are produced through increased lipolysis of stored TG in adipose tissue, which in turn further inhibit the antilipolytic effect of insulin [79, 84].

On a molecular level, exposure of cells to increased levels of saturated fatty acids can impair proximal signaling of the insulin receptor through activation of JNK and IKKβ [78, 110, 111].

Besides free fatty acids, some of the adipokines secreted by the adipose tissue also affect insulin action: Pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin 6 (Il-6) constitute a major role in this process [79]. These adipokines will be discussed in the following section.

2.2.3 Chronic low-grade Inflammation

Chronic low-grade inflammation is considered to play a crucial role in the pathogenesis of T2D [112] and CVD [113] (see **Table 1** for an overview of the role of selected immune mediators in T2D/CVD). Additionally, other diseases such as dementia [114], depressive disorders [115], certain types of cancer [116] and finally, an overall higher risk of all-cause mortality in old age [117] have been linked to a subclinical inflammatory state. Both obesity [118] and T2D [112] are now considered inflammatory diseases.

Table 1: Selected pro- and anti-inflammatory immune mediators and their potential role in type 2 diabetes mellitus and cardiovascular disease (adapted from [118-120]).

Immune mediator	Possible role in T2D/CVD
Primary pro-inflammatory cytokines (e.g. IL-1, TNF-α)	• mediate the attraction and migration of inflammatory cells into vascular tissue • induce expression of cellular adhesion molecules • induce "messenger cytokines" • TNF-α promotes IR, IL-1 regulates insulin secretion
"Messenger" cytokine IL-6	• principal procoagulant cytokine • can increase plasma concentrations of fibrinogen, PAI-1 and CRP • promotes IR
CRP	• amplifies inflammatory and procoagulant responses • predisposes to a state of hypercoagulability • downregulates nitric oxide, a vasoactive peptide that helps maintain vascular tone
PAI-1	• reduces plasma fibrinolysis • promotes atherothrombosis
Adiponectin	• anti-inflammatory adipose tissue hormone • promotes insulin sensitivity • stimulates fatty acid oxidation

Abbreviations: CRP, c-reactive protein; IL, interleukin; PAI-1, Plasmingen activator inhibitor type 1; TNF, tumor necrosis factor

Particularly the recognition of adipose tissue as an endocrine organ, secreting adipokines such as cytokines, has given further insight into the relation between obesity and IR [121]. The first clear link between obesity, diabetes, and chronic low-grade inflammation was provided by scientific findings related to the pro-inflammatory cytokine TNF-α: It was shown that this cytokine is overexpressed in the adipose tissue of obese mice, and furthermore, that the neutralization of TNF-α led to a decrease in IR [122, 123]. From then on, several other pro-inflammatory cytokines and mediators such as IL-6 have also been found to be overexpressed in adipose tissue in obese mice and humans [118] and their elevated circulating levels have been linked to obesity and incident T2D [124, 125]. In contrast, decreased circulating levels of the anti-inflammatory adipokine adiponectin have also been shown to correlate with greater adipocyte size [126]. Furthermore, it has been observed that macrophages accumulate in adipose tissue in excess [127, 128], probably driven by necrosis of hypertrophic adipocytes [129]. These macrophages are characterized by expression of pro-inflammatory cytokines such as TNF-α and IL-6, and reactive nitrogen species such as nitric oxide [15] and are thought to play an important role in influencing adipocyte biology and systemic IR [15, 130].

In addition to the induction through pro-inflammatory cytokines and macrophages, inflammatory signaling pathways can also be activated intracellularly through metabolic

stress [118]: the functional capacity of the ER can be overburdened in obesity causing ER stress. Particular in beta cells, an increased flux of proteins through the ER caused by elevated insulin production can lead to ER stress [131]. Moreover, several cell stressors, especially increased glucose metabolism, are associated with an increase in mitochondrial production of reactive oxygen species [132]. Besides activation of increased inflammatory pathways, oxidative stress can also impair insulin secretion, as beta cells are particularly vulnerable to oxidative stress because of their low levels of antioxidative enzymes [18, 112].

Of note, insulin displays anti-inflammatory actions in insulin-sensitive humans. However, a possible compensation of oxidative stress by insulin is lost in insulin-resistant individuals due to prolonged pro-inflammatory conditions [133]. This pro-inflammatory state is considered a primary cause of obesity-linked IR [118] and adipose tissue inflammation might furthermore be the main distinction between metabolically unhealthy and metabolically healthy obese individuals [134], perhaps explaining the increased CVD risk among some obese individuals [135].

Pro-inflammatory cytokines are able to inhibit insulin action in adipocytes via autocrine and paracrine signaling and are furthermore able to induce systemic IR via endocrine signals. Hence, supplementary to excess levels of free fatty acids, the circulating cytokines add further to the insulin resistant state [79, 118]. Thereby, as described for the other influencing factors, the inhibition of downstream signaling of the insulin receptor is the primary mechanism leading to inflammation induced IR [14, 130, 136].

2.2.4 Hepatic insulin resistance and nonalcoholic fatty liver disease

Besides adipose tissue, muscle cells, and pancreas, the liver is a major target of insulin action and particularly affected by obesity [14].

Nonalcoholic fatty liver disease (NAFLD) – a term used to describe a spectrum of hepatic diseases ranging from hepatic steatosis to cirrhosis, has received rising scientific attention as the hepatic manifestation of the metabolic syndrome [137]. In fact, NAFLD often accompanies abdominal adiposity [14] and hepatic fat accumulation is frequently observed in patients with IR or T2D [16].

The prevalence of NAFLD is increasing worldwide and is estimated to be 30% in adults and up to 10% in children and adolescents in developed countries [138-140]. Of particular concern is the rising prevalence of hepatic steatosis observed in children, which entails long-term detrimental health consequences [141]. Clinical diagnosis refers to the total liver fat content: If levels between 5 and 10% are exceeded or, alternatively, if cytoplasmatic TG droplets are present in more than 5% of hepatocytes and significant alcohol consumption can

be precluded, a NAFLD is diagnosed. If, additionally to fat accumulation, hepatocyte injury, infiltration of pro-inflammatory markers or fibrosis exists, this condition is termed nonalcoholic steatohepatitis (NASH) [142]. Hence, inflammatory processes are tightly linked to progression of fatty liver disease [14]. The gold standard for diagnosis of NAFLD is a liver biopsy. However, because of its invasive character, it is only conducted if clinical indications are present and thus inapplicable in epidemiological studies. Non-invasively, liver fat content can be estimated through different medical imaging techniques, e.g. sonography or magnetic resonance spectroscopy [142]. Furthermore, elevated liver enzymes can be used to assess liver health: If hepatocyte injury occurs, e.g. through impaired mitochondrial function due to oxidative stress, ALT and GGT are subsequently increasingly released [143]. Generally, it has to be noted that liver steatosis is often an asymptomatic disease and the majority of patients do not show any abnormalities during laboratory investigations [142].

The association between obesity, IR and hepatic steatosis can be largely explained by insulin actions and inflammatory processes: Hepatic steatosis arises if an imbalance exists between TG synthesis and removal. The fatty acids required for TG synthesis are thereby derived from three different sources: from the diet, de-novo lipogenesis, and adipose tissue [138]. In the condition of IR and obesity, increased levels of free fatty acids are delivered from adipose tissue, which are taken up by the liver and stored as TG. With the development of hepatic IR, insulin is no longer able to inhibit gluconeogenesis, leading to increased glucose levels, being stored as TG. However, due to a higher insulin sensitivity of the signaling molecule for β-oxidation (Foxa2), the inhibiting effect of insulin is retained intrahepatically during IR [144], leading to both hyperglycemia and hypertriglyceridemia [145]. Additionally, increased inflammatory gene expression has been observed in the liver with increasing adiposity [146], having two possible causes: First, lipid accumulation in the hepatocytes might be associated with a subacute inflammatory response similar to adipose tissue inflammation. Second, pro-inflammatory substances might reach the liver through the portal vein, where they initiate hepatic inflammation, activate Kupffer cells – the resident hepatic macrophages – and participate further in the development of IR [14, 146]. The fact that adipokines produced by visceral fat are – in contrast to peripheral fat cells – directly transported to the liver through portal circulation, highlights again the special role of visceral adipose tissue in metabolic disease development. Indeed, NAFLD often accompanies abdominal obesity [14].

2.2.5 Relevance in different stages of life

A persons' health status is traditionally thought to be the result of a complex interaction between genetical and environmental factors. The latter ones include a wide range of

influences, including diet, activity status as well as the socioeconomic and ecological environment. In the 1990ies, Barker et al. added an additional feature to this interaction: The concept of programming, which has been particularly extensively described for the perinatal period. Barkers concept originates from the observation that low birth weight was associated with later occurrence of T2D or CVD [147]. This simple epidemiological comparison was later extended to the whole period of early life, including both the perinatal phase as well as the time until the age of two years and onwards [148]. The concept of programming is based on the idea that lifetime consequences can result from stimuli or insights occurring during critical or sensitive periods early in life [149]. Subsequently, the characterization of specific critical periods for a later development of obesity or its complications, including T2D, have been extended to the period of adiposity rebound and adolescence [150].

However, not only in terms of potential programming effects it is of interest to consider e.g. diet and health outcome interactions during specific stages in life. This thesis builds on the assumption that during a lifetime, dietary habits and also activity levels may change: They are possibly firstly shaped during childhood and adolescence, adhered to during adulthood, but then maybe need to be modified again during the process of aging to adapt to changes in everyday life e.g. caused by retirement or impaired health status.

Adolescence

Puberty can be regarded a time frame which is of particular interest for carbohydrate metabolism. Besides pregnancy [151], puberty is the only period in life where a physiological IR occurs [19]. This decrease in insulin sensitivity, occurring during mid-puberty, is not related to body fat content, but leads to increased levels of fasting glucose and insulin. However, compared to the “pathological” IR, beta cell function is conserved, since the cells do not increase insulin secretion in the same proportion, leading to disproportionally low acute insulin responses. At the end of puberty, insulin sensitivity recovers again to pre-pubertal levels [19].

Additionally to changes in insulin sensitivity, puberty is also characterized by changes in levels of Insulin-like growth factor 1, growth hormones and sex steroids [152]. The occurring hormonal changes impact, among others, on appetite, satiety, and fat distribution and may predispose adolescents to later overweight and other related health outcomes. For instance, also adiponectin levels decrease and as this cytokine is involved in insulin metabolism and disposes anti-inflammatory effects, deviations in these changes might nevertheless have an impact on later health [153, 154].

Furthermore, puberty is characterized by behavioral changes in diet and activity levels: It has been frequently reported that on average adolescents decrease their physical activity and

prefer inactive pastimes such as watching TV [153]. Also, dietary quality is often reduced in puberty, particularly in regard to carbohydrates: generally, adolescents consume more added sugars, particularly as soft drinks, fast foods, and less fiber-rich products compared to younger children [155-157]. Importantly, these changes cannot be dismissed as short-term behavioral exceptions but instead may persist in the long-term [158, 159], underlining the importance to address this period and regard it as pivotal for public health approaches. Moreover, even if healthy normal-weight adolescents are able to adapt to these physiological and behavioral changes, this might not apply to those who enter puberty with excess body weight or emerging IR, exposing them to a higher risk for later development of T2D and CVD [160].

Older age

As stated by the IDF and Global Burden of Disease project, two of the factors leading to an increase in diabetes prevalence are the rising incidence due to demographic changes such as aging and the rising lifespan of patients with T2D due to better health care options [2]. Therefore, improved health care not only impacts on diabetes, but also prognosis of numerous other diseases, hence accounting for exactly these observed demographic changes [161].

An increased age is among the risk factors for several diseases, including T2D and NAFLD [2, 162]. It was shown that a state of chronic low-grade inflammation is involved in several processes of aging [52]. Hence, investigation of diet disease associations is not only of interest in older age groups because of higher disease prevalence, but also because metabolic adaptations might occur, which need to be specifically addressed. Finally, primary and secondary prevention approaches fitted for older age groups are essential to develop, since these age groups constitute a growing proportion of our population.

2.3 Evidence linking carbohydrate quality to risk markers of type 2 diabetes mellitus and related health outcomes

Carbohydrate quality plays a crucial role in the development of T2D and related diseases [5]. In this chapter, the evidence for the relation between dietary GI/GL, dietary fiber and whole grain intake and risk markers of T2D, obesity, low-grade inflammation, and hepatic steatosis will be discussed. For this purpose, each part will start with a short overview of the mechanisms proposed for the presented association.

2.3.1 Carbohydrate quality and risk markers for type 2 diabetes mellitus

Postprandial hyperglycemia and -insulinemia are tightly linked to diabetes pathogenesis. Particularly different aspects of carbohydrate quality have been shown to impact on glucose homeostasis [5]. While a lower blood glucose response is commonly attributed to a fiber- and whole grain-rich diet, the dietary GI is a somewhat newer and more precise parameter for estimating postprandial glucose excursions (see Chapter 2.1.3). For both aspects, relevant mechanisms and available evidence for adults as well as children/adolescents, will be presented – the latter in more detail, as this thesis focuses on the relevance in puberty.

Dietary glycemic index and glycemic load

Rapid increases in postprandial blood glucose levels in response to consumption of high-GI meals stimulate insulin release from beta cells, while the release of counter-regulatory hormones such as glucagon is suppressed. Of note, the incremental area under the blood glucose curve following a high-GI meal can be at least twice as high compared to a low-GI meal although containing the same nutrients and energy [13]. Hyperinsulinemia, caused by postprandial hyperglycemia, may in turn induce downregulation of the insulin-receptor which results in IR [163, 164]. This is considered to eventually lead to a cycle of compensatory hyperinsulinemia and IR, enhancing the strain on beta cells. Additionally, IR can be caused by counter-regulatory hormone secretion as well as increased levels of free fatty acids in the late postprandial phase. Elevations in blood glucose and free fatty acid levels can furthermore impair beta cell function – conditions which have been termed “glucotoxicity” and “lipotoxicity” [13]. It could be shown that healthy non-obese children are capable to increase insulin sensitivity of their peripheral tissues and thus adapt to postprandial glycemia [165]. However, obese adolescents, who are often less insulin sensitive and not able to increase their insulin sensitivity, have to increase their insulin secretion to achieve a comparable decrease in glucose levels [166]. While beta cell mass is able to compensate changes in metabolic load in most healthy individuals, regular consumption of a high-GI meal can worsen IR and contribute to an overwhelming metabolic load in susceptible individuals. The beta cell mass may eventually fail to compensate for IR caused by a possible increased beta cell apoptosis and T2D develops [5, 17, 18] (**Figure 3**). Another point to be mentioned in relation to dietary GI and diabetes risk is the “second meal effect,” which describes an improved carbohydrate tolerance approximately four to five or ten to twelve hours after a previous meal. This beneficial effect could be shown for low-GI carbohydrates such as lentils, barley and oats, and is mainly described for low-GI whole grain foods [167-169]. Thereby, the low-GI feature appears to particularly impact on the shorter term period, for example a low-GI breakfast impacts on the glucose response at lunch [31, 170].

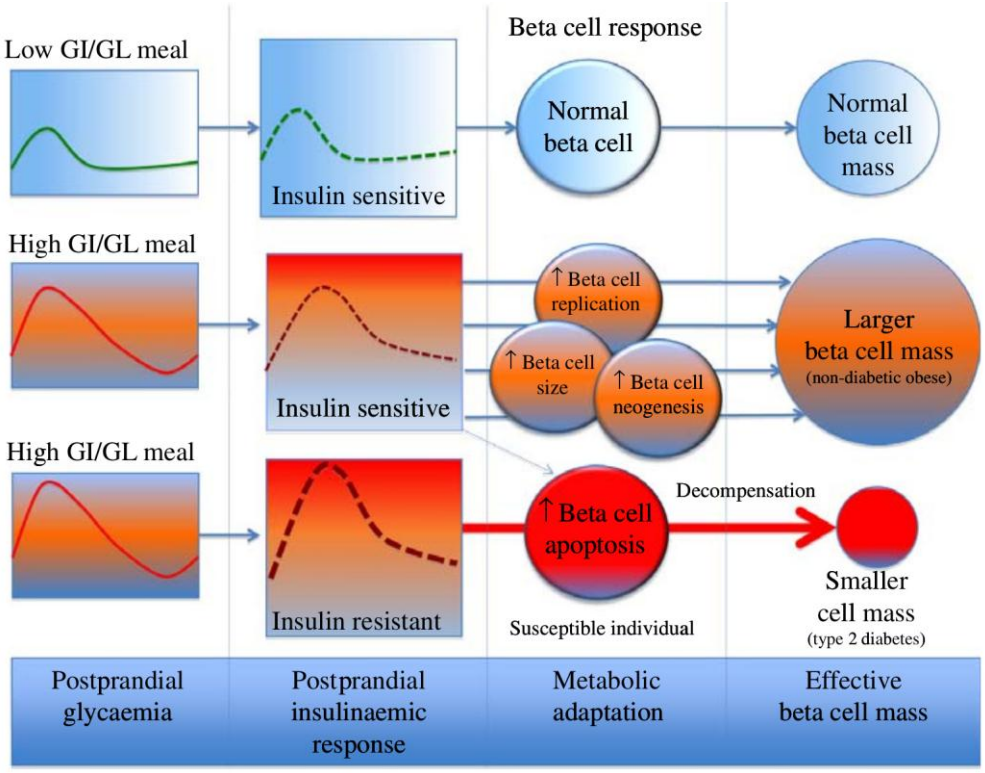

Figure 3: Effect of a high-GI meal on the development of type 2 diabetes mellitus, from [5].

There exist five meta-analyses of prospective cohort studies conducted on **adults** on the relevance of dietary GI/GL for T2D development [171-175]. All of them consistently show a protective effect of a low dietary GI/GL on later diabetes risk. Additionally, meta-analyses demonstrated a link between higher dietary GI/GL and an increased CVD risk, a common consequence of T2D [171, 176-179]. Furthermore, two recently published meta-analyses showed for the first time a dose-response relationship between dietary GI/GL and T2D risk: For a 100-g increment in dietary GL, the risk for T2D increased by 45% in the meta-analysis by Livesey et al.[175]. In the meta-analysis by Greenwood et al., a 5-unit increase in GI and a 20-unit increase in GL was associated with an 8% and 3% increased T2D risk, respectively [173]. However, the evidence-based dietary guideline “carbohydrate intake and prevention of nutrition-related diseases,” published by the DGE, only rated the evidence for the association between dietary GI/GL and T2D as possible (evidence grades: inconclusive, possible, probable, and convincing) [23]. This decision was explained by the heterogeneity observed among published studies. However, three of the meta-analyses were published [172, 173, 175] after the guideline publication, and one of them explained the sources of heterogeneity among the included studies [175]. This indicates that the conclusion on evidence rating may need to be revised in an updated version.

Regarding evidence for **children or adolescents**, there do not exist studies with “hard endpoints” such as manifest T2D and there is only one **observational study** assessing the

relevance of dietary GI/GL for HOMA-IR as a marker of IR [180] and thus a risk marker for disease development. For the present literature overview, HOMA-IR as well as liver enzymes (ALT and GGT) were chosen as endpoints since the analyses included in this thesis also focused on these parameters as risk markers for T2D (see Chapter 3 and OA2, Appendix 2). In the Danish part of the European Youth Heart Studies, no prospective association was found between the dietary GI at 8 to 10 years of age, assessed by a single 24h recall, and HOMA-IR values six years later in 233 girls and boys [180] (**Table 2**).

Table 2: Observational study in children and adolescents examining the relation between dietary GI and GL and IR (measured by HOMA-IR)[1]

First author, Year, Country	Population, recruitment, name of study[2]	Follow-up	Exposure: assessment method, average baseline values	Outcomes: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[3]
Kynde 2010 [180] Denmark	233 participants Danish part of the European Youth Heart Studies % female: 59% Ø age: 9.7 years Ø BMI: 17.1	6 years	24h recall interview supplemented with a qualitative food record from the same day mean (SD) dietary **GI**: girls: 60[4] (5), boys: 58 (5)[4]	Mean (SD) **HOMA-IR**: girls: 1.8 (1.0), boys: 1.7 (0.9)	age, sexual maturity (at baseline and follow-up), BMI, physical activity, mother`s education, school location, baseline HOMA z-scores	• no association between dietary GI at baseline and HOMA z-scores at follow-up (p for trend≥0.4) • cross-sectional analysis (including 651 adolescents) showed also no association (p for trend≥0.7)	~

Abbreviations: Ø average value, mean or median as provided in the original publication; GI, glycemic index; GL, glycemic load; SD, standard deviation
[1] Only relevant exposures and outcomes are presented
[2] n number refers to those in final analysis
[3] ↑: direct association with GI or GL, (↑): trend for association with GI or GL, ~: no association with GI or GL,↓: inverse association with GI or GL
[4] original values based on white bread reference and were converted to glucose reference by dividing by 1.4286 [37]

Intervention studies examining the effect of dietary GI or GL on IR markers show inconsistent results: Ebbeling et al. [181] compared the effect of an ad libitum reduced-GL diet with an energy restricted low-fat diet on body composition (see Chapter 2.3.2) and IR after 6 months of intervention and further 6 months of follow-up. In the low-GL diet, lower HOMA-IR values were observed as compared to the low-fat diet independently of the BMI of the 14 participating obese adolescents. Another study showing a beneficial effect of dietary GI was conducted in Italy and included 26 obese girls and boys aged 7 to 13 years. In this randomized intervention trial, a low-GI hypocaloric and a high-GI hypocaloric diet were compared in relation to different cardiometabolic parameters. HOMA-IR values were significantly reduced after 6 months in the low-GI group only. However, no between-group differences were provided [182]. In a pediatric weight management center in the US, a portion controlled diet was retrospectively compared to a healthy eating plan with and without assistance of a dietitian. According to the healthy eating plan, less than 10% of energy should be consumed from saturated fat and the GI should be below 50. ALT levels decreased significantly in the group with the healthy eating plan supported by a dietitian. However, again differences between groups were not provided. Furthermore, compliance or dietary intake was not assessed; hence the conclusions rely only on the advices given. In addition, it was not mentioned whether potential confounding factors were considered, further impairing the study results, particularly as the participants' families could elect their diet of choice by themselves rather than being randomized to one of the groups [183]. The two interventional trials from Mirza et al. [184] and Ramon-Krauel et al. [185] did not find effects of dietary GI or GL: In the first study, 64 obese Hispanic US children and adolescents took part, who were randomized to either a low-GL or a low-fat diet for two years. Dietary counseling was conducted in the first 12 weeks only. After two years, and also after in-between measurements every three months, no difference in HOMA-IR was observed between the two intervention groups [184]. Ramon-Krauel et al. conducted their study in girls and boys, who were obese similar to the participants in the presented intervention studies but were also diagnosed to have fatty liver. In this study, both a high-GL and a low-fat diet led to reductions in ALT concentrations and hepatic lipid content after six months, with no difference between the two groups. HOMA-IR reductions tended to be greater after the low-fat diet. However, baseline levels in this group were almost twice as high compared to the low-GL group [185] (**Table 3**).

Table 3: Intervention studies in children and adolescents examining the effect of dietary GI and GL on IR (measured by HOMA-IR and ALT)[1]

First author, Year, Country	Participant characteristics[2]	Study design	Dietary Intervention	Outcome: baseline levels and primary endpoints	Results	+/-[3]
Ebbeling 2003 [181] USA	14 obese participants % female: 69% Ø age: 16.1 years Ø BMI: 36	randomized controlled trial, 6 months intervention, 6 months follow-up	**reduced GL diet (RGL)** (n=7)**:** ad libitum, mean (SEM) GI: 53 (3), mean (SEM) GL (g/1000kcal): 69 (6) **reduced fat diet (RFD)** (n=7)**:** energy restricted (-250 to 500 kcal/d), mean GI: 56 (2), mean GL: 79 (7)	mean (SEM) **HOMA-IR**: RGL: 3.5 (0.7) RFD: 4.3 (0.7)	• at 12 months, HOMA-IR had increased significantly less in the RGL compared to the RFD group (p for difference=0.02) • results remained unchanged after additional adjustment for BMI (p for difference=0.03)	+
Iannuzzi 2009 [182] Italy	26 obese participants % female: 54% age: 7-13 years Ø BMI: 28.3	randomized intervention trial**,** 6 months duration	**low-GI diet (LGI)** (n=13): mean estimated GI: 60 **high-GI diet (HGI)** (n=13): mean estimated GI: 90 both diets hypocaloric (30% less energy) dietary counseling, meal plan provided, recording of consumed foods	mean (SD) **HOMA-IR**: LGI: 3.1(1.5) HGI: 3.2 (1.6)	• HOMA-IR significantly reduced only in the LGI-group (p=0.04, for HGI: p=1.0) • no differences between groups assessed	(+)
Mirza 2013 [184] USA	64 obese Hispanic participants % female: 44% Ø age: 11.7 years Ø BMI: 30.6 (BMI-z score: 2.24)	randomized controlled trial, 24 months intervention	**low-GL diet (LGD)** (n=33): mean (SE) GI after 3 months: 51 (1), after 24 months: 56 (1), mean (SE) GL after 3 months: 64 (3), after 24 months: 77 (4) **low-fat diet (LFD)** (n=31): mean GI after 3 months: 55 (1), after 24 months: 54 (2), mean GL after 3 months: 74 (3), after 24 months: 74 (3) dietary counseling first 12 weeks	mean (SE) **HOMA-IR**: LGD: 3.35 (0.4) LFD: 3.35 (0.3) (values derived from total study sample; completers and non-completers)	• no differences between groups for changes in HOMA-IR (p for difference>0.1)	-

Table 3: *continued.*

First author, Year, Country	Participant characteristics[2]	Study design	Dietary Intervention	Outcome: baseline levels and primary endpoints	Results	+/-[3]
Ramon-Krauel 2013 [185] USA	17 obese participants with fatty liver % female: 18% Ø age: 11.9 years Ø BMI: 32.7 (BMI-z score: 2.39)	randomized pilot intervention trial, 6 months duration	**low-GL diet (LGD)** (n=8): mean (SD) GI after 6 months: 55 (7), mean (SD) GL (g/1000kcal): 56 (13) **low-fat diet (LFD)** (n=9): mean GI after 6 months: 60 (7), mean GL: 70 (8) dietary counseling	mean (SD) **HOMA-IR**: LGD: 4.5 (1.6) LFD: 9.3 (8.1) mean (SD) **ALT** (IU/l): LGD: 63.3 (36.0) LFD: 75.9 (50.9)	• trend for greater decrease in HOMA-IR in LFD-group (p for difference=0.08), but this group had higher baseline values • decrease in ALT-concentrations in both groups, with no difference between them (p for difference=0.4)	-
Siegel 2011 [183] USA	64 participants % female: 64% Ø age: 11.3 years Ø BMI: 32.8	retrospective study, 4.5 months	**portion controlled diet (PC)** (n=28) **healthy eating plan (HEP)** (n=21) **HEP without seeing a dietician (n=15)** HEP recommends <10% of calories from saturated fat and low GI (≤ 50)	mean **ALT** (U/l): PC: 50.0 HEP plus dietitian: 30.6 HEP : 23.8	• ALT levels decreased significantly in the HEP plus dietitian group (p<0.005) • no differences between groups assessed	(+)

Abbreviations: Ø average value, mean or median as provided in the original publication; ALT, alanine aminotransferase; GI, glycemic index, GL, Glycemic load; SD, standard deviation; SE, standard error; SEM, standard error of the mean

[1] Only relevant exposures and outcomes are presented

[2] n number refers to those who completed the study

[3] +: effect of dietary intervention on body fat measures, -: no effect of dietary intervention on body fat measure

Dietary fiber / whole grain

Regarding the association between dietary fiber and its effect on postprandial glycemic excursions, it is crucial to consider the food structure. As described in Chapter 2.1.1, intact kernels and milled flour – although both whole grain foods by definition and high in dietary fiber – have completely different postprandial glycemic responses [31]. In fact, an Australian analysis demonstrated that the fiber content of 121 tested foods and 13 mixed meals could not predict postprandial glycemia or insulinemia. In turn, dietary GI and GL were the best predictors for blood glucose responses [45]. Nonetheless, viscous fibers, like those in oats or barley, have gel-like properties, and are able to delay gastric emptying, leading to slower rates of glucose appearance in the blood with subsequently decreased insulin secretion. These beneficial effects are of relevance particularly for diabetic patients, for whom they are best described [186]. According to a relatively new investigation, dietary fiber from cereals might influence protein absorption and digestion in the small bowel. As presented in Chapter 2.1.4, protein intake induces increases in insulin concentration. Hence, the concomitant consumption of fiber- and protein-rich foods could possibly counteract these insulin rises [187].

Regarding beneficial effects of whole grain foods on diabetes risk, the high magnesium content is also relevant: Magnesium enhances insulin secretion and could thus improve glucose clearance from the blood [188].

Finally, the second meal effect, which has already been mentioned in the section referring to the GI, is discussed for most low-GI whole grains such as intact barley or rye kernels. In contrast to the low-GI feature, the fermentation of indigestible carbohydrates in the colon might be responsible for an improved carbohydrate tolerance after the longer interval between meals (ten to twelve hours), i.e. between breakfast and dinner [31].

Results from meta-analyses on prospective cohort studies in adults support a beneficial role of a high fiber and whole grain intake: Particularly a high intake of cereal fiber [189, 190] and whole grains [191-193] is consistently related to a decreased risk of T2D [190, 191, 193] and CVD [189, 192, 193]. The evidence-based dietary guideline from the DGE concluded that fiber from cereals and whole grains reduce the risk for T2D and scored the evidence for this association as probable. For total fiber consumption it was however stated that there is possibly no association to diabetes risk. Likewise, no relationship seems to exist with soluble fiber (possible evidence) as well as fiber from fruit and vegetables (probable evidence). Evidence on the relevance of insoluble fiber is considered inconclusive [23].

There are only four studies among **children and adolescents** regarding the role of dietary fiber and whole grain intake on diabetes risk factors and they are all **observational**: The

study from Denmark mentioned above also investigated fiber intake and its association with HOMA-IR levels after six years. However, as for dietary GI, no prospective association was observed. In the cross-sectional analysis, baseline fiber intake was inversely related to baseline HOMA-IR levels in girls only [180]. A similar observation has been made in a Finish study, which aimed to assess the effect of a lifelong dietary counseling to meet the Nordic Nutrition Recommendations on insulin sensitivity. It was observed that girls, who had a high fiber intake at the age of 15, had lower HOMA-IR levels at the age of 20 years. For boys, no such association was observed [194]. However, a US study including 16 overweight Latina adolescents did not support a role of dietary fiber for IR among girls: In this study, 12-week changes in fiber intake were not related to changes in HOMA-IR [195]. Also, a British study including adolescent girls only, found no clear association between dietary fiber intake and later IR: In this study, fiber intake was divided into soluble and insoluble fiber. Neither insoluble fiber intake at 16/17 years of age, nor changes in intake between 16/17 and 18/19 years were related to 3-year changes in HOMA-IR levels, whereas an increase in soluble fiber intake between 16/17 and 18/19 years of age was related to a decrease in HOMA-IR levels. Nonetheless, soluble fiber intake at the age 16/17 was not related to changes in HOMA-IR levels [196] (**Table 4**).

There are two other studies, which did not study HOMA-IR or liver enzymes but HbA1c and insulin sensitivity measured by euglycemic insulin clamp: In a Dutch study, fiber intake during adolescence was not prospectively related to HbA1c levels at age 36, which were measured as part of different metabolic syndrome components [197]. Furthermore, a higher whole grain intake was cross-sectionally related to greater insulin sensitivity among US adolescents [198].

Table 4: Observational studies in children and adolescents examining the relation between dietary fiber and whole grain intake and IR (measured by HOMA-IR)[1]

First author, Year, Country	Population, recruitment, name of study[2]	Follow-up	Exposure: assessment method, average baseline values	Outcomes: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[3]
Davis 2007 [195] USA	16 overweight girls re-analysis of Adolescent Latinas Adjusting Sugars (ALAS) Study Ø age: 14.5 years Ø BMI Z-score: 2.0	12 weeks	3-day diet records mean (SD) dietary **fiber**: 15.4g (10.0), 8.1g/1000kcal (3.0) mean (SD) **whole-wheat grains** (servings/d): 1.1 (1.3)	mean (SD) **HOMA-IR**: 3.8 (2.5)	age, baseline HOMA-IR, baseline fiber	• changes in fiber intake were not related to changes in HOMA-IR	~
Kynde 2010 [180] Denmark	233 participants Danish part of the European Youth Heart Studies (EYHS) % female: 59% Ø age: 9.7 years Ø BMI: 17.1	6 years	24h recall interview supplemented with a qualitative food record from the same day mean (SD) dietary **fiber** (g/MJ): girls: 2.1 (0.7), boys: 2.1 (0.7)	mean (SD) **HOMA-IR**: girls: 1.8 (1.0), boys: 1.7 (0.9)	age, sexual maturity (at baseline and follow-up), BMI, physical activity, mother`s education, school location, baseline HOMA z-scores	• no association between dietary fiber intake at baseline and HOMA z-scores at follow-up (p for trend ≥0.2) • among girls but not boys, baseline fiber intake was inversely associated with baseline HOMA z-score (p for trend=0.03 and 0.5, respectively); non-significant after Bonferroni correction	~ $girls_{cs}$ (↓)
Oranta 2013 [194] Finland	518 participants re-analysis from the Special Turku Coronary Risk Factor Intervention Project (STRIP) Study % female: 47% Ø age: 15 years Ø BMI: 20.5	5 years	4-day food record mean **fiber** intake: girls: 15.7g, 2.1g/1000kcal; boys: 17.7g, 2.0g/1000kcal	mean **HOMA-IR**: girls: 1.69, boys: 1.72	study group, age, sex, BMI	• in girls but not in boys, fiber intake (g/1000kcal) was inversely associated with HOMA-IR (p for trend<0.0001, and 0.8. respectively); also after adjustment for BMI	boys ~ girls ↓

Table 4: *continued*.

First author, Year, Country	Population, recruitment, name of study[2]	Follow-up	Exposure: assessment method, average baseline values	Outcomes: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[3]
White 2012 [196] UK	774 girls National Heart, Lung, and Blood Institute Growth and Health Study Ø age: 16.0 years Ø BMI: 23.8	3 years	food diary on three days mean (SD) **soluble fiber** (g) at age 16/17 years: 4.00 (1.80), at age 18/19 years: 4.39 (2.03) mean (SD) **insoluble fiber** (g) at age 16/17 years: 7.34 (3.34), at age 18/19 years: 7.69 (3.80)	mean (SD) **HOMA-IR**: 2.62 (3.38)	age at entry, race, change between the ages of 16/17 and 18/19 years in height and menstrual status, parental education	• no prospective association between intake of soluble or insoluble fiber at age 16/17 years and changes in HOMA-IR (p for trend>0.05) • increases in grams of soluble fiber between ages 16/17 and 18/19 years were associated with a decrease in HOMA-IR (p for trend<0.05) • no cc association for insoluble fiber (p for trend>0.05)	~ soluble $fiber_{cc}$ ↓

Abbreviations: Ø average value, mean or median as provided in the original publication; cc, concurrent; cs, cross-sectional; SD, standard deviation

[1] Only relevant exposures and outcomes are presented

[2] n number refers to those in final analysis

[3] ↑: direct association with dietary fiber/whole grain intake, (↑): trend for association with dietary fiber/whole grain intake, ~: no association with dietary fiber/whole grain intake,↓: inverse association with dietary fiber/whole grain intake

2.3.2 Carbohydrate quality and obesity

Different aspects of carbohydrate nutrition have been related to an unfavourable development of body composition. This chapter will describe the relevance of the dietary GI on body fat, as this will be one focus of the present thesis. So far, mechanisms and evidence for these associations have been debated controversially. Comparable to Chapter 2.3.1, emphasis lies on evidence regarding children and adolescents.

Dietary glycemic index and glycemic load

After consumption of a high-GI meal, blood glucose levels increase rapidly, but decrease equally steep, often below baseline levels, two to four hours afterwards – a condition termed "reactive hypoglycemia." This hypoglycemic state is characterized by the coexistence of high insulin and low glucagon levels inducing uptake of glucose – and also fatty acids – in muscle, fat, and liver cells while reducing fat oxidation. Hence, two of the main circulating metabolic fuels are decreased, leading to enhanced levels of counter-regulatory hormones which stimulate hunger and food intake to restore energy homeostasis [13, 199, 200]. Decreased fat and increased carbohydrate oxidation has been related to weight gain, while the counter-regulatory hormone-response is associated with loss of lean body mass through its proteolytic effects [200-202]. Conversely, low-GI meals have been associated with increased satiety, delayed return of hunger, as well as reduced ad libitum food consumption [203]. It could be shown that these meals lead to lower 10-hour blood glucose and insulin levels as well as higher concentrations of the satiety hormone cholecystokinin compared to high-GI meals [204]. Compared to fully gelantized starches, which are present in the majority of high-GI foods such as breads and breakfast cereals, low-GI foods are digested more slowly and reach lower parts of the ileum where they stimulate satiety signals [200, 205-207]. Studies assessing the effect of low- versus high-GI breakfast and/or lunch on subsequent energy intake in children and adolescents observed a significantly greater energy intake after consumption of a high-GI meal [199, 208]: In obese boys, a high-GI breakfast and lunch was associated with a higher voluntary energy intake as compared to moderate- or low-GI meals. In accordance with this result, hormonal and metabolic changes that promote greater food intake were observed [199]. Likewise, the consumption of a high-GI breakfast was related to higher lunch intake as compared to low-GI as well as low-GI with added sucrose breakfasts in normal and overweight children. Furthermore, in this study, children consuming a high-GI breakfast reported greater hunger ratings at lunchtime compared to a low-GI breakfast, although immediate satiety following breakfast was comparable [208]. It has moreover been proposed that the occurrence of both hyperinsulinemia and hypoglycemia might favor the consumption of high-GI foods, which would subsequently induce another hypoglycemic condition [199, 209].

One meta-analysis of intervention studies compared the effects of high- and low-GI/GL diets on parameters of obesity and obesity-related risks in overweight and obese **adults.** In total, 14 studies were included. No difference was observed comparing the effects of these two diets on change in body weight (14 studies) or waist circumference (10 studies). Only three studies assessed the effect on fat free mass: Unexpectedly, low-GI/GL diets induced a significantly greater decrease in fat free mass compared to high-GI/GL diets. [210]. The DGE came to the conclusion that in women a higher dietary GI is related to higher risk of adiposity with a possible evidence – for men, the evidence for such a relation was considered inconclusive. Regarding dietary GL it was concluded that there is possibly no relationship with adiposity risk [23].

In the evidence-based dietary guidelines the available data on adiposity risk is presented for **children/adolescents** and adults separately, as there are more studies available for this disease outcome for both age groups compared to the other diseases presented. However, regarding the role of dietary GI/GL on childhood and adolescent adiposity development, the available evidence until 2010 was termed inconclusive [23]. Four prospective **observational studies** addressed the relevance of dietary GI/GL on later body fat development, but not all support a strong role for GI or GL. Two of these identified studies were conducted in the DONALD Study, examining a prospective relevance both in childhood [211] and adolescence [212]. The first included 380 young children and analysed the prospective association between dietary GI and GL at an age of two and percentage body fat at an age of seven as well as the impact of the change of dietary GI and GL during the five years on changes in body fat. Neither a prospective nor a concurrent relevance could be shown for dietary GL. Baseline dietary GI tended to be prospectively related to percentage body fat at an age of seven. However, because multiple comparisons were done and the concurrent analysis showed no association, the authors concluded that overall there appeared to be no association [211]. Likewise, Cheng et al. [212] did not find a cross-sectional or 4-year concurrent relationship in an analysis including 215 adolescents from the DONALD Study. However, a stratified analysis revealed that overweight adolescents with a higher dietary GI at baseline tended to have higher %BF and BMI-SDS at baseline, whereas no association was observed for normal weight adolescents. In contrast, a US study including 85 overweight Latino adolescents observed no correlation between dietary GI or GL and adiposity variables including total fat mass [213]. An Australian study, assessing the association between carbohydrate nutrition and development of adiposity in 856 participants from the Sydney Childhood Eye Study, indicated – similar to adult cohort studies – a relevance for girls only. However, only a statistical trend was observed for a relation between baseline GL and 5 year changes in body fat, while the concurrent analyses also showed no relevance of dietary GL

in girls. Dietary GI was not related to later body fat, neither in girls nor in boys [214] (**Table 5**).

Table 5: Observational studies in children and adolescents examining the relation between dietary GI and GL and body fat measures[1]

First author, Year, Country	Population, recruitment, name of study[2]	Follow-up	Exposure: assessment method, average baseline values	Outcomes: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[3]
Buyken 2008 [211] Germany	380 participants DONALD Study % female: 47% Ø age: 2 years	5 years	3-day weighed dietary records mean (SD) dietary **GI** at age 2 years: 51.7 (3.5); at age 7 years: 55.8 (3.0) mean (SD) dietary **GL** (g/d) at age 2 years: 62.8 (14.6); at age 7 years: 112.8 (24.4)	mean (SD) **%BF** (estimated using skinfold measurements): at age 2 years: 18.9 (3.1); at age 7 years: 17.5 (4.6)	age, age^2, age^3, sex, maternal overweight, year of birth, birth weight, rapid weight gain between birth and age 2, intakes of energy, protein, fiber, and added sugar	• trend for prospective associations between GI and %BF (p for trend=0.07) • no prospective associations between GL and %BF (p for trend=0.4) • no concurrent associations between GI/GL and %BF (p for trend≥0.4)	~ GI_{pros} (↑)
Cheng 2009 [212] Germany	215 participants DONALD Study % female: 54% Ø age: 9.4 years	mean: 4 years	3-day weighed dietary records mean (SD) dietary **GI** at baseline: 55.7 (3.4); at endpoint: 56.5 (3.7) mean (SD) dietary **GL** (g/d) at baseline: 120.1 (29.7); at endpoint: 151.8 (42.8)	median (IQR) **%BF** (estimated using skinfold measurements): at baseline: 16.3 (12.6, 22.6); at endpoint: 17.5 (13.7-23.7)	age, age^2, age^3, sex, maternal overweight, breastfeeding, energy, and fiber intake	• no cs or cc associations between GI/GL and %BF (p for trend≥0.4) • interaction for %BF with overweight status (p for interaction: cs: 0.04, cc: 0.03); statistical trend only for overweight adolescents at baseline: a higher dietary GI at baseline tended to be related to a higher %BF at baseline (p for trend=0.05)	~ overweight: GI_{cs} (↑)

Table 5: *continued.*

First author, Year, Country	Population, recruitment, name of study[2]	Follow-up	Exposure: assessment method, average baseline values	Outcomes: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[3]
Davis 2009 [213] USA	85 overweight participants Study of Latino Adolescents at Risk for Diabetes cohort % female: 44% Ø age: 14.2 years	mean: 1.5 years	multiple pass 24h dietary recalls mean (SD) dietary **GI** at baseline: 59 (6) mean (SD) dietary **GL** (g/d) at baseline: 133 (51)	mean (SD) **total fat mass** (DEXA: kg): 28.7 (9.6)	sex, Tanner stage, time between visits, baseline visceral adipose tissue, energy and fiber intake, and baseline subcutaneous abdominal adipose tissue	• GI/GL were not significantly correlated to changes in adiposity variables	~
Gopinath 2013 [214] Australia	856 participants Sydney Childhood Eye Study % female: 49% Ø age: 12 years	5 years	120-item self-administered FFQ Mean (SD) dietary **GI** at baseline: girls: 54.3 (3.4), boys: 54.3 (3.2) Mean (SD) dietary **GL** (g/d) at baseline: girls: 138.0 (53.3), boys: 145.1 (54.2)	mean (SD) **%BF** (BIA): at baseline: girls: 23.9 (9.0), boys: 15.8 (7.4)	age, sex, ethnicity, parental education, exposure to passive smoking, change in height, screen viewing time, time spent in physical activity, and energy intake	• trend for prospective associations between GL at baseline and change in %BF (p for trend=0.07) in girls • no prospective relation between GI and %BF in girls (p for trend=0.17) • change in GI/GL not related to cc change in %BF in girls (p for trend≥0.15) • among boys, no association between GI/GL and change in %BF (prospective and cc analyses)	~ girls: GL_{pros}(↑)

Abbreviations: Ø average value, mean or median as provided in the original publication; %BF, percentage body fat; BIA, bioelectrical impedance analysis; cc, concurrent; cs, cross-sectional; DEXA, dual-energy X-ray absorptiometry; FFQ, food frequency questionnaire; GI, glycemic index, GL. Glycemic load; IQR, interquartile range; SD, standard deviation

[1] Only relevant exposures and outcomes are presented

[2] n number refers to those in final analysis

[3] ↑: direct association with GI or GL, (↑): trend for association with GI or GL, ~: no association with GI or GL,↓: inverse association with GI or GL

The four identified **intervention studies** also showed inconsistent results for an effect of dietary GI/GL on body fat in children or adolescents: Two of the studies were already mentioned in Chapter 2.3.1, as they addressed both measures of IR and adiposity [181, 184]. Besides beneficial effects on HOMA-IR, the reduced-GL diet in the study by Ebbeling et al. [181] also led to greater reductions in fat mass over the duration of 12 months compared to the reduced-fat diet. Moreover, the authors conducted a post-hoc analysis of the study, pooling the data from both groups: These results showed that the change in dietary GL was a strong predictor of change in %BF among both groups. Likewise, the results of the study from Mirza et al. [184] on the outcomes IR and BMI were similar (results for body fat were not presented): Both the low-GL and the low-fat diet prescribed to obese Hispanic children and adolescents did not differ in their effect on BMI reductions. Another US study on 85 obese girls and boys compared the effect of a low-carbohydrate, and a low-GL diet (both ad libitum) to an energy-restricted portion controlled diet. Percentage body fat decreased in all three groups over the three months intervention period and remained low after nine months of follow-up. However, like in the aforementioned study, no significant group differences were observed [215]. In turn, in 465 European children from the Diet, Obesity and Genes (DiOGenes) family-based study, a low protein diet accompanied by a high-GI was associated with the greatest increases in %BF and the counterpart, a high-protein, low-GI diet was protective against the development of obesity. However, no isolated effect of dietary GI was observed, which might be due to the little difference achieved in GI (2.3 instead of the aimed 15 points difference between the groups). It should furthermore be noted that the DiOGenes study is a family intervention study which primarily focussed on the obese/overweight parents. Among the included children the baseline prevalence of obesity/overweight was 48% - hence these children represent an at-risk population [216] (**Table 6**).

In addition, two retrospective studies including obese US children do not investigate the effect on body fat mass (one main focus of this thesis) but on body weight or BMI: The study by Siegel et al. [183] was already mentioned in Chapter 2.3.1: Their results showed that those children following a low-GI healthy eating plan for three months, additionally supported by a dietician, decreased their BMI compared to participants on a healthy eating plan without support by a dietician or on a portion controlled diet [183]. The second study included ten year old children who were either assigned to an ad libitum low-GI/GL (n=64) or an energy-restricted low-fat diet (n=43). Participants following the former diet had greater decreases in their BMI and weight compared to participants in the low-fat group. However, these results cannot be attributed to GI only, as the macronutrient composition of the diets was not matched [217].

Table 6: Intervention studies in children and adolescents examining the effect of dietary GI and GL on body fat measures[1]

First author, Year, Country	Participant characteristics[2]	Study design	Dietary Intervention	Outcomes: baseline levels and primary endpoints	Results	+/-[3]
Ebbeling 2003 [181] USA	14 obese participants % female: 69% Ø age: 16.1 years	randomized controlled trial, 6 months intervention, 6 months follow-up	**reduced GL diet (RGL)** (n=7)**:** ad libitum, mean (SEM) GI: 53 (3), mean (SEM) GL (g/1000kcal): 69 (6) **reduced fat diet (RFD)** (n=7)**:** energy restricted (-250 to 500 kcal/d), mean GI: 56 (2), mean GL: 79 (7) dietary counseling	mean (SEM) **total fat mass** ((kg): RGL: 38.8 (2.6) RFD: 48.5 (3.0)	• at 12 months, fat mass had decreased significantly more in the RGL compared to the RFD group (p for difference=0.01, ITT analysis) • pooled post-hoc analysis: GL was a significant predictor of change in %BF (0-6 months) among both groups (p for trend=0.006)	+
Papadaki 2010 [216] Netherlands, Denmark, UK, Greece, Germany, Spain, Bulgaria, and Czech Republic	465 participants DiOGenes % female: 57% Ø age: 12.4 years	randomized intervention trial, 6 months intervention	**low-protein/low-GI (LP/LGI)** (n=102), **LP/high-GI (LP/HGI)** (n=87), **high-protein /LGI (HP/LGI)** (n=92), **HP/HGI** (n=96), **control diet** (n=88) target difference: 15 GI points, achieved difference: 2.3 GI points dietary counseling	mean (SD) **%BF** (DEXA: kg): girls: 30.0 (8.9) boys: 25.8 (11.8)	• %BF increased significantly more in the LP/HGI group than in the other groups (p for difference=0.04) • percentage of overweight/obese children decreased significantly in the HP/LGI group (p for difference=0.03)	+

Table 6: *continued.*

First author, Year, Country	Participant characteristics[2]	Study design	Dietary Intervention	Outcomes: baseline levels and primary endpoints	Results	+/-[3]
Kirk 2012 [215] USA	85 obese participants % female: 58% Ø age: 9.8 years	randomized clinical trial, 3 months intervention, 9 months follow-up	**low-carbohydrate diet (LC)** (n=35): ad libitum, mean (SD) GL (g/1000kcal) at baseline: 73.2 (11.4) **reduced GL diet (RGL)** (n=36): ad libitum, mean GL (g/1000kcal) at baseline: 76.5 (11.2) **portion controlled diet (PC)** (n=31): energy restricted (-500 kcal/d), mean GL (g/1000kcal) at baseline: 74.0 (12.6) dietary counseling	mean (SD) **%BF** (DEXA): 40.7 (3.7)	• no differences in %BF between the diet groups • in all diet groups, %BF decreased after 3 months of intervention (all p for difference ≤0.0002), remained reduced through to 12 months (all comparisons with baseline p for difference≤0.0002)	-
Mirza 2013 [184] USA	64 obese Hispanic participants % female: 44% Ø age: 11.7 years	randomized controlled trial, 24 months intervention	**low-GL diet (LGD)** (n=33): ad libitum; mean (SE) GI after 3 months: 51.3 (1.3), after 24 months: 55.5 (1.0), mean (SE) GL after 3 months: 63.8 (2.6), after 24 months: 77.2 (3.5) **low-fat diet (LFD)** (n=31): mean GI after 3 months: 55.0 (1.0), after 24 months: 54.4 (1.5), mean GL after 3 months: 73.8 (2.5), after 24 months: 73.6 (3.4) dietary counseling first 12 weeks	mean (SE) **%BF** (air-displacement plethysmography): LGD: 42.3 (0.8) LFD: 43.3 (0.7) mean (SE) **BMI z-score** : LGD: 2.25 (0.05) LFD: 2.24 (0.03) (values derived from total study sample; completers and non-completers)	• results for %BF not presented • no differences between groups in averaged mean BMI z-score at any of the measured time points or the overall decrease in BMI z-score (p for difference>0.1) • both dietary groups decreased their BMI z-scores at 3, 12, and 24 months post-intervention (p for difference to baseline<0.0001, 0.003, and 0.002, respectively)	-

Abbreviations: Ø average value, mean or median as provided in the original publication; %BF, percentage body fat; DEXA, dual-energy X-ray absorptiometry; GI, glycemic index; GL, glycemic load; ITT, intention to treat; SD, standard deviation; SE, standard error; SEM, standard error of the mean

[1] Only relevant exposures and outcomes are presented

[2] n number refers to those who completed the study

[3] +: effect of dietary intervention on body fat measures, -: no effect of dietary intervention on body fat measure

2.3.3 Carbohydrate quality and chronic low-grade inflammation

As described in Chapter 2.2, chronic low-grade inflammation is involved in the pathophysiology of obesity, T2D and hepatic steatosis and furthermore discussed as a main mechanism linking carbohydrate intake to the development of these diseases [52]. In the following section, the potential underlying mechanisms for the association between dietary GI/GL, fiber and whole grain intake and inflammatory markers will be outlined. Since the presentation and comparison of the evidence is part of the third research aim (see Chapter 3) the literature overview will not be presented here (for details see OA4, Appendix 4).

Dietary glycemic index and glycemic load

Rapid increases in blood glucose levels have been associated with generation of reactive oxygen species and superoxide radicals even in healthy individuals [133, 218]. On an intracellular level, hyperglycemia leads to overproduction of superoxide within mitochondria, while nitric oxide is increasingly generated under the influence of insulin. Their combinatory product, peroxynitrite, is associated with DNA damage and impairment of mitochondrial and cellular function [218]. Due to the anti-inflammatory effects of insulin, these effects are only of short duration in healthy, insulin sensitive individuals, but – as described in Chapter 2.2.3 – effects last longer in insulin resistant or obese people [133, 219]. This implies, that oxidative stress induced by high-GI meals is of particular relevance in persons with impaired glucose tolerance [5]. Of interest, it could be shown in individuals with T2D that acute glucose fluctuations are more closely linked to activation of oxidative stress compared to sustained hyperglycemia [220]. Furthermore, reactive hypoglycemia, already described in Chapter 2.3.2, leads to increased secretion of counter-regulatory hormones such as catecholamines as well as free fatty acids, which are thought to further intensify the pro-inflammatory state [133, 221]. NFκB is an important mediator of pro-inflammatory gene transcription, and its activation is closely linked to IR (see Chapter 2.2.1). It could be shown in healthy lean adults, that the consumption of 50g available carbohydrates as glucose or white bread lead to a three times higher NFκB activation compared to the consumption of pasta – a classical low-GI food. Interestingly, activation of NFκB paralleled glycemic but not insulinemic excursions, indicating that even in insulin sensitive subjects the pro-inflammatory effects of glucose are able to overwhelm the anti-inflammatory insulin actions (**Figure 4**) [222]. Moreover, the dietary GI also impacts on total antioxidant capacity, as could be shown in a feeding study with overweight men [223]: In those men following a high-GI diet over one week, an acute decline in antioxidant concentrations was observed as compared to those following a low-GI diet.

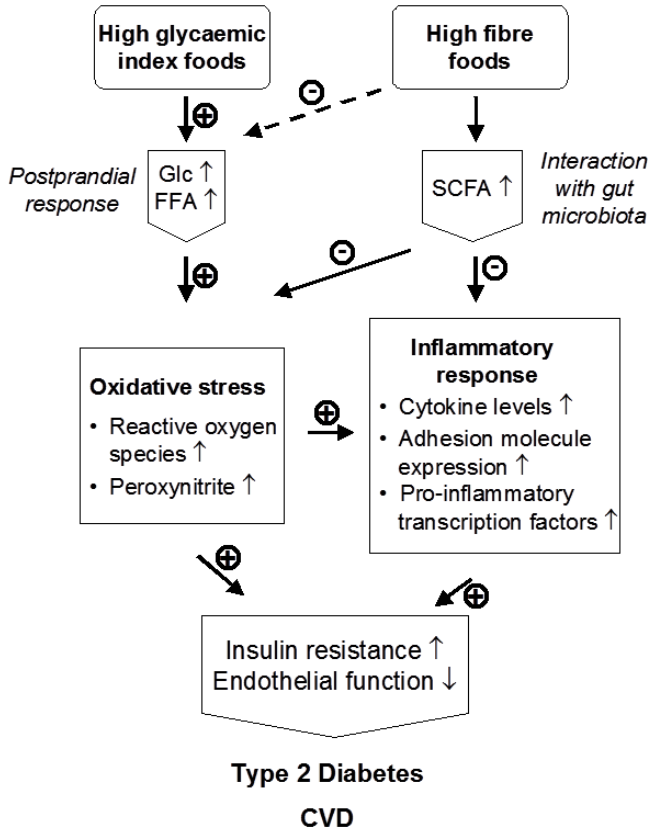

Figure 4: Proposed mechanisms linking high-GI and high-fiber foods to inflammation thereby contributing to type 2 diabetes mellitus and cardiovascular disease, from [224]
Abbreviations: CVD, cardiovascular disease; FFA, free fatty acid; Glu, glucose; SCFA, short chain fatty acid

Dietray fiber and whole grains

It has been proposed that dietary fiber interacts beneficially with gut microbiota and thereby influences inflammatory responses on a molecular level. Evidence from animal studies indicates that short chain fatty acids produced from fermentable fiber bind to receptors which are involved in inflammatory responses (**Figure 4**) [225]. Additionally, the inhibitory effect on nitric oxide synthase induction during fiber supplementation has been attributed to increased butyrate levels – one of the metabolic fiber products [226]. Another link between fiber-rich and whole grain products and inflammation are their high levels of bioactive compounds displaying anti-inflammatory effects: There are various antioxidants in cereal fibers with different mode of action. The main properties can be summarized as cofactors of antioxidant enzymes (e.g. zinc or selenium), radical scavengers (e.g. polyphenols or carotenoids), modification of the redox-status of tissues and cells, and protection of intestinal epithelium cells from oxygen-derived free radicals. The latter property also refers to the role of cereal antioxidants in thrombogenesis and platelet aggregation [31].

2.3.4 Carbohydrate quality and hepatic steatosis

The close link between liver fat accumulation and obesity as well as IR suggests a relevance of dietary factors associated with disturbances in glucose and insulin metabolism in NAFLD pathophysiology and prevention. To date, there are very few studies in humans assessing the direct effects of dietary modifications on markers of hepatic steatosis and of those who do, the inhibition of a further disease progression is often the primary aim. Hence, nutritional recommendations for NAFLD are mainly derived from approaches related to the closely associated conditions obesity and T2D, since weight loss and regain of glucose homeostasis are the major therapy aims for NAFLD [227]. Related mechanisms and evidence have been already described under Chapter 2.3.1 and 2.3.2. Noteworthy, Chapter 2.3.1 already includes intervention studies in children and adolescents with the liver enzymes ALT and GGT among the outcomes, as these are newly recognized markers for T2D risk [59-63]. Thus, these studies will only be briefly summarized in this chapter. This section will focus on mechanisms and evidence for the relevance of carbohydrate quality on different markers of hepatic steatosis in studies including adults. Also note that studies on GI/GL and dietary fiber/whole grain will be presented in one table, as there are only few of them and some investigate both aspects.

Dietary glycemic index and glycemic load

It has been observed that a hypercaloric intake of carbohydrate-rich foods inducing increased postprandial glucose elevations leads to enhanced hepatic lipogenesis [228-230]. Hyperglycemia in response to consumption of a high-GI meal is associated with increased glucose uptake in the liver, where it is subsequently stored as TG after conversion through de novo lipogenesis [142]. Furthermore, hyperglycemia induced oxidative stress might also be relevant, since a pro-inflammatory state is also thought to be of importance in NAFLD pathophysiology, particularly in regard to the progression to NASH [143]. Considering the liver as a main tissue of lipid metabolism, dietary effects on serum lipids are also relevant: A high intake of carbohydrates is associated with increased levels of TG [231]. Moreover, increasing the proportion of carbohydrates in the diet for the expense of fat is related to reduced levels of high density lipoprotein (HDL) [232]. While not affecting TG and HDL-cholesterol, low-GI diets are in turn associated with reduced total and low density lipoprotein (LDL)-cholesterol [233, 234]. Thus, in terms of lipid metabolism, both carbohydrate content and its glycemic potency might be relevant – suggesting a potential role of dietary GL.

Regarding **observational evidence** on the association between dietary GI/GL and NAFLD, Fraser et al. [235] showed in a post-hoc analysis of an intervention trial, that a Mediterranean diet approach was associated with a greater reduction of ALT after six and twelve months compared to the traditional American Diabetes Association diet as well as a low-GI diet. The

study included 201 obese participants with diabetes and both the Mediterranean and the low-GI diet included the recommendation to consume low-GI carbohydrates with differing amounts of carbohydrates advised: While 50-55% of energy stemmed from carbohydrates in the low-GI diet, the Mediterranean diet included only 35% of energy from carbohydrates, implying a lower GL in this diet, which was however not stated. A cross-sectional study from Valtuena et al. [236] related the grade of steatosis from 241 apparently healthy Italians to different aspects of carbohydrate nutrition. They observed an increasing steatosis grade with an increasing dietary GI, whereas no association could be shown for dietary GL. Of interest, an additional stratified analysis revealed a relevance of the dietary GI particularly for insulin-resistant participants: Whereas no significant difference in steatosis grade could be observed between insulin-sensitive persons with a low/medium or a high dietary GI, those who were insulin-resistant and consumed a diet characterized by a high-GI had a prevalence of high-grade liver steatosis twice as high compared to a low/medium-GI diet (**Table 7**).

Two recent **intervention studies** also examined the effect of diets differing in GI on liver fat: A German study, including 32 young men, examined the effect of carbohydrate intake and dietary GI during a refeeding phase after preceding weight loss. For this purpose, one week of overfeeding was followed by three weeks of caloric restriction (halved energy intake) with a subsequent refeeding period. During the phase of caloric restriction, liver fat and TG-levels improved under both diets, suggesting that any potential effect of carbohydrate modification was overridden by the effect of weight loss. In the subsequent 2-week period of refeeding, carbohydrate intake but not dietary GI was positively associated with liver fat and TG-levels [237]. The other intervention trial included 35 older US-adults and compared a low-fat, low-saturated fat, low-GI diet with a high-fat, high-saturated fat, high-GI diet. No significant difference was observed between the two groups with respect to liver fat and ALT-levels. However, the absolute percentage of liver fat decreased significantly only in the diet characterized by a low-GI. In this regard, a higher baseline liver fat percentage was predictive of a greater decrease. Conversely, the diet characterized by a high-GI, but also a high-fat content (43 percentage of total energy intake (En%) compared to 23En%) led to improved TG- and HDL-cholesterol concentrations, supporting the detrimental effect of a high carbohydrate intake on these markers [238] (**Table 8**).

In **children and adolescents**, two intervention studies were identified that examined the effect of a low-GI diet on ALT concentrations: In a randomized pilot intervention trial including 17 obese adolescents, both a low-GL and a low-fat diet were associated with decreased ALT levels after six months, with no significant difference between the intervention groups [185]. In the retrospective study by Siegel et al. [183], ALT concentrations decreased significantly in the group following a low-GI healthy eating plan with assistance of a dietitian compared to

those following the plan without a dietitian or a portion controlled diet (see **Table 3** in Chapter 2.3.1).

Dietary fiber and whole grain

The role of dietary fiber and whole grain products for liver fat accumulation is thought to be attributed to beneficial effects on blood lipids and/or oxidative stress. Regarding the former mechanism, particularly a high intake of viscous fiber is related to lower levels of total and LDL cholesterol [239-241]. It could be shown that soluble fibers such as ß-glucans reduce bile acids and cholesterol re-absorption from the ileum, thereby inhibiting cholesterol synthesis in the liver [234, 242, 243]. The regulating effect on bile acid metabolism might also be associated with a reduction in hepatic fat accumulation [142]. Fiber and whole grain products are furthermore rich in antioxidants (see Chapter 2.3.3), which may exert beneficial effects in the pathogenesis of steatohepatitis and fibrosis [142, 244].

In **observational studies** on the relation between dietary fiber or whole grain intake and markers of liver fat accumulation, a common approach is to compare the diets of patients with and without NAFLD, as done by Zelber-Sagi et al. [245]. In this cross-sectional study, including 340 Israelis (30% of them diagnosed with NAFLD) fiber intake did not differ between the healthy participants and those with fatty liver [245]. In a Japanese study, NAFLD-patients were compared to those with diagnosed NASH instead of healthy persons, in order to find potential dietary strategies to inhibit disease progression. Although fiber intake was lower in NASH-patients, the difference between these two patient groups was not statistically significant [246]. Additionally, in the study by Valtuena et al. [236] no association between grade of steatosis among quartiles of fiber intake was observed (**Table 7**).

Even if the **intervention study** by Utzschneider et al. [238] was not designed to create a difference in dietary fiber, significant differences were observed in fiber intake which resulted from the difference in carbohydrate intake. Therefore, the beneficial effect on absolute percentage of liver fat after the low-fat/low-saturated fat/low-GI diet might possibly also be attributed to the higher fiber content (**Table 8**).

No studies in **children or adolescents** were identified reporting on the relevance of fiber or whole grain intake for liver enzyme concentrations.

Table 7: Observational studies in adults examining the relation between dietary GI/GL, dietary fiber and whole grain intake and markers of hepatic steatosis[1]

First author, Year, Country	Population, recruitment, name of study[2]	Follow-up	Exposure: assessment method, average baseline values	Outcomes: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[3]
Fraser 2008 [235] Israel	201 obese participants with diabetes post-hoc analysis of a quasi-randomized controlled trial % female: 49% Ø age: 56.0 years Ø BMI: 31.5	12 months	dietary counseling, FFQ, 24-h recall **American Diabetes Association Diet (ADA)** (n=64): **low-GI diet (LGI)** (n=73): **modified Mediterranean diet (MMD)** (n=64): also low-GI carbohydrates (no GI values provided)	mean (SD) **ALT** (U/l): ADA: 25.0 (1.5) LGI: 22.8 (1.4) MMD: 24.5 (1.6)	baseline measurements	• ALT-concentrations decreased in all diet groups (p for difference to baseline<0.001) • MMD diet was associated with the lowest ALT-concentrations after 6 and 12 months (p for difference <0.001)	(↑)
Toshimitsu 2007 [246] Japan	46 partcicpants (28 with NASH, 18 with simple fatty liver (FL)) % female: 37% Ø age: 47.9 years Ø BMI: 27.6	-	3-day dietary records mean **fiber** (g): NASH: 12.9 FL: 15.1	mean (SD) **steatosis** (%): NASH: 55 (21) FL: 43 (19) mean (SD) **ALT** (IU/l): NASH: 119 (88) FL: 75 (53) mean (SD) **TG** (mg/dl): NASH: 162 (100) FL: 168 (95) mean (SD) **HDL-cholesterol** (mg/dl): NASH: 54 (34) FL: 48 (14)	-	• no significant difference between NASH- and FL-patients in fiber intake	~

Table 7: *continued.*

First author, Year, Country	Population, recruitment, name of study[2]	Follow -up	Exposure: assessment method, average baseline values	Outcomes: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[3]
Valtuena 2006 [236] Italy	241 participants % female: 43% Ø age: 59.8 years Ø BMI: 27.1	-	3-day dietary records quartile ranges **GI**: Q1: 49.2-53.6 Q2: 53.7–55.6 Q3: 55.7–57.9 Q4: 58.1–68.5 quartile ranges **GL** (g/d): Q1: 75–147 Q2: 147–181 Q3: 181–211 Q4: 212–352 quartile ranges **fiber**(g/d): Q1: 7.3–15.2 Q2: 15.3–19.5 Q3: 19.5–23.2 Q4: 23.3–40.3	mean (SD) **ALT** (U/l): GI Q1-Q3: 21 (9) GI Q4: 24 (12) mean (SD) **TG** (mmol/l): GI Q1-Q3: 0.86 (0.60) GI Q4: 0.94 (0.79) mean (SD) **HDL-cholesterol** (mmol/l): GI Q1-Q3: 1.54 (0.44) GI Q4: 1.52 (0.61)	sex, waist circumference, IR, energy intake, and other nutritional factors	• ALT concentrations were higher in the 4. GI-quartile, but not TG- or HDL-cholesterol concentrations • prevalence of high-grade liver steatosis (measured by ultra-sonography) increased with GI-quartiles (p for trend=0.03), but not with GL-, or fiber-quartiles (p for trend>0.2) • stratification by IR-status: IR-individuals: prevalence of high-grade liver steatosis twice as high in high-GI (Q4) as compared to low/moderate-GI (Q1-Q3) group; insulin-sensitive individuals: no significant difference	ALT: GI: ↑ GL, fiber: ~
Zelber-Sagi 2007 [245] Israel	349 partcipants (30% NAFLD prevalence) % female: 47% Ø age: 50.7 years Ø BMI: 27.2	-	semi-quantitative FFQ mean (SD) **fiber** (g): NAFLD: 27.0 (12.1) healthy: 26.4 (12.7)	mean (SD) **ALT** (U/l): 22.0 (10.0) mean (SD) **TG** (mg/dl): 117.0 (61)	age, gender, BMI and total calorie intake, other nutritional factors	• fiber intake was not related to significant differences in ALT- and TG-concentrations between participants with and without NAFLD	~

Table 7: *continued.*

Abbreviations: Ø average value, mean or median as provided in the original publication; ALT, alanine-aminotransferase; FFQ, food frequency questionnaire; FL, fatty liver; GI, glycemic index, GL, glycemic load; HDL, high density lipoprotein; NAFLD, Nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; Q, quartile; SD, standard deviation; TG, triglyceride

[1] Only relevant exposures and outcomes are presented

[2] n number refers to those who completed the study

[3] ↑: direct association with GI,GL, dietary fiber or whole grain intake, (↑): trend for association with GI,GL, dietary fiber or whole grain intake, ~: no association with GI,GL, dietary fiber or whole grain intake, ↓: inverse association with GI,GL, dietary fiber or whole grain intake

Table 8: Intervention studies in adults examining the effect of dietary GI/GL, dietary fiber and whole grain intake on markers of hepatic steatosis[1]

First author, Year, Country	Participant characteristics[2]	Study design	Dietary Intervention	Outcome: baseline levels and primary endpoints	Results	+/-[3]
Lagerpusch 2013 [237] Germany	32 men Ø age: 25.5 years Ø BMI: 23.5	controlled, parallel-group intervention trial, 6 weeks duration; study protocol: 1week overfeeding (50 or 65% CHO), 3 weeks caloric restriction, 2 weeks refeeding (50 or 65% CHO with either high or low GI)	**low-GI diet (LGI)** (n=16): mean GI=41 **high-GI diet (HGI)** (n=16): mean GI=74 all foods/beverages provided, intake supervised by nutritionists	mean **liver fat** (%): 6.21 mean **TG** (mg/dl): 93.9	• no effect of dietary GI was observed on liver fat or TG levels, neither in the caloric restriction nor in the refeeding phase	-
Utzschneider 2013 [238] USA	35 participants % female: 63% Ø age: 69 years Ø BMI: 29.0	randomized, double-blind, parallel design intervention study, 4 weeks duration	**low-fat/low-saturated fat/low-GI diet (LSAT)** (n=20): average (SEM) daily GI <55, mean fiber (g/d): 40.0 (1.9) **high-fat/high-saturated fat/high-GI diet (HSAT)** (n=15): average daily GI >70, mean fiber (g/d): 13.3 (0.7) all foods provided, dietary records to monitor compliance	median (IQR) **liver fat** (% by weight): LSAT: 2.2 (3.1) HSAT: 1.2 (4.1) mean (SEM) **ALT** (IU/l): LSAT: 20.5 (1.4) HSAT: 19.8 (1.0) mean (SEM) **TG** (mmol/l): LSAT: 1,29 (0.16) HSAT: 1,26 (0.10) mean (SEM) **HDL-cholesterol** (mmol/l): LSAT: 1.56 (0.11) HSAT: 1.55 (0.10)	• no effect on diet group on liver fat or ALT-levels (p for difference≥0.1) • significant decrease in absolute percentage of liver fat only after LSAT diet (p for difference<0.05), higher liver fat at baseline was thereby predictive of a greater absolute decrease • HSAT-diet lead to significantly improved TG- and HDL-cholesterol concentrations compared to LSAT (p for difference≤0.01)	(-)

Table 8: continued.

Abbreviations: Ø average value, mean or median as provided in the original publication; ALT, alanine-aminotransferase; GI, glycemic index, GL, glycemic load; HDL, high density lipoprotein; IQR, interquartile range; SEM, standard error of the mean; TG, triglyceride
[1] Only relevant exposures and outcomes are presented
[2] n number refers to those who completed the study
[3] +: effect of dietary intervention on liver fat measures, -: no effect of dietary intervention on liver fat measures

2.4 Conclusive Considerations

In adults, evidence from prospective cohort studies supports a beneficial role of both a high cereal fiber and whole grain intake and a low dietary GI/GL for T2D risk (see Chapter 2.3.1). However, proposed mechanisms differ and the relevance in childhood and adolescence has to be regarded as inconclusive. Particularly the relevance of puberty as a potentially critical period for later disease development with the physiologically occurring IR has yet to be addressed: carbohydrate nutrition during this period might be of specific relevance and the detection of associations with later T2D risk markers would be pivotal for dietary preventive approaches, which should start early in life. Moreover, it is of great relevance to also consider newly emerging risk factors for T2D such as liver enzymes, so that investigations cover both systemic and hepatic IR (A*im 2*). As obesity is the major risk factor for T2D development (see Chapter 2.2.2), clarification of the question whether postprandial rises in glucose or insulin during puberty are related to later development of an unfavorable body composition is of importance (*Aim 1*). One of the main discussed mechanisms linking carbohydrate quality to T2D and CVD is chronic low-grade inflammation (see Chapter 2.3.1-2.3.3). Given a potential role for risk markers of T2D, it would be interesting from a mechanistic point of view, whether this role also extends to inflammation. Thus, the long term relevance of adolescent carbohydrate nutrition also needs to be determined for inflammatory markers (Aim 3, 1st research question). In adults, numerous observational and interventional studies have recently been conducted in the emerging research field of chronic low-grade inflammation. A systematic review of these studies, comparing both the relevance of dietary GI and GL as well as fiber and whole grain and the results from observation and intervention studies, needs yet to be done (A*im 3, 2nd research question*). Scientific evidence on the role of carbohydrate quality for chronic disease risk focuses mainly on studies in middle-aged adults, implying the outlined need for long-term studies, starting e.g. in puberty. On the other hand, older adults represent a growing proportion of our population with a higher prevalence of most chronic diseases. Additionally, metabolic alterations may occur as part of the aging process, including a higher pro-inflammatory state (see Chapter 2.2.5). Hence, investigating the role of carbohydrate nutrition in an older population is crucial for dietary recommendations adapted to special age groups. The accumulation of liver fat seems to coexist with IR and obesity (see Chapter 2.2.4). However, the role of carbohydrate nutrition and liver function has not been fully elucidated yet (*Aim 4*).

3. AIMS AND RESEARCH QUESTIONS

As outlined in the previous chapters, carbohydrate quality plays a crucial role in the development of T2D and related health outcomes. However, prospective evidence in certain periods of life such as puberty or older age is lacking. Furthermore, a systematic compilation of the available literature on the relevance of carbohydrate quality on chronic low-grade inflammation has not been done yet. To fill in these gaps, the following four aims were formulated for this thesis:

Research aim 1: To examine carbohydrate nutrition and body composition

Is a diet inducing higher levels of postprandial glycemia or insulinemia during puberty prospectively related to body composition in young adulthood?

Postprandial excursions in blood glucose or insulin levels during critical periods of physiological IR such as puberty could impact on later overweight development. As overweight is one proposed mechanism linking dietary GI to T2D risk, investigating the prospective association between dietary GI and insulin index during puberty and body composition in young adulthood is of great interest. Applying the new concept of the FII to dietary data from the DONALD Study furthermore allows to distinguish between postprandial glycemia and insulinemia and their potentially different relevance for later overweight development.

Research aim 2: To examine carbohydrate nutrition and type 2 diabetes mellitus risk markers

Is the quantity and/or the quality of carbohydrate intake during puberty prospectively related to risk markers of type 2 diabetes mellitus in younger adulthood?

Carbohydrate nutrition during the critical phase of puberty may be of particular relevance for later risk of T2D. Using data from the DONALD Study, the habitual carbohydrate intake – both in its quantity and quality – during puberty will be related to different risk markers for T2D in younger adulthood.

Research aim 3: To examine carbohydrate nutrition and chronic low-grade inflammation

Is the quantity and/or the quality of carbohydrate intake during puberty prospectively related to inflammatory markers in younger adulthood?

Chronic low-grade inflammation contributes to the development of many chronic diseases and represents a likely intermediary in the relationship between carbohydrate nutrition and both T2D and CVD. Therefore, analyzing the prospective associations between carbohydrate nutrition during puberty and inflammatory markers in younger adulthood will give further mechanistic insights into the overall relevance of carbohydrate quality for T2D risk.

Is carbohydrate quality of relevance for low-grade inflammation in adults? Evidence from observational and interventional studies

The potential effects of carbohydrate nutrition on chronic inflammation have been investigated in a number of observational and interventional studies. A comparative assessment of the evidence from these two types of studies may be particularly insightful since evidence appraisal on the relevance of carbohydrate nutrition for chronic diseases (i.e T2D, CVD, cancer etc.) almost exclusively draws on observational studies. Hence, the evidence from published observational and interventional studies conducted in adults on the relevance of fiber intake, whole grain consumption and dietary GI/GL for markers of chronic low-grade inflammation (hsCRP and IL-6) will be systematically evaluated.

Research aim 4: To examine carbohydrate nutrition and liver function

Is carbohydrate quality longitudinally associated with markers of liver function in an older Australian population?

Alterations in liver function such as hepatic fat accumulation are thought to play a causal role in the development of T2D and the metabolic syndrome. Indeed, the liver enzymes ALT and GGT are newly recognized risk markers for T2D. The close relationship with IR and obesity suggests a link to carbohydrate induced disturbances in glucose and insulin metabolism. Therefore, the cross-sectional and longitudinal associations between carbohydrate quality and markers of liver function will be examined using data from the BMES, as these comorbidities become more frequent in older age groups.

4. GENERAL METHODOLOGY

The proposed research questions will be investigated on the basis of data from two observational studies: The DONALD Study, providing longitudinal data from young Germans, and the BMES, which includes data on an older Australian population. Furthermore, to give an overview on the current evidence for the relevance of carbohydrate quality on chronic low-grade inflammation, a systematic review was conducted. This chapter provides a brief overview on the study designs of the DONALD Study and BMES and describes the procedure of the systematic literature search.

4.1 The DONALD Study

The DONALD Study is an ongoing open cohort study conducted at the Research Institute of Child Nutrition in Dortmund, Germany [247, 248]. The study focuses on nutritional behavior, food consumption, growth, development, metabolism and health from infancy to adulthood. Since 1985, participants are recruited in the city of Dortmund and surrounding communities via personal contacts, maternity wards or pediatric practices. Eligible are healthy babies of Caucasian decent whose mothers and/or fathers are willing to participate in a long-term study and of whom at least one parent has sufficient knowledge of the German language. 35 to 40 infants are newly recruited every year and first examined at the age of 3 months. Each child returns for 3 more visits during the first year, 2 in the second and then annually until adulthood. Since 2005, participants from the age of 18 years are invited for subsequent examinations with fasting blood withdrawal – until then the study is purely observational and non-invasive. Furthermore, data on parental socio-demographic characteristics, lifestyle, health status and anthropometry are obtained every four years up to study participants' age of 18 years in a personal examination and by questionnaire. The study was approved by the Ethics Committee of the University of Bonn, and all examinations are performed with written parental and participants' consent.

At each visit the assessments always include a medical examination, anthropometric measurements, questionnaires, and detailed 3-day weighed dietary records [247] (**Figure 5**).

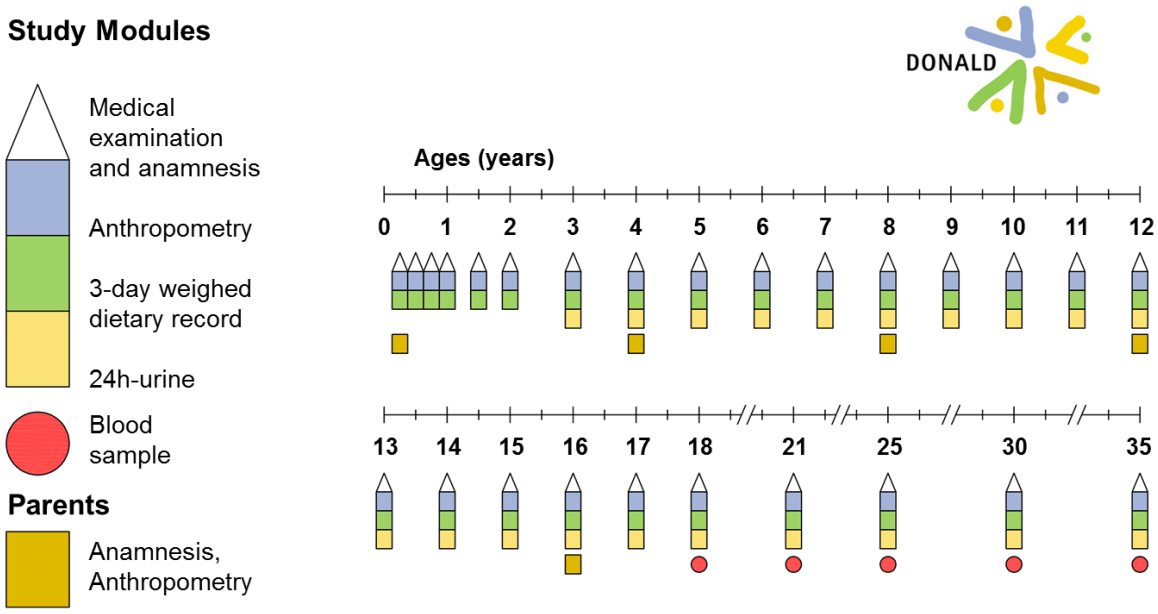

Figure 5: Examination schedule of the DONALD Study, from [248].

For the dietary records, all foods and beverages consumed as well as leftovers are weighed and recorded on three days by the participants themselves (or by the parents of the younger participants) to the nearest 1g using electronic food scales (initially Soehnle Digita 8000; Leifheit SG, Nassau; Germany; now WEDO digi 2000; Werner Dorsch GmbH, Münster/Dieburg, Germany). Recording household measures, such as number of spoons or scoops, is allowed when weighing is not possible. All foods consumed by the participants are listed in the in-house food and nutrient database LEBTAB (Lebensmitteltabelle). LEBTAB is based on the German standard food tables [249], contains data obtained from commercial food products, and is continuously updated to include all recorded food items [250]. It allows examining intake of both macronutrients and different parameters of carbohydrate quality such as added sugar, dietary fiber and whole grain. To further allow the examination of dietary GI, GL and insulin demand, the database was extended to include the dietary GI according to existing standard procedures [251] and FII, for which a standardized assignment procedure was developed and implemented (see OA1, Appendix 1)[1].

Anthropometric measurements are performed according to standardized procedures: At each visit, body weight and height is recorded to the nearest 100g and 0.1cm, respectively, and from the age of 6 months onward skinfold thickness is measured twice at four different sites (supra-iliacal, subscapular, biceps, triceps) on the right side of the body for estimation of body fat.

[1] Contribution of Janina Goletzke: Complementation of assignment of GI values (approximately 500) and complete revision of the existing GI database, assignment of all FII values to the 3-day weighed dietary records (together with Gesa Joslowski)

Venous blood samples are drawn after an overnight fast, centrifuged within 15 minutes and frozen at –80°C in the Research Institute. Noteworthy, work presented in this thesis was part of two projects funded by the Federal Office for Agriculture and Food and the World Cancer Research Fund. Within the first project, examining the long-term relevance of carbohydrate nutrition during puberty for IR and inflammation in younger adulthood, serum and plasma samples were transported to the technical laboratory of the German Diabetes Center to determine serum activities of the liver enzymes ALT and GGT as well as concentrations of inflammatory markers. Serum insulin concentrations were measured in the Laboratory for Translational Hormone Analytics in Paediatric Endocrinology at the University of Giessen within the WCRF funded project.

Notably, puberty was defined according to chronological age. Chronological age might be confounded because children of the same age may differ substantially in their pubertal stage. However, the chronological age range we used starts at the same time point at which DONALD participants on average are undergoing puberty according to the age at take-off (onset of pubertal growth spurt). Furthermore, the chronological age range ends where most girls and boys included in the DONALD study have already experienced their first menarche and their voice break, respectively [252, 253]. Therefore, we supposed that chronological age as defined, adequately covers the period of puberty. In addition, preliminary analyses using age at take-off and peak height velocity to define puberty were run and yielded similar results for the relationships of the dietary GI, GL, and insulin demand with %BF. Thus, chronological age was used uniformly to define puberty in order to not reduce the sample sizes too much.

4.2 The Blue Mountains Eye Study

The BMES is a population-based cohort-study of vision, common eye diseases, and other health outcomes in an urban, predominantly Caucasian Australian population aged 49 years and older, which was initiated in 1992 with three 5-year follow-up examinations. In the baseline examination participated 3654 eligible residents of two postcodes of the Blue Mountains region, west of Sydney, Australia (1992-1994; BMES-1). Of these, 2335 took part in the second examination (1997-99; BMES-2) and 1952 in the third (2002-04; BMES-3). The study was conducted in accordance with the recommendations of the Helsinki Declaration and was approved by the University of Sydney and the Sydney West Area Health Service Human Research Ethics Committees. Written informed consent was obtained from all participants. BMES data could be used for the present thesis because of a collaboration between the Research Institute of Child Nutrition in Dortmund (Dr. Buyken) and the University of Sydney (Prof. Brand-Miller, Prof. Mitchell).

In the BMES, liver enzymes – which are relevant for this thesis – were initially analyzed in the first follow-up (BMES-2). Hence, baseline examination data could not be used and thus, BMES-2 data were termed baseline and BMES-3 data (second re-examination) as the respective 5-year follow-up.

Dietary data was collected using a 145-item FFQ modified for the Australian diet and vernacular from an early Willett questionnaire [254]. This FFQ was validated against 4-day weighed food records collected on three occasions during one year (n=79) and showed moderate-to-good agreement for ranking individuals according to their dietary GI, dietary fiber, and total carbohydrate intake [255]. Nutrient intakes were estimated using the Australian Tables of Food Composition (NUTTAB95) and published GI values with the glucose=100 scale [256]. Additional GI data was obtained from the Sydney University Glycemic Index Research Service online database (www.glycemicindex.com).

Fasting blood specimens were drawn, centrifuged on site and then sent within the same day to the Westmead Hospital, Sydney, for analysis of – among others – the liver enzymes ALT and GGT as well as fasting triglycerides and HDL cholesterol, which were of interest for the fourth research aim.

4.3 Systematic literature search

As part of the third research aim, a systematic review was conducted according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement [257]: For this purpose, the databases MEDLINE, EMBASE and Cochrane Library (Cochrane Central Register of Controlled Trials (CENTRAL)) were systematically searched for observational and interventional studies assessing the relevance of dietary GI/GL, fiber or whole grain intake for concentrations of the inflammatory markers hsCRP or IL-6. To be included in the review, different pre-specified inclusion criteria had to be met, e.g. only studies conducted in adults who were either healthy, overweight, had features of the metabolic syndrome or T2D were considered [2] (for details see OA4, Appendix 4).

[2] Contribution of Janina Goletzke: Formulation of inclusion and exclusion criteria (together with Anette Buyken), completion of the literature search (together with Gesa Joslowski and Anna Felbick), conduction of the updated search, data extraction (together with Anna Felbick), contacting the authors to request additional information

5. ORIGINAL ARTICLES

Aim 1: To examine carbohydrate nutrition and body composition

The research question of Aim 1 assessed whether *a diet inducing higher levels of postprandial glycemia or insulinemia during puberty is prospectively related to body composition in young adulthood.* This aim was addressed using DONALD data: All participants having at least two 3-day weighed dietary records available during puberty and anthropometric measurements taken in young adulthood were included in this analysis. Addressing the question whether postprandial glycemia or insulinemia is more relevant for later unfavorable body composition development, the dietary GI and GL as well as the dietary insulin index and insulin load – novel measures of insulin demand – were examined. While dietary GI values were available for the majority of carbohydrate containing foods recorded from the participants, the dietary insulin index had to be newly assigned to all carbohydrate- protein-, or fat-providing foods.

In this group of 262 DONALD participants, the dietary insulin index during puberty was prospectively related to higher levels of percentage body fat during young adulthood. No association with later BMI was observed. Neither the dietary GI nor the dietary GL were related to body composition in young adulthood.

The abstract of this analysis is presented below (see Appendix 1 for the full publication).

OA1 Prospective associations of dietary insulin index, glycemic index, and glycemic load during puberty with body composition in young adulthood.

Joslowski G, Goletzke J[3], Cheng G, Günther ALB, Bao J, Brand-Miller JC, Buyken AE. IJO 2012, 36, 1463-1471

Background: Puberty is a so-called critical period for overweight development and characterized by physiological IR during mid-puberty. This study addressed the hypothesis that habitual consumption of a diet inducing higher levels of postprandial glycemia or insulinemia during puberty may have an unfavorable effect on body composition in young adulthood.

Methods: Multivariate regression analysis were performed on 262 DONALD participants with at least two 3-day weighed dietary records during puberty (baseline: girls 9-14years; boys 10-15years) and anthropometric measurements in young adulthood (18-25years). A published dietary glycemic index was assigned to each carbohydrate containing food. Similarly, each food was assigned a food insulin index (insulinemic response to a 1MJ portion of food relative to 1MJ of glucose) using 121 values measured at Sydney University.

Results: Dietary glycemic index or glycemic load during puberty were not related to body composition in young adulthood. In contrast, a higher dietary insulin index (II) during puberty was associated with higher levels of percentage of body fat (%BF) in young adulthood, even after adjustment for early life, socioeconomic and nutritional factors; %BF in energy-adjusted tertiles of dietary II were 23.1 (95%CI: 21.9, 24.4), 24.4 (23.2, 25.7), 24.8 (23.6, 26.0) %, p_{trend} =0.02. Adjustment for baseline %BF attenuated this relationship (p_{trend}=0.1). Dietary II was not related to BMI.

Conclusion: This study suggests a prospective adverse influence of dietary II during puberty on %BF in young adulthood. Postprandial increases in insulinemia rather than increases in glycemia appear to be implicated in an unfavorable development of body composition.

[3] Contribution of JG: assignment of all FII values to the 3-day weighed dietary records (together with GJ), interpretation of the data (together with all co-authors)

Aim 2: To examine carbohydrate nutrition and risk of type 2 diabetes mellitus

To answer the research question of Aim 2, whether *the quantity and/or the quality of carbohydrate intake during puberty is prospectively related to risk markers of type 2 diabetes mellitus in younger adulthood,* data from 226 DONALD participants was used. Compared to the first research aim, a smaller number of adult participants was considered for this investigation – even if the age group was extended to include adults beteen18 and 36 years. The reason for this was that in contrast to fasting blood samples, which were only added to the DONALD protocol in 2005, anthropometric measurements were already part of the DONALD examination schedule prior to that and hence more frequently available. Evaluating nutritional data from at least two 3-day weighed dietary records, different parameters of carbohydrate nutrition (total carbohydrate intake, dietary GI, GL, added sugar, fiber and whole grain intake) were studied. Analyses of the fasting blood samples taken in younger adulthood from the DONALD participants provided measures of insulin sensitivity (HOMA-IR was calculated on the basis of fasting insulin and glucose values) and concentrations of the liver enzymes ALT and GGT. It was hypothesized that recurring postprandial glycemic excursions during a phase of physiological IR might be particularly relevant for later risk to develop T2D. As the dietary GI is a valid predictor of postprandial blood glucose excursions, it was postulated that associations are more pronounced for this aspect of carbohydrate quality compared to the other aspects investigated.

Indeed, the analysis revealed a consistent association between a higher dietary GI during puberty and increased levels of HOMA-IR, ALT and GGT in younger adulthood. Furthermore, a higher intake of added sugars from drinks was related to higher GGT levels during adulthood. No other examined parameter of carbohydrate nutrition was prospectively related to the risk markers of T2D in young adulthood.

Below, the abstract of the respective publication is presented (see Appendix 2 for the full publication).

OA2 A habitually higher dietary glycemic index during puberty is prospectively related to increased risk markers of type 2 diabetes in young adulthood.

Goletzke J[4], Herder C, Joslowski G, Bolzenius K, Remer T, Wudy SA, Rathmann SA, Roden M, Buyken AE. *Diab.Care 2013, Jul; 36(7):1870-6*

Background: Carbohydrate nutrition during periods of physiological IR such as puberty may impact on future risk of type 2 diabetes. This study examined whether the amount or the quality (dietary glycemic index, GI; glycemic load, GL; added sugar, fiber and whole grain intake) of carbohydrates during puberty is associated with risk markers of type 2 diabetes in younger adulthood.

Methods: The analysis was based on 226 participants (121 girls and 105 boys) from the DONALD Study with an average of five 3-day weighed dietary records (range 2-6) during puberty (girls: 9-14 years, boys: 10-15 years) and fasting blood samples in younger adulthood (18-36 years) (average duration of follow-up: 12.6 years). Multivariable linear regression was used to analyze the associations between carbohydrate nutrition and homeostasis model assessment IR (HOMA-IR) as well as the liver enzymes alanine-aminotransferase (ALT), and gamma-glutamyltransferase (GGT) (n=214).

Results: A higher dietary GI was prospectively related to greater values of HOMA-IR (p_{trend} =0.03), ALT (p_{trend} =0.02), and GGT (p_{trend} =0.04). After adjustment for sex, adult age, baseline BMI, early life and socioeconomic factors as well as protein and fiber intake, predicted mean HOMA-IR values in energy-adjusted tertiles of GI were 2.37 (95% confidence intervals: 2.16, 2.60), 2.47 (2.26, 2.71), and 2.59 (2.35, 2.85). The amount of carbohydrates, GL, added sugar, fiber and whole grain intake were not related to the analyzed markers.

Conclusion: Our data indicate that a habitually higher dietary GI during puberty may adversely affect risk markers of type 2 diabetes in younger adulthood.

[4] Contribution of JG: Complementation of assignment of GI values to the 3-day weighed dietary records (together with GJ), conduction of statistical analysis (together with KB), interpretation of the data (together with all co-authors), and drafting of the manuscript

Aim 3: To examine carbohydrate nutrition and chronic low-grade inflammation

In order to describe the association between carbohydrate nutrition and markers of low-grade inflammation, a two-step approach was used: An additional analysis was run with data from the DONALD Study to answer the first research question of Aim 3: *Is the quantity or the quality of carbohydrate intake during puberty prospectively related to inflammatory markers in younger adulthood?* For this purpose, 3-day weighed dietary records during puberty and blood samples in younger adulthood were used from 205 participants. Examined aspects of carbohydrate nutrition were total carbohydrate intake as well as dietary GI, dietary GL, added sugar, fiber, and whole grain intake and inflammatory markers were hsCRP, IL-6, IL-18 and adiponectin.

The results showed a relevance of a high total carbohydrate intake for later concentrations of IL-6. Separate analyses of carbohydrates from low and higher GI food sources did however reveal that only a high intake of the latter was related to higher IL-6 levels. Furthermore, a higher GL and a lower intake of whole grains during puberty were prospectively related to unfavorable levels of the pro-inflammatory cytokine IL-6. None of the other inflammatory markers was associated with carbohydrate nutrition.

A systematic literature search was conducted to answer the second question of Aim 3: *Is carbohydrate quality of relevance for low-grade inflammation in adults?* For this purpose, *evidence from both observational and interventional studies* was considered. Comparative assessment of studies relating dietary GI/GL, fiber or whole grain intake to markers of low-grade inflammation may be particularly insightful since chronic low-grade inflammation is related to the development of many chronic diseases such as T2D. Hence, dietary approaches to lower inflammatory markers could also be suitable in regard to preventive recommendations for T2D.

A total of 60 studies could be included assessing either the effect of carbohydrate quality on the inflammatory markers hsCRP or IL-6 – two commonly used markers in epidemiological and clinical studies. Regarding dietary GI, observational evidence is less consistent for a beneficial role on low-grade inflammation compared to a high dietary fiber and whole grain intake. However, several intervention studies do support a potential role of dietary GI or GL for low-grade inflammation, while the majority of intervention studies does not report a benefit of increasing fiber or whole grain intake. Anti-inflammatory benefits of higher dietary fiber and whole grain intakes suggested by observational studies are hence not supported by intervention studies, indicating that confounding is likely.

In the following, the abstracts of both publications are presented (see Appendices 3 and 4 for the full publications).

OA3 Prospective association between carbohydrate nutrition during puberty and markers of chronic low-grade inflammation in younger adulthood

Goletzke J[5], Buyken AE, Joslowski G, Bolzenius K, Remer T, Carstensen M, Egert S, Nöthlings U, Rathmann W, Roden M, Herder C (under review)

Background: Chronic low-grade inflammation represents a likely intermediary in the relationship between carbohydrate nutrition and both type 2 diabetes mellitus and cardiovascular disease. This study assessed the prospective association between carbohydrate quantity and quality (dietary glycaemic index, GI; glycaemic load, GL; added sugar; fibre and whole grain intake) during puberty, a potentially critical period for later disease, and low-grade inflammation in younger adulthood.

Design: The analysis was based on 205 participants (113 girls and 92 boys) from the DONALD study with at least two 3-day weighed dietary records during puberty (girls: 9-14 years, boys: 10-15 years) and blood samples in adulthood (18-36 years). Multivariable linear regression models were used to analyse the associations between carbohydrate nutrition and different pro- and anti-inflammatory immune mediators (high-sensitivity C-reactive protein, hs-CRP; IL-6; IL-18; adiponectin).

Results: A higher intake of carbohydrates during puberty (p_{trend}=0.005), particularly from higher GI food sources (p_{trend}=0.01), was prospectively related to higher levels of IL-6 in younger adulthood, independently of baseline BMI, early life, socioeconomic and other nutritional factors. Furthermore, a higher dietary GL (p_{trend}=0.002) and a lower intake of whole grains (p_{trend}=0.01) were independently associated with higher adult IL-6 levels. Dietary GI, added sugar and fibre intakes were not independently associated with IL-6 (p_{trend}≥0.09). Carbohydrate nutrition during puberty was not independently related to hs-CRP, IL-18 and adiponectin levels (all p_{trend}>0.1).

Conclusion: During puberty, a high intake of carbohydrates from higher GI food sources and a low whole grain consumption prospectively predict greater IL-6 concentrations in younger adulthood.

[5] Contribution of JG: Complementation of assignment of GI values to the 3-day weighed dietary records (together with GJ), conduction of statistical analysis (together with KB), interpretation of the data (together with all co-authors), and drafting of the manuscript

OA4 The role of carbohydrate quality in chronic low-grade inflammation – a systematic review on observational and intervention studies

Buyken AE, Goletzke J[6], Joslowski G, Felbick A, Cheng G, Herder C, Brand-Miller J.

AJCN (in press)

Background: Chronic low-grade inflammation is a likely intermediary between quality of carbohydrate and chronic disease risk. We conducted a systematic literature search to evaluate the relevance of carbohydrate quality on inflammatory markers in observational and intervention studies.

Methods: MEDLINE, EMBASE and the Cochrane Library were searched for studies on associations between glycemic index (GI), glycemic load (GL), dietary fiber or fiber supplements or whole grain intake and high-sensitivity C-reactive protein (hsCRP) or interleukin-6 (IL-6). Included studies had to be conducted on adults (healthy, overweight, with type 2 diabetes or metabolic syndrome features, but without inflammatory disease) with at least 20 participants and 3 weeks duration.

Results: In total, 22 of the 60 studies meeting our inclusion criteria examined GI/GL: 5 of 9 observational studies reported lower levels of hsCRP or IL-6 among persons with a lower dietary GI/GL; 3 of 13 intervention studies demonstrated significant anti-inflammatory effects of a low-GI/GL diet and 4 further studies were suggestive of beneficial effects (trends or effects in a subgroup). For fiber intake, 13 of 16 observational studies reported an inverse relationship with hsCRP or IL-6, but only 1 of 11 intervention studies demonstrated a significant anti-inflammatory effect of fiber intake and a further trial reported a beneficial trend. For whole grain intake, 6 of 7 observational studies observed an inverse association with inflammatory markers, but only 1 of 7 intervention studies reported significant anti-inflammatory effects, one further study was suggestive (in a subgroup) and another study found an adverse effect (trend only).

Conclusion: In summary, evidence from intervention studies for anti-inflammatory benefits is less consistent for higher fiber or whole grain diets than for low-GI/GL diets. Benefits of higher fiber and whole grain intakes suggested by observational studies may reflect confounding.

[6] Contribution of JG: Completion of the literature search (together with GJ and AF) (including the updated search) and data extraction (together with AF), contacting of the authors to request additional information, interpretation of the results (together with all co-authors), and drafting of the manuscript (together with AEB)

Aim 4: To examine carbohydrate nutrition and liver function

In the fourth research aim, the following research question was addressed: *Is carbohydrate quality longitudinally associated with markers of liver function in an older Australian population?* For this purpose, data from the BMES was used. The possibility to use this study sample offered the advantage to investigate the stated associations in an age group where chronic disease incidence is more pronounced. Hence, preventive dietary approaches should be designed to especially target those groups. Recently, hepatic fat accumulation has been given much attention as a new mechanistic target regarding the pathophysiology of T2D and other chronic diseases. The current analyses were based on data from 866 BMES participants, for whom dietary data and information on ALT, GGT, TG, and HDL were available both on BMES2 and 3. ALT and GGT were used as non-invasive markers of liver fat and TG and HDL as measures of disturbances in lipid metabolism.

A high dietary fiber intake, especially from fruit sources, was cross-sectionally related to lower GGT- and TG-levels. Moreover, a higher dietary GI was associated with lower HDL-cholesterol levels in the cross-sectional analysis. In the 5-year change on change analyses, no associations were observed between aspects of carbohydrate quality and markers of liver function.

The abstract on these analyses is outlined on the next page (see Appendix 5 for the full publication).

OA5 Carbohydrate quality is not associated with markers of hepatic fat accumulation over 5 years in an older population.

Goletzke J[7], Buyken AE, Gopinath B, Rochtchina E, Barclay AW, Cheng G, Brand-Miller JC, Mitchell P. *Br.J.Nutr 2013, Jan 23:1-8*

Background: Nonalcoholic fatty liver disease (NAFLD) is closely associated with IR and obesity. Hence, carbohydrate quality could be of relevance to risk of NAFLD, but prospective data are lacking. The aim of this study was to investigate longitudinal associations between carbohydrate quality (including dietary glycemic index (GI), and intakes of sugar, starch, fiber) and markers of liver function in an older Australian population.

Methods: The analysis was based on 866 participants (□49 years) of the Blue Mountains Eye Study with fasting blood specimen and dietary intake data at baseline and 5-year follow-up. Multilevel mixed regression analysis was used to relate dietary GI and sugar, starch and fiber intake to the liver enzymes alanine-aminotransferase (ALT) and gamma-glutamyltransferase (GGT) as well as fasting TAG and HDL-cholesterol (HDL-C).

Results: After adjustment for potential confounding factors, a lower fiber intake was cross-sectionally related to higher GGT (p_{trend} =0.02) and fasting TAG (p_{trend} =0.002) levels, with fruit fiber being the most relevant fiber source (p_{trend} =0.095 for GGT; p_{trend} =0.003 for TAG). A higher dietary GI was associated with lower HDL-C (p_{trend} =0.046). Changes in carbohydrate quality during 5 years were not related to changes in ALT, GGT, TAG or HDL-C (p_{trend} ≥0.08).

Conclusion: The absence of longitudinal associations between carbohydrate quality and liver enzymes and serum lipids in this older population does not support a major role of carbohydrate nutrition in liver function among elderly.

[7] Contribution of JG: conduction of statistical analysis (together with GC), interpretation of the data (together with all co-authors), and drafting of the manuscript

6. GENERAL DISCUSSION

The overall aim of this thesis was to investigate the relevance of different aspects of carbohydrate quality during selected periods of life for risk markers of T2D and related health outcomes i.e. obesity, chronic low-grade inflammation and hepatic fat accumulation. Regarding the period of puberty, the results indicated that a habitual higher dietary GI during adolescence was prospectively associated with lower risk markers of T2D (Study II) but not with body composition in young adulthood (Study I). Furthermore, both a high intake of carbohydrates from higher GI food sources and a lower whole grain intake during puberty appeared to be detrimental for chronic low-grade inflammation in younger adulthood (Study III). For adults, the systematic literature search in observational and interventional studies showed that there is less consistent evidence from intervention studies for anti-inflammatory benefits of higher fiber or whole grain diets than there is for low-GI/GL diets. This indicates that benefits of higher fiber and whole grain intakes suggested by observational studies may reflect confounding (Study IV). Moreover, carbohydrate quality was not prospectively related to markers of liver function among elderly (Study V).

Below, the central findings of the Studies I-V will be related to the current scientific discussion. Also, general methodological issues regarding exposure and outcome assessment as well as concerning the two study populations from the DONALD Study and BMES will be discussed. Finally, the public health relevance of the present findings and possible practical implications will be presented.

6.1 Research Aims

In the following chapters, the findings related to the four research aims will be discussed in the context of the current nutritional recommendations. For a detailed discussion of the respective results, please see the corresponding publications (Appendices 1 to 5).

6.1.1 Research Aim 1: To examine carbohydrate nutrition and body composition

With regard to the first research question, a novel link was found between postprandial insulinemia, but not glycemia, during puberty, and %BF in young adulthood. The dietary insulin index and insulin load represent a relatively new concept for estimating the dietary insulin demand, which considers not only carbohydrate rich foods, but in contrast to the dietary GI also those high in protein or fat. However, it should be noted that the dietary insulin index might be particularly useful to disentangle diet-disease related mechanisms while its practicability might be limited: Dietary GI values are similar in healthy and insulin resistant subjects and are hence unaffected by a person's metabolic status. In contrast, Lan-

Pidhainy and co-authors showed that the dietary insulin index is more dependent on insulin sensitivity and glycemic control. As the insulin index is hence subject dependent and not a food's property, this observation suggests, according to the authors, a limited clinical utility [258]. However, as the DONALD population consists of healthy young Germans and hence a very homogeneous sample, comparing the dietary insulin index/insulin load to the dietary GI/GL represent useful concepts to examine the different effects of dietary postprandial glycemia and insulinemia.

While dietary insulin demand has not yet been related to body composition, one human [259] and one animal study [259, 260] suggest a special relevance of early rises in postprandial insulin levels for later obesity risk. From a mechanistic point of view, high postprandial insulin levels may lead to a preferred direction of nutrients away from oxidation in muscle towards storage in fat [203], to a suppression of lipolysis [199], and to reduction in insulin sensitivity, leading in turn to increased insulin secretion [13]. This implies that long-term exposure of the beta cells to an increased postprandial demand may promote IR and the development of higher %BF [261, 262].

Overall, the role of insulin for body weight is controversially discussed: Gary Taubes, one popular advocate in this discussion, recently published an essay claiming that "lipophilia" but not the "energy balance hypothesis" explains overnutrition and obesity [263]. According to this theory, an increased insulin response due to a high intake of carbohydrate rich foods is causal for overweight development by triggering fat accumulation. Particularly because he completely refuses a causal impact of overeating in obesity development, Taube's essay has been debated controversially. Taubes opponents mainly highlight the interaction of both hypotheses as well as the impact of other factors such as physical activity [263-268]. According to the lipophila hypothesis, hyperinsulinemia is causal for overweight development. Other researchers – among them Robert Lustig – declare IR as a defence mechanism to prevent further weight gain, although obesity, hyperinsulinemia, and hence IR are closely linked conditions [269-272]. According to this theory, the explanation for this paradox is that not all tissues are equally insulin-resistant in obesity: while the adipose tissue remains largely insulin sensitive, the liver as the primary target of insulin becomes resistant which has substantial consequences (Lustig 2008): This was shown in animal studies where isolated hepatic IR promoted adipogenesis and peripheral IR (Cai, Yuan et al. 2005). Of interest, a recent animal study [273] shed further light on the role of insulin in overweight development: By taking advantage of the fact that mice have two insulin genes, the researchers created a line that was genetically limited in the amount of insulin they could produce. After being fed a high-fat diet, these mice did not gain weight, and had less liver fat and inflammation compared to those mice with higher insulin levels. The authors found that

the low insulin levels in the former haploid mice contributed to a greater energy expenditure due to changes occurring in white adipose tissue: it took on energy burning attributes which are normally associated with brown adipose tissue. This study thus indicates that hyperinsulinemia leads to obesity not by causing fat accumulation directly but by decreasing energy expenditure and thus allowing energy storage [273, 274].

If, as the present results suggests, dietary insulinemia is indeed more important than glycemia regarding body composition, this might explain why studies relating dietary GI/GL to body composition are reporting such inconsistent results: dietary GI/GL would then only be crude estimates, as they do not consider protein and fat which are other important contributors to insulin demand [49]. Indeed, a lower FII can be achieved by choosing foods with a lower GL – which in turn can be obtained by lowering the GI or the carbohydrate content – but also by substituting carbohydrate rich foods for those rich in protein and/or fat. Of interest, a low-GI, high-protein diet as employed in the DiOGenes study is characterized by a lower insulin demand as insulin demanding carbohydrates are substituted for less insulin-demanding protein (particularly non-dairy protein). Findings from this study revealed that adherence to this diet was associated with the most successful 2-year weight loss maintenance among overweight adults [275] and the prevention of overweight and obesity over a 6 month period in children and adolescents at risk for overweight development [216] (see Chapter 2.3.2). Based on these results, a new large multicenter intervention study – the PREVIEW (PREVention of diabetes through lifestyle Intervention and population studies in Europe and around the World) study – will examine whether this diet is also successful in preventing the manifestation of T2D in pre-diabetic participants.

As a high dietary insulin demand might be particularly detrimental for adolescents entering puberty with excess body fat, a subsequent analysis was done using data from the RESIST study: the results indicated that in obese adolescents at risk for T2D, adherence to an energy restricted low-GL and insulin load diet might assist in weight loss [276].

Overall, the present findings revealed that postprandial insulinemia is associated with an unfavorable development of body composition. While being important from a mechanistic point of view, more evidence is needed to draw final conclusions regarding the relevance of the FII concept and its possible inclusion into dietary recommendations.

6.1.2 Research Aim 2: To examine carbohydrate nutrition and type 2 diabetes mellitus risk markers

The analysis regarding the relevance of carbohydrate nutrition during puberty for risk markers of T2D in younger adulthood provides new epidemiological evidence of a

detrimental role of recurring postprandial glycemic excursions during puberty for later levels of HOMA-IR, ALT, and GGT. These results are in accordance with observational evidence in adults showing an association between a higher dietary GI and an increased diabetes risk, but are the first to suggest that this association emerges already during puberty. While it has already been proposed that the dietary GI might be particularly important for insulin-resistant persons [5], our data indicates that this may extend to physiologically occurring IR experienced during puberty. Of note, the observed relation was partly attributable to adult body composition (the significant associations were attenuated towards a trend after additional inclusion of adult waist circumference in the final models; for further detail see OA2, Appendix 2); however, a trend remained, suggesting an additional mechanism such as oxidative stress, which is addressed in the third research aim. In Study I (see previous chapter), no independent association has been observed between dietary GI and adult body composition. A possible explanation for this slight inconsistency could be the different study sample.

In the present analysis, the dietary GI was the only aspect of carbohydrate nutrition that was consistently related to the different risk markers for T2D, pointing to a specific role of postprandial glycemia during puberty on later T2D risk. Intervention studies assessing the effect of a low-GI/GL diet in children and adolescents on T2D risk markers however provide only some support (see Chapter 2.3.1). Particularly further long term evidence, e.g. from large-scale prospective studies preferably in at-risk populations such as overweight or insulin-resistant adolescents, is still lacking and would be of great relevance to further support the present results.

Health authorities consistently judge the evidence for an impact of the dietary GI or GL on different health outcomes as inconclusive, although they do acknowledge a potential relation particularly for the risk of T2D. In the 2003 report of a joint WHO/FAO expert consultation on “Diet, nutrition and the prevention of chronic diseases,“ the evidence for an association between dietary GI and weight gain and T2D was ranked as possible [10]. The US Dietary Guidelines Advisory Committee (DGAC) concludes that strong and consistent evidence shows that GI and GL are not related to body weight. Regarding T2D, the evidence for an association between dietary GI and T2D is rated moderate, while convincing evidence shows, according to the DGAC, that there is no relation to dietary GL [8]. The European Food and Safety Authority (EFSA) acknowledges that there is some support for a role of a lower GI and GL for the development and treatment of T2D and also for possible favorable effects on some metabolic risk factors. However, the available evidence until 2009 was still regarded inconclusive [9]. Likewise, the DGE concluded that a higher dietary GI is possibly associated with the development of obesity in women, and T2D in both men and women (see

Chapter 2.3.2 and 2.3.1). Evidence exists furthermore for a possible role of a higher dietary GI and GL in the development of coronary heart disease (CHD) in women only [23]. Interestingly, contrary to the national and international health agencies, the different diabetes associations are more supportive of the GI concept: They do acknowledge an additional benefit of considering the GI besides carbohydrate counting in dietary diabetes management [277-279].

The underlying reason for why the different health authorities judged the evidence regarding the GI as inconclusive and thus do not include the GI concept in dietary recommendations is the heterogeneity among the studies. However, as already mentioned, a meta-analysis from Livesey et al. [175] showed that heterogeneity among the included studies was almost exclusively attributable to differences in the studies regarding sex, ethnicity and dietary instrument validity. After accounting for these sources of heterogeneity, the authors showed a robust and consistent dose-response relation between a higher dietary GL and T2D risk.

To conclude, the present results are in accordance with observational evidence relating a higher dietary GI to an increased T2D risk in adults and extend this to puberty. Even if further large-scale studies in children and adolescents are needed to support the present findings, the inclusion of low-GI food choice advices in dietary recommendations might need to be considered.

6.1.3 Research Aim 3: To examine carbohydrate nutrition and chronic low-grade inflammation

Addressing the first research question of the third aim, again using data from the DONALD Study, it was shown that during puberty, a high intake of carbohydrates from higher GI food sources and low whole grain consumption appears to be detrimental for later low-grade inflammation. These results add to the accumulating evidence that a high carbohydrate intake may be detrimental if the carbohydrates stem from higher GI food sources [280-284]: In the EPICOR study (EPICOR is performed on Italian cohorts recruited as part of the European Prospective Investigation into Cancer and Nutrition (EPIC)), a high dietary GL and a high carbohydrate intake from high- but not low-GI foods was related to an increased risk of CHD in women but not in men [281]. Another study by Jacobsen et al. [280] reported that the replacement of saturated fat with carbohydrates with low-GI values was associated with a lower risk of myocardial infarction, while replacing saturated fat with carbohydrates with high-GI values was associated with a higher risk. Furthermore, results of a Finish cohort showed that the replacement of high-GI carbohydrates with medium-GI carbohydrates was associated with a reduced T2D risk among men, while there was no association observed when medium- or high-GI carbohydrates were replaced with low-GI carbohydrates [282]. Our

data extends these observations to chronic low-grade inflammation, a condition closely linked to chronic disease development.

While these results further indicate a relevance of the dietary GI during puberty (see previous Chapter 6.1.2), they also suggest a beneficial role of whole grain intake. Regarding the risk of obesity [23], T2D [190, 191, 193], and CVD [189, 192, 193] – as already mentioned in Chapter 2.3.1 – the majority of observational studies conducted in adults points to a beneficial role of a high fiber – particular cereal fiber – and whole grain intake. In contrast, only few studies exist in children and adolescents. Further, the results are not as supportive for a beneficial effect on obesity or T2D risk, and indicate that relevance might depend on baseline weight [23]. Regarding low-grade inflammation, there is only one observational study among US-adolescents which found an inverse association between fiber intake and CRP concentration [285].

In contrast to the dietary GI, which is, as explained above, not part of most of the current dietary recommendations, the recommendation for a high intake of dietary fiber and whole grain is part of most nutritional recommendations [1, 8, 9, 11, 286]. The WHO/FAO expert panel judged the evidence of dietary fiber being protective against obesity as convincing and against T2D and CVD as “probable” [10]. According to the DGAC, moderate evidence suggests that a higher intake of dietary fiber from whole grain foods is associated with reduced risk of obesity, T2D, and CVD. Regarding whole grain intake, the evidence is also judged as moderate for a beneficial effect on obesity and CVD, but only limited for T2D [8]. The EFSA concludes that a higher fiber intake is related to a decreased risk for T2D and CVD as well as improved weight maintenance and sustained weight loss in overweight subjects. However, they do acknowledge, that the contribution of dietary fiber per se to this beneficial effect remains to be established [9]. The DGE concluded that a higher dietary fiber intake reduces the risk for obesity and CVD with probable evidence. Furthermore, the beneficial effects of cereal fiber on T2D risk were rated probable. With regard to whole grain intake, according to the evidence appraisal of the committee, a higher intake reduces the risks of adiposity (possible), T2D (probable), and CHD (probable) [23].

The presented conclusions from the different health authorities on the relevance of dietary fiber and whole grain intake mainly build on evidence from observational studies. Notably, the WHO expert panel concluded that some discrepancy exists between observational and interventional studies in regard to relevant fiber sources for T2D prevention [10]. The systematic review conducted to address the second research question also revealed considerable discrepancies: While observational studies almost consistently suggested benefits of a high intake of fiber and whole grains for chronic low-grade inflammation, this was not verified by intervention studies. In turn, some but not all observational and

interventional studies are supportive of a protective role of a low dietary GI for inflammatory status. Thus, the discrepancies observed for dietary fiber and whole grain are not applicable to dietary GI and GL. Considering these results, the above presented conclusions and dietary recommendations may need to be revised: Fiber and whole grain notably correlate with other aspects of a healthier lifestyle [287], which cannot be completely adjusted for in observational studies. Hence, although observational studies on chronic low-grade inflammation (and also on T2D and CVD, see Chapter 2.3.1) indicate an inverse association, the beneficial effect of fiber or whole grain per se remains to be established – as concluded by the EFSA [9] – and might be rather overestimated.

Taken together, the present results extend the discussion that a high carbohydrate intake might actually be detrimental for chronic low-grade inflammation if the carbohydrates stem from high-GI food sources. Likewise, the beneficial effect of a high pubertal whole grain intake on later low-grade inflammation is in accordance with observational evidence in adults. However, in contrast to studies on dietary GI/GL, an inconsistency between observational and intervention studies reporting on fiber and whole grain intake was observed in adults, suggesting residual confounding and questioning current recommendations on high fiber and whole grain intakes which are almost solely based on observational evidence.

6.1.4 Research Aim 4: To examine carbohydrate nutrition and liver function

In the last research aim, the relevance of changes in carbohydrate nutrition on markers of liver function was examined in a population of older Australians. The lack of longitudinal associations and only small effect sizes suggest that carbohydrate nutrition is not of major relevance for liver function in this elderly population. In accordance, an intervention trial in older US-adults found no effect of a low-fat, low-saturated fat, low-GI diet over four weeks on ALT levels, but on the absolute percentage of liver fat when compared to its high-fat, high-GI counterpart. Of note, higher liver fat at baseline was predictive of a greater decrease in percentage of liver fat. [238]. However, regarding the present results, different aspects need to be kept in mind: The cohort of the BMES can be regarded comparably healthy (see also Chapter 6.2.3) and their diet is characterized by a high intake of fruits and low intake of sugar and soft drinks – thus by a good carbohydrate quality. This could indeed be one explanation for the fact that an association between dietary GI and the liver enzymes ALT and GGT was observed in the young DONALD cohort (see OA2, Appendix 2): The carbohydrate quality in younger age groups is less ideal now [155-157], indicating that this problem might not have reached older age groups yet. Furthermore, the healthy constitution of the BMES sample might also have precluded the detection of a strong association: Valtuena et al. [236]

suggested that the dietary GI was relevant for the risk of high-grade steatosis only in those who were insulin resistant. However, as only few participants in BMES had T2D or IFG, there was limited power to detect possible interactions with health status. Furthermore, the ALT values in the present sample were in the upper normal range at both examinations. Of interest, a recent publication showed an inverse relationship between ALT values in the normal range with total mortality, cardiovascular and non-cardiovascular events in middle-to-older aged participants from three large prospective cohort studies. These results suggest that associations between ALT levels and clinical outcomes are complex and that higher but still normal ALT levels might have a different predictive value compared to clinically elevated ALT levels [288]. Hence, although the present results are not indicative of an association, carbohydrate nutrition might still be of relevance for persons exhibiting clinically elevated liver enzyme levels.

Overall, this study does not suggest a role of carbohydrate nutrition in the studied elderly population. However, carbohydrate nutrition might be relevant for individuals with a diet characterized by an unfavorable carbohydrate quality and/or in those with clinically increased liver enzyme levels, which needs to be investigated in further studies.

6.2 Methodology and study population

This thesis builds on the data of two observational studies and one systematic review. Hence, there are different methodological issues to be considered regarding the aspects and markers chosen as exposure and outcome variables. These issues will be addressed in the following chapters.

6.2.1 Assessment of dietary predictors

Dietary assessment

Dietary intake in the DONALD Study is assessed with **3-day weighed dietary records**, the quasi-gold standard of dietary assessment methods [289]. This direct and prospective method does not rely on memory and portion sizes are assessed very precisely as they are not estimated but weighed. Additionally, for all foods, information on type and brand name are collected. In the DONALD Study, recipes as well as packages and labels are provided by the participants and their families for further information on the foods consumed, which are then added to the dietary record data [247]. The fact that all foods consumed need to be weighed and recorded implies that a high motivation is essential, and also exerts a substantial burden on the participants and their families and may thus inadvertently affect their usual food intake. Moreover, foods which are only rarely consumed such as fish, lentils

or seasonal fruits might not be captured by a 3-day dietary record. Hence, for estimation of the habitual dietary intake, records have to be collected repeatedly [92, 290]. In the DONALD Study, 3-day dietary records are collected annually during puberty, allowing thus a better estimation of the habitual diet.

By contrast, a **FFQ** was applied repeatedly to estimate dietary intake in the BMES. FFQs are commonly used in epidemiological studies as they are relatively inexpensive and easy to administer, particularly in large cohorts. However, as FFQs commonly capture periods of the previous 3 to 12 months, this method, while being able to estimate the habitual diet, relies on the participants' long-term memory and their ability to estimate and describe their usual food intake [92]. Furthermore, the list of foods enquired is relatively restricted, e.g. in the BMES, the FFQ comprises 145 items [255] and frequencies and average serving sizes need to be interpreted [92].

Overall, using a FFQ is applicable in this larger study sample, which was almost four-times bigger than the DONALD Study sample (comparing the subsamples used for this thesis). In addition, a FFQ was frequently applied in the studies assessing the association between carbohydrate quality and inflammatory markers included in the systematic review.

Dietary predictors

Regarding the different aspects of carbohydrate quality, the estimation of the diet's **GI** from individual foods is discussed controversially: Some authors argue that the glycemic response of a mixed meal cannot be accurately estimated by the GI values of its single components since other aspects such as the macronutrient composition influence blood glucose responses [291, 292]. In contrast, others have shown that the sum of GI values of individual foods can actually be used to estimate the GI of a whole diet or mixed meal [293-295]. Limitations of GI assignment comprise the fact that it is often based on GI values for similar foods only when no direct match is available and that it may vary between researchers. Furthermore, whereas weighed dietary records allow a direct assignment of GI values to all recorded foods, the GI assignment needs to be based on food groups if dietary intake is assessed by FFQ. This implies the additional risk of summarizing low- and high-GI foods in the same food group, e.g. whole kernel and wholemeal breads are both assigned the GI of wholemeal bread even though their glycemic response is different [31, 37]. To reduce the outlined shortcomings, a standardized assignment procedure, as used for the present studies (see OA 1, 2, 3 in Appendices 1, 2, 3) is of great importance. Furthermore, regarding FFQ data, a validation and provision of correlation coefficients is crucial for a correct interpretation of the results. This was done for the BMES where the FFQ showed moderate to good agreement for ranking individuals according to their GI as well as their total

carbohydrate, sugar, starch, and fiber intake, but not their GL [255]. Barkley et al. also showed that the majority of prospective studies investigating associations between total carbohydrates, GI, GL and chronic disease risk do not assess whether their FFQ is able to adequately rank individuals according to their GI and GL but rely on correlations with total carbohydrate intake only. However, the authors demonstrated that correlations for carbohydrates cannot be simply transferred to GI and GL but need to be assessed independently. Additionally, it has to be noted that in contrast to other FFQs, the FFQ used in BMES contained some additional questions on the type of breakfast cereals, which increased the accuracy of GI estimates [255]. Nonetheless, the FFQ was not designed to specifically assess dietary GI, which would be even more desirable and should, for instance, also include questions about the type of bread.

Livesey et al. [175] showed in their meta-analysis that the heterogeneity among the included studies was to a large extent attributable to the ability of the FFQ to correctly measure carbohydrate consumption. They concluded that a meta-analysis without appropriate covariates such as dietary instrument validity would thus underestimate the importance of GL in the contribution to risk of T2D. As mentioned before, only very few studies used dietary instruments specifically validated for GI/GL. Thus, for the assessment of dietary instrument validity, the surrogate measure total carbohydrate intake had to be used [175]. It is of note that also in the systematic review conducted as part of this thesis, two of the four studies reporting no associations for dietary GI/GL had employed an FFQ for which correlation coefficients with dietary records were comparably low for total carbohydrate intake.

An alternative and more exact approach to measure the glycemic impact of the diet would be to continuously monitor blood glucose concentrations [296]. However, this expensive approach would not be ethical in a young cohort like the DONALD Study, and moreover not feasible – especially in a large cohort like the BMES. Furthermore, as the sensors are only worn for a few days, habitual dietary intake cannot be estimated from these data, unless they would be applied repeatedly.

Regarding the **FII**, the same limitations apply as debated for GI estimation. Additionally, the FII assignment was based on 121 published values only, as compared to about 2800 values available for GI.

Comparing dietary GI and **dietary fiber and whole grain intake** as aspects of carbohydrate quality, there exist some differences which should be noted: Epidemiological studies on dietary GI/GL are not as amenable to residual confounding, since dietary GI/GL do not strongly correlate with healthy lifestyle behaviors as most populations are still largely unaware of what constitutes a low dietary GI [5]. In turn, it is well known that a higher fiber or

whole grain intake correlates notably with other aspects characterizing a healthier lifestyle and dietary recommendations to increase fiber intake are quite established [287] (see also Chapter 6.1.3). Hence, compared to dietary GI/GL, assessment of the fiber and whole grain intake might be more accurate but entails a higher possibility to reflect unmeasured confounding.

6.2.2 Outcome measurements

Regarding the different risk markers referred to in this thesis, some methodological considerations are warranted. Overall, it has to be noted, that the different outcome measures were only taken once in younger adulthood in the DONALD Study, compared to the repeated dietary assessment during puberty (two to six times). In BMES, dietary intake as well as liver enzymes and serum lipids were repeatedly measured (two times).

Anthropometry

In the DONALD Study, %BF is assessed by skinfold measurement, which is regarded a method susceptible to measurement error. Hydrostatic weighing would be more precise but is, however, not feasible in epidemiological studies. Another option would be the DEXA method, which has been regarded as a gold standard due to its practicability and high precision regarding measurements of body components [297]. However, in the DONALD Study, the method of choice has to be applicable on an annual measurement basis, precluding this option. A further alternative for the measurement of body fat would be the bioelectrical impedance method (BIA), which would be feasible in a cohort study due to its practicability and quick and safe handling [92]. Nevertheless, according to findings from Willett et al., BIA is not superior to BMI in predicting overall adiposity in the general population [298]. Overall, the skinfold technique is the most widely used technique in epidemiological studies [92] and equations of Durnin and Womersly [299], which were used in this thesis, on average agree very well with results from hydrostatic weighing [300]. An advantage of skinfold measurement is the low costs, but trained personnel is needed. In the DONALD Study, standardized procedures are applied by trained staff and an annual quality control is run to affirm quality standards.

Risk markers for type 2 diabetes mellitus

Diagnosis of diabetes is commonly based on 2-hour postprandial glucose values determined during an OGTT or, more recently, also on HbA1c values. The gold standard for measuring insulin sensitivity is the hyperinsulinemic euglycemic glucose clamp technique. However, this method is regarded time-consuming, labor intensive, and expensive [55, 56, 92]. In analyses of DONALD data, HOMA-IR and the liver enzymes ALT and GGT were used as T2D risk

markers. An OGTT has been newly introduced in the study since 2011, but data is not yet available for enough participants to be considered in the present analyses. Furthermore, for HbA1c measurement full blood samples are required, which are not obtained in the DONALD Study. The HOMA index is a simple surrogate index derived from fasting steady-state conditions, which has been widely used especially in large-scale epidemiological studies [56, 57]. Besides the quantitative insulin sensitivity check index (QUCKI), the HOMA index can be regarded the best and most extensively validated simple surrogate index [56, 57]. It may not give appropriate results in subjects with absent/severely impaired beta cell function [57], which does however not apply to the DONALD participants. Moreover, using the same metabolic parameters, i.e. fasting glucose and insulin levels, both HOMA-IR, estimating IR, and HOMA-ß, an estimate on insulin secretion, can be calculated, with the former index being more frequently used [56]. Besides these common indices, the liver enzymes ALT and GGT are recently recognized as risk markers for T2D because of the close link between hepatic and systemic IR [59-63] (see chapter 2.2.4).

Inflammatory markers

To examine the impact of carbohydrate nutrition on chronic low-grade inflammation in the DONALD Study, the pro-inflammatory markers IL-6, hsCRP, IL-18, and the anti-inflammatory adipose tissue hormone adiponectin were considered. The systematic review focused on studies reporting results for IL-6 and hsCRP, as these two inflammatory markers represent the most commonly measured immune mediators in clinical and epidemiological studies. Both CRP and IL-6 have been related to increased T2D [301, 302] and CVD risk [303-305] in cohort studies. However, while being frequently used, Mendelian randomization studies indicated that the association between CRP and risk of CVD is not causal [306, 307], but may have been biased by confounding or reverse causation. In contrast, evidence for a causal role in CVD development exists for IL-6 [308, 309]. These findings from Mendelian randomization analyses might be transferable to the inflammatory markers' role in T2D development. Regarding the relevance of adiponectin as a prognostic marker, meta-analyses showed a prospective association between decreased adiponectin levels and an elevated T2D risk [310], but no consistent relation with CHD and CVD risk [311-313]. Compared to these three markers, IL-18 is less frequently studied. However, results from the MONICA/KORA Augsburg Study showed a positive association between IL-18 concentrations and T2D risk [314], but not coronary events [304]. Hence, to better evaluate the usefulness of the different inflammatory markers in predicting T2D and CVD risk, further research is needed to assess their specific role in chronic disease development.

Markers of liver function

The gold standard for a diagnosis of NAFLD is liver biopsy [142]. To non-invasively estimate hepatic fat accumulation, the enzymes ALT and GGT are commonly used. Of note, GGT also serves as a marker for alcoholic fatty liver, which was the reason for excluding those BMES participants with a higher alcohol intake [137, 142, 315, 316] (for details see OA5, Appendix 5). Regarding the use in epidemiological studies, it has to be noted that raised liver enzymes can only reveal an increased risk, yet no quantitative conclusions about the extent of hepatic fat accumulation can be drawn. Indeed, NAFLD can be present without any elevations in liver enzymes. However, liver enzymes are commonly used as minimally invasive parameters to estimate fat accumulation and NAFLD risk [142, 315]. Additionally, serum lipids can be used as risk markers, since NAFLD is closely related to dyslipidaemia and the metabolic syndrome [137, 317, 318].

6.2.3 Study characteristics

Both the DONALD Study and the BMES are characterized by a prospective study design. Even if purely observational, this study design allows following a group of individuals over time and it is possible to study different exposures in order to determine how these factors are related to the development of specific outcomes. Hence, in contrast to cross-sectional approaches, prospective cohort studies are able to identify occurrences of diseases or their development. Regarding the particular examinations done in the context of this thesis, DONALD analyses focused on dietary intake during the critical period of puberty, while in BMES analyses, five year changes during older age were examined. Noteworthy, only subsamples of both cohorts were used for the present analyses.

Longitudinal cohort studies such as the present ones generally face the problem that only very interested participants will participate over the long-term, which in turn is often associated with a higher education and socioeconomic status. This may indeed introduce selection bias and hampers generalizability. However, it should be noted that the latter limitation is of minor importance when examining associations between exposure and outcome within a cohort, since it is unlikely to affect internal validity [247]. Unquestionably, the results presented cannot be generalized and thus require confirmation in other populations.

In the systematic review, the relevance of different aspects of carbohydrate quality from both observational and intervention studies was compared. The inclusion of both study designs served as an opportunity to compare their results and also revealed some discrepancies (see Chapter 6.1.3 and OA4, Appendix 4). As explained above, results from observational studies

can only give an indication for a causal relation which then needs to be further verified by intervention studies. However, it has to be noted that not all research questions can be answered by intervention studies, i.e., particularly in children and adolescents, the conduction of clinical trials is notoriously difficult, and often not ethically feasible.

DONALD Study

The prospective open cohort nature of the DONALD Study entails the rare and valuable possibility to cover the time from birth until adulthood. Moreover, the repeated, comprehensive measurements, taken in close intervals, allow to examine different associations between diet, anthropometrical characteristics, and risk markers for different health outcomes over the course of growth.

Even though the DONALD sample is very homogenous and can be regarded non-representative, comparison of the anthropometric characteristics with the German reference population shows only slight differences: Indeed, DONALD participants included in the different analyses of this thesis (Studies I, II, III) had slightly lower to comparable BMI values during puberty compared to the German reference population [102]. Participants included in the DONALD sub-sample for the body composition analyses (Study I) had a median %BF of 17% for men and 29% for women in young adulthood. However, since DEGS1 used BMI to identify overweight and obesity, no data on %BF were available for comparison. Regarding overweight and obesity prevalence in adulthood, on average 17.8% of the women were overweight and 5.4% of these were obese in Studies I, II, and III; among men, on average 30.7% were overweight and of these, 6.4% obese (average age: 21 years). Comparing this data to overweight and obesity prevalence in the DEGS1, the DONALD values are lower, especially for women: In DEGS1, 30.0% of the 18 to 29 years old women were overweight, and 9.6% obese. For men, the survey showed an overweight prevalence of 35.5% in 18 to 29 years old men, and an obesity prevalence of 8.6% [99]. This comparison further highlights the good health condition of the DONALD cohort, but also indicates the difficulty to apply the current findings to the growing proportion of overweight adolescents and young adults.

With regards to the daily macronutrient intakes, data from EsKiMo (Ernährungsstudie als KiGGS Modul), a nutrition module included in the KiGGS study, can be used as a reference. In this survey, information of the adolescents' diet was assessed with a personally conducted interview from twelve years onwards covering dietary intake of the last four weeks. The median energy intake during puberty was approximately 7MJ for female and 9MJ for male DONALD participants (in all three subsamples of Studies I, II, III) and hence around 1MJ lower as compared to girls and boys aged twelve years in EsKiMo. With a median intake of 36En% fat, 13En% protein, and 51En% carbohydrates of the DONALD participants during

puberty (in all three subsamples of Studies I, II, III)), the macronutrient proportions are comparable to twelve year old girls and boys in EsKiMO: These boys and girls had with 13En% a comparable median protein intake, but a lower fat intake (33En%), and a slightly higher carbohydrate intake (52.5En% for girls, and 51.8En% for boys in EsKiMo) [319].

BMES

In contrast to the DONALD Study, the BMES is a population-based cohort study including a representative older Australian community sample. Those 3654 participants, who started at baseline, where invited to three follow-up visits every five years. As participants died or were lost to follow-up, the sample size was reduced subsequently, introducing the possibility of selective survival and also affecting the representativeness (see above).

In the BMES subsample used in Study V, 68% of the 543 women and 70% of the 323 men were overweight or obese (median age 67 years; range: 54 to 94). Of note, data for overweight and obesity is only reported combined for a better comparability with the Australian Bureau of Statistics data. This prevalence data indicates that 70% of the women and 80% of the men aged 65 to 74 years were classified as overweight or obese. Hence, while prevalence rates were comparable for women, the male participants in the BMES are characterized by a lower overweight and obesity prevalence compared to the general Australian population in this age group [100].

Regarding dietary intake in the present BMES subsample, women and men consumed both 48En% carbohydrates, 31En% and 32En% fat, and 18En% and 17En% protein with a median energy intake of 8.0 and 8.9MJ, respectively. These intake values can only be compared to data from the National Nutrition Survey conducted in 1995 by the Australian Bureau of Statistics, as results from the 2011/13 Australian Health Survey are not yet available. Dietary intake was assessed by 24-h recalls and the results for the age group 55 to 64 years – the oldest group included in the survey – were as follows: With a median energy intake of 6.6MJ, women consumed 37En% fat, 45En% carbohydrates, and 17.3En% protein and men consumed 34En% fat, 45En% carbohydrates, and 17En% protein with a median energy intake of 9.4MJ (percentage of energy values calculated on the basis of gram and energy data) [320]. Hence, women in the BMES had a higher and men a slightly lower energy intake compared to the general Australian population. Furthermore, the BMES participants consumed less fat and more carbohydrates, while protein intake was comparable with the survey results.

6.3 Public Health relevance and possible practical implications

This thesis builds on the assumption that a high carbohydrate intake might be particularly detrimental not only for the growing number of overweight and insulin resistant individuals [5, 6], but also – as shown in this thesis – for adolescents facing a physiological IR. Even if not the amount of carbohydrates but their quality was the focus of the present thesis, some comments can be made: The present findings do not provide an indication for a beneficial effect of a carbohydrate-rich diet on risk markers of T2D. Furthermore, possible detrimental effects of a high carbohydrate intake from high-GI food sources on later low-grade inflammation are indicated. Hence, these results do not support the current recommendations to consume between 45 and 70% of energy as carbohydrates [8-10, 23] but are in accordance with the conclusion of the evidence-based dietary guideline of the German Nutrition society, stating that the total carbohydrate intake is not of relevance for the risk of T2D [23].

So far, while agreement exists on the importance to highlight carbohydrate quality in nutritional recommendations, the single aspects of carbohydrate quality have been included to a different extent:

As already summarized in Chapter 6.1.3, most health agencies recommend a high intake of **dietary fiber**. These recommendations are largely based on observational evidence. In the systematic review (Study IV), it has been observed that the strong observational evidence for a beneficial effect of a high fiber and whole grain intake on chronic low-grade inflammation was not supported by intervention studies, indicating potential residual confounding (see Chapter 6.1.3 and OA4, Appendix 4). This finding might also be applicable to the closely related health outcomes T2D and CVD, for which comparable strong observational evidence exists (see chapter 2.3.1). It should hence be considered that the current recommendations to increase the intake of dietary fiber and whole grain products, although contributing to a balanced diet, might probably not yield the expected health benefits. Indeed, the DGAC concluded that there was limited evidence showing an association between whole grain consumption and reduced incidence of T2D in large prospective cohort studies [8]. Furthermore, the FDA declared in a recent statement, that there is “very limited credible scientific evidence” for a health claim stating a relation between whole grain consumption and reduced risk of T2D. This statement was part of a letter sent to a food supplier (ConAgra) in response to his health claim petition. The FDA refused the suggested claims (stating a more consistent association between whole grains and T2D) after reviewing the petition, as well as the available literature on whole grain intake and T2D risk, and notes made during a public comment period. Confusingly enough, the FDA instead announced to consider exercising enforcement discretion for the following health claim to not mislead

consumers: “Whole grains may reduce the risk for T2D, although the FDA has concluded that there is very limited scientific evidence for this claim.” While the comprehensibility of this claim might be questioned, food manufacturers seem to be pleased to still be able to link whole grains to a reduced disease risk [321]. In this context, a recent study from Harvard revealed that grain products with the “Whole Grain Stamp,” one of the most commonly used front-of-package labels, were indeed higher in fiber and lower in trans-fats, but also higher in sugar and calories compared to products without the stamp. The other labels examined also had mixed performances for identifying healthier grain products. These results underline the need for a consistent, evidence-based standard for labeling whole grain foods [322]. Particularly, a common definition of what is regarded a whole grain food is desired in light of the diverse definitions currently existing (see Chapter 2.1.1), which would also simplify consumers’ food choices.

Concomitant with the recommendation to increase dietary fiber and whole grain intake, health agencies often advise a reduction in **sugar intake**, particularly from sugar-sweetened beverages [23, 323]. The observation that total sugar intake was not of relevance for T2D risk markers is in agreement with the evidence-based guideline from the German Nutrition Society [23]. Observational evidence however points to an unfavorable role of sugar intake from soft drinks [23] for which the present analyses show only a relevance for the liver enzyme GGT, one of the examined risk markers for T2D (see OA2, Appendix 2). However, comparable to dietary fiber and whole grain intake, soft drink consumption might be more a marker for an overall unhealthier nutrition and lifestyle, as large effects have only been shown in observational studies comparing extremes of dietary intake. A focus solely on soft drinks, which is observed in some of the recent health campaigns, e.g. the ban of all soft drinks above 473ml (16oz) in New York restaurants, might not relate to the desired decreases in disease prevalence rates. However, in light of an increase in sugar consumption, partly due to a higher availability in processed foods, and taking into account the fact that sugar does not possess any nutritional value, the recommendation for a reduced consumption is indeed reasonable [324-327].

In contrast to dietary fiber and sugar, the **dietary GI** is currently not included in most of the nutritional recommendations (see Chapter 6.1.2). However, the present results indicate a special role of postprandial glycemia during puberty for later low grade inflammation and T2D risk. For obesity, postprandial insulinemia was of greater relevance in our analyses. As the concept of the dietary insulin index is however very new, more studies are needed to confirm this. Regarding dietary GI, a consistent relevance for the different risk markers for T2D was shown in the present analyses. Even if available intervention studies in children and adolescents show inconsistent results for a relevance of dietary GI (see Chapter 2.3.1), no

unfavorable effects have been reported when following a low-GI diet. Therefore, the GI concept may need to be incorporated into dietary recommendations given to adolescents. So far, the main argument against its inclusion in dietary recommendations is the heterogeneity among the studies [9, 23, 328], which may however be based to a large extent on methodological problems [175] (see Chapter 6.1.2 and 6.2.1). Considering the GI in nutritional recommendations, a GI label on food products could promote a successful implementation as most people are largely unaware of the GI concept. However, so far GI claims on food labels are only authorized in Australia, New Zealand, and South Africa. Particularly in the EU, strict requirements might hamper the introduction of a low-GI label or health claim: For a qualified health claim, a single nutrient, substance, food or food category needs to be defined. As this is contrary to the whole GI concept, a permission of a GI health claim is very unlikely in the EU unless the regulations change. So far, the EFSA concluded in 2010 that low-GI carbohydrates were “not sufficiently characterized” and that their health benefits had not been established [329]. In this context, Aziz et al. [330] recently published an evaluation of the use of GI claims on food labels on behalf of Health Canada, which was also not supportive of an inclusion of the GI value on the product label. This evaluation has been criticized by the International Carbohydrate Quality Consortium (ICQC) committee [331]: In the response letter, the committee particularly highlighted the fact that the GI claim, as any other dietary claim, should not be used in isolation. A procedure similar to the Australian GI symbol was proposed, where the low-GI symbol requires the fulfillment of strict nutritional criteria. The Australian example furthermore shows that the GI concept is not too difficult to understand for consumers if communicated sufficiently: While the prediction of the dietary GI is rather sophisticated, the implementation can be done by using simple substitution tables (see **Table 9**), which can be used regardless of the implementation of a GI symbol. Indeed, one in four Australians choose low-GI products and substitute them for regular high-GI variants within a food group. Finally, the committee emphasized the need to distinguish high-GI whole grains from low-GI counterparts [331]. Indeed, analyses in the DONALD cohort also revealed that 76% of the whole grain foods consumed from the participants were of moderate- to high-GI (see OA3, Appendix 3), even though several low-GI options exist on the German food marked (e.g. wholemeal bread with intact kernels and unprocessed muesli with nuts etc.). It is thus pivotal to not only recommend the consumption of whole grain foods but also give specific dietary advice – preferably supported and facilitated by substitution tables or GI labels – which encourage a preferred selection of low-GI foods.

Table 9: Substitution of low-GI carbohydrate food sources for high-GI counterparts within the context of a prudent diet (modified from [38, 332])

High-GI Food	Low-GI Alternative
Bread—white or wholemeal	Breads containing intact whole grains; sourdough and pumpernickel breads
Processed breakfast cereals (i.e. cornflakes)	Unrefined cereals such as rolled oats or natural muesli, i.e. with nuts and dried fruits
Plain biscuits or crackers	Biscuits made with dried fruit, oats, and whole grains, nuts
Cakes and muffins	Make them with fruit, oats, oat bran, rice bran, and psyllium husks
Potato: mashed potatoes, oven potatoes	Pasta, lentils or low-GI potato varieties/preparations (i.e. baby new potatoes (small size), waxy potatoes, sweet potatoes, potato salad)
Rice: sticky white rice	Longer grain varieties such as Basmati, parboiled or brown rice

Overall, relating the results of this thesis to the current nutritional recommendation to consume a high-carbohydrate, high-fiber diet, the following modifications appear to be required: Since the present analyses indicate that it can be actually detrimental to have a high carbohydrate intake if the carbohydrates stem from higher GI food sources, the health agencies should either move away from recommending a minimum intake level or accompany their recommendation by detailed advice to consume not only fiber/whole grain-rich but also low-GI carbohydrate foods.

7. CONCLUSION AND PERSPECTIVES

To conclude, the results presented in this thesis indicate a particular relevance of the diets' blood glucose rising effect during the critical period of puberty for later risk markers of T2D. Since both obesity and diabetes prevalence are increasing also in younger age groups, preventive strategies starting early in life are urgently needed. As the present analyses indicate a special relevance of the diets' glycemic potency, efforts to improve carbohydrate quality – particularly in adolescents – should not focus solely on a high whole grain intake, but need to be complemented by an advice for a preferred selection of low-GI foods.

Future intervention studies should aim to disentangle the effect of fiber and whole grain intake per se on different health outcomes, leading either to a confirmation or revision of the current recommendations. Also, more well-conducted studies using dietary assessment methods being able to estimate the GI correctly, which assess the long-term effect of low-GI diets, are urgently needed to overcome the current methodological problems hampering the formulation of conclusive remarks regarding the relevance of the GI concept.

The inclusion of OGTT measurements in the DONALD protocol will furthermore lead to a more precise evaluation of T2D risk in this cohort. Overall, the present results need to be verified by other prospective studies, ideally encompassing a longer time span, i.e. until middle adulthood. Moreover, further studies are needed to examine the impact of carbohydrate quality in the elderly, preferably in cohorts characterized by less favorable nutritional habits or a higher prevalence of IR or T2D.

Importantly, any public health strategy to improve carbohydrate quality during adolescence should be accompanied by advice on the overall diet quality and regular physical activity. Successful prevention strategies to reduce later risk for obesity and diabetes should furthermore not focus on adolescents only but involve all age groups including, for example, a family based approach.

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International Journal of Obesity (2012) **36,** 1463 – 1471

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ORIGINAL ARTICLE

Prospective associations of dietary insulin demand, glycemic index, and glycemic load during puberty with body composition in young adulthood

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BACKGROUND: Puberty is a so-called critical period for overweight development and is characterized by physiological insulin resistance during mid-puberty. This study addressed the hypothesis that habitual consumption of a diet inducing higher levels of postprandial glycemia or insulinemia during puberty may have an unfavorable effect on the body composition in young adulthood.
METHODS: Multivariate regression analysis was performed on 262 participants of the Dortmund Nutritional and Anthropometric Longitudinally Designed Study with at least two 3-day weighed dietary records during puberty (baseline: girls 9 – 14 years; boys 10 – 15 years) and anthropometric measurements in young adulthood (18 – 25 years). A published dietary glycemic index was assigned to each carbohydrate-containing food. Similarly, each food was assigned a food insulin index (insulinemic response to a 1 MJ portion of food relative to 1 MJ of glucose) using 121 values measured at Sydney University.
RESULTS: Dietary glycemic index or glycemic load during puberty was not related to body composition in young adulthood. In contrast, a higher dietary insulin index and a higher dietary insulin load during puberty were associated with higher levels of percentage of body fat (%BF) in young adulthood, even after adjustment for early life, socioeconomic and nutritional factors; %BF in energy-adjusted tertiles of dietary insulin index were 22.9 (95% confidence intervals (CI): 21.6, 24.1), 24.5 (23.2, 25.7), 24.7 (23.5, 25.9) %, $P_{\text{for trend}} = 0.01$; %BF in energy-adjusted tertiles of dietary insulin load were 22.8 (95% CI: 21.5, 24.0), 24.5 (23.2, 25.7), 24.8 (23.6, 26.0) %, $P_{\text{for trend}} = 0.01$. Adjustment for baseline %BF attenuated these relationships ($P_{\text{for trend}} = 0.1$ and $= 0.08$, respectively). Dietary insulin demand was not related to body mass index.
CONCLUSION: This study suggests a prospective adverse influence of dietary insulin demand during puberty on %BF in young adulthood. Postprandial increases in insulinemia rather than increases in glycemia appear to be implicated in an unfavorable development of body composition.

International Journal of Obesity (2012) **36,** 1463 – 1471; doi:10.1038/ijo.2011.241; published online 17 January 2012

Keywords: dietary insulin index; dietary insulin demand; dietary glycemic index; dietary glycemic load; body composition; body fat

INTRODUCTION

Over the previous years, the relevance of the dietary glycemic index (GI) for the development of obesity has been controversially debated. Among adults, prospective cohort studies suggest a role of the dietary GI for body composition.[1-4] However, similar associations have not been observed among healthy children and adolescents[5,6] or overweight Latino adolescents.[7]

Intervention studies in overweight and obese adults suggest a specific efficacy of low-GI weight-loss diets[8,9] for persons with already increased insulin secretion levels.[9] Puberty is a so-called 'critical period' for overweight development, which is characterized by physiological insulin resistance and changes in levels of various hormones, including insulin-like growth factor (IGF)-1, growth hormones as well as sex steroids.[10] In fact, IGF-1 levels rise steeply during puberty and peak before the end of puberty, whereas the development of the insulin sensitivity follows the reverse course.[11,12]

It is possible that postprandial glycemia and insulinemia are relevant targets during puberty so as to prevent the development of an unfavorable body composition. Mechanisms linking the habitual consumption of high-GI foods to body composition include reduced satiety signaling, as fully gelatinized starches in high-GI foods do not reach the lower parts of the ileum, and enhanced carbohydrate oxidation and decreased fat oxidation in response to habitual postprandial glycemia and insulinemia.[13] In addition, reactive hypoglycemia in the late postprandial phase has been proposed to induce hunger and higher voluntary energy intakes.[14] Counter-regulatory hormone responses following this reactive hypoglycemia may have proteolytic effects, favoring the loss of lean body mass and a reduction of resting energy expenditure.[13] Finally, elevated IGF-1 levels may predispose to obesity later in life,[15] and the GI of a meal has been found to acutely affect the IGF-1 axis.[16]

As high-GI foods influence both blood glucose and insulin levels, it is not clear which of these postprandial changes is potentially more relevant for an unfavorable development of body composition. Insulin secretion is also stimulated by dietary protein and, moreover, dietary protein and fat may both act synergistically with carbohydrates, raising insulin levels and reducing postprandial glycemia.[17-19] The food insulin index (FII) compares the

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Received 20 May 2011; revised 27 October 2011; accepted 5 November 2011; published online 17 January 2012

postprandial insulin response to any food relative to a reference food (glucose) and also, unlike the GI, considers foods with no or low amounts of carbohydrates.[20]

This study addressed the hypothesis that habitual consumption of a diet inducing higher levels of postprandial glycemia or insulinemia during puberty may have an unfavorable effect on body composition in young adulthood.

METHODS

Study population

The present study was ancillary to the Dortmund Nutritional and Anthropometric Longitudinally Designed Study (DONALD Study), an ongoing, open cohort study conducted at the Research Institute of Child Nutrition in Dortmund, Germany. Details on this study have been described elsewhere.[21]

Briefly, as the recruitment began in 1985, detailed data on diet, growth, development, and metabolism between infancy and adulthood have been collected from >1300 healthy children. Every year, an average of 50 infants are newly recruited and first examined at the age of 3 months. Each child returns for three more visits in the first year, two in the second and then once annually until adulthood. The study was approved by the Ethics Committee of the University of Bonn, and all examinations are performed with parental consent.

The children who were initially recruited for the DONALD Study differed considerably in age. Because of the open cohort design, many children have not yet reached young adulthood. In total, 394 subjects were aged 18 years or older by the time of this analysis. They were term (37–42 week gestation) singletons with a birth weight $\geqslant 2500$ g and had at least one anthropometric measurement in young adulthood. As we were interested in the long term-relevance of dietary parameters during adolescence for adult body composition, we regressed dietary intake on the last anthropometric measurement available during young adulthood ($\geqslant 18$ and $\leqslant 25$ years of age, mean age $= 20.3$ years). Of these, 308 participants had provided at least two 3-day weighed dietary records at baseline (puberty was defined by chronological age: girls 9–14 years, boys 10–15 years), allowing the estimation of habitual dietary intake during puberty. Participants who were identified to consistently underreport their energy intake (that is, all food records were implausible or they had provided more implausible than plausible food records) were excluded from the study ($n = 23$). A 3-day weighed dietary record was considered plausible when the total recorded energy intake was adequate in relation to the basal metabolic rate (estimated from the Schofield equations[22]) using modified cut-offs from Goldberg *et al*.[22,23] Overall, 1379 records were included (2–7 records per participant). Furthermore, participants had to have anthropometric data available at baseline and information on relevant covariates such as early life (for example, breast feeding) and socioeconomic factors (for example, maternal overweight). This resulted in a final sample of 262 participants (53.6% female, 46.4% male).

Anthropometric measurements

Participants are measured at each visit according to standard procedures,[24] dressed in underwear only and barefoot. From the age of 2 years onward, standing height is measured to the nearest 0.1 cm using a digital stadiometer (Harpenden Ltd., Crymych, UK). Body weight is measured to the nearest 100 g using an electronic scale (Seca 753E; Seca Weighing and Measuring Systems, Hamburg, Germany). Skinfold thicknesses are measured from the age of 6 months onward at four different sites (supra-iliacal, subscapular, biceps, triceps) on the right side of the body to the nearest 0.1 mm using a Holtain caliper (Holtain Ltd., Crosswell, United Kingdom). Since 2005, waist circumference is also routinely measured according to World Health Organization recommendations at the midpoint between the lower rib margin and the iliac crest.[25] The three trained nurses who perform the measurements undergo an annual quality control, conducted in six to eight healthy young adult volunteers. Average inter- and intra-individual variation coefficients obtained in the last 6 years (2005–2010) were 0.7 and 1.8% for waist circumference, 7.9 and 12.7% for biceps, 5.4 and 6.2% for triceps, 5.2 and 7.8% for subscapular, and 7.5 and 9.1% for supra-iliacal skinfolds.

Anthropometric calculations

Regarding body mass index (BMI, $kg\,m^{-2}$) in puberty, sex- and age-independent standard deviation scores were calculated using the German reference curves for BMI.[26] Percentage body fat (%BF) was derived using equations of Slaughter *et al*.[27] for pubescent children, which consider triceps and subscapular skinfolds. Overweight during puberty was defined according to values proposed by the International Obesity Task Force, which correspond to an adult BMI of $25\,kg\,m^{-2}$.[28] The reference values for %BF published by McCarthy *et al*.[29] were used to determine pubertal participants with excess body fatness, that is, %BF above the 85th percentile.[29]

Regarding anthropometric data in young adulthood, BMI was calculated and %BF was estimated from skinfolds using Durnin and Womersley equations,[30] which are based on triceps, biceps, scapular and iliacal skinfolds.

Nutritional assessment

Food consumption in the DONALD Study is assessed annually using 3-day weighed dietary records. All foods and beverages consumed are weighed and recorded, as well as leftovers, to the nearest 1 g over 3 days using electronic food scales (initially Soehnle Digita 8000; Leifheit SG, Nassau; Germany; now WEDO digi 2000; Werner Dorsch Gmbh, Muenster/Dieburg, Germany). For this analysis, dietary variables were calculated as individual means of the 3-day weighed dietary records using LEBTAB,[31] the in-house database, which is continuously updated to include all recorded food items. LEBTAB is based on the German standard food tables[32] and data obtained from commercial food products. Currently, LEBTAB contains more than 13 100 entries, including additives, supplements and medicine, that is, 1207 basic food items and 10 832 composite foods.

To better describe the habitual dietary intake during puberty, an individual average intake was calculated from at least two records during puberty.

Dietary GI and insulin index

Dietary GI is defined as the incremental area under the curve of glucose response following the intake of 50 g of carbohydrate from a test food as compared with area under the curve of glucose response induced by the same amount of carbohydrate ingested as glucose in 5–10 separate individuals.[33] A published GI value[34] was assigned to each carbohydrate-containing food recorded in the dietary records (based on glucose as the reference food) according to a standardized procedure.[35] The carbohydrate content (in grams) of each consumed food was then multiplied by the food's GI to obtain its glycemic load (GL). The sum of these GL values for each subject corresponds to the total daily GL. The overall GI is obtained by dividing the total daily GL by the total daily carbohydrate intake.

The FII is defined as the insulinemic response (area under the curve) following the intake of 1000 kJ of a food relative to the insulinemic response to glucose that is, the reference food (FII $= 100$).[20] Foods originally tested against a white-bread standard were converted to the glucose standard by a conversion factor of 0.73. For the present analysis, 121 FII values measured at Human Nutrition Unit School of Molecular and Microbial Biosciences University of Sydney, Australia in groups of 10 individuals[36] were available to assign a FII value to each food recorded in the dietary records according to a standardized procedure similar to that established for GI assignment (Figure 1). The dietary GL was the principal consideration when matching foods rich in carbohydrates with an available FII, as it is the best predictor of FII.[20,36] The protein content was used as a guide to find the best match when carbohydrate content was low. A published FII or a close match was available for 36% of the foods (steps 1 and 2), a weighted mean was calculated for another 33% (step 4) and 18% of the foods were assigned the mean FII of the respective food group (step 3). For 11% of the foods the FII value was assigned

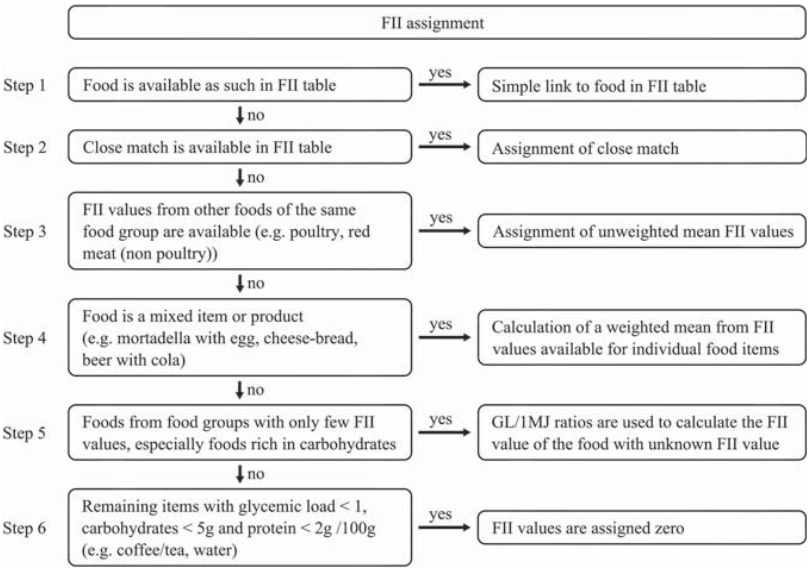

Figure 1. Flowchart for the assignment of FII values to food items recorded in the 3-day weighed dietary records (FII values measured at Human Nutrition Unit School of Molecular and Microbial Biosciences University of Sydney, Australia).

zero (step 6) and the GL ratio was used to calculate the FII of 3% foods (for example, $FII_{sucrose} = GL_{sucrose}/GL_{glucose} \times FII_{glucose}$; step 5). The average dietary insulin load was calculated by summing the product of FII, energy content and consumption frequency over all recorded food items in the 3-day dietary records. The average dietary insulin index was calculated by dividing the insulin load by total energy intake.[37]

Potentially confounding factors

On their child's admission to the study, parents are interviewed by the study pediatrician, and weighed and measured by the study nurses using the same equipment as for children from 2 years onward. Information on the child's birth characteristics are abstracted from the 'Mutterpass', a standardized document given to all pregnant women in Germany. The duration of full breastfeeding (no solid foods and no liquids other than breast milk, tea or water) is inquired by the pediatricians at the first visits until complementary feeding is initiated. For this analysis the following characteristics were considered: breastfeeding status (ever fully breastfed (yes/no) was defined as fully breastfed >2 weeks), maternal overweight status (BMI $\geqslant 25\,kg\,m^{-2}$), high maternal educational status ($\geqslant 12$ years of schooling) and smoking in the household (yes/no).

Statistical analysis

To analyze the potential relation of dietary insulin index, insulin load, GI and GL during puberty with body composition in young adulthood, the distribution of these dietary variables was grouped into tertiles (T1–T3). Tests for differences were performed among the tertiles of dietary insulin index, insulin load, GI and GL using ANOVA for normally distributed continuous variables, Kruskal–Wallis test for non-normally distributed continuous variables and χ^2-test for categorical variables. Analysis of the association between diet during puberty and body composition in young adulthood was performed by multiple linear regression analysis. As BMI was not normally distributed it was log-transformed before the analysis. Each potential confounder was initially considered separately and included if it modified the respective association substantially. Thus, sex was retained in the basic model (model A). In a further step, we also adjusted for early life (breastfeeding) and socioeconomic factors (maternal overweight) as well as other nutritional factors (model B). In a final model, we controlled for confounding by body composition at baseline (model C). All dietary variables except dietary insulin index and GI were energy adjusted using the residual method.[38] To account for age-dependent changes in intake levels all variables were standardized by age group and sex (mean = 0, s.d. = 1).[6]

The adjusted means (that is, least-squares means predicted by the model when the other variables were held at their mean values) are presented with their 95% confidence interval by tertiles. *P*-value <0.05 was considered as statistically significant. All statistical analyses were carried out using SAS procedures (version 9.1.3, SAS Institute, Cary, NC, USA).

RESULTS

Subjects who were excluded from the study sample ($n = 132$) did not differ in sex, birth weight or length, gestational age, BMI and %BF in young adulthood from those who were included ($n = 262$) (data not shown).

Baseline characteristics of the 262 healthy participants did not differ across tertiles of GI and GL. However, subjects with a diet in the lowest tertile of insulin index and insulin load were less likely to be overweight at baseline and those in the lowest tertile of insulin load tended to have lower levels of %BF at baseline. Furthermore, participants in the lowest tertile of the dietary insulin load were less likely to have had mothers with a high level of education (Table 1). Mean BMI–standard deviation scores during puberty were close to zero, indicating that the BMI values at baseline were comparable to the German reference population.

Participants in the highest dietary insulin index and insulin load tertile had lower of total and saturated fat, total and animal protein, but higher intakes of vegetable protein, carbohydrate, and added sugar (% of total energy, %E) as well as higher dietary GI and GL compared with participants in the lowest dietary insulin index and insulin load tertile (Table 2). Comparable differences were seen across tertiles of GL. A higher dietary GI was related to lower intakes of total and animal protein as well as fiber, and higher added sugar intake (%E), a higher dietary insulin index and a higher GL.

Overall, dietary insulin index, insulin load, GI and GL during puberty were not related to BMI in young adulthood, (Table 3) and dietary GI and GL during puberty were not related to %BF in young adulthood (Table 4). However, a higher dietary insulin index

Table 1. Demographic, anthropometric, birth, and socioeconomic characteristics by energy-adjusted tertiles of dietary insulin index, insulin load, GI, and GL at baseline ($n = 262$), DONALD Study, Germany

	Subjects	*Dietary insulin index at baseline*				*Dietary insulin load at baseline*				*Dietary GI at baseline*				*Dietary GL at baseline*			
	n	*T1*	*T2*	*T3*	P *value*[a]	*T1*	*T2*	*T3*	P *value*[a]	*T1*	*T2*	*T3*	P *value*[a]	*T1*	*T2*	*T3*	P *value*[a]
Female (*n* (%))	262	46 (53.5)	47 (53.4)	47 (53.4)	>0.9	46 (53.5)	47 (53.4)	47 (53.4)	>0.9	46 (53.5)	47 (53.4)	47 (53.4)	>0.9	46 (53.5)	47 (53.4)	47 (53.4)	>0.9
Age (years)[b]	262	9.8	9.9	9.3	0.7	9.8	9.9	9.3	0.6	9.9	9.5	9.2	0.7	9.8	9.8	9.6	>0.9
Weight (kg)[b]	262	32.3	34.6	34.5	0.5	33.1	33.0	35.0	0.5	34.9	33.1	32.7	0.3	33.5	34.9	33.4	0.7
Height (m)[b]	262	139.7	142.1	142.2	0.6	139.9	141.3	142.5	0.8	142.0	140.0	142.1	0.2	141.0	142.1	141.1	0.7
BMI-SDS	262	-0.12	-0.02	0.06	0.4	-0.08	-0.10	0.10	0.3	0.10	-0.03	-0.15	0.2	0.06	-0.05	-0.09	0.5
BMI (kg m^{-2})[b]	262	16.6	16.7	16.8	0.6	16.9	16.5	16.9	0.3	17.2	16.7	16.1	0.2	17.0	16.7	16.5	0.5
Overweight (*n* (%))[c]	262	6 (7.0)	12 (13.6)	16 (18.2)	0.09	6 (7.0)	12 (13.6)	16 (18.2)	0.09	11 (12.8)	13 (14.8)	10 (11.4)	0.8	10 (11.6)	11 (12.5)	13 (14.8)	0.8
Body fatness (%)[b,d]	262	15.1	16.3	17.4	0.1	15.1	16.3	17.5	0.09	15.4	16.4	16.0	0.9	16.3	15.9	16.0	0.7
Excess body fat (*n* (%))[e]	262	11 (12.8)	15 (17.1)	19 (21.6)	0.3	12 (14.0)	14 (15.9)	19 (21.6)	0.4	14 (16.3)	15 (17.1)	16 (18.2)	0.9	15 (17.4)	12 (13.6)	18 (20.5)	0.5
Birth weight (g)	262	3443	3429	3542	0.2	3458	3423	3533	0.2	3506	3473	3436	0.6	3477	3481	3456	0.9
Birth length (cm)[b]	262	51.5	52.0	52.0	0.5	52.0	51.0	52.0	0.7	52.0	52.0	51.0	0.5	52.0	52.0	52.0	0.8
Pregnancy duration (weeks)[b]	262	40.0	40.0	40.0	0.6	40.0	40.0	40.0	0.2	40.0	40.0	40.0	0.5	40.0	40.0	40.0	0.2
Breast feeding (>2weeks (*n* (%))[f]	262	58 (67.4)	66 (75.0)	61 (69.3)	0.5	56 (65.1)	69 (78.4)	60 (68.2)	0.1	62 (72.1)	56 (63.6)	67 (76.1)	0.2	60 (69.8)	61 (69.3)	64 (72.7)	0.9
Maternal overweight (*n* (%))[g]	262	22 (25.6)	31 (35.2)	31 (35.2)	0.3	22 (25.6)	31 (35.2)	31 (35.2)	0.3	24 (27.9)	27 (30.7)	33 (37.5)	0.4	23 (26.7)	30 (34.1)	31 (35.2)	0.4
Maternal education (*n* (%))[h]	260	30 (35.3)	44 (50.0)	41 (47.1)	0.1	28 (32.9)	47 (54.0)	40 (45.5)	0.02	40 (46.5)	37 (42.5)	38 (43.7)	0.9	30 (35.3)	44 (50.6)	41 (46.6)	0.1
Smoking in the household (*n* (%))	208	25 (34.7)	22 (31.9)	23 (34.3)	0.9	25 (34.2)	20 (28.6)	25 (37.3)	0.5	15 (22.7)	29 (40.3)	26 (37.1)	0.07	23 (31.9)	23 (32.4)	24 (36.9)	0.8

Abbreviations: BMI, body mass index; GI, glycemic index; GL, glycemic load; SDS, standard deviation scores; T, tertile. [a]Significant differences between the tertiles were tested using analysis of variance for normally distributed continuous variables, Kruskal-Wallis test for not normally distributed continuous variables and χ^2-test for categorical variables. Values are means unless indicated as medians[b] or otherwise. [c]Derived from the age- and sex-specific cut-points proposed by the International Obesity Task Force, which are linked to the adult cut-off point of a BMI of 25 kg m^{-2}.[28] [d]Calculated according to Slaughter *et al.*[27] [e]Derived from age-specific cut-points proposed by McCarthy *et al.*,[29] the 85th percentile of body fat was used as cut-off for excess of body fat. [f]Breast feeding categories: ⩽2 weeks, >2 weeks of full breastfeeding. [g]Maternal BMI ⩾25 kg m^{-2}. [h]School education for at least 12 years.

Table 2. Baseline nutritional data by energy-adjusted tertiles of dietary insulin index, insulin load, GI, and GL at baseline ($n = 262$), DONALD Study, Germany

Subjects	*Dietary insulin index at baseline*				*Dietary insulin load at baseline*				*Dietary GI at baseline*				*Dietary GL at baseline*			
	T1	*T2*	*T3*	P *value*[a]	*T1*	*T2*	*T3*	P *value*[a]	*T1*	*T2*	*T3*	P *value*[a]	*T1*	*T2*	*T3*	P *value*[a]
All (*n*)	86	88	88		86	88	88		86	88	88		86	88	88	
Total energy (MJ per day)[b]	7.9	7.9	7.9	>0.9	7.9	7.8	7.9	0.8	7.9	7.6	8.1	0.7	7.9	7.5	8.0	0.5
Fat (% of energy)	38.7	35.2	33.2	<0.0001	38.7	35.3	33.2	<0.0001	35.7	36.0	35.4	0.6	39.0	35.6	32.6	<0.0001
Saturated fatty acid (% of energy)	16.9	15.4	14.6	<0.0001	16.9	15.4	14.6	<0.0001	15.9	15.8	15.3	0.2	17.1	15.7	14.1	<0.0001
Protein (% of energy)	13.8	12.9	12.4	<0.001	13.8	12.9	12.5	<0.0001	13.7	12.9	12.5	<0.0001	13.9	13.0	12.3	<0.0001
Animal protein (% of energy)	9.2	8.1	7.4	<0.0001	9.2	8.0	7.5	<0.0001	9.0	8.0	7.7	<0.0001	9.3	8.0	7.4	<0.0001
Vegetable protein (% of energy)	4.6	4.9	5.0	0.001	4.6	4.9	5.0	0.0008	4.8	4.9	4.8	0.4	4.6	5.0	4.9	0.01
Consumers of alcohol (*n* (%))	7 (8.1)	8 (9.1)	7 (8.0)	>0.9	7 (8.1)	8 (9.1)	7 (8.0)	>0.9	13 (15.1)	7 (8.0)	2 (2.3)	0.009	7 (8.1)	8 (9.1)	7 (8.0)	>0.9
Carbohydrate (% of energy)	47.4	51.8	54.3	<0.0001	47.5	51.8	54.2	<0.0001	50.5	51.0	52.0	0.07	47.0	51.3	55.1	<0.0001
Added sugar (% of energy)	12.6	14.7	16.1	<0.0001	12.7	14.6	16.2	<0.0001	12.4	13.9	17.1	<0.0001	11.8	14.2	17.5	<0.0001
Dietary insulin index	39	42	45	<0.0001	39	42	45	<0.0001	41	42	43	<0.0001	40	42	44	<0.0001
Dietary insulin load	319	338	359	0.0002	321	333	362	<0.0001	332	334	350	0.1	322	336	358	0.0006
Dietary GI	55.0	56.1	56.7	<0.0001	55.1	56.1	56.7	<0.0001	53.3	56.0	58.5	<0.0001	54.4	56.1	57.3	<0.0001
Dietary GL (g)[b]	124.4	133.0	143.4	<0.0001	123.8	132.1	144.9	<0.0001	126.6	131.9	144.9	0.0001	122.7	131.6	149.5	<0.0001
Fiber (g)[b]	18.9	19.2	18.4	0.9	18.9	19.1	18.4	0.9	20.4	18.5	17.5	0.0006	19.3	19.0	18.3	0.6

Abbreviations: GI, glycemic index; GL, glycemic load; T, tertile. [a]Significant differences between the tertiles were tested using analysis of variance for normally distributed continuous variables, Kruskal–Wallis test for not normally distributed continuous variables and χ^2-test for categorical variables. Values are means unless indicated as medians[b] or otherwise.

Table 3. Relation of dietary insulin index, insulin load, GI, and GL at baseline to body mass index (kg m^{-2}) in young adulthood ($n = 262$), DONALD Study, Germany

	T1	*T2*	*T3*	$P_{for\ trend}$
Insulin index				
Model A	22.5 (21.9, 23.2)	22.5 (21.8, 23.2)	22.8 (22.2, 23.5)	0.5
Model B	23.1 (22.3, 23.8)	23.0 (22.3, 23.7)	23.3 (22.6, 24.0)	0.5
Model C	23.0 (22.4, 23.6)	22.7 (22.2, 23.3)	22.9 (22.4, 23.5)	0.8
Insulin load				
Model A	22.6 (21.9, 23.3)	22.3 (21.6, 22.9)	23.0 (22.3, 23.7)	0.5
Model B	23.1 (22.4, 23.8)	22.9 (22.2, 23.6)	23.4 (22.7, 24.1)	0.6
Model C	22.9 (22.3, 23.5)	22.7 (22.2, 23.3)	23.0 (22.4, 23.6)	0.9
GI				
Model A	22.8 (22.1, 23.5)	22.5 (21.8, 23.2)	22.5 (21.8, 23.2)	0.8
Model B	23.0 (22.3, 23.7)	23.0 (22.3, 23.7)	23.3 (22.6, 24.0)	0.4
Model C	22.8 (22.2, 23.4)	22.8 (22.2, 23.3)	23.1 (22.5, 23.7)	0.4
GL				
Model A	23.2 (22.5, 23.9)	22.6 (21.9, 23.3)	22.1 (21.4, 22.8)	0.3
Model B	23.3 (22.6, 24.1)	23.1 (22.5, 23.8)	22.8 (22.1, 23.5)	0.4
Model C	23.3 (22.7, 23.9)	22.9 (22.3, 23.5)	22.5 (21.9, 23.1)	0.6

Abbreviations: GI, glycemic index; GL, glycemic load; T, tertile. Values are means and 95% confidence interval. Model A: adjusted for sex. Model B: adjusted for sex, early life factors (breast feeding), socioeconomic factors (maternal overweight) and nutritional factors (insulin index: energy; insulin load: energy; GI: energy, fiber, protein; GL: energy, fiber, protein). Model C: Model B + adjustment for baseline (body mass index).

and insulin load during puberty was associated with a higher %BF in young adulthood, even after adjustment for early life, socioeconomic and nutritional factors (model B for insulin index and insulin load, both $P_{for\ trend} = 0.01$). Additional consideration of baseline %BF attenuated these relationships (model C, $P_{for\ trend} = 0.1$ for insulin index and $P_{for\ trend} = 0.08$ for insulin load). Model B did not include fiber as a covariate because it did not affect the associations between dietary insulin index or insulin load and body composition. Intakes of carbohydrate, protein or fat were not considered because those macronutrients contribute to the dietary insulin index and insulin load. However, as protein may also conduce higher lean mass,[39] we included this macronutrient as a covariate in a further step (data not shown) and observed a similar association between higher dietary insulin index and insulin load during puberty, and higher %BF in young adulthood (insulin index: $P_{for\ trend} = 0.0965$; insulin load: $P_{for\ trend} = 0.07$).

In an additional analysis we included carbohydrates and protein to address the effect of qualitative changes in dietary insulin index only, by holding the macronutrient intake constant, that is, the effect of substituting carbohydrate- and protein-rich foods of a high insulin demand for carbohydrate- and protein-rich foods with a low insulin demand on %BF in young adulthood. Using this qualitative approach, a higher dietary insulin index (Figure 2, Panel A) and a higher dietary insulin load (Figure 2, Panel B) were both related to a higher %BF in young adulthood even when controlling for baseline %BF ($P_{for\ trend} = 0.04$ and $P_{for\ trend} = 0.03$,

Table 4. Relation of dietary insulin index, insulin load, GI, and GL at baseline to percentage body fat in young adulthood ($n = 262$), DONALD Study, Germany

	T1	*T2*	*T3*	$P_{for\ trend}$
Insulin index				
Model A	22.3 (21.1, 23.4)	23.8 (22.7, 25.0)	24.2 (23.0, 25.3)	0.01
Model B	22.9 (21.6, 24.1)	24.5 (23.2, 25.7)	24.7 (23.5, 25.9)	0.01
Model C	23.2 (22.1, 24.3)	24.2 (23.1, 25.3)	24.2 (23.1, 25.3)	0.1
Insulin load				
Model A	22.2 (21.1, 23.4)	23.7 (22.5, 24.9)	24.3 (23.2, 25.5)	0.007
Model B	22.8 (21.5, 24.0)	24.5 (23.2, 25.7)	24.8 (23.6, 26.0)	0.01
Model C	23.1 (22.0, 24.2)	24.2 (23.1, 25.3)	24.3 (23.3, 25.4)	0.08
GI				
Model A	23.3 (22.1, 24.5)	23.5 (22.4, 24.7)	23.5 (22.3, 24.7)	0.9
Model B	23.5 (22.2, 24.9)	24.1 (22.9, 25.3)	24.4 (23.1, 25.6)	0.7
Model C	23.7 (22.5, 24.8)	24.0 (22.9, 25.1)	24.0 (22.9, 25.1)	>0.9
GL				
Model A	23.8 (22.6, 25.0)	23.6 (22.5, 24.8)	22.8 (21.7, 24.0)	0.8
Model B	24.3 (23.0, 25.6)	24.3 (23.1, 25.5)	23.5 (22.2, 24.8)	0.4
Model C	24.3 (23.1, 25.5)	24.2 (23.1, 25.3)	23.2 (22.1, 24.3)	>0.9

Abbreviations: GI, glycemic index; GL, glycemic load; T, tertile. Values are means and 95% confidence intervals. Model A: adjusted for sex. Model B: adjusted for sex, early life factors (breast feeding), socioeconomic factors (maternal overweight) and nutritional factors (insulin index: energy; insulin load: energy; GI: energy, fiber, protein; GL: energy, fiber, protein). Model C: Model B + adjustment for baseline (percentage body fat).

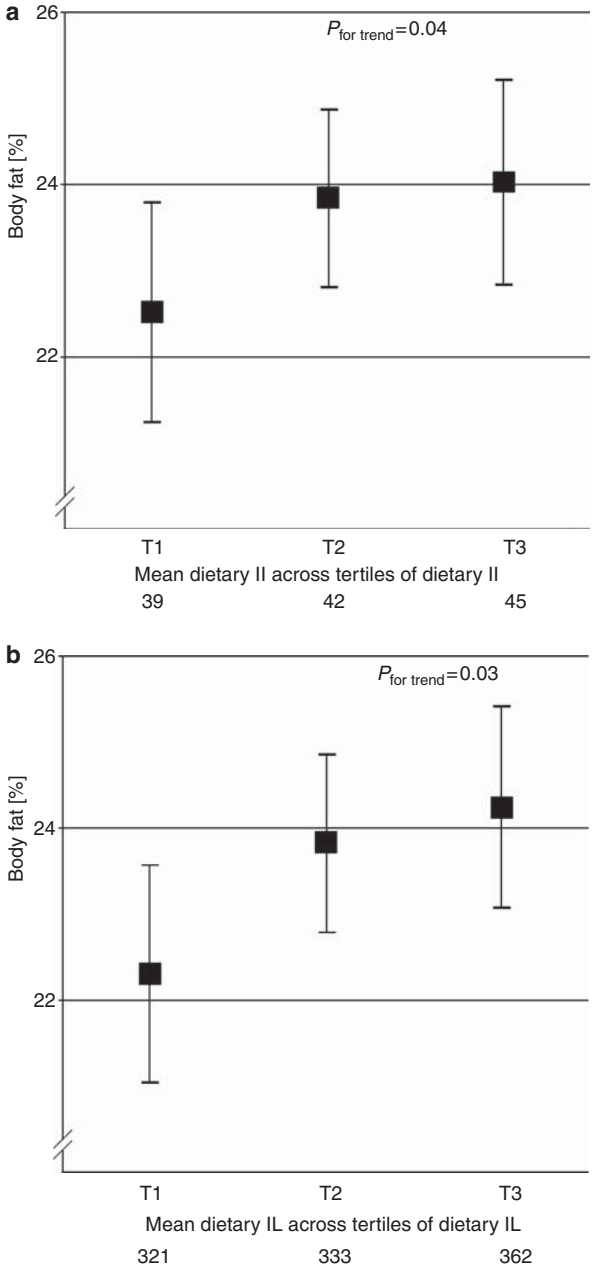

Figure 2. Percentage body fat in young adulthood by energy-adjusted tertiles of dietary insulin index (II) (**a**) and insulin load (IL) (**b**) during puberty (baseline) for 262 subjects. Data are means (95% CI) adjusted for sex, early life factors (breast feeding), socioeconomic factors (maternal overweight), nutritional factors (energy, carbohydrates, protein) and percentage body fat at baseline. *P* for trend refers to the *P* value obtained in linear regression models with percentage body fat as continuous variable. T, tertile.

respectively). Similar results were obtained when adjusting for intakes of carbohydrates and fat, or intakes of protein and fat (data not shown).

Dietary insulin index, insulin load, GI and GL were not related to waist circumference, which was, however, available for a subsample of 196 participants only (data not shown).

We performed a number of additional analyses using

- the minimum number of two dietary records per subject only, randomly selecting two records for those participants who had provided more than two records ($n = 262$)
- the first anthropometric measurement in young adulthood as an outcome ($n = 262$)
- anthropometric measurements at the age of 18 years as an outcome ($n = 218$)

All approaches yielded similar results for the relationships of the dietary insulin index or insulin load to %BF (data not shown).

DISCUSSION

To the best of our knowledge, the present study provides new epidemiological evidence on a prospective relevance of dietary insulin demand during puberty for %BF in young adulthood among a healthy free-living population. Although our data are purely observational and hence need to be interpreted cautiously, our study suggests that postprandial rises in insulinemia rather than glycemia may have adverse consequences for the development of body composition in early adulthood.

The mechanistic role of high postprandial insulin levels for a specific gain in %BF may be traced to the preferential direction of nutrients away from oxidation in muscle and toward storage in fat.[40] In line with this, Chaput *et al*.[41] reported that postprandial hyperinsulinemia at 30 min strongly predicted weight gain and change in waist circumference over 6 years in adults, especially among those consuming lower-fat diets. Furthermore, high insulin and low plasma glucagon levels may restrain hepatic glucose production and suppress lipolysis.[42] Thus, over a longer term, consistently high postprandial demand on the beta-cells may eventually reduce insulin sensitivity[14] and also promote the development of higher %BF.[43,44]

Another plausible mechanism by which a high dietary insulin index or insulin load (that is, insulin demand) may contribute to a higher %BF may work through cross-stimulation of both insulin and IGF-1 secretion.[45] *In vitro* studies using cultures of adipocyte precursor cells found a stimulatory effect of higher levels of IGF-1 on the proliferation of preadipocytes, which may therefore contribute to body-fat formation. Furthermore, IGF-1 stimulated the cellular glucose uptake in preadipocytes and adipocytes, increased lipogenesis and inhibited lipolysis in adipocytes.[46] We speculate that the physiological insulin resistance and the concurrent elevations of IGF-1 levels during puberty may work together to increase the susceptibility to postprandial insulinemic spikes and thus contribute to the development of high body fat.

In our view, it is plausible that we did not observe an association between dietary GI and body composition in our cohort of relatively lean subjects with physiological insulin resistance affecting peripheral tissues,[47] as a higher dietary GI may be of relevance primarily among persons who already respond with exaggerated insulin responses.[9] This may also explain why other studies reported associations between dietary GI and body composition mainly among overweight and less insulin-sensitive persons.[8,9] Conversely, we had expected to find at least a tendency for a comparable relation between dietary GL and body composition, as dietary GL has recently been identified as the best indirect predictor of the postprandial insulin response.[36] However, although the main contribution to the insulin responses arises from carbohydrate-rich foods, Bao *et al*.[36] reported dietary GL to explain only 46% of the observed variability in insulin responses, that is, foods with little or no carbohydrates and a higher protein and fat content make additional important contributions.

It could be argued that the association between dietary insulin demand and unfavorable body composition may be primarily attributable to one macronutrient only (for example, carbohydrates). However, our additional analysis adjusting for protein, carbohydrates and energy suggests that in particular substitutions of carbohydrate- and protein-rich foods with a higher insulin demand for carbohydrate- and protein-rich foods with a lower insulin demand are the relevant principle for the associations with body fat. In addition, further adjustment for protein enhanced the association between dietary insulin demand and body fat. This may reflect a bi-directional relevance of dietary protein, which may contribute to a higher lean body mass on the one hand[39] and a higher insulin secretion[17] or lower insulin clearance on the other hand.[48]

The relationship between a higher dietary insulin demand during puberty and a higher %BF in young adulthood was attenuated by the additional consideration of baseline %BF. While this confirms the prevailing long-term relevance of %BF already in childhood, we may have also corrected for earlier effects of dietary insulin demand on body composition. It may be that the dietary insulin demand has a more important role in adolescents who are overweight or have a higher %BF. In our sample we did not find a consistent interaction between overweight or excess body fat at baseline and dietary insulin demand concerning %BF in young adulthood, but this may be attributable to the fact that our sample is comparatively healthy with lower prevalences of overweight or excess body fat.

Our study has several limitations. First, we applied the FII concept, developed to quantify the insulin response to foods – to estimate the dietary insulin demand. Hence, the limitations debated for the estimations of dietary GI[49–51] also apply to the estimation of dietary insulin demand. As the FII assignment was based only on 121 published FII values it must be considered crude, yet allowing a classification of foods in FII groups.[52] Second, %BF was estimated from skinfold thickness measurements, which are known to be more susceptible to measurement error than are specialized research-based techniques. Other more accurate methods to estimate %BF, such as hydrostatic weighing, may be preferable to estimate body fat,[53] but the skinfold equations of Durnin and Womersley[30] are feasible and agree, on average, very well with results from hydrostatic weighing.[53] Furthermore, measurements were conducted by trained and quality-monitored personnel, which has been shown to reduce intra- and inter-observer variability considerably,[54] as was the case in the present study. Third, the DONALD population has a relatively high socioeconomic status,[55] as reflected by the parental educational level. It is possible that the relative homogeneity of the healthy DONALD sample means that extremes of diet or behavior are not represented. However, non-representativeness is less relevant for the present analysis and will likely result in underestimation rather than overestimation of the true associations. On the other hand, the homogeneity of our sample might have reduced our vulnerability to residual confounding. Finally, we examined the long-term relevance of the dietary insulin demand, GI and GL on body composition at a single point in young adulthood only, as presently only 141 participants had at least two anthropometric measurements in both adolescence and young adulthood. In the future, continued follow-up of our participants will also allow analyses of growth trajectories.

A clear strength of our study is its prospective nature and the carefully collected, repeated data on growth, the availability of data on several possible confounders, such as parental characteristics and repeated dietary data. Overall the analyses were based on 1379 weighed 3-day dietary records, that is, on average each subject had provided 5 dietary records during puberty (2–7 records). A further advantage lies in the use of 3-day weighed dietary records, which permitted a particularly detailed assignment of dietary GI values for each carbohydrate-containing and FII values for all foods.

In conclusion, our analysis indicates that postprandial increases in insulinemia rather than glycemia are implicated in an unfavorable development of body fat in the critical period of puberty.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank the staff of the Research Institute of Child Nutrition for carrying out the anthropometric measurements, and for collecting and coding the dietary records. We also thank all the participants of the DONALD Study. The DONALD study is supported by the Ministry of Science and Research of North Rhine Westphalia, Germany, and this analysis was partially funded by the World Cancer Research Fund International (grant no. 2010/248).

AUTHOR CONTRIBUTIONS

GJ conducted the statistical analysis and wrote the manuscript; GJ and AEB conceived the research project; GJ and JG assigned all FII values to the 3-day weighed dietary records; all authors made substantial contributions to the interpretation of the results.

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Clinical Care/Education/Nutrition/Psychosocial Research

ORIGINAL ARTICLE

Habitually Higher Dietary Glycemic Index During Puberty Is Prospectively Related to Increased Risk Markers of Type 2 Diabetes in Younger Adulthood

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OBJECTIVE—Carbohydrate nutrition during periods of physiological insulin resistance such as puberty may affect future risk of type 2 diabetes. This study examined whether the amount or the quality (dietary glycemic index [GI], glycemic load [GL], and added sugar, fiber, and whole-grain intake) of carbohydrates during puberty is associated with risk markers of type 2 diabetes in younger adulthood.

RESEARCH DESIGN AND METHODS—The analysis was based on 226 participants (121 girls and 105 boys) from the Dortmund Nutritional and Anthropometric Longitudinally Designed Study (DONALD) with an average of five 3-day weighed dietary records (range 2–6) during puberty (girls, age 9–14 years; boys, age 10–15 years) and fasting blood samples in younger adulthood (age 18–36 years) (average duration of follow-up 12.6 years). Multivariable linear regression was used to analyze the associations between carbohydrate nutrition and homeostasis model assessment–insulin resistance (HOMA-IR) as well as the liver enzymes alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) (n = 214).

RESULTS—A higher dietary GI was prospectively related to greater values of HOMA-IR (P_{trend} = 0.03), ALT (P_{trend} = 0.02), and GGT (P_{trend} = 0.04). After adjustment for sex, adult age, baseline BMI, and early life and socioeconomic factors as well as protein and fiber intake, predicted mean HOMA-IR values in energy-adjusted tertiles of GI were 2.37 (95% CI 2.16–2.60), 2.47 (2.26–2.71), and 2.59 (2.35–2.85). The amount of carbohydrates, GL, and added sugar, fiber, and whole-grain intake were not related to the analyzed markers.

CONCLUSIONS—Our data indicate that a habitually higher dietary GI during puberty may adversely affect risk markers of type 2 diabetes in younger adulthood.

Diabetes Care **36:1870–1876, 2013**

Concern has been raised that the commonly advocated low-fat, high-carbohydrate diet may be detrimental for the growing number of persons with impaired glucose tolerance even among youths, since it induces postprandial rises in glucose and insulin and may thereby increase the risk the risk of developing type 2 diabetes (1,2). Observational evidence suggests that dietary glycemic index (GI) and glycemic load (GL) are related to risk of type 2 diabetes (3,4), yet it remains to be determined whether the relevance of postprandial rises in glucose and insulin extends to puberty—a period characterized by a physiological insulin resistance (5).

Chronic postprandial hyperglycemia and hyperinsulinemia can also exacerbate hepatic insulin resistance: enhanced glucose uptake by the liver subsequently leads to increased hepatic fat accumulation through upregulated de novo lipogenesis. In fact, hepatic fat accumulation is frequently observed in patients with insulin resistance or type 2 diabetes (6). The liver enzymes alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) are commonly used as surrogate parameters for hepatic fat content and are now recognized as risk markers for type 2 diabetes (7,8). Furthermore, preliminary evidence supports a role of carbohydrate nutrition for hepatic steatosis and these indirect markers of liver fat (9).

This study addressed the hypothesis that recurring postprandial glycemic excursions during puberty are of specific relevance for later risk of type 2 diabetes. Since calculated dietary GI is a valid predictor of glycemic responses (10,11), we postulate that dietary GI estimated from 3-day dietary records repeatedly collected during puberty is a better predictor of type 2 diabetes risk in younger adulthood than intakes of dietary fiber, whole grain, or added sugar. This hypothesis was addressed using data from a cohort of healthy young Germans. The homeostasis model assessment–insulin resistance (HOMA-IR) index and the liver enzymes ALT and GGT was used as risk markers of type 2 diabetes.

RESEARCH DESIGN AND METHODS

The present analysis is based on data from the Dortmund Nutritional and Anthropometric Longitudinally Designed Study (DONALD), an ongoing open cohort study conducted at the Research Institute of Child Nutrition in Dortmund, Germany (12). This study has previously been described in detail (12). Briefly, since 1985, detailed data on diet, growth, development, and metabolism

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Received 9 October 2012 and accepted 18 December 2012.
DOI: 10.2337/dc12-2063

Table 1—*Demographic, anthropometric, birth, and socioeconomic characteristics by sex-specific tertiles of dietary glycemic index: DONALD, Germany*

		Dietary GI			
	n	T1 (*n* = 75)	T2 (*n* = 76)	T3 (*n* = 75)	*P*[a]
Female (%)	226	53.3	54.0	53.3	1.0
Early life factors					
Birth year[b]	226	1,986	1,987	1,987	0.3
Birth weight (g)	226	3,485	3,496	3,471	0.9
Birth length (cm)	226	51.7	51.8	51.6	0.8
Pregnancy duration (weeks)[b]	225	40	40	40	0.4
Breast-feeding (>2 weeks) (%)[c]	226	74.7	64.5	74.7	0.3
Data from puberty					
Age (years)[b]	226	9.8	9.1	9.9	0.8
BMI SDs	226	0.16	0.03	−0.08	0.2
BMI (kg/m^2)[b]	226	17.5	16.6	16.4	0.2
Overweight (%)[d]	226	16.0	15.8	12.0	0.7
%BF[b,e]	226	16.3	16.0	16.2	0.7
Excess body fat (%)[b,f]	226	18.7	17.1	18.7	1.0
Socioeconomic factors (%)					
Maternal overweight[g]	222	27.4	33.8	34.7	0.6
Maternal education[h]	226	53.3	42.1	45.3	0.4
Maternal occupation[i]	226	50.7	51.3	50.7	1.0
Smoking in the household	221	20.8	42.7	39.2	0.01
Data from younger adulthood					
Age (years)[b]	226	21.9	22.4	21.5	0.5
BMI (kg/m^2)[b]	226	23.2	22.2	22.2	0.5
%BF[b,j]	226	26.9	27.5	26.1	1.0
Waist circumference (cm)[b]	226	76.7	76.1	75.1	0.8
ALT (units/L)[b]	214	14.9	15.6	17.7	0.02
GGT (units/L)[b]	214	13.7	14.7	16.3	0.07
TG (mmol/L)[b]	214	1.09	1.14	1.14	0.7
Glucose (mmol/L)[b]	226	5.11	5.11	5.22	0.4
Insulin (mU/L)[b]	226	76.8	81.5	81.2	0.2
HOMA-IR[b]	226	2.46	2.52	2.58	0.1
HDL (mmol/L)[b]	222	1.45	1.47	1.53	0.9
LDL (mmol/L)[b]	222	2.40	2.38	2.53	0.4

Data are means unless otherwise indicated. T, tertile. [a]Significant differences between the tertiles were tested using ANOVA for normally distributed continuous variables, Kruskal-Wallis test for non–normally distributed continuous variables, and χ^2 test for categorical variables. [b]Values are means unless indicated as medians. [c]Breast-feeding categories: ≤2 vs. >2 weeks of full breast-feeding. [d]Derived from the age- and sex-specific cut points proposed by the International Obesity Task Force, which are linked to the adult cutoff point of BMI 25 kg/m^2 (Cole et al., 2000 [ref. 16]). [e]Calculated according to Slaughter et al. (1988 [ref. 17]). [f]Derived from age-specific cut points proposed by McCarthy et al. (2006 [ref. 18]); the 85th percentile of body fat was used as cutoff for excess of body fat. [g]Maternal BMI ≥25 kg/m^2. [h]School education for at least 12 years. [i]Maternal occupation (yes/no). [j]Calculated according to Durnin and Womersley (ref. 19).

have been collected from >1,300 healthy children. Participants are recruited in the city of Dortmund and surrounding communities via personal contacts, maternity wards, or pediatric practices. On average, 40 infants are newly recruited every year and first examined at the age of 3 months. Each child returns for three more visits during the first year, two in the second, and then annually until adulthood. Since 2005, participants over the age of 18 years are invited for subsequent examinations with fasting blood withdrawal. The study was approved by the ethics committee of the University of Bonn, and all examinations are performed with written parental and adult participants' consent (12).

Because of the open cohort design, many children had not yet reached younger adulthood, and among those who did age varied from 18 to 36 years. At the time of this analysis, one measurement of insulin and glucose was available for 319 participants (mean age 22.7 years), who were term (36–43 weeks' gestation) singletons with a birth weight ≥2,500 g. ALT and GGT values were available for 309 participants. Of these, 229 participants (for HOMA analysis) and 221 (for ALT and GGT analysis), respectively, had provided at least two plausible 3-day weighed dietary records during the adolescent baseline period (chronological age: girls 9–14 years, boys 10–15 years), allowing the estimation of habitual dietary intake. Participants who consistently underreported their energy intake (i.e., they had provided more implausible than plausible food records) were excluded from the study (*n* = 20) (13). A 3-day weighed dietary record was considered plausible when the total recorded energy intake was adequate in relation to the basal metabolic rate (13). For inclusion in the study sample, participants also had to have anthropometric measures taken in adolescence and adulthood as well as information on relevant covariates. This resulted in a final sample of 226 participants for analysis of insulin or related outcomes and of 214 for the liver enzymes.

Blood analysis

Venous blood samples were drawn after an overnight fast, centrifuged within 15 min, and frozen at –80°C in the Research Institute. For the present analysis, blood samples were transported to the technical laboratory of the German Diabetes Center to determine serum activities of ALT and GGT using the COBAS C311 analyzer (Roche, Mannheim, Germany). Serum insulin concentrations were measured with an immunoradiometric assay in the Laboratory for Translational Hormone Analytics in Pediatric Endocrinology at the University of Giessen. Based on these values, HOMA-IR and secretion (HOMA of β-cell function [HOMA-β]) were calculated (14).

Anthropometric measurements

From the age of 2 years onward, standing height is measured to the nearest 0.1 cm using a digital stadiometer (Harpenden, Crymych, U.K.). Body weight is measured to the nearest 100 g with an electronic scale (Seca 753E; Seca Weighing and Measuring Systems, Hamburg, Germany). Measurements are taken at each visit according to standard procedures. Skinfold thicknesses are measured from the age of 6 months onward at four different sites (suprailiacal, subscapular, biceps, and triceps) on the right side of the body to the nearest 0.1 mm using a Holtain caliper (Holtain, Crosswell, U.K.).

Table 2—*Baseline nutritional data by sex-specific tertiles of dietary glycemic index: DONALD, Germany*

	Dietary GI (n = 226 subjects)			
	T1 (n = 75)	T2 (n = 76)	T3 (n = 75)	P[a]
Dietary GI	53.4	56.1	58.4	<0.0001
Total energy (MJ/day)[b]	7.95	7.55	7.96	0.5
Fat (% energy)	35.4	36.5	35.4	0.2
Saturated fatty acid	15.8	16.2	15.4	0.08
Protein (% energy)	13.4	13.0	12.5	0.0007
Animal protein	8.69	8.10	7.62	0.0001
Vegetable protein	4.72	4.88	4.85	0.4
Carbohydrate (% energy)	51.1	50.5	52.1	0.08
Added sugar (% energy)	13.1	14.2	17.0	<0.0001
From drinks	3.21	4.03	6.07	<0.0001
From sweets	6.05	6.28	7.16	0.03
From other sources	3.81	3.92	3.73	0.8
Dietary GL (g)[b]	131.0	131.3	140.4	0.02
Fiber (g)[b]	20.6	18.9	17.3	0.0001
Fiber (g/MJ)	2.63	2.51	2.31	0.0005
From bread and cereals	1.30	1.30	1.30	1.0
From vegetables	0.55	0.50	0.48	0.06
From fruits	0.58	0.57	0.37	<0.0001
Whole grain (g)[b]	29.1	19.2	16.3	0.0004
Whole grain (g/MJ)	4.43	3.36	2.9	0.003

Data are means unless otherwise indicated. [a]Significant differences between the tertiles were tested using ANOVA for normally distributed continuous variables, Kruskal-Wallis test for non–normally distributed continuous variables, and χ^2 test for categorical variables. [b]Values are means unless indicated as medians.

Waist circumference in younger adulthood was measured at the midpoint between the lower rip and the iliac crest to the nearest 0.1 cm. Sex- and age-specific SD scores (SDs) were calculated for the adolescent BMI values using the German BMI standards (15). For definition of overweight during puberty, values proposed by the International Obesity Task Force were used (16). Percentage body fat (% BF) for pubescent children was derived using the equations of Slaughter et al. (17), and excess body fatness was defined according to the %BF standard (18). For estimation of %BF in adulthood, equations of Durnin and Womersley were used (19).

Dietary assessment

During 3 days, the participants or their parents weighed and recorded all foods and beverages consumed as well as leftovers to the nearest 1 g using electronic food scales (initially, Soehnle Digita 8000; Leifheit, Nassau, Germany; now, WEDO digi 2000; Werner Dorsch, Münster/Dieburg, Germany). For this analysis, dietary variables were calculated as individual means of the 3-day weighed dietary records using LEBTAB (20), the in-house database. As we aimed to describe the habitual dietary intake, an individual average intake during puberty was calculated from at least two records (average of 5 records per participant).

Each carbohydrate-containing food recorded in the dietary records was assigned a published GI value (21) (based on glucose as a reference food) according to a standardized procedure (22). The carbohydrate content (in grams) of each consumed food was then multiplied by the food's GI to obtain the respective GL. The overall dietary GI is obtained by dividing total daily GL by total daily carbohydrate intake.

The following foods were defined as added sugars: white sugar, brown sugar, raw sugar, corn syrup, corn syrup solids, high-fructose corn syrup, malt syrup, maple syrup, pancake syrup, fructose sweetener, liquid fructose, honey, molasses, anhydrous dextrose, and crystal dextrose (23). Fruit syrups commonly used as sweeteners in Germany also were considered added sugars. Dietary fiber content was calculated using the LEBTAB database. Whole-grain intake was estimated by assigning whole-grain content in grams to each carbohydrate-containing food using the respective recipe and ingredient information available at the time of recording. The definition of whole grain followed the whole-grain label statements of the U.S. Food and Drug Administration (24).

Statistical analysis

Baseline characteristics of the study population are presented by sex-specific tertiles of dietary GI. Tests for differences between these tertiles were performed using ANOVA for normally distributed continuous variables, Kruskal-Wallis test for non–normally distributed continuous variables, and χ^2 test for categorical variables.

For analysis of the prospective association between carbohydrate nutrition during puberty and risk markers for type 2 diabetes in younger adulthood, multivariable linear regression models were used. As the outcome variables were not normally distributed, HOMA-IR was log transformed prior to analysis, and liver enzymes ALT and GGT were log transformed twice to obtain normal distribution. All dietary variables except dietary GI were energy adjusted using the residual method. To account for age-dependent nutritional differences, we standardized all variables by age-group and sex (mean ± SD 0 ± 1).

Covariates considered as potentially affecting the association between carbohydrate nutrition and risk markers of type 2 diabetes were birth weight, gestational age, breast-feeding for >2 weeks, first-born child (yes/no), BMI SDs or %BF at baseline, maternal overweight (BMI ≥25 kg/m^2), high maternal educational status (≥12 years of schooling), maternal occupation (yes/no), smoking in the household, parental history of diabetes (yes/no [questionnaire based]), physical activity level (light, moderate, or high [questionnaire based]), and intakes of protein (total, animal, or vegetable) and fat (total and saturated fat). Vice versa adjustment for added sugar, fiber, and GI was also considered. Each potential confounder was initially examined separately and included only if it *1*) substantially altered the association of the principal dietary variables with the outcome in the unadjusted models (>10%), *2*) significantly predicted the outcome, or *3*) improved the coefficient of determination (>5%). In the basic model (model A), sex and age were included, since age at blood

Table 3—*HOMA-IR in younger adulthood by tertiles of carbohydrate nutrition parameters during puberty*

	HOMA-IR (n = 226)			
	T1	T2	T3	P_{trend}
Carbohydrate (energy %)				
Model A	2.54 (2.31–2.78)	2.66 (2.42–2.91)	2.54 (2.32–2.78)	0.5
Model B	2.48 (2.24–2.75)	2.53 (2.31–2.77)	2.41 (2.18–2.65)	0.6
Conditional model	2.43 (2.20–2.67)	2.54 (2.33–2.77)	2.43 (2.21–2.67)	0.5
Glycemic Index				
Model A	2.44 (2.23–2.67)	2.59 (2.37–2.83)	2.71 (2.47–2.96)	0.03
Model B	2.37 (2.16–2.60)	2.47 (2.26–2.71)	2.59 (2.35–2.85)	0.03
Conditional model	2.39 (2.19–2.61)	2.49 (2.28–2.72)	2.54 (2.32–2.79)	0.09
Glycemic load				
Model A	2.58 (2.35–2.82)	2.52 (2.30–2.75)	2.64 (2.41–2.89)	0.6
Model B	2.46 (2.23–2.72)	2.38 (2.17–2.61)	2.57 (2.34–2.83)	0.4
Conditional model	2.44 (2.21–2.68)	2.39 (2.19–2.61)	2.58 (2.36–2.83)	0.6
Added sugar (energy %)				
Model A	2.61 (2.39–2.86)	2.64 (2.41–2.89)	2.48 (2.26–2.71)	0.7
Model B	2.57 (2.32–2.86)	2.57 (2.34–2.82)	2.29 (2.07–2.54)	0.3
Conditional model	2.53 (2.29–2.80)	2.56 (2.35–2.80)	2.33 (2.11–2.57)	0.4
Added sugar from drinks (energy %)				
Model A	2.71 (2.47–2.96)	2.52 (2.30–2.76)	2.51 (2.29–2.75)	0.8
Model B	2.64 (2.39–2.92)	2.45 (2.23–2.69)	2.35 (2.13–2.60)	0.5
Conditional model	2.60 (2.36–2.86)	2.43 (2.22–2.65)	2.40 (2.18–2.63)	0.8
Fiber (g/1,000 kcal)				
Model A	2.67 (2.44–2.92)	2.65 (2.43–2.90)	2.41 (2.20–2.64)	0.3
Model B	2.51 (2.29–2.77)	2.56 (2.34–2.80)	2.36 (2.15–2.60)	0.4
Conditional model	2.50 (2.28–2.74)	2.53 (2.32–2.75)	2.40 (2.19–2.63)	0.6
Whole grain (g/1,000 kcal)				
Model A	2.60 (2.38–2.85)	2.61 (2.38–2.85)	2.52 (2.30–2.76)	0.8
Model B	2.42 (2.20–2.66)	2.56 (2.34–2.80)	2.45 (2.23–2.69)	0.9
Conditional model	2.38 (2.17–2.60)	2.56 (2.35–2.79)	2.47 (2.26–2.71)	0.7

Values are means (95% CI) unless otherwise indicated. Model A, adjusted for sex, age (categorical: ≤19, >19, ≤25, and >25 years), and energy (residuals). Model B, model A plus early life factors (firstborn), BMI SDs at baseline, socioeconomic factors (maternal education), and nutritional factors (carbohydrate, GI, GL, and sugar adjusted for fiber and protein; fiber adjusted for GI and protein). Conditional model, additional inclusion of waist circumference in younger adulthood in model B.

withdrawal in younger adulthood varied considerably (18–35 years). In a second model (model B), we further adjusted for early life and socioeconomic as well as other nutritional factors. Finally, we ran a conditional model (additionally including waist circumference in younger adulthood) to assess whether the observed associations are partly attributable to effects of carbohydrate nutrition on body composition. Verification of the linear regression modeling assumptions showed that these were appropriate for the analyzed longitudinal data.

As associations between carbohydrate nutrition and risk markers of type 2 diabetes did not differ by sex (P for interaction >0.2), data were pooled for analysis. The adjusted means are presented by tertiles with the corresponding 95% CIs. P values <0.05 were considered statistically significant. All statistical analyses were carried out using SAS procedures (version 9.1.3; SAS Institute, Cary, NC).

RESULTS—Subjects who were excluded from the study sample because of missing information (dietary intake data or covariates) (n = 93) did not differ from those included (n = 226) with respect to early life factors or anthropometric or metabolic characteristics in younger adulthood (data not shown).

Participants with a higher dietary GI during adolescence were more likely to be exposed to smoking in the household (Table 1). There were no other differences in anthropometric, early life, or socioeconomic factors during puberty between the dietary GI tertiles. Regarding data from younger adulthood, participants with a higher dietary GI during puberty had higher ALT and GGT values (Table 1). In terms of nutritional intake data during puberty, those in the higher dietary GI tertiles consumed less (animal) protein, (fruit) fiber, and whole grain, as well as more added sugar, especially from drinks (Table 2).

The amount of carbohydrates, dietary GL, added sugar, fiber, and whole-grain intake during puberty was not associated with HOMA-IR in younger adulthood (Table 3). A higher dietary GI during puberty was prospectively related to higher values of HOMA-IR in multivariable analysis (P for trend = 0.03 [model A]). This association was not explained by baseline BMI, early life or socioeconomic factors, or protein or fiber intake (P for trend = 0.03 [model B]). No prospective associations were observed between carbohydrate nutrition and HOMA-β (P for trend ≥0.2) (data not shown).

A higher dietary GI was also independently associated (adjustment for baseline BMI and socioeconomic and nutritional factors) with higher values of both ALT

(*P* for trend = 0.02 [model B]) and GGT (*P* for trend = 0.04 [model B]) (Fig. 1). Amount of carbohydrates, dietary GL, total added sugar, dietary fiber, and whole-grain intake were not related to liver enzymes. Higher intakes of added sugar from drinks during puberty were independently related to higher levels of GGT in adulthood (*P* for trend = 0.04 [model B]) (data not shown).

We also examined the association between carbohydrate nutrition and fasting insulin levels; similarly, this analysis revealed a prospective positive relation for dietary GI only (*P* for trend = 0.045). Further adjustment for breast-feeding status, birth weight, physical activity level, or parental history of type 2 diabetes did not change any of the results.

The additional inclusion of waist circumference in adulthood attenuated the associations between dietary GI and risk markers of type 2 diabetes toward a trend (conditional model [Table 3]). The corresponding mean predicted ALT and GGT values in sex-specific tertiles of GI were 16.7 units/L (95% CI 15.3–18.4), 16.3 units/L (15.0–17.8), and 18.0 units/L (16.4–19.9) (*P* for trend = 0.07) and 14.1 units/L (12.7–15.7), 14.0 units/L (12.6–15.5), and 16.6 units/L (14.8–18.7) (*P* for trend = 0.09), respectively.

CONCLUSIONS—This study provides new epidemiological evidence of a detrimental role of postprandial glycemic excursions during puberty for risk markers of type 2 diabetes in younger adulthood. Dietary GI was the only feature of carbohydrate nutrition that was consistently related to different diabetes risk markers. As a low-GI diet is characterized by an average of ≤45 (25), the dietary GI in the present sample (56.0 ± 2.4) can be considered moderate.

The association between dietary GI and diabetes risk seen in our study is in accordance with observational evidence in adulthood linking dietary GI to risk of developing type 2 diabetes (3,4). Our study is, however, the first to suggest that this association emerges already during puberty. In view of the relatively large 95% CIs, the observed associations have to be interpreted cautiously. In our study, a 5-unit increase of dietary GI was accompanied by a 9% increase in HOMA-IR and an 11% increase in ALT values. This is in line with evidence from large observational studies, where moderate GI differences between extreme quantiles were also associated with relatively large differences in type 2 diabetes risk (3). Importantly, there was no strong correlation between HOMA-IR, ALT, and GGT in our study ($r < 0.4$), which argues against the possibility of chance findings.

Of note, the relation between dietary GI and diabetes risk markers appeared to be partly attributable to body composition, since associations were attenuated toward a trend in the conditional model. Nonetheless, a trend was maintained, suggesting an additional mechanism independent of body composition. In fact, a previous analysis of ours did not reveal an independent association between GI during puberty and body composition in younger adulthood (26). Another mechanism by which dietary GI may affect diabetes risk independently of body composition is oxidative stress: Increased postprandial glycemia can exert prooxidative and proinflammatory effects (27). Hyperglycemia-induced oxidative stress could impair mitochondrial function (28). In turn, impaired mitochondrial function may cause both hepatocyte injury and subsequently increased release of ALT and GGT (28) and contribute to insulin resistance independently of hepatic lipid content (29). Moreover, excessive postprandial glycemia increases the strain on β-cell mass, which can be particularly detrimental in a phase of decreased insulin sensitivity such as puberty (30). Our data indicate a long-term relevance of dietary GI for both systemic and hepatic insulin resistance, as reflected by associations with HOMA-IR and insulin as well as GGT and ALT. Moreover, in our healthy sample, habitual dietary GI seems to be of long-term relevance for insulin sensitivity only, since GI was not prospectively related to β-cell function (e.g., HOMA-β).

The results of our study dismiss the relevance of total carbohydrate intake for later insulin sensitivity and corroborate the rising awareness that carbohydrate quality is more important for risk of type 2 diabetes than carbohydrate quantity—at least for healthy persons. We cannot, however, exclude the possibility that lower carbohydrate intake may offer some benefits for obese adolescents, since they cannot adapt appropriately to high-carbohydrate diets by increasing their insulin sensitivity and may, hence, need to increase insulin secretion further (31).

We observed no prospective association between consumption of added sugar from drinks or fiber intake and adult type 2 diabetes risk markers except for an association between added sugar from drinks and GGT. Observational studies in adults support a relation of both consumption of sugar-sweetened beverages (32) and cereal fiber (33) to type 2 diabetes risk, while mechanistic studies point to specific benefits of viscous fiber on insulin sensitivity (34). This discrepancy may to some degree

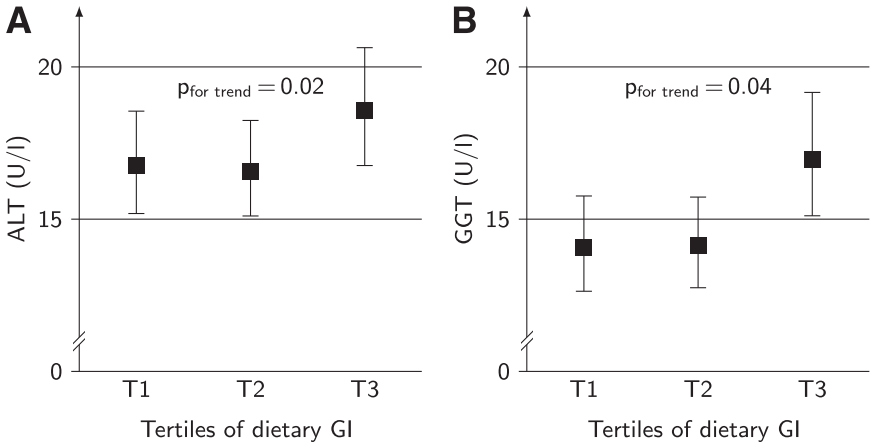

Figure 1—*ALT (units/L)* (A) *and GGT (units/L)* (B) *levels in younger adulthood by energy-adjusted tertiles of dietary glycemic (GI) (mean dietary GI across tertiles [T]: tertile 1, 53.5; 2, 56.2; and 3, 58.5) during puberty (baseline) for 214 subjects. Data are geometric means (95% CI) adjusted for sex, age (categorical ≤19, >19, ≤25, and >25 years), BMI SDs at baseline, socioeconomic factors (maternal overweight), energy (residuals), and protein and fiber intake. See the text for results from the conditional model additionally considering waist circumference in younger adulthood. Note that the slight U-shape in* A *results from illustration of least square means by GI tertiles, the association is linear, and all assumptions of linear regression modeling are met. (See the* STATISTICAL ANALYSIS.*)*

result from residual confounding. In the present analysis, confounding is less likely because the DONALD population is comparably homogeneous with a higher socioeconomic status. In addition, benefits of higher fiber intakes are partly attributed to lower postprandial glycemia. This response is, however, better described by dietary GI: In a recent study using 121 foods and 13 meals, postprandial glycemia was related to GI and GL but not fiber content (35). It is therefore possible that exposure to postprandial glycemia during puberty (as estimated by dietary GI) is of particular relevance for diabetes risk in younger adulthood, whereas other mechanisms linking fiber intake to diabetes risk become more important in later adulthood.

The main strengths of our study are its prospective design and the detailed repeated measurements of dietary intake during puberty. Assessment of dietary intake during puberty is notoriously difficult, but the present analysis was based on an average of five dietary records during puberty (range 2–6 per participant), which allowed estimation of habitual dietary intake. Comparisons of our carbohydrate-intake data with other studies in adolescents showed similar intake levels with respect to total carbohydrate, added sugar (36,37), and dietary GI (38). The availability of data on several potential confounders, such as parental characteristics, including self-reported parental history of type 2 diabetes, further strengthens our analysis. However, we cannot preclude residual confounding, resulting from imprecisely measured or unmeasured confounding factors. Importantly, only crude questionnaire-based data were available for physical activity levels.

Our study also has several limitations. First, risk markers of type 2 diabetes were only measured once in younger adulthood. Second, the relatively elaborate DONALD study design results in a socioeconomic status above average, and extremes of diet or behavior might not be represented, which is likely to introduce selection bias. Thirdly, estimation of the dietary GI from the GI values of individual foods is discussed controversially (10,39). However, in contrast to most epidemiological studies using food-frequency questionnaires, the GI estimates in this study stem from direct assignment of GI values to all carbohydrate-containing foods recorded during 3 days (22).

Relating our results to those from other studies, the lack of data on the longer-term influence of adolescent nutrition on later health becomes very evident. Our study provides new evidence for a long-term impact of postprandial glycemic excursions during puberty on later diabetes risk. The absence of such associations for other measures of carbohydrate quality suggests that advice focusing solely on dietary fiber and added sugar intake is insufficient. Further large-scale studies, preferably in at-risk populations (e.g., overweight or insulin-resistant adolescents) are needed to support the present findings and confirm their public health relevance.

In conclusion, our data indicate that a habitually higher dietary GI during puberty may adversely affect risk markers of type 2 diabetes in younger adulthood. Advice for preferred selection of low-GI carbohydrates during puberty may need to be incorporated into preventive dietary recommendations given to adolescents.

Acknowledgments—This work was financially supported by the German Federal Ministry of Food, Agriculture, and Consumer Protection through the Federal Office for Agriculture and Food (Grant 2810HS035). Furthermore, insulin measurements were funded by the Wereld Kanker Onderzoek Fonds (WCRF NL) (Grant 2010/248). The DONALD study is supported by the Ministry of Science and Research of North Rhine Westphalia, Germany. The German Diabetes Center is funded by the German Federal Ministry of Health, the Ministry of School, Science, and Research of the State of North-Rhine-Westphalia, and the German Center for Diabetes Research.

No potential conflicts of interest relevant to this article were reported.

J.G. assigned the GI values, conducted the statistical analysis, wrote the manuscript, contributed to the interpretation of the results, critically revised the manuscript, and approved the final version of the manuscript. C.H. gave detailed assistance in the drafting process, contributed to the interpretation of the results, critically revised the manuscript, and approved the final version of the manuscript. G.J. assigned the GI values, contributed to the interpretation of the results, critically revised the manuscript, and approved the final version of the manuscript. K.B. conducted the statistical analysis, contributed to the interpretation of the results, critically revised the manuscript, and approved the final version of the manuscript. T.R. contributed to the interpretation of the results, critically revised the manuscript, and approved the final version of the manuscript. S.A.W. ensured correct determination of plasma glucose, contributed to the interpretation of the results, critically revised the manuscript, and approved the final version of the manuscript. Triglycerides, ALT, and GGT values were measured in the laboratory of M.R., and M.R. contributed to the interpretation of the results, critically revised the manuscript, and approved the final version of the manuscript. W.R. contributed to the interpretation of the results, critically revised the manuscript, and approved the final version of the manuscript. A.E.B. conceived the research project, supervised the project, gave detailed assistance in the drafting process, contributed to the interpretation of the results, critically revised the manuscript, and approved the final version of the manuscript. A.E.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Parts of the study were presented as a poster session at the 48th Annual Meeting of the European Association for the Study of Diabetes, Berlin, Germany, 1–5 October 2012.

The authors thank the staff of the Research Institute of Child Nutrition for carrying out the anthropometric measurements and for collecting and coding the dietary records. The authors also thank all the participants of the DONALD study. The authors thank the staff of the technical laboratory of the German Diabetes Center, Düsseldorf, and the Laboratory for Translational Hormone Analytics in Paediatric Endocrinology, Giessen, for carrying out blood analysis.

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The Journal of Nutrition. First published ahead of print July 30, 2014 as doi: 10.3945/jn.114.193391.

The Journal of Nutrition
Nutritional Epidemiology

Increased Intake of Carbohydrates from Sources with a Higher Glycemic Index and Lower Consumption of Whole Grain during Puberty Are Prospectively Associated with Higher IL-6 Concentrations in Younger Adulthood among Healthy Individuals[1–3]

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Abstract

Chronic low-grade inflammation represents a likely intermediary in the relation between carbohydrate nutrition and both type 2 diabetes and cardiovascular disease. This study assessed the prospective association between carbohydrate quantity and quality [dietary glycemic index (GI), glycemic load (GL), and added sugar, fiber, and whole-grain intake] during puberty, a potentially critical period for later disease, and low-grade inflammation in younger adulthood. The analysis was based on 205 participants (113 girls and 92 boys) from the DONALD (Dortmund Nutritional and Anthropometric Longitudinally Designed) study with at least 2 3-d weighed dietary records during puberty (girls: 9–14 y, boys: 10–15 y) and blood samples in younger adulthood (18–36 y). Multivariable linear regression models were used to analyze the associations between carbohydrate nutrition and circulating concentrations of pro- and anti-inflammatory immune mediators [high-sensitivity C-reactive protein (hs-CRP), interleukin (IL) 6, IL-18, and adiponectin]. A higher intake of carbohydrates during puberty (P-trend = 0.005), particularly from higher-GI food sources (P-trend = 0.01), was prospectively related to higher concentrations of IL-6 in younger adulthood, independently of baseline BMI and early life, socioeconomic, and other nutritional factors. Furthermore, a higher dietary GL (P-trend = 0.002) and a lower intake of whole grains (P-trend = 0.01) were independently associated with higher IL-6 concentrations in adults. Dietary GI and added sugar and fiber intakes were not independently associated with IL-6 (P-trend $\geq$ 0.09). Carbohydrate nutrition during puberty was not independently related to hs-CRP, IL-18, and adiponectin concentrations (all P-trend > 0.1). During puberty, a higher intake of carbohydrates from higher-GI food sources and lower whole-grain consumption prospectively predict greater IL-6 concentrations in young adulthood. These data support the hypothesis that diet during puberty influences later inflammation and metabolic dysfunction. J. Nutr. doi: 10.3945/jn.114.193391.

Introduction

Carbohydrate quality plays a crucial role in the development of type 2 diabetes (T2D)[11] and cardiovascular disease (CVD) (1). Observational evidence suggests that a high intake of whole-grain products or dietary fiber, particularly from cereal sources, protects against T2D and CVD (2,3). In addition, the glycemic potency of carbohydrates as measured by the dietary glycemic index (GI) and glycemic load (GL) was linked to the risk of T2D (4,5) and CVD (6).

[1] The results presented in this article are part of a project funded by the German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) through the Federal Office for Agriculture and Food (BLE), grant 2810HS035. The DONALD study is supported by the Ministry of Science and Research of North Rhine Westphalia, Germany. The German Diabetes Center is funded by the German Federal Ministry of Health, the State Ministry of School, Science and Research of the State of North-Rhine-Westphalia, and in part by grants to the German Center for Diabetes Research (DZD e.V.).

[2] Author disclosures: J. Goletzke, A. E. Buyken, G. Joslowski, K. Bolzenius, T. Remer, M. Carstensen, S. Egert, U. Nöthlings, W. Rathmann, M. Roden, and C. Herder, no conflicts of interests.

[3] Supplemental Tables 1 and 2 and Supplemental Figure 1 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

[11] Abbreviations used: CVD, cardiovascular disease; DONALD, Dortmund Nutritional and Anthropometric Longitudinally Designed; GI, glycemic index; GL, glycemic load; hs-CRP, high-sensitivity C-reactive protein; T2D, type 2 diabetes.

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Manuscript received March 5, 2014. Initial review completed April 16, 2014. Revision accepted July 7, 2014.
doi: 10.3945/jn.114.193391.

Chronic low-grade inflammation is discussed as a main mechanism linking carbohydrate intake to the development of these chronic diseases (7). Although the evidence on the impact of total carbohydrate intake on inflammatory markers is inconclusive (8), the majority of studies point to the relevance of carbohydrate quality: in a large intervention study, a diet characterized by a lower GI led to a reduced inflammatory status (9) and prospective studies observed an association between high dietary fiber (10) and whole-grain intake (11) and decreased high-sensitivity C-reactive protein (hs-CRP) and IL-6 concentrations.

Primary prevention of metabolic chronic diseases should start early in life. The fact that puberty associates with physiologic insulin resistance (12) suggests that insulin metabolism might be particularly sensitive to carbohydrate nutrition during this period. Indeed, we previously demonstrated a direct association between a higher dietary GI during puberty and T2D risk markers (HOMA-IR and liver enzymes) in younger adulthood (13). It remains to be determined whether carbohydrate quantity and quality during this period are also of prospective relevance for chronic low-grade inflammation. Therefore, the present study analyzes the prospective association between the amount (% of total energy) and the quality (dietary GI, GL, and added sugar, fiber, and whole-grain intakes) of habitual dietary carbohydrate consumption during puberty and proinflammatory markers (hs-CRP, IL-6, IL-18), as well as the anti-inflammatory adipose tissue hormone adiponectin in younger adulthood in a cohort of healthy young Germans. hs-CRP, IL-6, IL-18, and adiponectin were selected because of their previously reported associations with risk of T2D and/or CVD in large meta-analyses (14–16).

Participants and Methods

Study population. The present analysis is based on data from the DONALD (Dortmund Nutritional and Anthropometric Longitudinally Designed Study) study, an ongoing open cohort study conducted at the Research Institute of Child Nutrition in Dortmund, Germany. Since 1985, detailed data on diet, growth, development, and metabolism have been collected from >1500 apparently healthy children. On average, 40 infants are newly recruited every year and first examined at the age of 3 mo. Each child returns for 3 more visits during the first year, 2 in the second year, and then annually until adulthood (17). Since 2005, participants >18 y are invited for subsequent examinations with fasting blood withdrawal. The study was approved by the Ethics Committee of the University of Bonn, and all examinations are performed with written parental and adult participants' consent.

Because of the open cohort design, many children have not yet reached younger adulthood, and among those who did, age varies from 18 to 36 y. At the time of this analysis, 1 measurement of inflammatory markers was available for 308 participants, who were term (36–43 wk gestation) singletons with a birth weight ≥2500 g. Of these, 220 participants had provided at least 2 plausible 3-d weighed dietary records during the puberty baseline period (chronologic age: girls 9–14 y, boys 10–15 y), allowing the estimation of habitual dietary intake. Participants who were identified to consistently under-report their energy intake (i.e., all food records were implausible or they had provided more implausible than plausible food records) were excluded from the study (n = 7). A 3-d weighed dietary record was considered plausible when the total recorded energy intake was adequate in relation to the basal metabolic rate [estimated from the Schofield equations (18) by using modified cutoffs from Goldberg et al. (19)]. For inclusion in the study sample, participants also had to have anthropometric measures taken in puberty and adulthood as well as information on relevant covariates such as early life and socioeconomic factors. This resulted in a final sample of 205 participants for analysis with an average follow-up duration between puberty and younger adulthood of 12.7 y.

Blood analyses. In younger adulthood, venous blood samples were drawn after an overnight fast, centrifuged within 15 min, and frozen at –80°C in the Research Institute of Child Nutrition. For the present analysis, serum and plasma samples were transported to the German Diabetes Center. Concentrations of plasma hs-CRP were measured by using the Roche/Hitachi Cobas c311 analyzer (Roche Diagnostics). Plasma concentrations of IL-6 and total adiponectin were determined by using the Human IL-6 Quantikine HS and the Human Total Adiponectin/Acrp30 Quantikine ELISA kits, respectively, from R&D Systems. Serum IL-18 was quantified with the ELISA kit from Medical and Biological Laboratories. Intra-assay CVs for hs-CRP, IL-6, IL-18, and total adiponectin were 1.0%, 7.2%, 3.7%, and 3.8%, respectively. Interassay CVs for hs-CRP, IL-6, IL-18, and total adiponectin were 2.6%, 11.8%, 7.1%, and 8.0%, respectively.

Anthropometric measurements. From the age of 2 y onward, standing height is measured to the nearest 0.1 cm by using a digital stadiometer (Harpenden). Body weight is measured to the nearest 100 g with an electronic scale (Seca 753E; Seca Weighing and Measuring Systems). Measurements are taken at each visit according to standard procedures, with the participants dressed in underwear only and barefoot. Skinfold thicknesses are measured twice from the age of 6 mo onward at 4 different sites (suprailiacal, subscapular, biceps, triceps) on the right side of the body to the nearest 0.1 mm by using a Holtain caliper. Sex- and age-specific SD scores were calculated for the adolescent BMI values by using the German BMI standards (20). To define overweight during puberty, values proposed by the International Obesity Task Force were used, which correspond to an adult BMI of 25 kg/m^2. On the basis of triceps and subscapular skinfold thicknesses, percentage of body fat for pubescent children was derived by using the equations of Slaughter et al. (21). Excess body fatness in puberty was defined according to body fat reference curves by McCarthy et al. (22). For estimation of percentage body fat in adulthood, equations of Durnin and Womersly (23) were used, which are based on triceps, biceps, scapular, and iliac skinfold thicknesses.

Dietary assessment. Three-day weighed dietary records are used to assess nutritional intake in the DONALD study. All foods and beverages consumed by the participants as well as leftovers are weighed and recorded to the nearest 1 g by using electronic food scales (initially Soehnle Digita 8000; Leifheit SG; now WEDO digi 2000; Werner Dorsch). Recording household measures, such as number of spoons or scoops, is allowed when weighing is not possible. Furthermore, recipes as well as packages and labels are provided by the participants and their families for further information on the foods consumed. For this analysis, dietary variables were calculated as individual means of the 3-d weighed dietary records by using LEBTAB, the in-house database (24). LEBTAB is based on the German standard food tables and data obtained from commercial food products and is continuously updated to include all recorded food items. Because we aimed to describe the habitual dietary intake, an individual average intake during puberty was calculated from at least 2 records (on average, 5; range: 2–6). Each carbohydrate-containing food recorded in the dietary records was assigned a published GI value (25) (based on glucose as a reference food) according to a standardized procedure.

The carbohydrate content of the food was the principal consideration when matching a particular food with one listed in the tables; additional factors considered comprised food group, regional origin of the food, mode of preparation, main ingredients, and sugar content. The assignment process is updated on a regular basis to incorporate newly published GI values. The value of carbohydrate content (in g) of each consumed food was then multiplied by the respective GI to obtain the respective GL. The overall dietary GI is obtained by dividing total daily GL by total daily carbohydrate intake. To distinguish carbohydrate intake from higher and low GI food sources, a GI of 55 was chosen as the cutoff. Foods with a GI <55 are defined as low-GI foods (25).

The following foods were defined as added sugars: white sugar, brown sugar, raw sugar, corn syrup, corn syrup solids, high-fructose corn syrup, malt syrup, maple syrup, pancake syrup, fructose sweetener, liquid fructose, honey, molasses, anhydrous dextrose, and crystal dextrose (26). Fruit syrups commonly used as sweeteners in Germany also were considered added sugars. Conversely, naturally occurring sugars such as lactose in milk or fructose in fruits were not included.

Dietary fiber content was calculated by using the LEBTAB database. Whole-grain intake was estimated by assigning whole-grain content in

grams to each carbohydrate-containing food using the respective recipe and ingredient information available at the time of recording. The definition of whole grain followed the Whole Grain Label Statements of the US FDA: "Cereal grains that consist of the intact, ground, cracked or flaked caryopsis, whose principal anatomical components—the starchy endosperm, germ and bran—are present in the same relative proportions as they exist in the intact caryopsis" (27).

Statistical analysis. Baseline characteristics of the study population are presented for the total study population. To analyze the prospective association between carbohydrate nutrition during puberty and low-grade inflammation in younger adulthood, multivariable linear regression models were used. Because the outcome variables were not normally distributed, hs-CRP, IL-6, and IL-18 were log-transformed prior to analysis; and for adiponectin, the square root was extracted to obtain normal distribution. All dietary variables except for dietary GI were energy-adjusted by using the residual method. To account for age-dependent nutritional differences, all variables were standardized by age group and sex (mean = 0, SD = 1).

Covariates considered as potentially affecting the association between carbohydrate nutrition and inflammatory markers were gestational weight gain (kg), birth weight (continuous and < or ≥3000 g), gestational age (wk), breastfeeding for >2 wk (yes or no), firstborn child (yes or no), BMI SD score or % of body fat at baseline, maternal overweight (BMI ≥25 kg/m^2; yes or no), high maternal educational status (≥12 y of schooling; yes or no), maternal occupation (higher, other, or no), smoker in the household (yes or no), parental history of diabetes (yes or no; questionnaire-based), physical activity level (light, moderate, or high; questionnaire-based), and intakes of protein (total, animal, or vegetable) or fat (total and saturated fat). Vice versa adjustment for whole grain and GI was also considered. Each potential confounder was initially examined separately and included only if it *1*) substantially altered the association of the principal dietary variables with hs-CRP, IL-6, IL-18, or adiponectin in the models (>10%); *2*) significantly predicted the outcome variable; or *3*) improved the coefficient of determination (>5%). In the basic model (model A), sex and age (categorical: ≤19 or >19 y and ≤25 or >25 y) were included, because age at blood withdrawal in younger adulthood varied considerably (18–36 y). In a second model (model B), we further adjusted for early life and socioeconomic factors as well as for other nutritional factors. Finally, for significant findings we also conducted conditional models (additionally including waist circumference in younger adulthood) to examine whether the observed associations were independent of adult body composition.

Because other studies indicate sex differences regarding the association between dietary GI or GL and both T2D and CVD (*5*,*6*,*28*,*29*) and some inflammatory markers differ physiologically between women and men, additional sex-stratified analyses were performed. However, tests for interaction were not significant (all *P*- interaction > 0.3).

In addition to the slope of the regression, the adjusted means of the respective inflammatory marker (i.e., least-squares means predicted by the model when the other variables are held at their mean values) are presented by tertiles of the carbohydrate nutrition parameters with the corresponding 95% CIs. All *P* values presented in the tables or figures are based on tests for trend over the entire respective samples by using the continuous data. All models conform to the assumptions of linear regression models (linearity, normality and homoscedasticity of residuals, absence of multicollinearity). *P* values <0.05 were considered significant. All statistical analyses were carried out by using SAS procedures (version 9.1.3; SAS Institute).

Results

The participants who were excluded from the final study sample because of missing dietary or covariable information (n = 103) did not differ from those who were included (n = 205) with regard to early life and adult anthropometric characteristics or inflammatory markers (*P*-difference > 0.1).

Girls and boys did not differ with regard to early life or socioeconomic characteristics. Female participants were younger at baseline and had a lower BMI but a higher percentage of body fat. This persisted to younger adulthood, when women were additionally less likely to be overweight and to have an increased waist circumference. Also, women had higher concentrations of hs-CRP and adiponectin (**Table 1**). With regard to nutritional intake data during puberty, energy intake and dietary GL were lower in girls because they consumed more fiber, particularly from fruits, and had a higher magnesium intake compared with boys (**Table 2**). Overall, the participants' diet during puberty can be considered a high-carbohydrate diet with an energy percentage of >50%, mostly derived from foods with a GI >55, yet relatively rich in fiber and whole grains (Table 2).

A higher intake of carbohydrates during puberty was prospectively related to higher concentrations of the plasma IL-6 in younger adulthood (*P*-trend = 0.009, model A). Separate consideration of carbohydrates from higher- and low-GI food sources indicated that only carbohydrate intake from higher-GI sources was of relevance for later IL-6 concentrations (*P*-trend = 0.009, model A). Additional consideration of body composition during puberty and early life, parental socioeconomic, and other nutritional factors did not affect these associations (model B). Furthermore, a higher dietary GL (*P*-trend = 0.002, model B) and a lower intake of whole grains (*P*-trend = 0.01, model B) during puberty were independently associated with higher concentrations of IL-6 in younger adulthood. No independent associations were seen with dietary GI or added sugar or fiber intake (all *P*-trend ≥ 0.09, model B) (**Table 3**).

The additional inclusion of waist circumference in adulthood in a conditional model only slightly affected the associations observed between aspects of pubertal carbohydrate nutrition and adult IL-6 concentrations (total carbohydrates: *P*-trend = 0.006; carbohydrates from higher-GI food sources: *P*-trend = 0.02; dietary GI: *P*-trend = 0.12; dietary GL: *P*-trend = 0.003; whole grains: *P*-trend = 0.02, conditional model) (Table 3).

None of the analyzed markers of carbohydrate nutrition during puberty was independently related to hs-CRP (**Table 4**), IL-18 (**Supplemental Table 1**), or adiponectin (**Supplemental Table 2**) in younger adulthood (all *P*-trend > 0.1). Moreover, added sugar consumption from drinks was not related to any of the inflammatory markers (all *P*-trend > 0.3) (data not shown).

Sex-stratified analysis revealed similar findings for both male and female participants: carbohydrate nutrition was unrelated to hs-CRP, IL-18, or adiponectin (data not shown). In both sexes, pubertal dietary GL and total carbohydrate intake were related to adult IL-6 concentrations (women: *P*-trend = 0.02 and 0.05, respectively; men: *P*-trend = 0.049 and 0.06, respectively), whereas carbohydrates from low-GI food sources and added sugar and fiber intake were unrelated to later IL-6 concentrations (data not shown). Of note, associations of carbohydrates from higher-GI foods, dietary GI, and whole grains with adult IL-6 concentrations were significant only among women (**Supplemental Fig. 1**). However, these sex differences were not significant in formal tests for interaction (all *P* > 0.3).

Further adjustment for breastfeeding status, pubertal physical activity level, parental history of T2D, smokers in the household, or smoking status in adulthood as well as the inclusion of interaction terms for sex in the final models did not change any of the results (data not shown). A subgroup analysis that included only those participants aged 18–25 y showed similar results (data not shown).

Discussion

This study provides novel epidemiologic evidence for a sustained adverse effect of a higher carbohydrate intake from higher-GI

TABLE 1 Demographic, anthropometric, birth, and socioeconomic characteristics for participants of the DONALD study[1]

	Participants		
	F (n = 113)	M (n = 92)	P^2
Early life factors			
Birth year	1986 (1982, 1989)	1987 (1982, 1988)	0.8
Birth weight, *g*	3410 (3100, 3750)	3460 (3190, 3840)	0.2
Breast-fed >2 wk, *n (%)*[3]	80 (70.8)	62 (68.1)	0.7
Data from puberty			
Age, *y*	9.0 (9.0, 9.1)	10.0 (10.0, 10.1)	<0.0001
BMI SDS	−0.03 (−0.74, 0.63)	0.10 (−0.62, 0.63)	0.7
BMI, *kg/m²*	16.4 (15.1, 18.0)	17.4 (15.7, 18.8)	0.02
Overweight,[4] *n (%)*	17 (15.0)	11 (12.0)	0.5
Body fat,[5] *%*	17.6 (14.4, 23.5)	13.6 (11.3, 20.0)	<0.0001
Excess body fat,[6] *n (%)*	19 (16.8)	18 (19.6)	0.6
Socioeconomic factors, *n (%)*			
Maternal overweight[7]	37 (32.7)	26 (28.3)	0.5
Maternal education[8]	50 (44.3)	45 (48.9)	0.5
Maternal occupation[9]	57 (50.4)	46 (50.0)	0.9
Smokers in household	37 (32.7)	30 (32.6)	1.0
Data from younger adulthood			
Age, *y*	22.2 (18.1, 24.7)	21.9 (18.1, 23.8)	0.6
BMI, *kg/m²*	22.0 (20.6, 24.5)	23.2 (21.5, 25.8)	0.01
Overweight,[7] *n (%)*	22 (19.5)	29 (31.5)	0.047
Body fatness,[10] *%*	30.7 (27.8, 33.8)	17.7 (13.9, 22.9)	<0.0001
Waist circumference, *cm*	72.0 (68.0, 77.0)	80.3 (76.1, 87.5)	<0.0001
Plasma metabolites			
hs-CRP, *mg/dL*	0.13 (0.06, 0.30)	0.05 (0.03, 0.11)	<0.0001
IL-6, *pg/mL*	0.68 (0.47, 1.01)	0.68 (0.48, 1.00)	0.7
Adiponectin, *μg/mL*	8.80 (6.82, 12.50)	3.89 (3.92, 9.29)	<0.0001
Serum IL-18, *pg/mL*	244 (209, 326)	244 (198, 300)	0.4

[1] Values are medians (25th, 75th percentile) unless otherwise indicated. DONALD, Dortmund Nutritional and Anthropometric Longitudinally Designed; hs-CRP, high-sensitivity C-reactive protein, SDS, SD score.
[2] Significant differences between female and male participants were tested by using ANOVA for normally distributed continuous variables, Kruskal-Wallis test for non-normally distributed continuous variables, and chi-square test for categorical variables.
[3] Breastfeeding categories: ≤2 wk or >2 wk full breastfeeding; n = 91 for male participants.
[4] Derived from the age- and sex-specific cutoffs proposed by the International Obesity Task Force, which are linked to the adult cutoff of a BMI of 25 kg/m².
[5] Calculated according to reference 21.
[6] Derived from age-specific cutoffs proposed by McCarthy et al. (22); the 85th percentile of body fat was used as the cutoff for excess of body fat.
[7] BMI ≥25 kg/m².
[8] School education for at least 12 y.
[9] Maternal occupation: yes or no.
[10] Calculated according to reference 23.

food sources (GI >55) and a higher dietary GL during puberty on adult IL-6 concentrations, although higher pubertal whole-grain consumption appears to be beneficial.

Evidence on the relevance of carbohydrates and their glycemic potency is inconsistent. Although low-carbohydrate diets per se might be able to reduce inflammatory markers in intervention studies (8), it remains questionable whether this effect is attributable to weight loss only (30). Cross-sectional observational evidence for a role of GI and/or GL (31) was not confirmed by the only 2 prospective studies in partly overweight adults (32) or in those at risk of CVD (33). However, longer-term intervention trials suggest benefits of reducing GL (34) or GI (9). In our study, a 9% higher intake of carbohydrates was associated with a 4.8% increase in plasma IL-6 concentrations during adulthood (values calculated on the basis of differences between tertiles 1 and 3). In line with this, a 26-g increase in dietary GL, which captures carbohydrates adjusted for GI, was accompanied by a 4.4% increase in plasma IL-6. Separate analyses of carbohydrates from higher- and low-GI food sources did, however, reveal that the relevance of overall carbohydrate intake was attributable to those carbohydrates with a blood glucose–increasing effect only: a 5% increase in these carbohydrates was related to an increase of 5% in IL-6 concentrations.

Of note, the prospective associations between pubertal carbohydrate nutrition and adult plasma IL-6 concentrations were largely independent of adult body composition. The significant relations of total carbohydrate intake, carbohydrates from high-GI food sources, GL, and whole-grain intake to adult IL-6 concentrations were only slightly attenuated in conditional models that additional accounted for adult waist circumference. This is in line with our previous observation that pubertal GI and GL were unrelated to adult body composition (35).

Oxidative stress in response to postprandial glycemic excursions may be another main mechanism driving this association. A possible compensation of oxidative stress by anti-inflammatory effects of insulin in insulin-sensitive humans could be lost in insulin-resistant individuals due to prolonged proinflammatory conditions (36). Such a higher vulnerability to oxidative stress may also extend to puberty, because exaggerated compensatory insulin excursions in response to postprandial glycemia during

TABLE 2 Baseline nutritional data during puberty for participants of the DONALD study[1]

	Participants		
Nutritional variables	F (n = 113)	M (n = 92)	P^2
Total energy, *kJ/d*	7166 (6532, 7952)	8921 (8025, 9935)	<0.0001
Fat, *%en*	36.3 (33.7, 38.3)	36.1 (33.1, 38.2)	0.6
SFAs	15.9 (14.3, 17.3)	15.7 (14.4, 17.0)	0.4
MUFAs	14.8 (13.5, 15.9)	14.8 (13.8, 15.7)	0.8
PUFAs	5.3 (4.7, 5.9)	5.1 (4.4, 5.7)	0.2
Protein, *%en*	12.6 (11.5, 14.0)	13.3 (12.1, 14.0)	0.2
Carbohydrate, *%en*	51.1 (48.5, 53.6)	51.1 (48.2, 53.8)	1.0
Higher-GI carbohydrate,[3] *%en*	30.1 (26.3, 33.1)	30.3 (27.6, 32.9)	0.5
Low-GI carbohydrate,[3] *%en*	21.1 (18.1, 23.7)	20.5 (17.5, 23.8)	0.4
≥50% of carbohydrates from higher-GI food sources, *n (%)*	95 (84.1)	83 (90.2)	
Dietary GI	56.6 (54.9, 57.9)	57.0 (55.4, 58.1)	0.09
Dietary GL, *g*	124 (110, 136)	156 (136, 176)	<0.0001
Added sugar, *%en*	14.8 (11.2, 18.2)	14.8 (12.1, 18.8)	0.3
Fiber, *g/1000 kJ*	2.49 (2.19, 2.86)	2.29 (2.02, 2.68)	0.003
From cereals	1.24 (1.00, 1.57)	1.19 (1.03, 1.47)	0.9
From vegetables	0.54 (0.40, 0.68)	0.47 (0.36, 0.60)	0.08
From cereals	0.49 (0.37, 0.68)	0.38 (0.26, 0.53)	0.0006
Whole grain, *g/1000 kJ*	3.13 (1.67, 5.40)	2.60 (1.01, 4.83)	0.4
Magnesium, *mg*	242 (218, 276)	293 (252, 347)	<0.0001
Thiamin, *mg*	1.40 (1.10, 1.67)	1.68 (1.44, 1.97)	<0.0001
Riboflavin, *mg*	1.69 (1.43, 2.17)	2.18 (1.81, 2.46)	<0.0001
Vitamin B-6, *mg*	1.94 (1.58, 2.39)	2.24 (1.92, 2.75)	<0.0001
Folate, *μg*	339 (273, 437)	397 (341, 502)	<0.0001
Vitamin C, *mg*	148 (127, 200)	159 (127, 211)	0.5

[1] Values are medians (25th, 75th percentile) unless otherwise indicated. Nutritional data were averaged from 2–6 3-d weighed dietary records. DONALD, Dortmund Nutritional and Anthropometric Longitudinally Designed; GI, glycemic index, GL, glycemic load; %en, percentage of total energy intake.
[2] Significant differences between girls and boys were tested by using ANOVA for normally distributed continuous variables, Kruskal-Wallis test for non-normally distributed continuous variables, and chi-square test for categorical variables.
[3] Distinction between carbohydrate intake from higher- and low-GI food sources with GI of 55 as the cutoff.

TABLE 3 Prospective associations of variables of carbohydrate quality at baseline with plasma IL-6 (pg/mL) in young adulthood[1]

	T1	T2	T3	β (SE)	*P*-trend
Carbohydrate					
Model A	0.58 (0.49, 0.69)	0.67 (0.56, 0.79)	0.81 (0.68, 0.97)	0.190 (0.072)	0.009
Model B	0.58 (0.49, 0.69)	0.68 (0.57, 0.80)	0.83 (0.70, 0.98)	0.197 (0.069)	0.005
Conditional model	0.57 (0.48, 0.68)	0.68 (0.58, 0.81)	0.83 (0.70, 0.98)	0.193 (0.069)	0.006
Carbohydrate, higher GI[2]					
Model A	0.61 (0.51, 0.73)	0.64 (0.54, 0.76)	0.80 (0.67, 0.95)	0.189 (0.072)	0.009
Model B	0.62 (0.52, 0.74)	0.64 (0.54, 0.76)	0.80 (0.68, 0.95)	0.183 (0.072)	0.01
Conditional model	0.63 (0.53, 0.75)	0.63 (0.53, 0.75)	0.80 (0.67, 0.95)	0.161 (0.073)	0.02
Carbohydrate, low GI[2]					
Model A	0.73 (0.62, 0.88)	0.65 (0.54, 0.77)	0.66 (0.56, 0.79)	−0.037 (0.074)	0.6
Model B	0.73 (0.61, 0.86)	0.65 (0.55, 0.77)	0.68 (0.57, 0.81)	−0.013 (0.074)	0.9
Conditional model	0.72 (0.60, 0.85)	0.65 (0.55, 0.77)	0.69 (0.58, 0.82)	0.013 (0.074)	0.9
GI					
Model A	0.58 (0.48, 0.69)	0.70 (0.59, 0.83)	0.78 (0.66, 0.93)	0.176 (0.078)	0.03
Model B	0.57 (0.47, 0.68)	0.68 (0.57, 0.81)	0.73 (0.61, 0.87)	0.136 (0.080)	0.09
Conditional model	0.57 (0.48, 0.69)	0.69 (0.58, 0.82)	0.72 (0.61, 0.86)	0.115 (0.080)	0.12
GL					
Model A	0.57 (0.48, 0.67)	0.69 (0.58, 0.82)	0.82 (0.69, 0.97)	0.231 (0.073)	0.002
Model B	0.55 (0.46, 0.65)	0.66 (0.55, 0.79)	0.80 (0.67, 0.94)	0.226 (0.072)	0.002
Conditional model	0.55 (0.46, 0.65)	0.67 (0.56, 0.79)	0.80 (0.67, 0.94)	0.213 (0.072)	0.003
Added sugar					
Model A	0.62 (0.52, 0.74)	0.63 (0.53, 0.75)	0.80 (0.67, 0.95)	0.158 (0.067)	0.02
Model B	0.62 (0.52, 0.75)	0.62 (0.51, 0.74)	0.74 (0.62, 0.88)	0.101 (0.072)	0.2
Conditional model	0.62 (0.52, 0.75)	0.62 (0.52, 0.74)	0.74 (0.62, 0.88)	0.101 (0.072)	0.2
Fiber					
Model A	0.81 (0.68, 0.97)	0.68 (0.57, 0.81)	0.58 (0.49, 0.68)	−0.148 (0.066)	0.03
Model B	0.77 (0.64, 0.92)	0.65 (0.55, 0.78)	0.59 (0.49, 0.71)	−0.111 (0.069)	0.11
Conditional model	0.77 (0.64, 0.92)	0.65 (0.55, 0.77)	0.60 (0.50, 0.72)	−0.105 (0.068)	0.12
Whole grain					
Model A	0.84 (0.71, 1.00)	0.66 (0.56, 0.79)	0.56 (0.47, 0.67)	−0.196 (0.067)	0.004
Model B	0.78 (0.65, 0.93)	0.66 (0.56, 0.79)	0.56 (0.46, 0.67)	−0.177 (0.069)	0.01
Conditional model	0.77 (0.64, 0.91)	0.66 (0.57, 0.79)	0.56 (0.46, 0.67)	−0.166 (0.069)	0.02

[1] Values are means (95% CIs); nutritional variables are residuals; $n = 205$. Model A: adjusted for sex, age (categorical: ≤19 or >19 y and ≤25 or >25 y), and energy (residuals). Model B: model A plus early life factors (gestational weight gain), socioeconomic factors (maternal overweight), BMI SD score at baseline, and nutritional factors (carbohydrates, GI, GL, and added sugar: whole grain; fiber and whole grain: GI). Conditional model: model B plus adult waist circumference to investigate the possibility of a mediation of any observed association by adult waist circumference. GI, glycemic index, GL, glycemic load; T, tertile.
[2] Distinction between carbohydrate intake from higher- and low-GI food sources with a GI of 55 as the cutoff.

this period could increase the strain on β-cells, which are particularly sensitive to oxidative stress (37). Unfortunately, biomarkers of oxidative stress are notoriously difficult to measure, and the remaining serum and plasma samples from the DONALD study did not allow these measurements because of low long-term stability of markers of oxidative stress even during storage at −80°C.

In rodents it has furthermore been shown that long-term exposure to a high-GI diet leads to a delayed switch to both carbohydrate and fat oxidation in the postprandial state. Of interest, in a mouse model prone to obesity, FA oxidation was impaired already 3 wk after the start of a high-GI diet, whereas phenotypic markers were comparable in the low- and high-GI groups, indicating that reduced metabolic flexibility might precede changes and causally affect the development of an obese insulin-resistant phenotype (38).

In our study we observed no independent effect of added sugar intake or consumption of added sugar from drinks on later inflammatory markers. Studies on the effect of a single glucose challenge reported increased oxidative stress (36). Also, in a study in healthy young men, a high consumption of soft drinks, sweetened with glucose, fructose, or sucrose, over 3 wk led to increased hs-CRP concentrations (39). In our cohort, added sugar mainly comprised sucrose, and consumption amounts—in total and from drinks—can be considered average, albeit not high (40). Moreover, glycemic excursions in response to sucrose are moderate rather than high as reflected by a GI of 65 (25). Hence, the glycemic and, in turn, possibly proinflammatory potential of the diet might be better described by dietary GI.

In contrast to whole-grain intake, which was inversely associated with later IL-6 concentrations, dietary fiber was not of independent relevance in our study. A recent cross-sectional study in adolescents found an inverse association between fiber intake and CRP concentrations (41). Among adults, most observational studies also reported reduced low-grade inflammation among those consuming more whole grain and dietary fiber, although the evidence is less consistent in intervention studies (42,43). Dietary fiber may reduce chronic inflammation by beneficially interacting with gut microflora and decreasing lipid oxidation (44), but whole grains possess several additional components displaying diverse anti-inflammatory effects, such as free radical scavenging or antioxidant enzyme activation (45). Our results point to a relevance of whole grains, which may better represent the combination of different anti-inflammatory effects.

Our study adds to the current discussion that a high carbohydrate intake may have detrimental health effects if the carbohydrates

TABLE 4 Prospective association of variables of carbohydrate quality at baseline with plasma hs-CRP (mg/dL) in young adulthood[1]

	T1	T2	T3	β (SE)	*P*-trend
Carbohydrate					
Model A	0.10 (0.08, 0.13)	0.08 (0.06, 0.10)	0.08 (0.07, 0.11)	−0.033 (0.106)	0.8
Model B	0.10 (0.08, 0.13)	0.08 (0.06, 0.10)	0.09 (0.07, 0.11)	−0.021 (0.106)	0.8
Conditional model	0.10 (0.07, 0.13)	0.08 (0.06, 0.10)	0.08 (0.07, 0.11)	−0.032 (0.104)	0.8
Carbohydrate, higher GI[2]					
Model A	0.09 (0.07, 0.11)	0.09 (0.07, 0.11)	0.09 (0.07, 0.12)	0.027 (0.107)	0.8
Model B	0.09 (0.07, 0.11)	0.09 (0.07, 0.12)	0.09 (0.07, 0.12)	0.022 (0.109)	0.9
Conditional model	0.09 (0.07, 0.12)	0.08 (0.06, 0.11)	0.09 (0.07, 0.11)	−0.044 (0.108)	0.7
Carbohydrate, low GI[2]					
Model A	0.11 (0.08, 0.14)	0.07 (0.06, 0.10)	0.09 (0.07, 0.11)	−0.073 (0.108)	0.5
Model B	0.11 (0.08, 0.14)	0.08 (0.06, 0.10)	0.09 (0.07, 0.12)	−0.057 (0.110)	0.6
Conditional model	0.10 (0.08, 0.13)	0.08 (0.06, 0.10)	0.09 (0.07, 0.12)	0.002 (0.109)	1.0
GI					
Model A	0.10 (0.08, 0.13)	0.07 (0.06, 0.09)	0.10 (0.08, 0.13)	−0.013 (0.116)	0.9
Model B	0.10 (0.08, 0.13)	0.07 (0.06, 0.10)	0.10 (0.08, 0.13)	−0.029 (0.120)	0.8
Conditional model	0.10 (0.07, 0.13)	0.07 (0.06, 0.10)	0.09 (0.07, 0.12)	−0.087 (0.119)	0.5
GL					
Model A	0.09 (0.07, 0.12)	0.09 (0.07, 0.11)	0.08 (0.06, 0.11)	−0.053 (0.109)	0.6
Model B	0.09 (0.07, 0.12)	0.09 (0.07, 0.12)	0.09 (0.07, 0.11)	−0.045 (0.110)	0.7
Conditional model	0.09 (0.07, 0.12)	0.09 (0.07, 0.12)	0.08 (0.06, 0.11)	−0.080 (0.108)	0.5
Added sugar					
Model A	0.09 (0.07, 0.11)	0.09 (0.07, 0.11)	0.09 (0.07, 0.12)	0.086 (0.098)	0.4
Model B	0.09 (0.06, 0.11)	0.09 (0.07, 0.12)	0.09 (0.07, 0.12)	0.080 (0.109)	0.5
Conditional model	0.08 (0.06, 0.11)	0.09 (0.07, 0.12)	0.09 (0.07, 0.12)	0.081 (0.106)	0.4
Fiber					
Model A	0.09 (0.07, 0.11)	0.09 (0.07, 0.12)	0.09 (0.07, 0.11)	−0.051 (0.097)	0.6
Model B	0.09 (0.07, 0.11)	0.09 (0.07, 0.12)	0.09 (0.07, 0.12)	−0.041 (0.103)	0.6
Conditional model	0.08 (0.06, 0.11)	0.09 (0.07, 0.11)	0.09 (0.07, 0.11)	−0.025 (0.100)	0.8
Whole grain					
Model A	0.11 (0.08, 0.14)	0.08 (0.06, 0.10)	0.09 (0.07, 0.11)	−0.063 (0.100)	0.5
Model B	0.11 (0.08, 0.14)	0.08 (0.06, 0.10)	0.09 (0.07, 0.12)	−0.067 (0.104)	0.5
Conditional model	0.10 (0.08, 0.13)	0.08 (0.06, 0.10)	0.09 (0.07, 0.11)	−0.038 (0.102)	0.7

[1] Values are means (95% CIs); nutritional variables are residuals; n = 205. Model A: adjusted for sex, age (categorical: ≤19 or >19 y and ≤25 or >25 y), and energy (residuals). Model B: model A plus early life factors (gestational weight gain), socioeconomic factors (maternal overweight), BMI SD score at baseline, and nutritional factors (carbohydrates, GI, GL, and added sugar: whole grain; fiber and whole grain: GI). Conditional model: model B plus adult waist circumference to investigate the possibility of a mediation of any observed association by adult waist circumference. GI, glycemic index, GL, glycemic load; hs-CRP, high-sensitivity C-reactive protein; T, tertile.
[2] Distinction between carbohydrate intake from higher- and low-GI food sources with GI of 55 as the cutoff.

stem from higher-GI food sources (46) and extends this observation to chronic low-grade inflammation. This is of public health relevance because low-grade inflammation is linked to the development of a range of chronic diseases including T2D, CVD, depression, dementia, some types of cancers, frailty in old age, and mortality (7) and because most free-living populations, such as the DONALD adolescents, consume >50% of their calories as carbohydrates (47,48). Of note, in the present study, 59% of the carbohydrates and 76% of the whole grains came from foods with a moderate or high GI. Hence, efforts to improve carbohydrate quality among adolescents should not focus solely on encouraging a higher whole-grain intake but may need to be complemented by specific advice for a preferred selection of low-GI carbohydrates (e.g., pasta, legumes) and low-GI whole grains (e.g., muesli, pumpernickel).

Although the associations between carbohydrate nutrition and IL-6 in the present study can be considered robust, it is not clear why these relations did not extend to other inflammatory markers. Importantly, previous studies that reported associations between dietary components such as GL and inflammatory biomarkers including CRP, IL-18, and adiponectin mainly focused on individuals with T2D or at high cardiometabolic risk (31,32, 49,50) and who were not comparable to our study participants with regard to age and overall health status. Furthermore, the chemical complexity of foods summarized as whole grain or determining GI and GL make it extremely difficult to identify molecular pathways that may be responsible for the associations observed for IL-6 in our study compared with the lack of effects on hs-CRP, IL-18, and adiponectin.

Although our data do not allow us to explain why we observed associations only for IL-6, we believe that our findings may be of clinical relevance. We acknowledge that IL-6 is a complex cytokine in the pathophysiology of T2D. On the one hand, IL-6 is involved in the induction of obesity-associated insulin resistance in liver and adipose tissue (51). On the other hand, IL-6 is released from skeletal muscle after exercise with potentially beneficial paracrine and endocrine effects (52). Recent mouse studies indicated that IL-6 may be implicated in the regulation of insulin secretion (53) and the limitation of inflammatory processes (54). Despite the controversy that the aforementioned studies indicate, it is important to emphasize that IL-6 concentrations are consistently related to increased T2D risk in many epidemiologic studies (16). In addition, Mendelian randomization analyses showed that IL-6 but not CRP is a causal factor in the development of coronary heart disease (55,56), underpinning the potential public health relevance of

our findings. Of interest, a cross-sectional study analyzing the association between several inflammatory markers and insulin resistance in adolescents observed the strongest association for IL-6, whereas associations with IL-18 or adiponectin were not significant after adjustment for confounders (57). Nonetheless, further studies are needed to explore the potential vulnerability of adolescents to carbohydrate-induced postprandial glycemia and the potentially protective effects of whole grains during puberty on inflammatory markers both in the short and the long term.

The main strengths of our study are its prospective design and the detailed repeated assessments of dietary intake during puberty. For the present analysis, an average of 5 dietary records were available (range: 2–6 records per participant) from which habitual dietary intake could be estimated. This repeated collection of dietary records further attenuates the risk of not capturing seldom-consumed foods and also improves overall reliability. Another strength is the availability of information on early life and socioeconomic factors, which could potentially have confounded the examined associations. However, residual confounding, resulting from imprecisely measured or unmeasured confounding factors, cannot be precluded.

Our study also has several limitations. First, in contrast to the repeatedly measured dietary intake data, inflammatory markers were measured only once in younger adulthood. Second, because of the relatively elaborate DONALD study design, the socioeconomic status of the study population is above average and extremes of diet or behavior might not be represented, which could introduce selection bias yet reduces our vulnerability to residual confounding. Third, the estimation of the dietary GI from the GI values of individual foods is regarded controversial (58). However, although most epidemiologic studies use FFQs, the GI estimates in this study were directly assigned to all carbohydrate-containing foods recorded during 3 d. Moreover, numerous factors exist influencing the glycemic response to a food, such as heating or ripening (59). Variability of glycemic response is, however, not only a problem of GI but of other nutrients as well (25,60).

In conclusion, our study provides novel evidence that a higher intake of carbohydrates from higher-GI food sources may be detrimental and higher whole-grain consumption may be beneficial for adult IL-6 concentrations. These data support the hypothesis that diet during puberty influences later inflammation and metabolic dysfunction.

Acknowledgments

The authors thank Birgit Holtermann, Ute Kahrweg, Martina Okunek, and Sabine Twenhöven for carrying out the anthropometric measurements and Christa Chahda and Ruth Schäfer for collecting and coding the dietary records. They also thank Ulrike Poschen and Gabi Gornitzka from the German Diabetes Center, Düsseldorf, for carrying out the blood analyses. A.E.B. conceived the research project and had primary responsibility for the final content; A.E.B. and C.H. supervised the project and provided detailed assistance in the drafting process; M.C. measured the inflammatory markers in the laboratory of C.H.; J.G. and G.J. assigned the GI values; J.G. and K.B. performed the statistical analysis; J.G. wrote the manuscript; and J.G., A.E.B., G.J., K.B., T.R., M.C., S.E., U.N., W.R., M.R., and C.H. made substantial contributions to the interpretation of the results. All authors read and approved the final manuscript.

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AJCN. First published ahead of print February 19, 2014 as doi: 10.3945/ajcn.113.074252.

Association between carbohydrate quality and inflammatory markers: systematic review of observational and interventional studies[1–3]

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ABSTRACT

Background: Chronic low-grade inflammation is a likely intermediary between quality of carbohydrate and chronic disease risk.

Objective: We conducted a systematic literature search to evaluate the relevance of carbohydrate quality on inflammatory markers in observational and intervention studies.

Design: MEDLINE, EMBASE, and the Cochrane Library were searched for studies on associations between glycemic index (GI), glycemic load (GL), dietary fiber or fiber supplements or whole grain intake, and high-sensitivity C-reactive protein (hsCRP) or interleukin 6 (IL-6). Included studies had to be conducted on adults (healthy, overweight, with type 2 diabetes or metabolic syndrome features, but without inflammatory disease) with ≥20 participants and a 3-wk duration.

Results: In total, 22 of the 60 studies that met our inclusion criteria examined GI/GL: 5 of 9 observational studies reported lower concentrations of hsCRP or IL-6 among persons with a lower dietary GI/GL; 3 of 13 intervention studies showed significant antiinflammatory effects of a low-GI/GL diet, and 4 further studies suggested beneficial effects (trends or effects in a subgroup). For fiber intake, 13 of 16 observational studies reported an inverse relation with hsCRP or IL-6, but only 1 of 11 intervention studies showed a significant antiinflammatory effect of fiber intake, and a further trial reported a beneficial trend. For whole-grain intake, 6 of 7 observational studies observed an inverse association with inflammatory markers, but only 1 of 7 intervention studies reported significant antiinflammatory effects, 1 further study was suggestive (in a subgroup) of such, and another study found an adverse effect (trend only).

Conclusions: Evidence from intervention studies for antiinflammatory benefits is less consistent for higher-fiber or whole-grain diets than for low-GI/GL diets. Benefits of higher fiber and whole-grain intakes suggested by observational studies may reflect confounding. *Am J Clin Nutr* doi: 10.3945/ajcn.113.074252.

INTRODUCTION

Chronic, low-grade inflammation is now considered to be intimately linked to the development of diabetes (1) and cardiovascular disease (CVD)[4] (2). In addition, subclinical activation of the immune system has been found to be associated with a range of other diseases, such as dementia (3), depressive disorders (4), and certain types of cancer (5). Finally, low-grade inflammation is associated with a higher risk of all-cause mortality in old age (6). Thus, modifiable risk factors that effectively reduce chronic inflammation can be expected to contribute substantially to the prevention of chronic disease.

In this context, different aspects characterizing carbohydrate quality have recently received considerable interest. Dietary fiber intake is considered to reduce chronic inflammation by decreasing lipid oxidation (7) and beneficially interacting with gut microflora via regulatory influences of short-chain fatty acids produced from colonic fermentation of fiber (8). Whole-grain foods are additionally rich in several bioactive compounds with antiinflammatory properties, such as free radical scavenging, antioxidant enzyme activation, or modification of the redox status of tissues and cells (7). In addition, viscous fiber from oats or barley may slow the rates of glucose appearance in the blood (9). Postprandial glycemic response to a food is best captured by the glycemic index (GI, a ranking of carbohydrate foods by their glycemic potency) (10) and the glycemic load (GL, defined as the mathematical product of the GI and carbohydrate content) (11). Excessive postprandial blood glucose excursions are considered to yield nitric oxide generation, which in turn combines with superoxide to produce peroxynitrite—a potent long-lived pro-oxidant molecule (12). Hence, consumption of high-GI foods may contribute to oxidative stress and both acute and chronic low-grade inflammation (13).

These potential effects of carbohydrate quality on chronic inflammation have recently been investigated in many observational

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[2] The DONALD study is supported by the Ministry of Science and Research of North Rhine Westphalia, Germany. The German Diabetes Center is funded by the German Federal Ministry of Health; the Ministry of School, Science and Research of the State of North-Rhine-Westphalia; and the German Center for Diabetes Research (Deutsches Zentrum für Diabetesforschung).

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[4] Abbreviations used: CRP, C-reactive protein; CVD, cardiovascular disease; FFQ, food-frequency questionnaire; GI, glycemic index; GL, glycemic load; hsCRP, high-sensitivity C-reactive protein.

Received August 23, 2013. Accepted for publication January 23, 2014.
doi: 10.3945/ajcn.113.074252.

and interventional studies. Comparative assessment of the evidence from these 2 types of studies may be particularly insightful because evidence appraisal on the relevance of carbohydrate quality for chronic diseases (eg, type 2 diabetes, CVD, cancer) almost exclusively draws on observational studies. Thus, the aim of the current systematic review was to evaluate the evidence from currently published observational and interventional studies conducted in adults who were either healthy, were overweight, or had features of the metabolic syndrome or type 2 diabetes regarding the relevance of fiber intake, whole-grain consumption, and dietary GI/GL for markers of chronic low-grade inflammation. We selected high-sensitivity C-reactive protein (hsCRP) and IL-6 because they represent the most commonly measured immune mediators in clinical and epidemiologic studies. Currently, there is more evidence that IL-6 is causal for the development of inflammation-related diseases such as CVD (14, 15), whereas C-reactive protein (CRP) concentrations may be a "bystander" rather than a true risk factor. However, CRP has been frequently measured in studies with robust associations with many health outcomes and is therefore a useful prognostic biomarker.

SUBJECTS AND METHODS

Study selection

We conducted a systematic literature search of the MEDLINE (http://www.ncbi.nlm.nih.gov/pubmed/), EMBASE (http://www.elsevier.com/online-tools/embase), and Cochrane Library [Cochrane Central Register of Controlled Trials (CENTRAL); http://onlinelibrary.wiley.com/cochranelibrary/search/] databases from January 1990 through September 2012 (updated June 2013). The search was limited to this time frame because hsCRP assays were first available in the early 1990s. The following terms were used to identify all potentially relevant publications published as conference abstracts or complete manuscripts in the English or German language: glyc(a)emic index/load, whole grain(s), fiber/fiber, carbohydrate quality together with (hs-) CRP, (high-sensitivity) C-reactive protein, IL-6, and interleukin 6 (*see* "Supplemental data" in the online issue). The search was restricted to human studies carried out in adults (≥18 y). Inclusion criteria for epidemiologic studies (cross-sectional or prospective cohort studies) were as follows: dietary GI, GL, whole grain or dietary fiber intake as a predictor, hsCRP, or IL-6 among the outcomes and information on the dietary assessment method. Inclusion criteria for intervention studies were as follows: a randomized controlled or a crossover design, information on adherence to the intervention diets, and data on changes in BMI or body weight.

Because we were interested in the specific effects of GI/GL, whole grain, or fiber on low-grade inflammation, we excluded studies that analyzed dietary patterns, treatment studies, or studies on pregnant women. To this end, we excluded intervention studies on participants with inflammatory diseases other than type 2 diabetes or the metabolic syndrome at baseline, ie, diseases such as arthritis, pneumonia, or Alzheimer disease for which inflammation or oxidative stress represent relevant components in their development and/or progression.

Furthermore, studies lasting <3 wk or including <20 participants (10 individuals per treatment group) were not considered to provide sufficient information for the research questions under investigation. The literature search was conducted independently by 3 investigators (JG, GJ, and AF). The identification process is illustrated in **Figure 1**. In addition, a manual search of references cited by the published original studies and relevant review articles was performed (cross-references). This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (16).

Data extraction

Two investigators (JG and AF) independently reviewed and extracted relevant data from each report. Any disagreement between reviewers was resolved by consensus and if necessary referred to the senior researcher (AEB). Extracted data included information on study design, duration, location, sample size, participant characteristics (sex, age, BMI, health status), nutritional assessment, type of intervention (eg, dietary counseling or provision of foods) and weight maintenance or loss, GI, GL, whole grain and/or fiber intakes, and adjustments for potentially confounding factors (*see* Supplemental Tables 1–4 under "Supplemental data" in the online issue). For studies that provided data on GL and carbohydrate intake only, the dietary GI was calculated [GI = GL/carbohydrate (in g) × 100]. GI values referring to white bread as the reference food (= 100) (17–21) were transformed to the glucose scale by dividing the respective GI and GL values by 1.4286 (22). Data on hsCRP and IL-6 were extracted as the inflammatory markers of interest. We extracted data from baseline and change and/or endpoint of these outcome measurements. Where available, results from intention-to treat analyses are reported to reduce attrition bias.

RESULTS

The study selection process is shown in Figure 1. Of the 970 reports identified by the search, 864 were excluded based on title and abstract. The remaining 106 reports were reviewed in full, which led to the exclusion of a further 49 reports. The literature update conducted in June 2013 revealed 3 more eligible studies. Of the 60 studies included in the systematic review, 22 addressed dietary GI or GL, 27 fiber, and 14 whole grain intake. Qi et al (23) addressed all 3 aspects of carbohydrate quality considered in this review, and Murakami et al (24) examined both dietary GI/GL and dietary fiber intake.

Dietary GI and GL

Epidemiologic studies

Nine epidemiologic studies were identified that addressed dietary GI and/or GL as a nutritional exposure variable (17–19, 23–28) (**Table 1**). Overall, the studies included 26,131 participants (range: 171–18,137) aged 20–67 y with a BMI (in kg/m^2) ranging from 21 to 30. Four of the studies included women only (19, 23, 24, 27). Dietary GI ranged from 52 to 71 and dietary GL from 96 to 179 g, mostly assessed by means of a food-frequency questionnaire (FFQ) (n = 7 studies). Details on population, assessment method, average baseline concentrations of exposures, and covariates considered in the analysis and results are shown elsewhere (*see* Supplemental Table 1 under "Supplemental data" in the online issue).

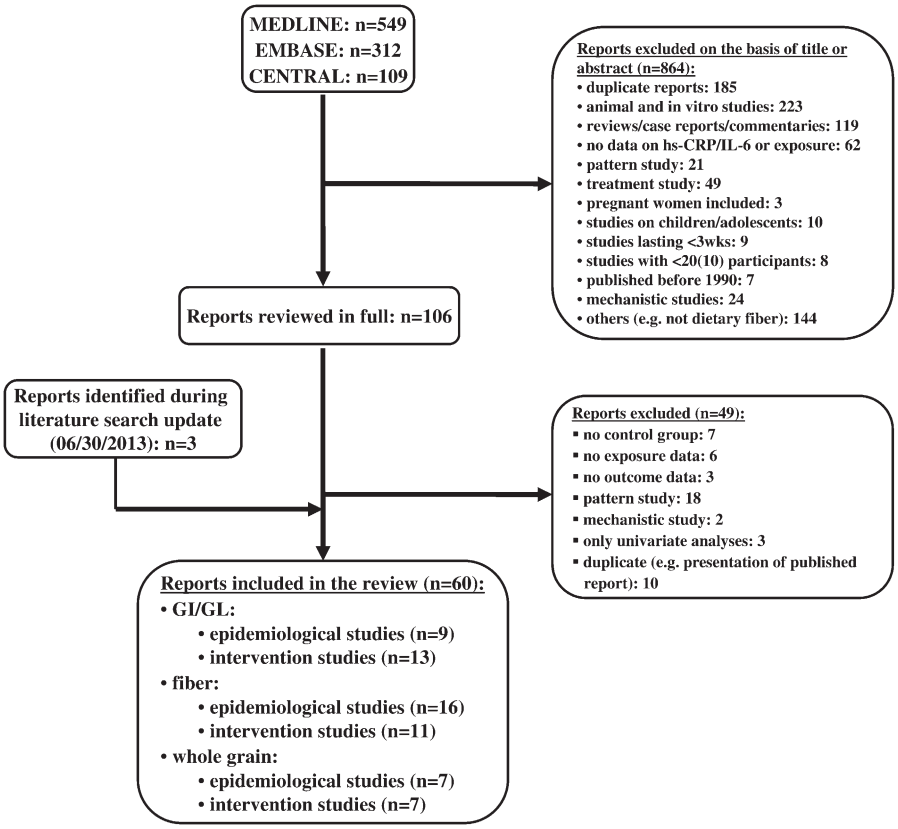

FIGURE 1. Flowchart of the study selection process. The literature search was conducted during September 2012, was updated in June 2013, and included all studies published from January 1990 onward. Note that 2 studies addressed more than one aspect of carbohydrate quality. MEDLINE: http://www.ncbi.nlm.nih.gov/pubmed/; EMBASE: http://www.elsevier.com/online-tools/embase; CENTRAL: http://onlinelibrary.wiley.com/cochranelibrary/search/. GI, glycemic index; GL, glycemic load.

One cross-sectional study among 244 healthy US women reported a strong association with hsCRP for both dietary GI and GL (19). Similarly, in a subsample of 974 Dutch participants drawn from 2 population-based cohorts, higher concentrations of GI and GL tended to be related to higher concentrations of hsCRP (P = 0.05 and P = 0.09, respectively) (26). Three cross-sectional studies reported associations confined to either GI or GL: in >18,000 postmenopausal US women (27) and a sample of 891 US women with type 2 diabetes (23), a higher dietary GI, but not a higher dietary GL, was related to increased hsCRP concentrations. Conversely, in a subsample of 4366 Dutch participants drawn from a population-based cohort, a direct association was seen for dietary GL only (28). In two 1-y prospective studies among 511 Spanish participants at high CVD risk (25) and 582 healthy Americans (17), indications of a cross-sectional association between GI and IL-6 (25) or GL and hsCRP (17) were not confirmed in longitudinal analyses. Finally, 2 studies conducted in 136 overweight Americans (18), and 443 healthy young Japanese women (24) found no associations between dietary GI or GL and hsCRP (Table 1).

Intervention studies

Thirteen intervention studies met all the inclusion criteria (20, 21, 29–39) (**Table 2**). The included studies lasted 4–52 wk and included a total of 2237 participants (range: 15–932) aged 30–66 ywith a BMI ranging from 28 to 36. Two studies included male participants only (32, 37). The dietary GI (estimated from 10 studies) in the intervention (low-GI or GL) and control (high-GI or GL) groups ranged from 33 to 57 and 58 to 86, respectively, and the corresponding dietary GL ranges (estimated from 11 studies) were 36–158 and 68–250 g, respectively. Three studies were designed as weight-loss trials (21, 30, 36), and 2 additional studies offered advice on weight loss if desired (20, 39), but weight changes were similar in the intervention and control groups. Details on participant characteristics, dietary interventions, primary endpoints, and the analysis and results are shown elsewhere (*see* Supplemental Table 2 under "Supplemental data" in the online issue).

Three GI/GL intervention studies reported that reductions in hsCRP (21, 31) or IL-6 (34) in the low-GI/GL group were significantly larger than changes in the control group. In the Diet, Obesity and Genes study, conducted in 932 overweight participants from 8 European countries, groups assigned to a low-GI diet had notably larger reductions in hsCRP concentrations after the 26-wk weight-maintenance period than did those assigned to a high-GI diet (31), whereas a higher protein intake significantly increased hsCRP. Two smaller intervention studies including obese persons reported larger reductions in IL-6 in response to a low-GI diet (34) and larger reductions in hsCRP in response to a low-GL diet (21) when compared with changes under a high-GI or low-fat control diet, respectively. In 4 further studies, overall changes were not significantly

TABLE 1
Dietary GI/GL and hsCRP and IL-6: epidemiologic studies[1]

First author, year, country	Study population, characteristics, name of study	Follow-up	Exposure: assessment method	Outcome: average (baseline) concentration	Results	Association[2]
Bullo, 2011, Spain (25)	511 participants with high CVD risk, 56% female, age 67.2 y*, BMI 29.2 kg/m^2*, PREDIMED trial	1 y	FFQ	Mean IL-6: 9.97 pg/mL	• No prospective associations between GI/GL and IL-6 ($P > 0.3$) • Trend for direct cross-sectional association between GI and IL-6 ($P = 0.05$)	GI/GLprosp ∼, GIcross (↑IL-6)
Du, 2008, Netherlands (26)	786 participants (30% T2D, 23% IGT), 47% female, age 65 y*, BMI 27.8 kg/m^2*, CoDAM and Hoorn Study	Cross	FFQ	Median hsCRP: 2.0 mg/dL	• Trend for direct association between GI and hsCRP ($P = 0.05$) • Trend for direct association between GL and hsCRP ($P = 0.09$)	GI/GLcross (↑CRP)
Griffith, 2008, USA (17)	582 participants (64% overweight or obese), 48% female, age 48 y*, BMI 27.4 kg/m^2*, SEASONS	1 y	24-h dietary recall (4×)	Mean hsCRP: 1.8 mg/L	• No longitudinal associations between GI/GL and hsCRP ($P > 0.16$) • Trend for direct cross-sectional association between GL and hsCRP ($P = 0.07$) • Direct cross-sectional association between GL and hsCRP among obese participants ($P = 0.04$)	GI/GLprosp ∼, GIcross (↑CRP)
Huffman, 2007, USA (18)	171 sedentary participants with overweight to mild obesity and dyslipidemia, 51% female, age 53 y*, BMI 29.6 kg/m^2*	Cross	FFQ	Geometric mean hsCRP: 2.2 mg/L	• No association between GI/GL and hsCRP ($P > 0.18$)	GI/GLcross ∼
Levitan, 2008, USA (27)	18,137 postmenopausal women, age 54.8 y*, BMI 25.7 kg/m^2*, Women's Health Study	Cross	FFQ	Geometric mean hsCRP[3]: 1.80 mg/L	• Higher hsCRP concentrations among persons with a higher dietary GI ($P < 0.001$) • No association between GL and hsCRP ($P = 0.2$)	GIcross ↑CRP, GLcross ∼
Liu, 2002, USA (19)	244 healthy women, age 59 y*, BMI 26 kg/m^2*, Women's Health Study	Cross	FFQ	Median hsCRP: 2.8 mg/L	• Higher hsCRP concentrations among persons with a higher dietary GI ($P < 0.01$) and higher GL ($P < 0.01$)	GI/GLcross ↑CRP
Murakami, 2008, Japan (24)	443 healthy women, age 19.5 y*, BMI 21.3 kg/m^2*	Cross	Diet-history questionnaire	Mean hsCRP: 0.30 mg/L	• No association between GL and hsCRP ($P > 0.3$)	GI/GLcross ∼

(Continued)

TABLE 1 (*Continued*)

First author, year, country	Study population, characteristics, name of study	Follow-up	Exposure: assessment method	Outcome: average (baseline) concentration	Results	Association[2]
Qi, 2006, USA (23)	891 women with T2D, age 58.5 y*, BMI 29.7 kg/m^2*, Nurses' Health Study	Cross	FFQ	Geometric mean CRP[3]: 5.75 mg/L	• Higher CRP concentrations among persons with a higher dietary GI (P = 0.04) • No association between GL and CRP (P = 0.17)	GIcross ↑CRP, GLcross ~
Van Woudenbergh, 2011, Netherlands (28)	4366 participants, 60% female, age 67.3 y*, BMI 26.2 kg/m^2*, Rotterdam Study	Cross	FFQ	Median hsCRP: 1.65 mg/L	• Higher hsCRP concentrations among persons with a higher dietary GL (P = 0.01) • No association between GI and hsCRP (P = 0.9)	GLcross↑CRP, GIcross ~

[1] *See* "Supplemental data" in the online issue for details on population, assessment method, average baseline concentrations of exposures, covariates considered in analysis, and results. *Average value (mean or median as provided in the original publication). CoDAM, Cohort Study Diabetes and Atherosclerosis Maastricht; Cross, cross-sectional; CVD, cardiovascular disease; FFQ, food-frequency questionnaire; GI, glycemic index; GL, glycemic load; hsCRP, high-sensitivity C-reactive protein; IGT, impaired glucose tolerance; IL-6, interleukin 6; PREDIMED, Prevencion con dieta mediterranea; prosp, prospective; SEASONS, Seasonal Variation of Blood Cholesterol Study; T2D, type 2 diabetes mellitus.

[2] ↑: Direct association with GI or GL; (↑): trend for association with GI or GL; ~: no association with GI or GL; ↓: inverse association with GI or GL.

[3] Estimated from values given per quantiles.

different between the treatment groups. However, results suggested a treatment effect because the authors reported a trend for a between-group difference of change (29) or a treatment effect (35), a pronounced reduction in the low-GL study arm only (36, 39), or a between-group difference of change confined to participants with high body fat mass (35). In 6 further studies, lasting 4–40 wk, low-GI/GL diets did not result in larger reductions in hsCRP concentrations (20, 30, 32, 33, 37, 38) or IL-6 (37, 38) in comparison with reductions observed under various control diets with a higher GI and GL (Table 2).

Dietary fiber

Epidemiologic studies

In total, 16 epidemiologic studies were identified that reported on the association between dietary fiber intake and hsCRP or IL-6 (23, 24, 40–53) (**Table 3**, top). Overall, these studies included 39,893 participants (range: 87–9895) aged 20–69 y (data from 12 studies) and a BMI of 21–31 (data from 12 studies). Three studies included women only (23, 24, 51); one study was performed in men only (53). Dietary fiber intake was assessed through an FFQ (n = 8 studies), 24-h recalls (n = 5 studies), or other methods (n = 3), and the studies varied notably in the presentation of dietary fiber intake data (*see* Supplemental Table 3 under "Supplemental data" in the online issue).

Overall, 13 of these 16 studies reported an inverse association between the intake of dietary fiber and concentrations of hsCRP (23, 40–42, 45, 47–50, 53) or IL-6 (43, 46, 51, 53). Of note, inverse associations with these markers were also seen in longitudinal analyses of 4 studies including healthy individuals (51), persons with a metabolic syndrome (41), and CVD risk factors or type 2 diabetes (45, 46), who were followed up after 3 mo (45) or 1 y (41, 46, 51). In 3 further cross-sectional studies including <2000 participants, dietary fiber intake was not related to hsCRP concentrations (24, 44, 52) (Table 3, top).

Intervention studies

Eleven intervention studies addressing the effect of dietary fiber intake or fiber supplements on hsCRP and/or IL-6 were identified (60–70) (**Table 4**, top). These studies lasted 3–16 wk and included a total of 690 participants (12–166) aged 38–63 y with a BMI range from 25 to 34 (data from 10 studies). One study was performed in men only (70). In most of the studies, fiber supplements were added to the habitual diet (61, 63, 66–70), 2 used special fiber-enriched study foods (60, 62), and 1 study compared a high-fiber Dietary Approaches to Stop Hypertension (DASH) diet and a fiber-supplemented diet (65). Four studies reported the fiber dose of the administered supplements only (63, 68–70). Overall, information on fiber intake differed notably, precluding the calculation of mean intakes. Three of the studies were designed as weight-loss trials (63, 69, 70), but a trend toward a greater BMI reduction in the intervention group was observed in one study only (63) (*see* Supplemental Table 4 under "Supplemental data" in the online issue).

In one crossover trial examining participants with mild hypercholesterolemia, hsCRP but not IL-6 concentrations were reduced to a larger extent in the 5-wk high-fiber period when compared with the low-fiber period (64). In a 3-wk randomized crossover study among

TABLE 2
Dietary GI/GL and hsCRP and IL-6: intervention studies[1]

First author, year, country	Participants' characteristics	Study design, dietary interventions	Outcome: baseline concentrations	Results[2]	Effect or no effect of intervention[3]
Ebbeling, 2012, USA (29)	21 participants with overweight and obesity, 38% female, age 30.3 y*, BMI 34.4 kg/m^2*	Randomized, controlled, crossover, 4-wk duration, low-GI vs low-fat vs very-low CHO diet	Mean hsCRP: 1.75 mg/L	• hsCRP not significantly different between test diet periods ($P = 0.13$) • *P*-linear trend from low-fat to low-GI to very-low CHO diet: 0.05	Low-GI/GL: (+CRP)
Fabricatore, 2011, USA (30)	79 participants with T2D, 64.9% female, age 52.5 y*, BMI 36.3 kg/m^2*	Parallel-group study with balanced randomization, 40-wk duration, low-GL ($n = 39$) vs low-fat ($n = 40$)	Mean hsCRP[4]: 7.7 mg/L	• No between-group difference for change in hsCRP after 40 wk ($P = 0.8$)	Low-GL: −CRP
Gögebakan, 2011 (31)[5]	932 healthy participants, 65% female, age 41 y*, BMI 34 kg/m^2*, DiOGenes study	RCT, 26-wk duration, low-GI/low-protein ($n = 150$) vs low-GI/high-protein ($n = 315$) vs high-GI/low-protein ($n = 155$) vs high-GI/high-protein diet ($n = 155$) vs control group ($n = 154$)	Mean hsCRP: 2.78 mg/L	• Decrease in hsCRP significantly larger in low-GI groups than decrease in high-GI groups ($P < 0.001$) • Low-GI groups more likely to achieve a >15% hsCRP reduction than high-GI groups ($P = 0.007$)	Low-GI: +CRP
Hartman, 2010, USA (32)	64 men at risk of colorectal cancer, age 54 y*, BMI 28.7 kg/m^2*	Randomized, crossover, 4-wk duration, legume-rich/low-GI vs isocaloric diet	Mean hsCRP: 1.28 mg/L	• No between-group difference for change in hsCRP ($P = 0.9$)	Low-GI: −CRP
Jebb, 2010, UK (33)	522 participants at risk of metabolic syndrome, 58% female, age 51.5 y*, BMI 28.5 kg/m^2*	5-center parallel-design RCT, 24-wk duration, high-MUFA/LGI ($n = 108$) vs high-MUFA/HGI ($n = 107$) vs low-fat/LGI ($n = 119$) vs low-fat/HGI ($n = 109$) vs control high-SFA/HGI ($n = 79$)	Mean hsCRP[4]: 0.53 mg/L	• No between-group difference for relative change in hsCRP ($P = 0.9$)	Low-GI: −CRP
Jenkins, 2008, Canada (20)	210 participants with T2D, 39% female, age 60.5 y*, BMI 30.9 kg/m^2*	Parallel, randomized, 6-mo duration, low-GI ($n = 106$) vs high-cereal fiber ($n = 104$)	Mean hsCRP[4]: 4.61 mg/L	• No between-group difference for change in hsCRP ($P = 0.8$)	Low-GI: −CRP

(Continued)

TABLE 2 (*Continued*)

First author, year, country	Participants' characteristics	Study design, dietary interventions	Outcome: baseline concentrations	Results[2]	Effect or no effect of intervention[3]
Kelly, 2011, USA (34)	28 previously sedentary participants with insulin resistance and obesity, 46% female, age 66 y*, BMI 34.2 kg/m^2*	RCT, 12-wk duration, low-GI (n = 13) vs high-GI (n = 15)	No data	• Reduction in IL-6 significantly larger in low-GI than reduction in high-GI diet group (P = 0.01)	Low-GI: +IL-6
Neuhouser, 2012, USA (35)	80 participants with overweight and obesity, 59% female, age 29.5 y*, BMI 27.5 kg/m^2*	Randomized, crossover, 28-d duration, low-GL vs high-GL	Mean hsCRP: 2.13 mg/L, mean IL-6: 1.79 pg/mL	• No treatment effect on hsCRP, trend for higher IL-6 after low-GL diet period (P = 0.09) • Treatment effect among those with high baseline body fat mass (n = 51): low-GL diet period [0.7 (0.5–0.8)], high-GL diet period [0.9 (0.7–1.1) mg/L; P = 0.02)]	Low-GL (+CRP subgroup)
Pereira, 2004, USA (21)	39 participants with overweight or obesity, 15% female, age 30.7 y*, BMI ≥27 kg/m^2	Parallel, randomized, 9–10-wk duration, low-GL (n = 22) vs low-fat (n = 17)	Mean hsCRP[5]: 0.24 mg/dL	• Relative reduction in hsCRP significantly larger in low-GL diet group than relative change in low-fat diet group (P = 0.03)	Low-GL: +CRP
Pittas, 2006, USA (36)	34 participants with overweight, % female: 75.5%, age 34.6 y*, BMI 27.5 kg/m^2*	RCT, 6-mo duration, low-GL (n = 16) vs high-GL (n = 1)	Mean hsCRP[4]: 2.7 mg/L	• No between-group difference for change in hsCRP (P = 0.13) • Mean hsCRP decreased from baseline in low-GL group ($P < 0.01$) but not in high-GL group	Low-GL: (+CRP)

(Continued)

TABLE 2 (*Continued*)

First author, year, country	Participants' characteristics	Study design, dietary interventions	Outcome: baseline concentrations	Results[2]	Effect or no effect of intervention[3]
Shikany, 2009, USA (37)	24 men with overweight and obesity, age 34.5 y*, BMI 27.8 kg/m^2*	Randomized, crossover, 4-wk duration, low-GI/GL vs high-GI/GL	Mean CRP[4]: 1.7 mg/L, mean IL-6[4]:2.9 ng/L	• Changes in CRP or IL-6 did not differ between the 2 diet periods (P = 0.7 and P = 0.6, respectively)	Low-GI/GL: –CRP, −IL-6
Vrolix, 2010, Netherlands (38)	15 participants with overweight, 40% female, age 52.5 y*, BMI 31 kg/m^2*	Randomized, double-blind, crossover, 11-wk duration, decreased GI vs increased GI	No data	• No treatment effect on hsCRP or IL-6 (P = 0.3 and P = 0.9, respectively).	Low-GI: –CRP, −IL-6
Wolever, 2008, Canada (39)	162 persons with T2D, 54% female, age 59.9 y*, BMI 30.9 kg/m^2*	Multicenter, RCT, 1-y duration, low-GI (n = 55) vs high-GI (n = 48) vs low-CHO (n = 53	Mean CRP[4]: 2.6 mg/L	• CRP change tended to differ between the diet groups (P = 0.064) (low-GI group: decrease; high-GI group: increase; low-CHO group: intermediate) • Proportion of participants with CRP-concentration ≥3 mg/L at 1 y significantly different between low-GI, high-GI, and low-CHO groups (33%, 80%, and 33%, respectively)	Low-GI: (+CRP)

[1] *See* "Supplemental data" in the online issue for details on participant characteristics, dietary interventions, primary endpoints, analysis, and results. *Average value (mean or median as provided in the original publication). CHO, carbohydrate; DiOGenes, Diet, Obesity and Genes; GI, glycemic index; GL, glycemic load; HGI, high glycemic index; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; LGI, low glycemic index; RCT, randomized controlled trial; T2D, type 2 diabetes mellitus.

[2] P values of between-group differences for changes in inflammatory markers are reported for RCTs; P values for treatment effects are reported for crossover trials.

[3] +: Effect of dietary intervention on hsCRP or IL-6; (+): trend for effect on hsCRP or IL-6; −: no effect of dietary intervention on hsCRP or IL-6.

[4] Weighted mean value calculated from baseline concentrations for treatment groups. *See* "Supplemental data" in the online issue for baseline concentrations in the treatment groups.

[5] Denmark, the Netherlands, the United Kingdom, Greece (Crete), Germany, Spain, Bulgaria, and the Czech Republic.

35 healthy, obese, or hypertensive participants, an hsCRP reduction tended to be more pronounced under the high-fiber-supplemented diet when compared with the high-fiber DASH diet (*P*-treatment effect = 0.09) (59). In none of the other included intervention studies did the fiber supplementation or the fiber-enriched study foods result in greater reductions of hsCRP or IL-6 when compared with changes under the control diets (60–63, 66–70) (Table 4, top). Strictly speaking, the intervention by Jenkins et al (20) also constitutes a fiber-intervention study. However, because fiber intake was higher in the low-GI control diet group than in the high-cereal-fiber diet group, this study was reviewed in the GI/GL section only.

Whole grain

Epidemiologic studies

Seven observational studies were identified reporting on the association between whole grain intake and hsCRP and/or IL-6 (23, 54–59) (Table 3, bottom). The overall number of participants in these studies was 11,295 (259–5496); they were aged 27–62 y and had a BMI of 24–31. Two of the studies included women only (23, 54). Definition and assessment of whole grain intake, mostly by means of an FFQ (n = 6 studies), differed notably across the studies (*see* Supplemental Table 3 under "Supplemental data" in the online issue).

Five studies observed a significant association between a higher whole-grain consumption and lower hsCRP or IL-6 concentrations (23, 54, 56–59), and one study reported only a trend for the relation between whole grain and hsCRP (56). In 2 further studies, whole-grain intake was not related to hsCRP or IL-6 concentrations (55). Of note, whole-grain intake appeared to be of specific relevance for inflammatory markers among persons with type 2 diabetes (23, 57) (Table 3, bottom).

Intervention studies

Seven studies were identified that examined the effect of whole-grain intake on hsCRP or IL-6 concentrations (71–77) (Table 4, bottom). The duration of these intervention studies ranged from 3 to 16 wk. Studies included a total of 742 participants (15–266) aged 46–60 y with a BMI range of 27 to 36. One study included only women (76). Two studies were designed as weight-loss trials (75, 76). Although weight loss did not differ between the groups, one study observed a greater loss in percentage body fat in the intervention group (whole-wheat group) (76). Definition and assessment of whole-grain intake was again notably different between the studies (*see* Supplemental Table 4 under "Supplemental data" in the online issue).

In one study (75) based on 50 obese individuals with the metabolic syndrome, who were instructed to either avoid whole grains or to consume all grains by means of whole grains, significantly larger reductions in hsCRP were observed with the whole-grain treatment. In a further study, significant improvements in hsCRP in response to a whole-grain diet when compared with changes under a control diet were confined to patients not using statins (73). In addition, in a trial among 72 overweight postmenopausal women, increases in IL-6 concentrations were more pronounced in the whole-wheat group than in the refined-wheat food group, whereas hsCRP concentrations were unaffected (76). The remainder of 4 studies, in which whole-grain foods were provided, did not observe a treatment effect on hsCRP or IL-6 concentrations (71, 72, 74, 77) (Table 4, bottom).

Qualitative comparison did not provide an indication that sex, age, or health status of the study population influenced the results of the 31 intervention studies identified in this systematic review.

DISCUSSION

The current systematic review identified 29 observational studies that addressed the relevance of dietary GI/GL, fiber, and whole grain to chronic low-grade inflammation as assessed by hsCRP or IL-6. The observational studies addressing dietary fiber or whole-grain intake almost unanimously suggest a benefit of a higher consumption on low-grade inflammation, whereas evidence is less consistent for a beneficial role of a lower GI or GL. However, considering the evidence from 31 intervention studies, a different picture emerges because most of the intervention studies do not report a benefit of increasing fiber or whole-grain intake for low-grade inflammation, whereas several intervention studies do support a potential role of dietary GI or GL.

The current review shows considerable heterogeneity among observational studies regarding associations between dietary GI/GL and markers of chronic inflammation. This is in line with the heterogeneity reported for observational studies linking GI/GL to chronic disease outcomes such as type 2 diabetes (78, 79) and CVD (80–83). A recent meta-analysis of the association between dietary GL and the development of type 2 diabetes showed that this heterogeneity was almost exclusively attributable to differences in the studies regarding sex, ethnicity, and the ability of the FFQ to correctly measure carbohydrate consumption (79). In the current review, 2 of the 4 studies reporting no associations had used an FFQ for which correlation coefficients with dietary records were <0.6 for total carbohydrate intake (24, 25) (*see* Supplemental Table 1 under "Supplemental data" in the online issue). The calculation of dietary GL and GI is based on data for total carbohydrate intake, which results in an insufficient estimation of dietary GI and GL. Additional methodologic limitations when estimating dietary GI and GL from FFQs include entry of low and high-GI foods into the same food grouping (eg, whole-kernel and whole-meal breads, respectively), assignment of GI values available for similar foods, and interresearcher variation in GI assignment (84). Of note, epidemiologic studies on dietary GI/GL are, however, not as amenable to residual confounding, because dietary GI/GL does not strongly correlate with healthy lifestyle behaviors given that most populations are still largely unaware of what constitutes a low dietary GI (85).

In line with evidence from observational studies, intervention studies provided some support for the relevance of the GI/GL concept on chronic inflammation. Associations were most evident in the largest (31) study, which used a low-GI diet rather than a low-GL diet, ie, diets that modified the quality of the consumed carbohydrates only. It is plausible that such a dietary approach would be most effective regarding the reduction of chronic inflammation because the avoidance of glycemic spikes is considered to be of primary relevance for oxidative stress, and this is well captured by the dietary GI in diets with at least a moderate to high carbohydrate content (86). In contrast, a lower carbohydrate intake may be associated with increased energy intake from protein and saturated fat, which might in turn be expected to increase

TABLE 3
Dietary fiber and whole grain intakes and hsCRP and IL-6: epidemiologic studies[1]

First author, year, country	Study population, characteristics, name of study	Follow-up	Exposure: assessment method	Outcome: average (baseline) concentration	Results	Association[2]
Dietary fiber intake: epidemiologic studies						
Ajani, 2004, USA (40)	3920 participants, 52% female, age 45.6 y*, BMI 28.6 kg/m^2*, NHANES (1999–2000)	Cross	24-h dietary recall	Mean CRP[3]: 4.3 mg/L	• Lower CRP concentrations among persons with higher dietary fiber intakes (P = 0.045) • Lower risk of CRP >3 mg/L among persons with higher dietary fiber intakes (P = 0.006)	Fibcross: ↓CRP
Bo, 2006, Italy (42)	1653 participants, 53% female, age 54.6 y*, BMI 26.5 kg/m^2*, representative sample of Italian province	Cross	Semiquantitative FFQ	Mean % of participants with hsCRP[3] ≥3 mg/L: 25%	• Lower risk of hsCRP ≥3 mg/L among persons with higher dietary fiber intakes (P = 0.003)	Fibcross: ↓CRP
Bo, 2008, Italy (41)	335 participants with metabolic syndrome, 58% female, age 55.7 y*, BMI 29.8 kg/m^2*, reanalysis of intervention study	1 y	Semiquantitive FFQ	Mean hsCRP[3]: 3.3 mg/L	• Increases in fiber intake associated with decreases in hsCRP concentrations (P = 0.03)	Fibprosp: ↓CRP
Chuang, 2011, Italy (43)	87 participants free of cancer, 39% female, age 54.0 y*, BMI 25.7 kg/m^2*, sampled from the EPIC-Italy cohort	Cross	Center-specific FFQ	Geometric mean IL-6[3]: 57.0 pg/mL	• Trend for inverse association between dietary fiber intake and IL-6 (P = 0.09)	Fibcross: (↓IL-6)
Diaz, 2005, USA (44)	1567 participants with overweight, 53% female, age 38.1 y*, BMI 30.6 kg/m^2*, NHANES (1999–2000)	Cross	24-h dietary recall	No data	• No association between dietary fiber intake and risk of hsCRP ≥0.3 mg/dL (NS)	Fibcross: ~
Estruch, 2009, Spain (45)	771 persons with diabetes or >3 CHD risk factors, 56% female, age 68.8 y*, BMI 29.9 kg/m^2*, reanalysis of intervention study	3 mo	FFQ	No data	• Increases in fiber intake associated with decreases in hsCRP concentrations (P = 0.004)	Fibprosp: ↓CRP

(Continued)

TABLE 3 (*Continued*)

First author, year, country	Study population, characteristics, name of study	Follow-up	Exposure: assessment method	Outcome: average (baseline) concentration	Results	Association[2]
Herder, 2009, Finland (46)	406 participants with overweight or obesity and IGT, 65.3% female, age 55.4 y*, BMI 31.2 kg/m^2*, reanalysis of the Finnish Diabetes Prevention Study	1 y	3-d food record	Median CRP[3]: 2.09 mg/L; median IL-6[4]: 1.76 pg/mL	• Changes in fiber (g/1000 kcal) correlated with changes in CRP concentrations ($P = 0.015$) • Changes in fiber (g) correlated with changes in IL-6 ($P = 0.008$)	Fibprosp ↓CRP, ↓IL-6
Kantor, 2013, USA (47)	9895 participants, mostly with overweight or obesity, 51.3% female, NHANES (1999–2000, 2001–2002, 2003–2004 cycles)	Cross	24-h dietary recalls	Geometric mean hsCRP[3]: 1.82 mg/L	• Lower ratio of the geometric mean hsCRP among those exposed to higher fiber intakes ($P < 0.0001$)	Fibcross: ↓CRP
King, 2003, USA (48)	4900 participants, 52.1% female, age ≥18 y, BMI 27.9 kg/m^2*, NHANES (1999–2000)	Cross	Recollection of food eaten the previous day	Median hsCRP: 2.0 mg/L	• Lower hsCRP concentrations among persons with higher dietary fiber intakes ($P < 0.05$) • Lower risk of hsCRP >3 mg/L among persons with higher dietary fiber intakes ($P < 0.05$)	Fibcross: ↓CRP
King, 2005, USA (49)	7891 persons with diabetes, hypertension or obesity, NHANES (1999–2002)	Cross	24-h dietary recall	No data	• Lower CRP concentrations among persons with higher dietary fiber intakes ($P < 0.05$) • Lower risk of CRP >3 mg/L among persons with higher dietary fiber intakes ($P < 0.05$)	Fibcross: ↓CRP

(Continued)

TABLE 3 (*Continued*)

First author, year, country	Study population, characteristics, name of study	Follow-up	Exposure: assessment method	Outcome: average (baseline) concentration	Results	Association[2]
Ma, 2006, USA (50)	524 participants, 49% female, age 48.3 y*, BMI 27.2 kg/m^2*	1 y	24-h dietary recalls (4×)	Geometric mean (±SD) CRP: 1.78 ± 1.66 mg/L	• Increases in fiber intake associated with decreases in CRP concentrations ($P = 0.03$) • Lower risk of CRP >3 mg/L among persons with higher dietary fiber intakes ($P = 0.01$)	Fibprosp: ↓CRP
Ma, 2008, USA (51)	1958 postmenopausal women, age 62.2 y*, BMI 28.8 kg/m^2*	Cross	FFQ	Geometric mean hsCRP: 2.01 mg/L; geometric mean IL-6: 1.90 pg/mL	• Increases in fiber intake associated with decreases in IL-6 concentrations ($P = 0.01$) • No association between dietary fiber intake and hsCRP ($P = 0.4$)	Fibcross: ↓IL-6, ∼CRP
Murakami, 2008, Japan (24)	443 female students, age 19.5 y*, BMI 21.3 kg/m^2*	Cross	Diet-history questionnaire	Mean hsCRP: 0.30 mg/L	• No association between dietary fiber intake and risk of hsCRP >1 mg/L) ($P > 0.3$)	Fibcross: ∼CRP
Oliveira, 2009, Portugal (52)	1060 participants, some with overweight, 64% female, age 54 y*, BMI <25 (n = 350) and ≥25 kg/m^2 (n = 710)	Cross	Semiquantitative FFQ	Median hsCRP: 1.9 mg/L (women), 1.4 mg/L (men)	• No association between dietary fiber intake and risk of increase in hsCRP (NS)	Fibcross: ∼CRP
Qi, 2006, USA (23)	1055 women with T2D, age 58.5 y*, BMI 29.8 kg/m^2*	Cross	Semiquantitative FFQ	No data	• Lower CRP concentrations among persons with higher cereal fiber intakes ($P = 0.03$) • No association between total fiber intake and hsCRP (NS)	Fibcross: ∼CRP; cereal fibcross: ↓CRP
Wannamethee, 2009, UK (53)	3428 men with no prevalent diabetes, age 60–79 y, British Regional Heart Study	Cross	Detailed 7-d recall, FFQ	Geometric mean hsCRP[4]: 1.70 mg/L	• Lower hsCRP and IL-6 concentrations among persons with higher dietary fiber intakes ($P < 0.0001$)	Fibcross: ↓CRP, ↓IL-6

(Continued)

TABLE 3 (*Continued*)

First author, year, country	Study population, characteristics, name of study	Follow-up	Exposure: assessment method	Outcome: average (baseline) concentration	Results	Association[2]
Whole-grain consumption: epidemiologic studies						
Gaskins, 2010, USA (54)	259 healthy women, age 27.3 y*, BMI 24.1 kg/m^2*	1 or 2 menstrualcycles	24-h dietary recall (≤4×/cycle)	Mean hsCRP: 0.6 mg/L	• Lower hsCRP concentrations among women with higher whole-grain consumption (P = 0.04)	WGprosp: ↓CRP
Jensen, 2006, USA (55)	938 healthy participants, 50.1% female, age (study 1: 60 y; study 2: 42.5 y)*, BMI (study 1: 25.6 kg/m^2; study 2: 24.4 kg/m^2)*, study 1: Health Professionals Follow-Up Study; study 2: Nurses' Health Study II	Cross	131-item FFQ	Mean CRP: 1.80 mg/L; mean IL-6: 1.53 pg/mL	• No association between whole grain intake and risk of increase in CRP or IL-6 (NS)	WGcross: ∼CRP, ∼IL-6
Lutsey, 2007, USA (56)	5496 healthy participants, 52.8% female, age 61.9 y*, BMI 27.9 kg/m^2*	Cross	Staff-assisted 127-item FFQ	Geometric mean hsCRP[3]: 3.26 mg/L	• Trend for inverse association between whole grain intake and hsCRP (P = 0.08) • No association between whole grain intake and IL-6 (P = 0.9)	WGcross: (↓CRP), ∼IL-6
Masters, unpublished data, USA (57)[4]	487 participants with T2D, 53.9% female, age 57.2 y*, BMI 31.4 kg/m^2*	Cross	FFQ	Median hsCRP[3]: 4.62 mg/L	• Lower hsCRP concentrations among persons with higher whole grain intake (P = 0.017) • Observed association independent of potential pathway nutrients (dietary fiber and magnesium intake)	↓CRP

(Continued)

TABLE 3 (*Continued*)

First author, year, country	Study population, characteristics, name of study	Follow-up	Exposure: assessment method	Outcome: average (baseline) concentration	Results	Association[2]
Masters, 2010, USA (58)	1015 healthy participants, 56.4% female, age 54.9 y*, BMI 28.4 kg/m^2*	Cross	114-item semiquantitive FFQ	Median hsCRP[3]: 1.72 mg/L	• Lower hsCRP concentrations among persons with higher whole grain intake (P = 0.03) • Inclusion of waist circumference, insulin sensitivity, 2-h glucose attenuated the results to nonsignificance • No association between refined grain intake and hsCRP (P = 0.6)	WGcross: ↓CRP
Montonen, 2012, Germany (59)	2198 participants, 60.9% female, age 50.4 y*, BMI 26.1 kg/m^2*, cohort from EPIC-Potsdam	Cross	148-item FFQ	Geometric mean hsCRP[3]: 0.80 mg/L	• Lower hsCRP concentrations among persons with higher whole-grain bread intake (P = 0.02) • Observed association independent of BMI and waist circumference	WGcross: ↓CRP
Qi, 2006, USA (23)	902 women with T2D, age 58.5 y*, BMI 29.7 kg/m^2*	Cross	Semiquantitative FFQ	Geometric mean hsCRP[3]: 5.75 mg/L	• Lower hsCRP concentrations among persons with higher whole-grain bread intake (P = 0.03)	WGcross: ↓CRP

[1] *See* "Supplemental data" in the online issue for details on population, assessment method, average baseline concentrations of exposures, and covariates considered in the analysis and results. *Average value (mean or median as provided in the original publication). Cross, cross-sectional; CHD, coronary heart disease; EPIC, European Prospective Investigation into Cancer and Nutrition; FFQ, food-frequency questionnaire; fib, dietary fiber intake; hsCRP, high-sensitivity C-reactive protein; IGT, impaired glucose tolerance; T2D, type 2 diabetes mellitus; WG, whole grain.

[2] ↑: Direct association with fiber or whole grains; (↑): trend for association with fiber or whole grains; ∼: no association with fiber or whole grains;↓: inverse association with fiber or whole grains.

[3] Estimated from values given per quantiles or averaged from the intervention and control groups (in post hoc analyses).

[4] Unpublished data, provided by authors.

TABLE 4
Fiber and whole grain and hsCRP and IL-6: intervention studies[1]

First author, year, country	Participants' characteristics	Study design, dietary interventions	Outcome: baseline concentration	Results[2]	Effect or no effect of intervention[3]
Fiber intake: intervention studies					
Biörklund, 2008, Sweden (60)	43 participants with mildly elevated serum cholesterol concentrations, 55.8% female, age 58 y*, BMI 25.0 kg/m^2*	Parallel, placebo-controlled, 5-wk duration, β-glucan (n = 22) vs placebo (n = 21)	Mean hsCRP[4]: 1.63 mg/L	• No between-group difference for change in hsCRP ($P > 0.05$)	β-Glucan: −CRP
Dall'Alba, 2013, Brazil (61)	44 participants with T2D and metabolic syndrome, 61.4% female, age 62 y*, BMI 29.8 kg/m^2*	RCT, 6-wk duration, guar gum (n = 23) vs control (n = 21)	Median hsCRP[4]: 2.37 mg/L	• No data on between-group difference for change in hsCRP (ie, no P value) • No significant change within each treatment arm ($P > 0.3$)	Guar gum: −CRP
Jenkins, 2002, Canada (62)	23 participants with T2D, 30% female, age 63 y*, BMI 26.7 kg/m^2*	Randomized crossover, 3-mo duration, wheat bran vs control	Mean hsCRP: 4.49 mg/L	• No between-group difference for change in hsCRP ($P = 0.4$)	Wheat bran: −CRP
Jensen, 2012, Denmark (63)	80 participants with obesity, 67.5% female, age 42.9 y*, BMI 34.2 kg/m^2*	Parallel, double-blind, placebo-controlled, 12-wk duration, alginate-fiber (n = 38) vs placebo (n = 42)	Mean hsCRP[4]: 4.0 mg/L	• No between-group difference for change in hsCRP ($P = 0.3$)	Alginate fiber: −CRP
Johansson-Persson, 2013, Sweden (64)	25 participants with mild hypercholesterolemia, 52% female, age 58.6 y*, BMI 26.6 kg/m^2*	Randomized, single-blind, crossover trial, 5-wk duration, high-fiber vs low-fiber	Mean CRP[4]: 1.7 mg/L; median IL-6[4]: 0.94 pg/mL	• Reduction in CRP significantly larger in high-fiber period than in low-fiber period (P = 0.017) • IL-6 not significantly different between diet periods ($P = 0.2$)	Dietary fiber: +CRP, −IL-6
King, 2007, USA (65)	35 participants (18 healthy and 17 with obesity and hypertension), 80% female, age 38.3 y*, BMI 28.4 kg/m^2*	Randomized, crossover, 3-wk duration, high-fiber DASH diet vs psyllium fiber	Mean hsCRP: 4.4 mg/L	• Trend for greater hsCRP reduction in fiber supplementation period ($P = 0.09$)	Psyllium fiber: (+CRP)
King, 2008, USA (66)	158 participants with overweight or obesity, 72.8% female, age 50.5 y*, BMI 33.4 kg/m^2*	RCT, 3-mo duration, high-fiber (psyllium) (n = 48) vs low-fiber (psyllium) (n = 53) vs control (n = 57)	Mean hsCRP[4]: 7.68 mg/L	• No difference in change between treatment groups and control group ($P > 0.05$)	Psyllium fiber −CRP

(Continued)

TABLE 4 (*Continued*)

First author, year, country	Participants' characteristics	Study design, dietary interventions	Outcome: baseline concentration	Results[2]	Effect or no effect of intervention[3]
Kohl, 2009, Germany (67)	12 participants with overweight or obesity and moderately increased concentrations of CRP (<5 mg/L), 67% female, age 49.7 y*, BMI 32.2 kg/m^2*	Randomized, double-blind, placebo-controlled crossover, 4-wk duration, β-glucan vs placebo	Mean CRP: 5.7 mg/L; no data for IL-6	• No treatment effect on CRP (P = 0.4) and IL-6 (P = 0.9)	β-Glucan, –CRP, −IL-6
Queenan, 2007, USA (68)	75 participants at risk of CVD (hypercholesterolemic), 67% female, age 44.9 y*, BMI <30 kg/m^2	Randomized, double-blind, parallel, 6-wk duration, oat β-glucan (n = 35) vs placebo (n = 40)	Mean CRP[4]: 0.37 mg/dL	• No between-group difference for change in CRP (P = 0.3)	Oat β-glucan: −CRP
Salas-Salvado, 2008, Spain (69)	166 participants with overweight or obesity, 78.3% female, age 47.9 y*, BMI 31.2 kg/m^2*	Parallel, double-blind, randomized, placebo-controlled, 16-wk duration, supplemented fiber twice a day (n = 53) vs supplemented fiber 3 times/d (n = 58) vs placebo (n = 55)	Mean hsCRP[4]: 0.77 mg/L	• No difference in change between treatment groups and control group (P = 0.5)	Fiber supplementation: –CRP
Wood, 2006, USA (70)	29 men with overweight, age 38.8 y*, BMI 29.7 kg/m^2*	Parallel, placebo-controlled, double-blind, 12-wk duration, soluble fiber (n = 14) vs placebo (n = 15)	Mean hsCRP[4]: 1.77 mg/L; mean IL-6[4]: 1.03 pg/mL	• No between-group difference for change in hsCRP and IL-6 ($P > 0.05$)	Soluble fiber supplementation: –CRP, −IL-6
Whole-grain consumption: intervention studies					
Andersson, 2007, Sweden (71)	30 healthy participants (women were postmenopausal), 73% female, age 59 y*, BMI 28.3 kg/m^2*	Randomized, nonblind, crossover, 6-wk duration, whole grain vs refined grain	Mean hsCRP[4]: 2.45 mg/L; mean IL-6[4]: 15.4 ng/L	• No treatment effect on hsCRP (P = 0.6) and IL-6 (P = 0.8)	WG: –CRP, −IL-6
Brownlee, 2010, UK (72)	266 participants with overweight or obesity, 49.8% female, age 45.7 y*, BMI 30.1 kg/m^2*	RCT, 16-wk duration, 60 g whole grain/d (n = 85) vs 60 g whole grain/d in first 8 wk, 120 g/d in last 8 wk (n = 81) vs control (n = 100)	Median CRP[4]: 2.6 mg/L	• No difference in change between intervention groups and control group ($P > 0.05$) ($P > 0.05$)	WG: −CRP

(Continued)

TABLE 4 (*Continued*)

First author, year, country	Participants' characteristics	Study design, dietary interventions	Outcome: baseline concentration	Results[2]	Effect or no effect of intervention[3]
de Mello, 2011, Finland (73)	103 participants with impaired glucose metabolism and features of the metabolic syndrome, 51% female, age 59 y*, BMI 31.1 kg/m^2*	Parallel design, RCT, 12-wk duration, healthy diet (n = 35) vs whole grain (n = 34) vs control (n = 34)	Median hsCRP[4]: 1.4 mg/L; median IL-6[4]: 1.4 ng/L	• No between-group difference for change in hsCRP (P = 0.3) and IL-6 (P = 0.7) • Significant improvements in hsCRP on whole-grain diet in comparison with control among patients not using statins ($P < 0.05$) (n = 76)	WG: − IL-6, (+CRP) subgroup
Giacco, 2010, Italy (74)	15 participants, some with overweight or obesity, 20% female, age 54.4 y*, BMI 27.4 kg/m^2*	Randomized, sequential, crossover, 3-wk duration, whole-meal wheat vs refined wheat	No data	• No treatment effect on hsCRP (P = 0.4)	Whole-meal wheat: −CRP
Katcher, 2008, USA (75)	50 participants with obesity and the metabolic syndrome, 50% female, age 46.6 y*, BMI 35.8 kg/m^2*	Randomized, open-label, parallel-arm, 12-wk duration, whole grain (n = 25) vs refined grain (n = 25)	Mean hsCRP: 6.0 mg/L; mean IL-6: 2.7 pg/mL	• Reduction in hsCRP significantly larger in whole-grain than in refined-grain group (P = 0.01) • No between-group difference of change in IL-6 (NS)	WG: +CRP, −IL-6
Kristensen, 2012, Denmark (76)	72 postmenopausal women with overweight or obesity, age 59.7 y*, BMI 30.2 kg/m^2*	Open-label, parallel, 12-wk duration, whole wheat (n = 38) vs refined wheat (n = 34)	Mean hsCRP[4]: 0.97 mg/L; mean IL-6[4]: 2.10 ng/L	• No between-group difference of change in hsCRP (P = 0.95) • Trend for greater IL-6 increase in whole-wheat group as compared with refined-wheat group (P = 0.09)	Whole wheat –CRP, (+IL-6 – adverse)
Tighe, 2010, Scotland (77)	206 healthy participants, some with signs of metabolic syndrome, 50% female, age 51.7 y*, BMI 27.7 kg/m^2*	Randomized, single-blind, controlled, 12-wk duration, whole wheat (n = 73) vs wheat + oats (n = 70) vs control (n = 63)	Median hsCRP[4]: 1.9 mg/L; median IL-6[4]: 1.2 pg/L	• No between-group difference of change in hsCRP (P = 0.5) and IL-6 (P = 0.3)	Whole wheat: –CRP, −IL-6

[1] *See* "Supplemental data" in the online issue for details on participant characteristics, dietary interventions, primary endpoints, analysis, and results. *Average value (mean or median as provided in the original publication). CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; RCT, randomized controlled trial; T2D, type 2 diabetes mellitus; WG, whole grain.

[2] P values of between-group differences for changes in inflammatory markers are reported for RCTs; P values for treatment effects are reported for crossover trials.

[3] +: Effect of dietary intervention on hsCRP or IL-6; (+): trend for effect on hsCRP or IL-6; −: no effect of dietary intervention on hsCRP or IL-6.

[4] Weighted mean value calculated from baseline concentrations for treatment groups. *See* "Supplemental data" in the online issue for baseline concentrations in the treatment groups.

inflammation (31). In line with this, the study by Ebbeling et al (29) observed the lowest hsCRP concentrations in the low-GI diet arm and higher hsCRP concentrations in the very low carbohydrate arm.

The current review identified a substantial discrepancy between observational and interventional studies with respect to the evidence from these studies linking fiber or whole grain intake to chronic low-grade inflammation. Similar discrepancies were previously reported (87) and appear to extend also to the preventive potential of whole-grain intake in the management of body weight (88). This may to some extent reflect the fact that relative differences in fiber or whole-grain intakes between the extreme quantiles in observational studies notably exceed relative differences commonly realized between treatment arms in intervention studies (*see* Supplemental Tables 3 and 4 under "Supplemental data" in the online issue). Alternatively, this discrepancy may arise from considerable confounding or from substantial methodologic limitations of the intervention studies.

It is well known that a higher fiber or whole-grain intake correlates notably with other aspects characterizing a healthier lifestyle (89), and it is very likely that observational studies only partly account for residual confounding because the assessed variables describing lifestyle may not reflect all relevant aspects (unmeasured confounding). Hence, observational studies may substantially overestimate the "true" beneficial effect of fiber or whole-grain intakes on low-grade inflammation.

Similarly, residual confounding may operate in observational studies linking higher fiber or whole-grain intakes to reduced risks of type 2 diabetes and CVD (90–94), because these associations are considered to be partly mediated by chronic low-grade inflammation (95). Hence, effective increases in whole grain or fiber intakes may not yield the health benefits currently expected based on observational evidence only. The fact that high-fiber diets were effectively used in landmark diabetes prevention studies (96, 97) does not contradict this concern, because the benefits observed in these studies stem from weight loss via integrated lifestyle modifications and cannot be directly attributed to the composition of the diet.

Concerning the intervention studies identified in this systematic review, note that most of them included $<$100 participants and were not designed to address chronic low-grade inflammation as a primary outcome (*see* Supplemental Tables 2–4 under "Supplemental data" in the online issue). Because most intervention studies may not have been sufficiently powered to detect smaller effects with statistical significance, our evidence appraisal also considered results suggestive of treatment effects (ie, trends) and/or effects in subgroups only. In addition, it could be argued that the included participants were too young or too healthy or that the time period covered by the intervention studies was too short for a beneficial effect on the inflammatory markers to establish. However, to address this concern we considered only studies lasting $\geq$3 wk, and qualitative comparisons did not suggest a link of effectiveness to age, health status of the participants, or study duration. Although weight-loss trials mostly observed comparable weight loss in both study arms, minor between-group differences in energy intake and hence minor differences in adiposity changes may nonetheless have confounded the findings. Moreover, some studies did not provide foods, but gave advice only. Generally, it is possible that the foods provided or selected were foods rich in dietary fiber or whole grain, yet had a high dietary GI because many whole-grain foods or foods rich in dietary fiber are characterized by a high dietary GI (eg, whole-meal breads or instant porridge oats). Beneficial components of high-fiber or whole-grain foods contributing to reduced chronic inflammation may have been counteracted by the higher postprandial glycemic excursions that these foods provoke. In this context, it is of interest that approaches directed at lowering the dietary GI appear to entail both a lower dietary GI and a higher dietary fiber intake, as evident in the study by Jenkins et al (20). Therefore, larger intervention studies using combinations of increases in whole-grain and fiber intakes and reductions in dietary GI/GL are needed to determine optimal dietary approaches to reduce chronic inflammation. The magnitude of differences observed in the studies reporting significant findings [with relative between-group differences of change ranging from 16% (31) to $\sim$40% (21, 64, 75)] supports the notion that such an approach can indeed yield clinically relevant findings.

Finally, CRP, although closely associated with the development of chronic diseases, may not be a causal risk factor, and the assays of both CRP and IL-6 are poorly standardized. Considerable variation for average concentrations of CRP and IL-6, which are most likely attributable to the type of assay, are shown in Tables 1–4. Whereas this should, in principle, not affect the effect sizes or estimates of intervention effects, it cannot be excluded that some assays may have been more precise than others [eg, interassay CVs ranged between 1.0% (42) and 10.8% (18) for CRP and reached up to 30.7% for IL-6 (43)], which could have led to an underestimation of associations in epidemiologic studies and of effects in intervention trials. We excluded studies that reported to be conducted in participants who had inflammatory diseases at baseline, but refrained from excluding studies on the basis of initial CRP concentrations alone because these may have depended more strongly on the type of assay than the participants' baseline status of acute or chronic inflammation. However, note that the diseases included in this review, such as diabetes or obesity, are also characterized by a proinflammatory component, precluding a strict exclusion of inflammatory diseases.

The strengths of this systematic review include its approach to consider the totality of evidence currently available from both observational and intervention studies for 3 major aspects of carbohydrate quality. Mechanisms discussed to link these carbohydrate quality measures to chronic inflammation show only some overlap, which justifies a separate consideration. Quantification of the observed associations would have been desirable; however, in our view, the data are too heterogeneous to justify a meta-analysis. In particular, the intervention studies differ notably in their design: the degree to which foods were provided, the use of supplements or dietary fiber, the definitions of whole-grain foods, and the broad variety of diets used as control diets. In view of this heterogeneity, the overall number of identified studies covering 3 aspects of carbohydrate quality and 2 study types is still considerably small. As larger intervention studies and prospective observational studies become available, future systematic reviews might be able to perform meta-analyses.

In conclusion, evidence from intervention studies for antiinflammatory benefits is less consistent for higher-fiber or whole-grain diets than for low-GI/GL diets. Antiinflammatory benefits of higher dietary fiber and whole grain intakes suggested by

observational studies are not supported by intervention studies, which indicates that confounding is likely and/or that the statistical power in intervention studies may have been too low to reveal small effects.

We are indebted to the participants and the authors of the original studies included in this review. We thank the following authors for answering our requests for additional information and for sometimes providing unpublished data: A Hanley (Masters et al 2009) M Golzarand, E Kantor, M Bullo, D Jacobs (Lutsey et al), E Rimm (Jensen et al 2006), and M Neuhouser.

The authors responsibilities were as follows—AEB and JCB-M: conceived the research project; JG: performed the literature search (including the updated search) and extracted the data from the literature; GJ and AF: performed independent searches; AF: performed the data extractions; AEB: resolved any disagreements in the search and extraction process; JG: contacted the authors to request additional information; CH: advised on queries related to the inflammatory markers; AEB and JG: wrote the manuscript; and AEB: had primary responsibility for the final content. All authors contributed to the interpretation of the results, critically revised the manuscript, and approved the final version of the article. JCB-M is the director of a not-for-profit GI-based food-endorsement program in Australia and manages the University of Sydney Glycaemic Index testing service. None of the other authors declared a conflict of interest.

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Detailed search strategy systematic review

1. "glycemic index" and CRP
2. "glycaemic index" and CRP
3. "glycemic load" and CRP
4. "glycaemic load" and CRP
5. "whole grain*" and CRP
6. fiber and CRP
7. fibre and CRP
8. "carbohydrate quality" and CRP
9. "glycemic index" and hs-CRP
10. "glycaemic index" and hs-CRP
11. "glycemic load" and hs-CRP
12. "glycaemic load" and hs-CRP
13. "whole grain*" and hs-CRP
14. fiber and hs-CRP
15. fibre and hs-CRP
16. "carbohydrate quality" and hs-CRP
17. "glycemic index" and "c-reactive protein"
18. "glycaemic index" and "c-reactive protein"
19. "glycemic load" and "c-reactive protein"
20. "glycaemic load" and "c-reactive protein"
21. "whole grain*" and "c-reactive protein"
22. fiber and "c-reactive protein"
23. fibre and "c-reactive protein"
24. "carbohydrate quality" and "c-reactive protein"
25. "glycemic index" and "high-sensitivity c-reactive protein"
26. "glycaemic index" and "high- sensitivity c-reactive protein"
27. "glycemic load" and "high-sensitivity c-reactive protein"
28. "glycaemic load" and "high- sensitivity c-reactive protein"
29. "whole grain*" and "high-sensitivity c-reactive protein"
30. fiber and "high-sensitivity c-reactive protein“
31. fibre and "high-sensitivity c-reactive protein"
32. "carbohydrate quality" and "high-sensitivity c-reactive protein"

33. "glycemic index" and IL-6
34. "glycaemic index" and IL-6
35. "glycemic load" and IL-6
36. "glycaemic load" and IL-6
37. "whole grain*" and IL-6
38. fiber and IL-6
39. fibre and IL-6
40. "carbohydrate quality" and IL-6
41. "glycemic index" and "interleukin 6"
42. "glycaemic index" and "interleukin 6"
43. "glycemic load" and "interleukin 6"
44. "glycaemic load" and "interleukin 6"
45. "whole grain*" and "interleukin 6"
46. fiber and "interleukin 6"
47. fibre and "interleukin 6"
48. "carbohydrate quality" and "interleukin 6"

We also worked with the NOT statement: e.g. for 2. "glycaemic index" and CRP not #1

Supplemental table 1: Dietary GI/GL and hsCRP/IL-6– epidemiological studies (detailed version)[1]

First author, Year, Country	Population, recruitment, Name of study	Follow-up	Exposure : assessment method, average baseline values	Outcome: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[2]
Bullo, 2013, Spain (25)	511 participants with high CVD risk PREDIMED trial % female: 56% Ø age: 67.2y Ø BMI: 29.2	1 year	FFQ, mean **GI** in GI quartiles: Q1: 61 Q2: 69 Q3: 74 Q4: 80 mean **GL** in GL quartiles: Q1: 110 Q2: 150 Q3: 190 Q4: 264 Correlation of carbohydrate intake from FFQ with food records: 0.56 (energy adjusted)	mean **IL-6**: 9.97 pg/ml	sex, age, changes in waist circumference, changes in BMI, intervention group, physical activity in leisure time, smoking, insulin use, presence of type 2 diabetes, n-3 fatty acid intake, fiber	<u>Adjusted cross-sectional estimates:</u> Baseline IL-6 (pg/ml) in **GI** quartiles (mean (95% CI)): Q1: 9.5 (8.2-10.9) Q2: 8.8 (7.7-10.1) Q3: 10.0 (8.7-11.5) Q4: 11.6 (10.1-13.4), $p_{for\ trend}$=0.05 Baseline IL-6 (pg/ml) in **GL** quartiles (mean (95% CI)): Q1: 9.6 (8.2-11.2) Q2: 9.2 (8.0-10.6) Q3: 9.6 (8.4-11.1) Q4: 11.4 (9.8-13.3), $p_{for\ trend}$=0.2 <u>Adjusted longitudinal estimates:</u> 1y changes in IL-6 (pg/ml) in **GI** quartiles (mean (95% CI)): Q1: 0 Q2: 1.5 (-1.8 - 4.7) Q3: -0.2 (-3.5 - 3.1) Q4: -1.1 (-4.4 - 2.1), $p_{for\ trend}$=0.3 1y changes in IL-6 (pg/ml) in **GL** quartiles: Q1: 0 Q2: -0.6 (-3.9 - 2.7) Q3: -1.8 (-5.1 - 1.5) Q4: 0.3 (-3.1 - 3.8), $p_{for\ trend}$=0.97	$GI/GL_{prosp.}$ ~ $GI_{cross.}$ ($\uparrow_{IL6}$)
Du 2008, Netherlands (26)	786 participants (30% T2D, 23% IGT) 321 from *CoDAM Study (*high risk for CVD population), 653 from *Hoorn Study (*general	cross	FFQ mean **GI**: 57 (SD: 4) mean **GL**: 130 (SD: 39) Correlation of carbohydrate intake from FFQ with food records: 0.75 (energy adjusted and de-attenuated)	median **hsCRP**: 2.0 (Q1-Q4: 1.2-3.6) mg/dl	age, sex, current smoking status, physical activity, cohort, total energy, alcohol, fiber, cholesterol, animal- and plant based protein, SFA. For GI additionally MUFA,	<u>Multiple regression of GI/GL with hsCRP (mg/dl):</u> hsCRP per 10-unit increase in **GI** ß=0.11 (SE:0.06); p=0.05 hsCRP per 50-unit increase in **GL** ß=0.08 (SE:0.05); p=0.09	$GI/GL_{cross.}$ ($\uparrow_{CRP}$)

First author, Year, Country	Population, recruitment, Name of study	Follow-up	Exposure : assessment method, average baseline values	Outcome: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[2]
	population) Persons with CRP > 10 mg/l excluded % female: 47% Ø age: 65y Ø BMI: 27.8				PUFA, polysaccharides, and mono-and disaccharides		
Griffith 2008, USA (17)	582 participants (64% overweight or obese) SEASON study % female: 48% Ø age: 48y Ø BMI: 27.4	1 year, quarterly assessments	24h-dietary recall, quarterly mean **GI**: 59[3] mean **GL**: 139[3]	mean **hsCRP**: 1.8 (min-max: 0.03- 9.6) mg/l	BMI, smoking status infection status	<u>Multiple regression of GI/GL with hsCRP (mg/l):</u> <u>Adjusted cross-sectional estimates:</u> hsCRP per **GI** unit: ß=0.002671 (SE=0.006269), p=0.7 hsCRP per **GL** unit: ß=-0.00096 (SE=0.000528), p=0.07 <u>Adjusted longitudinal estimates:</u> hsCRP changes associated with 3-mo change in GI: ß=-0.00396 (SE=0.002832), p=0.16 hsCRP changes associated with 3-mo change in GL; ß=-0.00012 (SE=0.000331), p=0.7 stratification by BMI category (18.5-24.9, 25.0-29.9, ≥30): cross-sectional association between GL and hsCRP among obese participants, p=0.04	$GI/GL_{prosp.} \sim GI_{cross.}$ ($\uparrow_{CRP}$)
Huffman 2007, USA (18)	171 sedentary participants with overweight to mild obesity and dyslipidemia 35 with hsCRP ≥10 mg/L excluded	cross	FFQ mean **GI** (SD): 53 (4)[3] mean **GL**: 109 (46)[3] Correlation of carbohydrate intake from FFQ with food records: 0.60-0.70 in age and sex-strata (energy adjusted)	geometric mean **hsCRP**: 2.2 (SD: 2.8) mg/l	HDL, energy intake	<u>Multiple regression of GI/GL with log-transformed hsCRP (mg/l):</u> hsCRP per **GI** unit: ß=0.003686, p=0.5 hsCRP per **GL** unit: ß=0.001610, p=0.18; stratification by sex showed similar	$GI/GL_{cross.} \sim$

First author, Year, Country	Population, recruitment, Name of study	Follow-up	Exposure : assessment method, average baseline values	Outcome: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[2]
	% female: 51% Ø age: 53y Ø BMI: 29.6					results	
Levitan 2008, USA (27)	18,137 postmenopausal women Women`s Health Study Ø age: 54.8y Ø BMI: 25.7	cross	FFQ, median **GI** in quintiles: Q1:49 Q2: 51 Q3: 53 Q4: 54 Q5: 57 median **GL** in quintiles: Q1: 92 Q2: 107 Q3: 117 Q4: 127 Q5: 143 Correlation of carbohydrate intake from FFQ with food records: 0.61 (energy adjusted)	geometric mean **hsCRP** 1.80 mg/l[4]	age, BMI, strenuous exercise, history of hypertension, postmenopausal hormone use, smoking status, intake of protein, saturated fat, trans fat, polyunsaturated fat,alcohol, cholesterol, fiber, magnesium, folate, total energy	<u>Adjusted geometric mean CRP (mg/l) in quintiles of **GI**:</u> Q1: 1.69 Q2: 1.83 Q3: 1.82 Q4: 1.79 Q5: 1.90; diff. Q5 - Q1: 1.12 (95% CI: 1.06-1.18), p-trend≤0.001 <u>Adjusted geometric mean CRP (mg/l) in quintiles of **GL**:</u> Q1: 1.78 Q2: 1.77 Q3: 1.80 Q4: 1.81 Q5: 1.86; diff. Q5 - Q1: 1.05 (95% CI: 0.97-1.14), p-trend =0.2	$GI_{cross.}$ ↑$_{CRP}$ $GL_{cross.}$ ~
Liu 2002, USA (19)	244 healthy women Women`s Health Study Ø age: 59y Ø BMI: 26	cross	FFQ, mean **GI** (SD): 53 (4)[3] mean **GL** (SD): 116 (22)[2] Correlation of carbohydrate intake from FFQ with food records: 0.61 (energy adjusted)	median **hsCRP**: 2.8 (IQR: 1.1–5.5) mg/l	age, treatment status, smoking status, BMI, physical activity, parental history of myocardial infarction, history of hypertension, diabetes, high cholesterol, hormone replacement therapy, alcohol intake, intakes of dietary fiber, folate protein, cholesterol and total energy	<u>Adjusted geometric mean hsCRP (95% CI) (mg/l) in **GI** quintiles:</u> Q1: 1.8 (1.3, 2.4) Q2: 2.5 (2.0; 3.2) Q3: 2.7 (2.0; 3.7) Q4: 2.5 (1.7; 3.3) Q5: 2.8 (2.0; 3.7); p-trend<0.01 <u>Adjusted geometric mean hsCRP (95% CI) (mg/l) in **GL** quintiles:</u> Q1: 1.4 (1.0; 2.0) Q2: 2.3 (1.8; 2.9) Q3: 2.9 (2.1; 4.1) Q4: 2.4 (1.7; 3.0) Q5: 3.8 (2.8; 5.2); p-trend<0.01	$GI/GL_{cross.}$ ↑$_{CRP}$
Murakami	443 healthy women	cross	Self-administered,	mean **hsCRP**:	residential block	<u>Adjusted ORs (95% CI) for</u>	GI/GL_{cross} ~

First author, Year, Country	Population, recruitment, Name of study	Follow-up	Exposure : assessment method, average baseline values	Outcome: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[2]
2008, Japan (24)	Ø age: 19.5y Ø BMI: 21.3		comprehensive diet history questionnaire, mean **GI**: 59[5] mean **GL**/4184 kJ: 80 (SD: 13); low GL: median: 71, high GL: median: 88 Correlation of carbohydrate intake from FFQ with food records: 0.48 (energy adjusted and de-attenuated)	0.30 (SD: 0.73) mg/l	(central or south Japan, size of residential area), current smoking, alcohol drinking, dietary supplement use, physical activity, BMI	<u>elevated hsCRP concentrations (>1mg/l) by low or high dietary GL</u> (median split): **low GL** (n=221): REF **high GL** (n=222): 1.16 (0.50-2.71), p>0.3	
Qi 2006, USA (23)	891 women with T2D Nurses` Health Study Ø age: 58.5y Ø BMI: 29.7	cross	FFQ, median **GI** in quintiles: Q1: 49 Q2: 51 Q3: 53 Q4: 54 Q5: 56 median **GL** in quintiles: Q1: 77 Q2: 89 Q3: 96 Q4: 104 Q5: 114 Correlation of carbohydrate intake from FFQ with food records: 0.61 (energy adjusted)	geometric mean **CRP**: 5.75 mg/l[4]	age, BMI, smoking, alcohol consumption, physical activity, aspirin use, HbA_{1C}, history of hypertension or hyper-cholesterolemia, postmenopausal hormone use, dietary fibers and magnesium	<u>Adjusted geometric mean CRP (mg/l) in quintiles of **GI**:</u> Q1: 5.05 Q2: 5.25 Q3: 6.55 Q4: 5.15 Q5: 6.68, p-trend=0.04 <u>Adjusted geometric mean CRP (mg/l) in quintiles of **GL**:</u> Q1: 5.02 Q2: 6.20 Q3: 5.57 Q4: 5.84 Q5: 6.15, p-trend=0.17	$GI_{cross.}$ ↑$_{CRP}$ $GL_{cross.}$ ~
Van Woudenbergh 2011, Netherlands (28)	4366 participants Rotterdam study % female: 60% Ø age: 67.3y Ø BMI: 26.2	cross	FFQ mean **GI** (SD): 59 (3) mean **GL**: 127 (22) Correlation of carbohydrate intake from FFQ with food records: 0.79	median **hsCRP**: 1.65 mg/l	age, sex,smoking, family history of diabetes, energy intake, protein, saturated fat, alcohol, fiber and BMI	<u>Multiple regression of GI/GL with log-transformed hsCRP (mg/l)</u> hsCRP per 10-unit increase in **GI** ß=0.005 (SEE: 0.04), p: 0.9 hsCRP per 50-unit increase in **GL** ß=0.11 (SEE: 0.04), p: 0.01	$GL_{cross.}$ ↑$_{CRP}$ $GI_{cross.}$ ~

First author, Year, Country	Population, recruitment, Name of study	Follow-up	Exposure : assessment method, average baseline values	Outcome: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[2]

[1] Abbreviations: cross, cross-sectional; CVD, cardiovascular disease; FFQ, food frequency questionaire; GI, glycemic index; GL, glycemic load; hsCRP, high-sensitivity C-reactive protein; prosp, prospective; Q, Quantile; T2D, type 2 diabetes mellitus

[2] ↑: direct association with GI or GL, (↑): trend for direct association with GI or GL, ~: no association with GI or GL,↓: inverse association with GI or GL

[3] original values base on white bread reference and were converted to glucose reference by multiplying by 1.4286 (22)

[4] estimated from values given per quantiles

[5] calculated on the basis of carbohydrate intake and dietary GL

Ø average value, mean or median as provided in the original publication.

Supplemental Table 2: Dietary GI/GL and hsCRP/IL-6 – intervention studies (detailed version)[1]

First author, Year, Country	Participants characteristics	Study design	Dietary Intervention	Outcome: baseline levels and primary endpoint	Results[2]	+/-[3]
Ebbeling 2012, USA (29)	21 participants with overweight and obesity % female: 38% Ø age: 30.3y Ø BMI: 34.4	randomized controlled crossover, 4 weeks duration	**low-GI**: mean GI: 33 (SD: 3), GL: 51 (6) **low-fat diet**: mean GI: 68 (3), GL: 185 (9) **very low CHO diet**: mean GI: 28 (9), GL: 4 (2) weight maintenance, foods provided	mean **hsCRP**: 1.75 (95% CI: 0.44-4.61) mg/l Primary endpoint: change in resting energy expenditure	<u>hsCRP (95% CI) (mg/l) after intervention:</u> **low-fat**: 0.78 (0.38-1.92) **low-GI**: 0.76 (0.50-2.20) **very low CHO diet**: 0.87 (0.57-2.69) p for difference between test diet periods: 0.13; p for linear trend from low fat to low GI to very low CHO diet: 0.05 Repeated-measures analysis adjusted for sex, age, order of diets, baseline weight, and mean weight during each period	Low GI/GL ($+_{CRP}$)
Fabricatore 2011, USA (30)	79 participants with T2D % female: 64.9% Ø age: 52.5y Ø BMI: 36.3	single-site parallel-group study with balanced randomization, 40 weeks duration	**low-GL (n=39)**: mean GI: 57, mean GL: 89 **low-fat (n=40)**: mean GI: 65, mean GL: 121 weight loss study, dietary advice based on "low-fat or low-GL pyramid", sample eating plans and recipes provided	mean **hsCRP**: low-GL: 8.0 (SE: 1.3) mg/l low-fat: 7.5 (1.8) mg/l Primary endpoint: weight change	<u>change in hsCRP (SE) (mg/l) after 40 weeks:</u> **low-GL**: -2.6 (2.3) **low-fat**: -3.3 (2.2) no between-group difference for change (p=0.8) no between-group difference for weight loss (p=0.3)	Low GL $-_{CRP}$
Gögebakan 2011, Netherlands, Denmark, UK, Greece, Spain, Germany, Bulgaria, Czech Republic (31)	932 healthy participants (some overweight) DiOGenes study % female: 65% Ø age: 41y Ø BMI: 34	RCT, 26 weeks duration	**low-GI/low-protein (n=150)** vs. **low-GI/high-protein (n=315)** vs. **high-GI/low-protein (n=155)** vs. **high-GI/high-protein diet (n=155)** vs. **control group (n=154)** target GI difference: 15 points weight maintenance, dietary instructions	mean **hsCRP** before weight loss: 2.78 (SD: 2.63) mg/l, p=0.8 for difference between groups Primary endpoint: weight change	<u>Mean (95%CI) change in hsCRP (mg/l) (ITT):</u> **low-GI groups**: -0.64 (-0.88 to –0.41) **high-GI groups**: -0.18 (0.41 to 0.05) **low-GI vs. high GI groups:** -0.46 (-0.79 to -0.13) p<0.001; Similar results in sensitivity and completion analyses. **low-GI** groups more likely to achieve an >15% CRP reduction than **high-GI** groups: OR: 1.57 (1.13-2.17), p=0.007 Analysis compares low-GI to high-GI diets combining information from all four study arms.	Low GI $+_{CRP}$
Hartman 2010, USA	64 men at risk for colorectal	randomized crossover,	**legume-rich/low-GI**: mean GI: 38 (SD: 2), GL: 84 (4)	mean **hsCRP**: 1.28 (SE: 1.24)	<u>Mean (SE) change in hsCRP (mg/l):</u> **legume diet:** -0.26 (0.13)	Low GI $-_{CRP}$

First author, Year, Country	Participants characteristics	Study design	Dietary Intervention	Outcome: baseline levels and primary endpoint	Results[2]	+/-[3]
(32)	cancer Ø age: 54y Ø BMI: 28.7	4 weeks duration (~2 weeks washout)	**isocaloric diet**: mean GI: 69 (3), GL: 152 (8), weight maintenance, foods provided	mg/l Primary endpoint: hsCRP, C-peptide power calculations for hsCRP	**control diet:** -0.23 (0.10) no between-group difference for change: -0.03 (0.17), p=0.9 Similar results for stratification by IR status or adjustment for age, BMI, baseline biomarker status,period, and treatment order	
Jebb 2010, UK (33)	522 participants at risk for metabolic syndrome % female: 58% Ø age 51.5y Ø BMI 28.5	5-center parallel-design RCT, 24 weeks duration	**high-MUFA/LGI (n=108)**: mean GI: 55, mean GL %en: 24 (48) **high-MUFA/HGI (n=107)**: mean GI: 63, mean GL %en: 28 (=55) (target GI difference: 11 points) **low-fat/LGI (n=119)**: mean GI: 56, mean GL: 29 %en (=50) **low-fat/HGI (n=109)**: mean GI: 64, mean GL: 33 %en (=61) (target GI difference: 13 points) **high-SFA/HGI (n=79)(control)**: weight maintenance, key sources of fat and CHO provided, additional dietary information	median **hsCRP**: high-MUFA/HGI: 0.54 (Q1, Q3: 0.20, 1.90) mg/l high-MUFA/LGI: 0.40 (0.14, 1.10) mg/l low-fat/HGI: 0.50 (0.10, 1.95) mg/l low-fat/LGI: 0.57 (0.16, 1.90) mg/l high-SFA/HGI: 0.70 (0.16, 2.30) mg/l Primary endpoint: change in insulin sensitivity	<u>% change (95% CI) in **hsCRP**:</u> **high-MUFA/HGI**: +3.8 (-21.4, 35.6) **high-MUFA/LGI**: +36.3 (3.0, 78.2) **low-fat/HGI**: +22.4 (-7.6, 60.3) **low-fat/LGI**: +8.0 (-13.5, 33.9), **high-SFA/HGI:** +21.3 (-5.8, 55.2) no between-group difference for change (p=0.9) Statistical test accounts for age, sex, center, ethnicity, baseline waist circumference, HDL cholesterol, and weight change	Low GI $-_{CRP}$
Jenkins 2008, Canada (20)	210 participants with T2D (treated with antihyperglycemic medications) % female 39% Ø age: 60.5y Ø BMI 30.9	parallel randomized, 6 months duration	**low-GI (n=106)**: mean GI: 48.7 (95% CI: 47.4, 50.0)[4], mean GL: 90.2 (84.3, 96.1)[4] **high-cereal fiber (n=104)**: mean GI (95% CI): 58.4 (57.7, 59.3)[4], mean GL: 116.2 (108.8, 123.5)[4] no weight loss study (participants who wished to lose weight were given advice on portion sizes and fat intake), checklists with food options provided	mean **hsCRP**: low-GI: 4.62 mg/l high-fiber: 4.59 mg/l Primary endpoint: change in HbA_{1c}	<u>Mean change (95% CI) in hsCRP (mg/l) until week 24 (ITT):</u> **low-GI**: -1.6 (-2.9, -0.3) **high-fiber**: -1.8 (-3.9, -0.4) no between-group difference for change (p= 0.8) change in body weight unrelated to hsCRP	Low GI $-_{CRP}$
Kelly 2011,	28 previously	RCT,	**low-GI (n=13)**: mean GI: 40.3	no data	<u>Change in IL-6 (ng/l)</u>	Low GI

First author, Year, Country	Participants characteristics	Study design	Dietary Intervention	Outcome: baseline levels and primary endpoint	Results[2]	+/-[3]
USA (34)	sedentary participants with insulin-resistance and obesity % female: 46% Ø age: 66y Ø BMI: 34.2	12 weeks duration	(SEM: 0.4), mean GL: 102 (9) **high-GI (n=15)**: mean GI: 80.2 (1.0), mean GL: 218 (24), diet and exercise intervention: 60 minutes aerobic exercise 5d/week in both groups weight maintenance, foods provided		reduction in IL-6 significantly larger in low-GI than reduction in high GI diet group p=0.01	$+_{CRP}$
Neuhouser 2012, USA (35)	80 participants with overweight and obesity % female: 59% Ø age 29.5y Ø BMI 27.5	randomized, crossover, 28 days duration (28 days washout)	**low-GL**: mean desired GL: 125 **high GL**: mean desired GL: 250, no data on GI weight maintenance, foods provided	mean **hsCRP**: 2.1 mg/l mean **IL-6**: 1.8 pg/ml Participants with hsCRP values >10 mg/l excluded prior to analysis Primary endpoint: biomarkers of inflammation and adiposity (among them: hsCRP and IL-6)	<u>Mean (95% CI) hsCRP (mg/l)</u>: **low-GL diet period**: 0.6 (0.5-0.7) **high-GL diet period**: 0.6 (0.6-0.7) p for treatment effect=0.9 <u>Mean (95% CI) IL-6 (ng/l)</u>: **low-GL diet period**: 1.3 (1.2-1.5) **high-GL diet period**: 1.2 (1.1-1.3) p for treatment effect=0.09 Treatment effect among those with high baseline body fat mass (n=51) (low-GL diet period: 0.7 (0.5-0.8), high-GL diet period: 0.9 (0.7-1.1) mg/l, p=0.02) Linear mixed models adjusted for baseline concentrations, diet sequence, feeding period, age, sex, and BMI	Low GL ($+_{CRP\ subgroup}$)
Pereira 2004, USA (21)	39 participants with overweight or obesity % female: 15% Ø age: 30.7y BMI: ≥ 27	parallel, randomized, time until 10% weight loss achievement (intervention group= mean 65 days, control	**low-GL (n=22)**: mean GI: 35.0[4], mean GL: 57.4[4] **low-fat (n=17)**: mean GI: 57.4[4], mean GL: 143.5[4] weight loss study, foods provided	mean **hsCRP:** low-GL: 0.28 (SD: 0.06) mg/dl low-fat: 0.19 (0.06) mg/dl Primary endpoint: resting energy expenditure	<u>Post-treatment mean (SE) hsCRP(mg/dl):</u> **low-GL**: 0.10 (0.03), %change (adjusted for baseline): -47.7 (11.9) **low-fat**: 0.13 (0.04), %change (adjusted for baseline): -5.1 (13.6) p between-group difference for change =0.03	Low GL $+_{CRP}$

First author, Year, Country	Participants characteristics	Study design	Dietary Intervention	Outcome: baseline levels and primary endpoint	Results[2]	+/-[3]
		group=69days)				
Pittas 2006, USA (36)	34 participants with overweight % female: 75.5% Ø age: 34.6y Ø BMI: 27.5	RCT, 6 months duration	**low-GL (n=16)**: mean GI: 53, mean GL: 88.5[5] **high-GL (n=16)**: mean GI: 86, mean GL: 228.1[5] weight loss study (30% caloric restriction), foods provided	mean **hsCRP**: low-GL: 3.1 (SEM: 0.7) mg/l high-GL: 2.2 (0.6) mg/l Primary endpoint: markers of glucose tolerance and hsCRP	Mean (SEM) hsCRP (mg/l) change at 6 months: **low-GL**: -1.44 (0.44) **high-GL**: 0.41 (0.91) no between-group difference for change p=0.13 mean hsCRP decreased from baseline by 35% in the low-GL group (p<0.01), and remained essentially unchanged in the high-GL group	Low GL ($+_{CRP}$)
Shikany 2009, USA (37)	24 men with overweight and obesity Ø age: 34.5y Ø BMI: 27.8	randomized, crossover, 4 weeks duration (4 weeks washout)	**low-GI/GL**: mean GI: 49.5 (SD: 3.3), mean GL: 158.3 (12.8), **high-GI/GL**: mean GI: 75.0 (4.2), mean GL: 245.5 (11.4), weight maintenance, foods provided	mean **CRP**: low-GI/GL: 1.3 (SD: 1.2) mg/l high-GI/GL: 2.1 (1.8) mg/l, mean **IL-6**: low-GI/GL: 2.5 (SD: 1.9) ng/l high-GI/GL: 3.2 (2.8) ng/l Primary endpoint: glucose levels	Mean (SD) CRP (mg/l) at 4 weeks: **low-GI/GL diet period:** 1.3 (1.2), change: 0.0 (0.7) **high-GI/GL diet period**: 1.7 (1.9), change: -0.4 (2.0) p for treatment differences: 0.7 Mean (SD) IL-6 (ng/l) at 4 weeks: **low-GI/GL diet period**: 2.3 (1.7), change: -0.2 (1.8) **high-GI/GL diet period**: 3.1 (3.4), change: -0.1 (3.5) p for treatment differences: 0.6	Low GI/GL $-_{CRP, IL6}$
Vrolix 2010, Netherlands (38)	15 participants with overweight %female: 40% Ø age: 52.5y Ø BMI: 31	randomized, double-blinded, crossover, 11 weeks duration (≥2 weeks washout)	**decreased GI**: mean GL: 36 (11) **increased GI**: mean GL: 68 (22) Data on GI not provided weight maintenance, replacement of regular foods (bread, fruit drink, cake, cookie for similar test foods with low GI	baseline hsCRP and IL-6 data not provided. Primary endpoint: HDL cholesterol	Mean (SD) hsCRP (mg/l) at 11 weeks: **decreased GI diet period**: 2.76 (1.85) **increased GI diet period**: 2.46 (1.71) Difference between diets: -0.30 (95%CI:-0.26, 0.86) mg/l, p=0.3 Mean (SD) **IL-6** (ng/ml)[6] at 11 weeks: **decreased GI diet period**: 1.41 (0.64) **increased GI diet period**: 1.42 (0.72) Difference between diet periods: 0.02 (-0.31, 0.72) ng/ml, p=0.9	Low GI $-_{CRP, IL6}$
Wolever 2008, Canada (39)	162 persons with T2D % female:	long-term multicenter, randomized, controlled,	**low-GI (n=55)**: GI: mean 55.1 (SEM: 0.4), GL: 133 (2) **high-GI (n=48)**: GI: 63.2 (0.4), GL: 135 (3)	mean **CRP**: **low-GI**: 2.64 (95% CI: 1.89, 3.72) mg/l, **high-GI**: 3.34 (2.56, 4.26)	Mean (95% CI) CRP (mg/l) after intervention: **low-GI**: 1.95 (1.68, 2.27); **high-GI**: 2.75 (2.33, 3.24); low CHO: 2.35 (2.01, 2.75)	Low GI ($+_{CRP}$)

First author, Year, Country	Participants characteristics	Study design	Dietary Intervention	Outcome: baseline levels and primary endpoint	Results[2]	+/-[3]
	54% Ø age: 59.9y Ø BMI: 30.9	1year duration	**low CHO (n=53)**: GI: 59.4 (0.4), GL: 120 (2) no weight-loss study (500kcal/d subtracted, if weight loss desired), key foods to choose were provided	mg/l low-CHO: 1.94 (1.48, 2.55) mg/l Primary endpoint: glycemic control (HbA_{1c})	CRP change tended to differ between the diet groups (p=0.064) (low-GI group: decrease, high-GI group: increase, low-CHO group: intermediate) Proportion of participants with CRP-level ≥3 mg/L at 1 year signficantly different between low-GI, high-GI and low-CHO group (33%, 80% and 33%, respectively). Linear mixed model regression analysis adjusted for BMI	

[1] Abbreviations: %en, percentage of total energy; CHO, carbohydrate; GI, glycemic index; GL, glycemic load; hsCRP, high-sensitivity C-reactive protein; IR, insulin resistance; ITT, intention to treat; RCT, randomized controlled trial; T2D, type 2 diabetes mellitus

[2] p-values of between-group differences for changes in inflammatory markers are reported for randomized controlled trials, p-values for treatment effects are reported for cross-over trials.

[3] +: effect of dietary intervention on hsCRP or IL-6, (+): trend for effect or effect in subgroup, -: no effect of dietary intervention on hsCRP or IL-6

[4] original values base on white bread reference and were converted to glucose reference by multiplying by 1.4286 (22)

[5] estimated from data on g/1000 kcal

[6] most likely pg/ml

Ø average value, mean or median as provided in the original publication.

Supplemental Table 3: Dietary fiber/whole grain intake and hsCRP/IL-6 – epidemiological studies (detailed version)[1]

First author, Year, Country	Population, recruitment, Name of study	Follow-up	Exposure : assessment method, average baseline values	Outcome: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[2]
Dietary fiber intake – epidemiological studies							
Ajani, 2004, USA (40)	3,920 participants NHANES (1999-2000) % female : 52% Ø age: 45.6y Ø BMI: 28.6	cross	24h dietary recall, mean fiber intake (g) in quintiles: Q1: 5 Q2: 10 Q3: 14 Q4: 18 Q5: 32	mean **CRP**: 4.3 mg/l[3]	Age, sex, race, education, smoking, physical activity, BMI, alcohol intake, fat intake, total energy	<u>Adjusted regression coefficients (SE) log-CRP (mg/l) by quintiles of fiber intake</u>: Q1: REF Q2: -0.19 (0.07) Q3: -0.19 (0.09) Q4: -0.12 (0.09) Q5: -0.31 (0.09); p-trend=0.045 <u>Adjusted OR (95% CI) for CRP >3 mg/l) Q5 vs.Q1:</u> 0.59 (0.41-0.85), p=0.006 exclusion of participants with cardiovascular conditions, diabetes or cancer did not alter the results	$Fib_{cross.}$ $\downarrow_{CRP}$
Bo, 2006, Italy (42)	1,653 participants representative sample of Italian province % female: 53% Ø age: 54.6y Ø BMI: 26.5	cross	Semi-quantitative FFQ (from the EPIC study) median fiber intake (g/d) in tertiles: T1: 13 T2: 19 T3: 28	mean % of participants with **hsCRP** ≥ 3 mg/l: 25%[2]	Age, sex, BMI, smoking, alcohol intake, physical activity, total calories, %en fat, magnesium intake	<u>Adjusted OR of hsCRP≥3 mg/l by tertiles of fiber intake:</u> T1: 1.83 (95% CI: 1.12-3.00) T2: 0.93 (0.63, 1.38) T3: REF p-trend=0.003 fiber association independent of magnesium intake; similar results in subgroup analyses (those with normal BMI and no metabolic abnormality)	$Fib_{cross.}$ $\downarrow_{CRP}$
Bo, 2008, Italy (41)	335 participants with metabolic syndrome Re-analysis of intervention study % female: 58%	1 year	Validated semi-quantitive FFQ, fiber intake (g/d): 19 (intervention group (n=169): 19 (SD 6), control group (n=166): 19 (8), p=0.8)	**hsCRP**: 3.3 mg/l (averaged from intervention and control group)	Age, sex and current BMI	<u>Adjusted longitudinal estimates</u> Log-transformed hsCRP changes (mg/l) associated with 1 year changes in fiber (g/day) ß: -0.01 (95%CI: -0.02 -0.002), p=0.03	$Fib_{prosp.}$ $\downarrow_{CRP}$

First author, Year, Country	Population, recruitment, Name of study	Follow-up	Exposure : assessment method, average baseline values	Outcome: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[2]
	Ø age: 55.7y Ø BMI: 29.8		lifestyle intervention (diet + exercise)				
Chuang, 2011, Italy (43)	87 participants free of cancer sampled within the EPIC-Italy cohort % female: 39% Ø age: 54.0y Ø BMI: 25.7	Cross	Centre-specific FFQ ranges of fiber intakes (g/d) in tertiles : T1: ≤17.52 T2: 17.52-24.03 T3: >24.03	Geometric mean **IL-6**: 57.0 pg/ml[3]	Age at recruitment, sex, centres (categorical), education, smoking status, alcohol drinking at baseline, BMI, physical activity, total energy intake	<u>Adjusted geometric mean levels of IL-6 (95% CI) (pg/ml) according to tertiles of total fiber intake:</u> T1: 91.8 (45.6, 184.9) T2: 45.2 (24.3, 83.1) T3: 34.1 (16.4, 71.5); p-trend=0.09 Additional analysis for cereal fiber intake: p=0.3	$Fib_{cross.}$ ($\downarrow_{IL6}$)
Diaz, 2005, USA (44)	1,567 participants with overweight NHANES (1999-2000) % female: 53% Ø age: 38.1y Ø BMI: 30.6	cross	24h dietary recall mean fiber intake (g): 14.53 (SD: 0.48)	no data	age, sex, BMI, smoking status, routine place of care, number of physician visits, exercise	<u>Adjusted OR (95% CI) for hsCRP ≥ 0.3 mg/dl by fiber strata:</u> <25 g/d fiber: REF ≥ 25 g/d fiber: 0.77 (0.44-1.35)	$Fib_{cross.}$ ~
Estruch, 2009, Spain (45)	771 persons with diabetes or more than 3 CHD risk factors Re-analysis of intervention study % female: 56% Ø age: 68.8y Ø BMI: 29.9	3 months	137 item FFQ mean fiber (g/d): 21.1 (SD: 7.6) Intervention: low fat vs. two Mediterranean-style diets	no data	age, sex, energy intake, intervention group, baseline	<u>3-months change in hsCRP (mg/l) by quintiles of change in dietary fiber intake:</u> Q1: -0.02 Q2: -0.03 Q3: -0.18 Q4: -0.21 Q5: -1.01, p-trend=0.04 change Q5 versus Q1: -1.08 (-1.80, -0.48), p for difference=0.004	$Fib_{prosp.}$ $\downarrow_{CRP}$
Herder, 2009, Finland (46)	406 participants with overweight or obesity and IGT Re-analysis of the Finnish Diabetes	1 year	3 day food record (pictures to estimate portion sizes) mean baseline fiber intake (g): intervention: 20.3 (SD: 7.3), control: 19.6 (SD: 7.9)	median **CRP**: 2.09 mg/l median **IL-6**: 1.76 pg/ml	age, sex, group, baseline BMI, change BMI	<u>Adjusted longitudinal estimates:</u> Correlation of changes in CRP and fiber intake changes: fiber (g): r= -0.08 p=0.11 fiber (g/1000 kcal): r=-0.122 p=0.015	$Fib_{prosp.}$ $\downarrow_{CRP, IL6}$

First author, Year, Country	Population, recruitment, Name of study	Follow-up	Exposure : assessment method, average baseline values	Outcome: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[2]
	Prevention Study % female: 65.3% Ø age: 55.4y Ø BMI: 31.2		Lifestyle intervention (including increase in fiber intake ≥15g/1000kcal)	values averaged from intervention and control group		Correlation of changes in IL-6 and fiber intake changes: fiber (g): r= -0.132, p=0.008 fiber (g/1000 kcal):r= -0.052, p=0.3	
Kantor, 2013, USA (47)	9,895 participants, mostly with overweight or obesity NHANES (1999-2000, 2001-2002, 2003-2004 cycles) % female: 51.3%	cross	24h dietary recalls (1 or 2 days included) fiber intake (g/d): <10: 27.1%, >10-20: 46.9%, >20-25: 12.1%, >25: 13.9%,	geometric mean hsCRP: 1.82 mg/l[3]	age, sex, race, education, smoking, BMI, physical activity, vitamin E supplement use, dietary fiber intake, dietary SFA, total energy intake, aspirin use, NSAID use, statin use, diabetes, CHD, regular use of glucosamine, chondroitin, fish oil	<u>Adjusted association of dietary fiber (g/d) with hsCRP ratio[4]</u> fiber <10: 1.00 (Ref), >10-20: 0.90 (0.83, 0.96), >20-25: 0.85 (0.76, 0.96) >25: 0.73 (0.66, 0.81), p<0.001	$Fib_{cross.}$ $\downarrow_{CRP}$
King, 2003, USA (48)	4,900 participants NHANES (1999-2000) % female: 52.1% age: ≥ 18 Ø BMI: 27.9	cross	recollection of food eaten the previous day by the respondent fiber intake in quartiles (g/d): Q1: < 8.4 Q2: 8.4-13.3 Q3: 13.3-19.5 Q4: >19.5	median **hsCRP** level 2.0 mg/l (64% of study population levels <3.0)	age, race, sex, BMI, smoking status, alcohol consumption, exercise, medications, total caloric intake	<u>Adjusted OR (95% CI) of elevated hsCRP (>3,0 mg/l) by fiber quartiles:</u> Q1: 1.0 (1.0) Q2: 0.75 (0.53-1.07) Q3: 0.64 (0.43-0.96) Q4: 0.58 (0.38-0.88), p<0.05 <u>Median (95%CI) hsCRP (mg/l) for fiber quartiles:</u> Q1: 2.3 (2.1-2.51) Q2: 2.04 (1.74-2.34) Q3: 1.89 (1.46-2.33) Q4: 1.76 (1.58-1.94), p<0.05	$Fib_{cross.}$ $\downarrow_{CRP}$
King, 2005, USA (49)	7,891 persons with diabetes, hypertension or obesity NHANES (1999-	cross	24h dietary recall fiber intake in quartiles (g/d): Q1: ≤8.8 Q2: 8.9-13.5	no data	age, race, sex, smoking status, alcohol consumption, exercise, medications, history of heart disease, total	<u>Median (SE) hsCRP (mg/l) level by quartiles of dietary fiber:</u> Q1: 2.39 (0.11) Q2: 2.23 (0.08) Q3: 2.05 (0.10)	$Fib_{cross.}$ $\downarrow_{CRP}$

First author, Year, Country	Population, recruitment, Name of study	Follow-up	Exposure : assessment method, average baseline values	Outcome: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[2]
	2002)		Q3: 13.6-19.9 Q4:≥ 20		serum cholesterol, total caloric intake	Q4: 1.52 (0.07), p<0.05 <u>OR (95%CI) of elevated hsCRP (>3,0 mg/l) by quartiles of fiber:</u> Q1:1.53 (1.29-1.8) Q2:1.53(1.31-1.8) Q3:1.41(1.17-1.69) Q4: Ref, p<0.05	
Ma, 2006, USA (50)	524 participants % female: 49 Ø age: 48.3y Ø BMI: 27.2	1 year	24h-dietary recalls (telephone-based) mean fiber intake (g/d): 16.1 (SD: 5.89) data collected quarterly	geometric mean **CRP**: 1.78 (SD: 1.66) mg/l	BMI, smoking status, age, current infection status, season of year at CRP measurement	<u>Adjusted cross-sectional estimates</u> Multiple regression of fiber with log CRP (mg/l): ß: -0.01 (SE 0.006), p=0.03 OR (95% CI) for elevated CRP values (>3.0 mg/l) Q1: REF Q2: 1.13 (0.58, 2.19) Q3: 0.75 (0.37, 1.52) Q4: 0.37 (0.16, 0.87), p-trend=0.01 <u>Adjusted longitudinal estimates</u> CRP changes associated with 3-mo change in dietary fiber ß:-0.008 (SE 0.004), p=0.03	$Fib_{prosp..}$ $\downarrow_{CRP}$
Ma, 2008, USA (51)	1,958 postmenopausal women Ø age 62.2y Ø BMI: 28.8y	cross	FFQ mean fiber intake (g/d): 15 (SD: 7)	geometric mean **hsCRP**: 2.01 (95% CI: 1.91-2.10) mg/l geometric mean **IL-6**: 1.90 (1.84-1.97) pg/ml	BMI, age, race, recreational physical activity, arthritis, smoking status, hormones therapy use in previous 3 mo, alcohol intake, and energy intake	<u>Geometric mean (95% CI) hsCRP (mg/l):</u> Q1: 2.31 (2.08-2.56) Q2: 1.85 (1.69-2.03) Q3: 1.87 (1.71-2.05) Q4: 2.04 (1.86-2.24) Q5: 1.98 (1.79-2.21), p-trend=0.4 <u>Geometric mean IL-6 (pg/ml):</u> Q1: 2.16 (1.99-2.35) Q2: 1.87 (1.74-2.02) Q3: 2.01 (1.87-2.16) Q4: 1.82 (1.69-1.96) Q5: 1.68 (1.55-1.83), p-trend=0.01	$Fib_{cross.}$ $\downarrow_{IL6,}$ $\sim_{CRP}$

First author, Year, Country	Population, recruitment, Name of study	Follow-up	Exposure : assessment method, average baseline values	Outcome: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[2]
Murakami, 2008, Japan (24)	443 female students Ø age: 19.5y Ø BMI: 21.3	cross	self-administered comprehensive diet history questionnaire dietary fiber intake: 7.1g/1000kcal (SD: 2.1)	mean **hsCRP**: 0.30 (SD: 0.73) mg/l	residential block (central or south Japan, size of residential area), current smoking, alcohol drinking, diaetary supplement use, physical activity, BMI	OR (95%CI) for elevated hsCRP concentrations (>1mg/l) by low or high dietary fiber (median split): low fiber: REF high fiber: 0.93 (0.41-2.12) p>0.3	$Fib_{cross.}$ ~$_{CRP}$
Oliveira, 2009, Portugal (52)	1060 participants, some with overweight % female: 64% Ø age: 54y BMI: <25: n=350; ≥25: n=710	cross	validated 82-item semi-quantitative FFQ intake of fiber (g/d) women: 21.4 men: 23.0 (averaged from values given for hsCRP categories)	median **hsCRP**: women. 1.9 (25^{th} –75^{th} P: 0.9-3.8) mg/l, men: 1.4 (0.7-3.0) mg/l	age, education, current smoking, regular exercise, total energy intake	OR (95% CI) for increase in hsCRP levels by one category (<1.00; 1.00-3.00; >3.00 to <10) associated with each 10 g increase in dietary fiber *Women:* BMI <25: 1.07 (0.71-1.61), ns BMI >25: 0.93 (0.71-1.23) , ns *Men:* BMI <25: 0.85 (0.50-1.44), ns BMI >25: 0.53 (0.37-0.76),n s	$Fib_{cross.}$ ~$_{CRP}$
Qi, 2006, USA (23)	1055 women with T2D Ø age: 58.5y Ø BMI: 29.8	cross	semi-quantitative FFQ no data	no data	age, BMI, smoking, alcohol consumption, physical activity, aspirin use, A1C, history of hypertension or hypercholesterolemia, postmenopausal hormone use, dietary fibers, magnesium	Increasing cereal fiber intake sig. associated with decreased hsCRP (p-trend=0.03) Intakes of total fiber, and fiber from other foods including fruits and vegetables were not associated with CRP (data not shown)	$Fib_{cross.}$ ~$_{CRP}$ Cereal fib_{cross} ↓$_{CRP}$
Wannamethee, 2009, UK (53)	3428 men with no prevalent diabetes British Regional Heart Study age: 60-79y	cross	detailed 7-day recall FFQ mean fiber intake (g/d): 25.9 (SD: 8.6); Q1: <20 Q2: 20.1-24.9 Q3: 25.0-30.9 Q4: >31.0	geometric mean **hsCRP**: 1.70 mg/l[3]	age, waist circumference, cigarette smoking, physical activity, social class, alcohol intake, preexisting myocardial infarction, stroke, use of statins, and total calorie intake	Bivariate associations of dietary fiber with hsCRP (mg/l) (p<0.0001) and IL-6 (pg/ml) (p<0.0001) persisted after adjustments (data not shown).	$Fib_{cross.}$ ↓$_{CRP, IL6}$

First author, Year, Country	Population, recruitment, Name of study	Follow-up	Exposure : assessment method, average baseline values	Outcome: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[2]
Whole grain consumption – epidemiological studies							
Gaskins 2010, USA (65)	259 healthy women Ø age 27.3y Ø BMI 24.1	1 or 2 menstruation cycles	24h dietary recall (≤4x/cycle) whole grain intake servings/d (1 serving=16g of a 100% whole grain food): median 0.5 (0.1, 1.3), 13.6 (6.0) g fiber/d Whole grain: bread, cereals, rice, pasta	mean **hsCRP:** 0.6 (SD: 1.2) mg/l	energy intake, age, race, BMI, illness during 7 d prior to visit, NSAID use the day before blood withdrawal	Predicted mean hsCRP (mg/l), % change from REF nonconsumers (n=123): 0.65, Ref. consumers <1 serving/d: (n=218): 0.58, - 10.5%, p=0.04 consumers ≥1 serving/d: (n=168): 0.57, -12.1%?, p=0.04 OR (95% CI) of increasing hsCRP levels from cycle 1 to cycle 2 by whole grain intake levels: OR for increase from low (<1 mg/L) to elevated (>3mg/L) hsCRP level 0 servings/d: REF 0.01-0.99 servings/d: 0.49 (0.20, 1.21) ≥1 servings/d: 0.11 (0.03, 0.41) OR for increase from moderate (≥1-≤ 3mg/L) to elevated (>3 mg/L) CRP level: 0 servings/d: REF 0.01-0.99 servings/d: 0.86 (0.36, 2.06) ≥1 servings/d: 0.27 (0.08, 0.96)	$WG_{prosp.}$ $\sim_{CRP}$ ↓
Jensen 2006, USA (66)	938 healthy participants: Study 1: Health Professionals Follow-Up Study, n=468; Study 2: Nurses Health Study II, n=470 % female: 50.1% Ø age: study 1: 60y,	cross	131-item FFQ, median whole grain intake : 22.3 g/d, women: 21.9, men: 23.4; median in quintiles: Q1: 8.2 Q2: 15.9 Q3: 22.3 Q4: 29.6 Q5: 43.8	mean **CRP:** 1.80 (SE: 0.12) mg/l mean **IL-6**: 1.53 (SE: 0.07) pg/ml	age, sex, total energy intake, alcohol intake, smoking, BMI, physical activity, hypercholesterolemia, fruit, vegetable, SFA, MUFA, PUFA intake	Geometric mean (SE) CRP (mg/l): Q1: 0.92 (1.107) Q2: 0.86 (1.07) Q3: 0.93 (1.07) Q4: 0.82 (1.07) Q5: 0.88 (1.07), p-trend=0.6 %diff Q1 vs Q5: -4.3% Geometric mean (SE) IL-6 (pg/ml): Q1: 1.20 (1.04) Q2: 1.07 (1.04)	$WG_{cross.}$ $\sim_{CRP, IL6}$

First author, Year, Country	Population, recruitment, Name of study	Follow-up	Exposure : assessment method, average baseline values	Outcome: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[2]
	study 2: 42.5y Ø BMI: study 1: 25.6 study 2: 24.4		Whole grain: whole wheat, whole-wheat flour, whole oats, whole-oat flour, whole cornmeal, corn flour, brown rice, brown rice flour, whole barley, whole rye, rye flour, bulgur, buckwheat, pop-corn, amaranth, and psyllium			Q3: 1.13 (1.05) Q4: 1.18 (1.05) Q5: 1.13 (1.05), p-trend=0.8 %diff Q1 vs Q5: -5.8%	
Lutsey 2007, USA (67)	5,496 healthy participants % female: 52.8% Ø age: 61.9y Ø BMI: 27.9	cross	staff-assisted 127 item FFQ, mean whole grain intake: 0.54 servings/d, median in quintiles: Q1: 0.02 Q2: 0.15 Q3: 0.39 Q4: 0.72 Q5: 1.39 Whole grain: cold whole grain cereals, oatmeal, dark bread, bran muffins, brown or wild rice	geometric mean **hsCRP**: 3.26 mg/l[3]	age, sex, race, education, survey centre, energy intake, current smoking, current alcohol use, dietary intake of fruit, vegetables, refined grains, dairy, fish and poultry, meat, leisure physical activity, sedentariness score, BMI, insulin	Geometric mean hsCRP (mg/l): Q1: 3.43 Q2: 3.23 Q3: 3.20 Q4: 3.24 Q5: 3.17, p-trend=0.08 Mean IL-6 (pg/ml): Q1: 1.54 Q2: 1.47 Q3: 1.45 Q4: 1.51 Q5: 1.51, p-trend=0.9	$WG_{cross.}$ $\sim_{CRP}$ ($\downarrow_{IL6}$)
Masters 2009**,** unpublished data, USA[3] (68)	487 participants with T2D % female: 53.9% Ø age: 57.2y Ø BMI: 31.4	cross	FFQ, median whole grain intake in tertiles (servings/day): T1: 0.15 (IQR: 0-0.43) T2: 0.72 (0.43-1.04) T3: 1.50 (1.04-4.15) whole grain: bread and cereals	median **hsCRP** 4.62 mg/l[3]	age, sex, ethnicity, total caloric intake, smoking status, total estimated energy expenditure, alcohol consumption category, oral hyperglycemic medication use and lipid-lowering medication use, vegetable intake, fruit intake, % energy from fat intake	Multiple regression of whole grain intake with hsCRP: ß: -0.151, SE: 0.063 p: 0.017 The significant association remained after inclusion of potential pathway nutrients (dietary fiber and magnesium intake) in the model.	$WG_{cross.}$ $\downarrow_{CRP}$
Masters 2010, USA (69)	1,015 healthy participants % female: 56.4%	cross	114 items semi-quantitive FFQ, median whole grain intake: 0.81 (SD: 0.73) servings/d;	median **hsCRP** 1.72 mg/l[3]	age, sex, ethnicity, total energy intake, smoking status, total energy expenditure, alcohol	multiple regression analysis for whole grain intakes with hsCRP mg/l (log) (n=932): ß: -0.102 (SEM: 0.048), p=0.03,	$WG_{cross.}$ $\downarrow_{CRP}$

First author, Year, Country	Population, recruitment, Name of study	Follow-up	Exposure : assessment method, average baseline values	Outcome: average (baseline) level	Covariates considered in analysis	Results	↑/~/↓[2]
	Ø age: 54.9y Ø BMI: 28.4		in quintiles: Q1: 0.04 Q2: 0.32 Q3: 0.65 Q4: 1.04 Q5: 2.00 whole grain: bread and cereals		consumption, refined grain, vegetable intake, fruit intake, percent energy from oleic acid, PUFA, SFA intake	After inclusion of waist circumference, insulin sensitivity, 2h glucose the results were no longer significant. No significant association between refined grain intake and hsCRP levels (p=0.6).	
Monto-nen 2012, Germany (70)	2,198 participants cohort from EPIC-Potsdam %female: 60.9% Ø age: 50.4y Ø BMI: 26.1	cross	148 item FFQ; median whole grain bread servings (50g)/d: Q1: 0.02 servings Q3: 0.52 servings Q5: 2.68 servings Whole grain: bread	geometric mean **hsCRP**: 0.80 mg/l[3]	age, sex, education, sport activity, occupational activity, smoking, alcohol intake, dietary variables (energy, red meat intake, coffee, and food items aggregating into the same pattern as whole gran bread (e.g. pasta, rice, pizza)	Geometric mean (95% CI) hsCRP (mg/l) by quintiles of whole grain bread intake: Q1: 0.77 (0.65-0.92) Q2: 0.86 (0.71-1.03) Q3: 0.91 (0.76-1.09) Q4: 0.79 (0.66-0.95) Q5: 0.68 (0.56-0.82), p trend=0.02 further adjustment for BMI and waist circumference did not change the results	$WG_{cross.}$ $\downarrow_{CRP}$
Qi 2006, USA (23)	902 women with T2D Ø age 58.5y Ø BMI: 29.7	cross	semi quantitative FFQ, median whole grain intake in quintiles Q1: 4.75 Q2: 9.82 Q3: 15.3 Q4: 22.8 Q5: 35.4 whole grain: whole wheat, whole-wheat flour, whole oats, whole-oat flour, whole cornmeal, corn flour, brown rice, brown rice flour, whole barley, whole rye, rye flour, bulgur, buckwheat, pop-corn, amaranth, and psyllium	geometric mean **hsCRP**: 5.75 mg/l[3]	age, BMI, smoking, alcohol consumption, physical activity, aspirin use, A1C, history of hypertension or hypercholesterolemia, postmenopausal hormon use, GI and magnesium	Geometric mean hsCRP(mg/l) according to quintiles of whole-grain: Q1: 6.60 Q2: 5.28 Q3: 5.76 Q4: 5.59 Q5: 5.52, p-trend= 0.03	$WG_{cross.}$ $\downarrow_{CRP}$

[1] Abbreviations: %en, percentage of total energy; cross, cross-sectional; CHD, coronary heart disease; fib, dietary fiber intake; FFQ, food frequency questionaire; hsCRP, high-sensitivity C-reactive protein; prosp, prospective; Q, Quantile; RC, regression coefficient; Ref, Reference; T2D, type 2 diabetes mellitus; WG, whole grain

[2] ↑: direct association with dietary fiber or whole grains, ~: no association with dietary fiber or whole grains, ↓: inverse association with dietary fiber or whole grains, (↓): trend for inverse association with dietary fiber or whole grains

[3] estimated from values given per quantiles

[4] CRP ratio=ratio of the geometric mean hsCRP among those exposed to those unexposed

Ø average value, mean or median as provided in the original publication.

Supplemental Table 4: Dietary fiber, fiber supplements and whole grain and hsCRP/IL-6 – intervention studies (detailed version)[1]

First author, Year, Country	Participants characteristics	Study design	Dietary Intervention	Outcome: baseline levels and primary endpoint	Results[2]	+/-[3]
Dietary fiber and fiber supplements – intervention studies						
Biörk-lund 2008, Sweden (54)	43 participants with mildly elevated serum cholesterol levels % female: 55.8% Ø age: 58y Ø BMI: 25.0	parallel, placebo-controlled, 5 weeks duration (3 wks run-in)	**ß-glucan enriched (n=22):** mean fiber (g): 18.7 (SD: 5.7) **control (n=21):** mean fiber (g): 17.4 (5.9) weight maintenance habitual diet plus study soup	mean **hsCRP**: ß-glucan: 1.31 (SD: 0.97) mg/l control: 1.97 (1.83) mg/l primary endpoint: LDL-cholesterol	Relative difference (%) in hsCRP (week 7-8 minus week 2-3): **ß-glucan**: -19.1 **control**: -30.8, no between group-difference for change p>0.05	ß-glucan $-_{CRP}$
Dall` Alba 2013, Brazil (55)	44 participants with T2D and metabolic syndrome % female: 61.4% Ø age: 62y Ø BMI: 29.8	randomized controlled trial, 6 weeks duration (2 weeks run-in)	**intervention**: mean fiber (g): 24.3 (SD: 5.4) **control**: mean fiber (g): 15.7 (6.3) weight maintenance, usual diet plus supplements in the intervention group (5g partially hydrolysed guar gum twice a day), white bread and soy bean oil supplied to avoid differences in carbohydrate and fat contents	median **hsCRP**: intervention: 2.8 (IQR: 1.1, 5.3) mg/l control: 1.9 (0.8, 5.2) mg/l primary outcome: metabolic syndrome components	Median (IQR) hsCRP (mg/l) after intervention: **intervention**: 2.1 (0.8, 7.9), p for difference to baseline=0.3 **control**: 1.5 (0.7, 4.0), p for difference to baseline=0.9 no data on between-group difference for change	Guar gum $-_{CRP}$
Jenkins 2002, Canada (56)	23 participants with T2D % female: 30% Ø age: 63y Ø BMI: 26.7	randomized crossover, 3 months (2 months wash-out)	**wheat bran:** mean fiber (g): 37.1 (SEM: 2.0), 21.3 g/1000kcal (0.8) **control:** mean fiber 21.0 (1.5), 11.7 g/1000kcal (0.7) weight maintenance habitual diet, conformed to the National Cholesterol Education Program, bread and cereals provided	mean **hsCRP**: wheat bran: 4.61 (SEM: 1.93) mg/l, control: 4.37 (1.91) mg/l endpoints: lipid and non-lipid CVD risk factors	Mean (SEM) hsCRP (mg/l) after intervention: **wheat bran**: 3.79 (1.56) **control**: 4.80 (2.01) no between-group difference for change in hsCRP -3.80 (-7.9%), p=0.4 Analysis adjusts for sex, sequence and baseline values	Wheat bran $-_{CRP}$
Jensen 2012, Denmark (57)	80 participants with obesity % female: 67.5% Ø age 42.9y	parallel, double blind, placebo-controlled, 12 weeks duration	**alginate-fiber** (n=38): 15g fiber supplement **placebo** (n=42): 0g fiber weight-loss study (-0.5 kg/ week)	mean **hsCRP**: alginate fiber: 2.7 (SEE: 0.4) mg/l control: 5.2 (1.0) mg/l	Mean (SEE) hsCRP difference (mg/l) after intervention: **alginate fiber** :-1.0 (0.5) **control**: -0.4 (0.5),	Alginate fib $-_{CRP}$

First author, Year, Country	Participants characteristics	Study design	Dietary Intervention	Outcome: baseline levels and primary endpoint	Results[2]	+/-[3]
	Ø BMI: 34.2		free choice of food items plus supplements	primary endpoint: weight change	no between-group difference for change in hsCRP (p= 0.3) Analysis adjusts for baseline values of lean body mass, body weight, and sex	
Johansson-Persson 2013, Sweden (58)	25 participants with mild hypercholesterolemia % female: 52% Ø age: 58.6y Ø BMI: 26.6 for CRP analyses: subjects with levels >10mg/l excluded	randomized, single-blinded, crossover trial, 5 weeks duration (3 weeks washout)	**high-fiber diet period**: mean fiber (g): 48.0 (SD: 6.8) **low-fiber diet period**: mean fiber: 30.2 (8.0) weight maintenance, usual diet plus study foods: one bread roll, one ready meal, two beverages, all with or without added fiber (rye bran, oat bran, sugar beet fiber)	mean **CRP**: high-fiber (n=24): 1.9 (SEM: 0.3) mg/l low-fiber (n=22): 1.5 (0.2) mg/l median **IL-6**: high-fiber (n=19): 0.73 (IQR: 1.6) pg/ml low-fiber (n=17): 1.17 (1.1) pg/ml primary endpoint: LDL-cholesterol	<u>mean (SEM) CRP (mg/l) after intervention:</u> **high-fiber** period (n=24): 1.5 (0.1) **low-fiber** period (n=22): 1.8 (0.3)l treatment difference (n=21): -0.71 (0.4), p-trend=0.017 <u>median (IQR) IL-6 (pg/ml) after intervention:</u> **high-fiber** period (n=19): 0.60 (1.4) **low-fiber** period (n=17): 0.99 (1.3), treatment difference (n=13): -0.14 (0.8), p-trend=0.2	Dietary fib $+_{CRP}$ $-_{IL6}$
King 2007, USA (59)	35 participants: 18 healthy and 17 with obesity and hypertension %female: 80% Ø age: 38.3y Ø BMI: 28.4	randomized, crossover, 3 weeks duration (3 wks run-in)	**high-fiber DASH-diet**: mean fiber intake: 27.7g/d (SD: 0.06) **psyllium fiber supplemented diet** (supplemented to reach 30g/d): mean fiber intake: 26.3 (0.4) weight maintenance dietary instructions	mean **hsCRP**: 4.4 (SD: 1.0) mg/l primary endpoint: hsCRP	<u>Difference (%) in hsCRP after each diet period:</u> **DASH-diet**:- 13.7 **supplemented diet**: -18.1 trend for difference between diet periods (0.051 (95% CI -0.008-0.111) p=0.09; supplementation minus DASH diet)	Psyllium fib ($+_{CRP}$)
King 2008, USA (60)	158 participants with overweight or obesity % female: 72.8% Ø age: 50.5y Ø BMI: 33.4	RCT, 3 months duration	**high fiber (n=48)**: mean fiber: 14.5 g/d (SD: 3.9) **low fiber (n=53)**: 13.5 (3.4) **control (n=57)**: 14.1 (4.4) weight maintenance 14, 7, or 0 g/d psyllium fiber	Mean **hsCRP**: high-fiber: 7.61 (SD: 5.8) mg/l low-fiber: 7.62 (6.7) mg/l control: 7.79 (7.5) mg/l primary endpoint: hsCRP power calculations for	<u>Mean (SD) changes in hsCRP (mg/l) (ITT):</u> **high-fiber**: 0.98 (4.57) **low-fiber**: -0.96 (4.45) **control**: 0.05 (7.87); no difference in change between treatment groups and control group	Psyllium fib $-_{CRP}$

First author, Year, Country	Participants characteristics	Study design	Dietary Intervention	Outcome: baseline levels and primary endpoint	Results[2]	+/-[3]
	women needed to have CRP concentrations >3mg/l, and men >2mg/l		supplement	hsCRP	(p>0.05)	
Kohl 2009, Germany (61)	12 participants with overweight or obesity and moderately increased levels of CRP (<5mg/l) % female: 67% Ø age 49.7y Ø BMI: 32.2	randomized, double-blind, placebo-controlled crossover, 4 weeks duration (3 wks washout)	**intervention**: 0.5g of ß-D-glucan, mean fiber 18.0 g/d **control**: nonfermentable waxy maize starch (placebo), mean fiber 18.8 weight maintenance usual diet plus supplements	mean **CRP**: 5.7 (SEM: 0.6) mg/l primary endpoint: CRP power calculations for CRP no data for mean **IL-6**	<u>Mean (SEM) CRP (mg/l) after 4 weeks:</u> **Intervention period**: 5.3 (0.8) **Control period**: 6.1 (1.2) No treatment effect (p=0.4) <u>IL-6:</u> no data No treatment effect (p=0.9, extracted from figure)	ß-glucan $-_{CRP}$
Queenan 2007, USA (62)	75 participants at risk for CVD (hypercholesterolemic) % female: 67% Ø age: 44.9y BMI <30	randomized, double-blind parallel, 6 weeks duration	**intervention (n=35)**: 6g/d concentrated oat ß-glucan, **placebo (n=40)**: dextrose no data on baseline fiber intake weight maintenance, but in both groups reduction of energy intake (-340-520kJ/d) usual diet plus supplements	mean **CRP**: intervention: 0.35 (SEM: 0.08) mg/dl placebo: 0.38 (0.06) mg/dl endpoints: cardiovascular endpoints (including CRP)	<u>Mean (SEM) changes in CRP (mg/dl):</u> **intervention**: -0.03 (0.06) **placebo**: 0.01 (0.04) no between-group difference for change in (p=0.5)	Oat ß-glucan $-_{CRP}$
Salas-Salvado 2008, Spain (63)	166 participants with overweight or obesity % female: 78.3% Ø age: 47.9y Ø BMI: 31.2	parallel, double-blind, randomized, placebo-controlled, 16 weeks duration	**fiber twice a day (2/d) (n=53)** **fiber three times a day (3/d) (n=58)** **placebo (n=55)** mixed fiber dose: 3g Plantago ovata husk and 1 g glucomannan (placebo: 3g microcrystalline cellulose) weight loss study, (2.5MJ energy reduction)	mean **hsCRP**: 2/d: 0.75 (SD: 1.39) mg/l 3/d: 0.86 (0.73) mg/l placebo: 0.70 (0.51) mg/l primary endpoint: weight change (no significant difference in weight change between groups)	<u>Mean (SD) change in hsCRP (mg/l):</u> **2/d**: -0.10 (0.15) **3/d**: -0.08 (0.10) **placebo**: 0.02 (0.05) <u>Mean differences to placebo:</u> **2/d**: -0.12 (95% CI: -0.42, 0.18) **3/d**: -0.10 (95% CI: -0.32, 0.12) No difference in change between treatment groups and control group (p=0.3)	Fib supp $-_{CRP}$

First author, Year, Country	Participants characteristics	Study design	Dietary Intervention	Outcome: baseline levels and primary endpoint	Results[2]	+/-[3]
			individual dietary advice plus supplements		Analysis adjusts for baseline value and BMI stratum	
Wood 2006, USA (64)	29 men with overweight Ø age: 38.8y Ø BMI: 29.7	parallel, placebo controlled, double blind, 12 weeks	**intervention (n=14)**: 3 g/d soluble fiber supplement **placebo (n=15)**: maltodextrin CHO-restricted diet (13% CHO, 60% fat, 27% protein) weight loss study dietary instructions plus supplements	mean **hsCRP**: intervention: 1.68 (SD: 1.5) mg/l placebo: 1.86 (1.29) mg/l mean **IL-6**: intervention: 1.31 (SD: 0.39) pg/ml control: 2.00 (1.62) pg/ml endpoints: cardiovascular risk factors (including hsCRP) changes in hsCRP were not correlated with change in body weight or fat mass	<u>Mean (SD) hsCRP (mg/l) week 12:</u> **intervention**: 1.35 (0.95) **control**: 1.55 (1.23) no between-group difference for change in (p>0.05) <u>Mean (SD) IL-6 (pg/ml) week 12:</u> **intervention**: 1.39 (0.50) **control**: 1.88 (1.07) no between-group difference for change in (p>0.05)	Fib supp -$_{CRP, IL6}$
Whole grain consumption – intervention studies						
Anders-son 2007, Sweden (71)	30 healthy participants (women were postmenopausal) % female: 73% Ø age: 59y Ø BMI: 28.3	randomized, non-blinded, crossover; 6 weeks duration (6 to 8 week wash out)	**whole grain:** 30.0 (SD: 4.9) g fiber **refined grain:** 17.3 (5.5) g fiber weight maintenance, habitual daily diet plus fixed amount of whole or refined grain products foods provided whole grain products defined as containing a minimum of 50% whole grain per dry substance	mean **hsCRP**: whole grain period: 2.03 (SD:1.62) mg/l refined grain period: 2.86 (2.96) mg/l mean **IL-6**: whole grain period: 14.8 (SD: 32.2) ng/l refined grain period: 15.9 (32.4) ng/l primary endpoint: insulin sensitivity	<u>mean hsCRP (mg/l) after intervention:</u> **whole grain period**: 2.38 (2.29) **refined grain period:**2.34 (1.57) no treatment effect.(p=0.6) <u>mean IL-6 (ng/l) after intervention:</u> **whole grain period:**15.2 (33.2) **refined grain period:**15.8 (30.9); no treatment effect.(p=0.8) Analysis adjusts for sequence and BMI	WG -$_{CRP, IL6}$
Brown-lee 2010, UK (72)	266 participants with overweight or obesity % female: 49.8%,	randomized controlled; 16 weeks duration	**intervention 1** (n=85): 60g whole grain/d (=3 servings/d) (mean: 74 (SD: 28.5) g/d) **intervention 2** (n=81): 60g whole grain/d in first 8 weeks, 120g/d in	median **CRP**: intervention 1: 2.4 (SD: 9.9) mg/l intervention 2: 3.2 (4.6) mg/l	<u>Median (SD) CRP (mg/l) at week 8:</u> **intervention 1**: 2.6 (2.5) **intervention 2**: 3.5 (7.2) **control:** 2.7 (2.8)	WG -$_{CRP}$

First author, Year, Country	Participants characteristics	Study design	Dietary Intervention	Outcome: baseline levels and primary endpoint	Results[2]	+/-[3]
	median age: 45.7y median BMI: 30.1 inclusion criteria: habitual consumption ≤30g whole grain/d		last 8 weeks (mean: 83 (31.1) g/d week 8, 115 (69.6) g/d week 16) **control group** (n=100): maintenance of current diet (mean 19 (19.9) g whole grain/d) weight maintenance, substitution of whole grain with refined grain foods to a prescribed amount, foods provided on demand	control: 2.4 (2.3) mg/l primary endpoint: LDL-cholesterol	Median CRP (mg/l) at week 16: **intervention 1**: 3.1 (4.3) **intervention 2**: 3.2 (5.9) **control**: 2.9 (3.5) No difference in change between intervention groups (average from two intervention groups) and control group -1.20 (-12.3-11.3), p>0.05	
de Mello 2011, Finland (73)	103 participants with impaired glucose metabolism and features of the metabolic syndrome % female: 51% Ø age: 59y, Ø BMI: 31.1	parallel design, RCT; 12 weeks	**healthy diet** (n=35): baseline: 29.3 g fiber/d (SD: 8.3), week 12: 36.5 (6.0) **whole grain** (n=34): baseline: 24.6 (7.0) g fiber/d, week12: 26.5 (5.4) **control** (n=34): baseline: 22.2 (6.9), week 12: 17.6 (4) weight maintenance, advice for replacement of usual cereal with at least 50% from whole grain source	median **hsCRP**: healthy: 1.4 (IQR: 0.7, 3.1) mg/l whole grain: 1.5 (0.7, 3.9) mg/l control: 1.4 (0.8, 2.3) mg/l median **IL-6**: healthy: 1.6 (IQR: 1.0, 2.6) ng/l whole grain: 1.4 (1.0, 2.3) ng/l control: 1.3 (0.8, 2.0) ng/l primary endpoint: inflammatory markers (including hsCRP and IL-6)	median change (IQR) (%) of hsCRP after intervention: **healthy diet:** -10 (-37, 41) **whole grain:** -20 (-40, 11) **control:** -8 (-35, 49) no between-group difference for change in (p=0.2) median change (IQR) (%) of IL-6 (ng/l) after intervention : **healthy diet**: -7 (-25, 13) **whole grain:** 3 (-15, 31) **control**: 3 (-11, 35) no between-group difference for change in (p=0.7) Significant improvements in hsCRP on whole grain diet in comparison to control among patients not using statins (p<0.05) (n=76)	WG: ($+_{CRP}$) subgroup
Giacco 2010, Italy (74)	15 participants, some with overweight or obesity % female: 20% Ø age: 54.4y	randomized sequential crossover, 3 weeks duration (2 wks run-in)	**Wholemeal wheat**: mean 32g (SD: 4) fiber, 23.1g (2.3) cereal fiber **refined wheat:** 20g (4) fiber, 9.8g (1.7) cereal fiber weight maintenance,	No data on baseline levels endpoints: metabolic markers (including hsCRP)	Mean (SD) hsCRP (mg/dl) after intervention: **wholemeal wheat period:** 1.8 (SD: 2.3) **refined wheat period:** 2.9 (4.1), no treatment effect (p=0.4)	Wholemeal wheat $-_{CRP}$

First author, Year, Country	Participants characteristics	Study design	Dietary Intervention	Outcome: baseline levels and primary endpoint	Results[2]	+/-[3]
	Ø BMI: 27.4		fixed amount of wholemeal or refined wheat products (wheat bread, pasta, rusks, and crackers), supplied by Barilla			
Katcher 2008, USA (75)	50 participants with obesity and the metabolic syndrome % female: 50% Ø age: 46.6y Ø BMI 35.8	randomized, open-label, parallel-arm, 12 weeks duration	diet composition at week 12: **whole grain** (n=25): mean 12.9 (SD: 2.2) g fiber/1000kcal **refined grain** (n=25): 9.7 (3.5) g fiber/1000kcal weight loss study (-500 kcal) dietary advice (list of foods)	mean **hsCRP**: whole grain: 6.0 (SD: 8.0) mg/l refined grain: 5.9 (6.0) mg/l mean **IL-6**: whole grain: 3.2 (6.3) pg/ml refined grain: 2.2 (1.3) pg/ml primary endpoint: weight loss	<u>Mean (SD) change in hsCRP (mg/l) after intervention:</u> **whole grain:** -2.4 (5.1) **refined grain:** 0.2 (2.9) significant between-group difference of change (p=0.01) <u>Mean (SD) change in IL-6 (pg/ml) after intervention:</u> **whole grain:** -0.9 (3.6) **refined grain:** -0.1 (0.4) no between-group difference of change (n.s.) Effects were independent of weight loss	WG $+_{CRP}$ $-_{IL6P}$
Kristen-sen 2012, Denmark (76)	72 postmenopausal women with overweight or obesity Ø age: 59.7y Ø BMI: 30.2	open-label parallel, 12 weeks duration (2 weeks run-in)	**whole wheat** (n=38): 105 g whole grains/d, 11.0 g fiber/d **refined wheat** (n=34): 0 g whole grains/d, 4.5 g fiber/d weight maintenance, intervention foods provided (bread, pasta, biscuits) whole-grain foods defined as containing a minimum of 50% of whole grain per dry matter, including the starchy endosperm, germ, and bran, in milled form .	mean **hsCRP**: whole wheat: 0: 0.95 (95%CI: 0.35, 1.55) mg/l refined wheat: 1.0 (0.42, 1.58) mg/l mean **IL-6**: whole wheat: 2.45 (95%CI: 2.13, 2.78) ng/l refined wheat: 1.70 (1.3, 2.0) ng/l primary endpoint: body weight and composition	<u>mean (95%CI) hsCRP(mg/l):</u> **whole wheat:** week 6: 0.98 (0.36,1.60), week 12: 0.85 (0.25,1.45) **refined wheat:** week 6: 1.06 (0.44,1.76), week 12: 1.07 (0.49,1.65) no between-group difference of change (p= 0.95) <u>mean (95% CI) IL-6 (ng/l) :</u> **whole wheat:** week 6: 2.59 (2.16, 3.02), week 12: 2.65 (2.15, 3.15) **refined wheat:** week 6: 1.84 (1.54, 2.14), week 12: 1.83 (1.51, 2.15), trend for between-group difference of change (p= 0.09)	Whole wheat $-_{CRP}$ $(+_{IL6})$

First author, Year, Country	Participants characteristics	Study design	Dietary Intervention	Outcome: baseline levels and primary endpoint	Results[2]	+/-[3]
					Analysis refers to completers-only and adjusts for age, baseline levels, baseline BMI and change in body weight from baseline No sig. difference in weight loss but in percentage body fat reduction between groups	
Tighe 2010, Scotland (77)	206 healthy participants, some with signs of metabolic syndrome % female: 50% Ø age: 51.7y Ø BMI: 27.7	randomized, single-blind, controlled; 12 weeks duration (4 wks run-in)	**whole wheat** (n=73): nonstarch polysaccharides: 18.5g (SD 0.5), **wheat+oats** (n=70): nonstarch polysaccharides:16.8g (0.5) **control** (n=63): nonstarch polysaccharides:11.3g (0.4) weight maintenance, refined cereal products substituted with 3 servings of whole wheat foods (bread, cereals) or 1 serving of whole wheat foods and 2 servings of oats - foods provided	median **hsCRP**: control:1.4 (IQR: 0.7, 2.7) mg/l whole wheat: 3.3 (0.5, 2.3) mg/l wheat+oat: 1.0 (0.4, 1.6) mg/l median **IL-6**: control:1.3 (IQR: 0.8, 2.3) pg/l whole wheat: 1.2 (0.9, 1.9) pg/l wheat+oat: 1.1 (0.8, 1.7) pg/l primary endpoints: total and LDL cholesterol	median (IQR) hsCRP (mg/l) at week 12: **whole wheat:** 0.9 (0.5, 1.9) **wheat+oat:**1.0 (0.6, 2.3), **control:** 1.1 (0.6, 3.0) no between-group difference of change (p=0.5) median (IQR) IL-6 (pg/l)[2] at week 12: **whole wheat:** 1.4 (1.0, 1.9) **wheat+oat:** 1.1 (0.8, 1.6), **control:** 1.4 (1.0, 2.4) no between-group difference of change (p=0.3)	Whole wheat -CRP, IL6

[1] Abbreviations: CHO, carbohydrate; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension, fib, dietary fiber intake; ITT, intention to treat; RCT, randomized controlled trial; supp, supplement; T2D, type 2 diabetes mellitus; WG: whole grain

[2] p-values of between-group differences for changes in inflammatory markers are reported for randomized controlled trials, p-values for treatment effects are reported for cross-over trials

[3] +: effect of dietary intervention on hsCRP or IL-6, -: no effect of dietary intervention on hsCRP or IL-6

Ø average value, mean or median as provided in the original publication.

British Journal of Nutrition, page 1 of 8
doi:10.1017/S0007114512005867

Carbohydrate quality is not associated with liver enzyme activity and plasma TAG and HDL concentrations over 5 years in an older population

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(Submitted 20 July 2012 – Final revision received 4 December 2012 – Accepted 4 December 2012)

Abstract

Non-alcoholic fatty liver disease (NAFLD) is closely associated with insulin resistance and obesity. Hence, carbohydrate quality could be of relevance to the risk of NAFLD, but prospective data are lacking. The aim of the present study was to investigate longitudinal associations between carbohydrate quality (including dietary glycaemic index (GI) and intakes of sugar, starch and fibre) and markers of liver function in an older Australian population. The analysis was based on 866 participants (≥49 years) of the Blue Mountains Eye Study with fasting blood specimens and dietary intake data at baseline and 5-year follow-up. Multi-level mixed regression analysis was used to relate dietary GI and sugar, starch and fibre intake to the liver enzymes alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT), as well as fasting TAG and HDL-cholesterol (HDL-C). After adjustment for potential confounding factors, a lower fibre intake was cross-sectionally related to higher GGT ($P=0{\cdot}02$) and fasting TAG ($P=0{\cdot}002$) levels, with fruit fibre being the most relevant fibre source ($P=0{\cdot}095$ for GGT; $P=0{\cdot}003$ for TAG). A higher dietary GI was associated with lower HDL-C ($P=0{\cdot}046$). Changes in carbohydrate quality during 5 years were not related to changes in ALT, GGT, TAG or HDL-C ($P\geq0{\cdot}08$). In conclusion, the absence of longitudinal associations between carbohydrate quality and liver enzymes and serum lipids in this older population does not support a major role of carbohydrate nutrition in liver function among the elderly.

Key words: Non-alcoholic fatty liver disease: Serum lipids: Glycaemic index: Dietary fibre

Non-alcoholic fatty liver disease (NAFLD) describes a condition of fat accumulation in hepatocytes in the absence of other causes of hepatic steatosis such as excess alcohol consumption. Recently, NAFLD has been recognised as an important risk factor for the development of both type 2 diabetes and CHD. In this context, NAFLD has also been termed as the hepatic manifestation of the metabolic syndrome, as it has been related to all its constituting features[1].

The close relationship with obesity and insulin resistance suggests a link to dietary factors associated with disturbances in glucose and insulin metabolism. Indeed, several recent cross-sectional[2,3] and case–control studies[4,5] have reported associations between carbohydrate quality and NAFLD risk in adults aged 50–60 years[2,3,5] as well as younger adults aged 30 years[4]. Both higher dietary glycaemic index (GI)[2] (which ranks carbohydrates according to their glycaemic potency) and added sugars[4,5], particularly from soft drinks, were related to increased NAFLD markers. By contrast, studies on the relevance of dietary fibre for liver fat content have yielded inconsistent results in middle-aged[6,7] and older adults[2]. Overall, evidence from prospective studies is lacking, but would be of importance to shed light on the longitudinal

Abbreviations: ALT, alanine aminotransferase; BMES, Blue Mountains Eye Study; GGT, γ-glutamyltransferase; GI, glycaemic index; HDL-C, HDL-cholesterol; NAFLD, non-alcoholic fatty liver disease.

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British Journal of Nutrition

relevance of carbohydrate quality for NAFLD risk. As the prevalence of NAFLD increases with age[1], investigating this relationship in older cohorts is relevant.

The liver enzymes alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) are commonly used as non-invasive estimates for hepatic fat accumulation, but in contrast to ALT, GGT also serves as a marker for alcoholic fatty liver[1,8–10]. Serum lipids can be considered additionally as risk markers, since NAFLD is closely related to dyslipidaemia and the metabolic syndrome[11,12]. Elevated hepatic lipogenesis leads to an overproduction of TAG and subsequently VLDL; hence, increased levels of fasting TAG may indicate metabolic alterations in the liver[13]. Low HDL-cholesterol (HDL-C) values serve as a further marker for disturbances in lipid metabolism, and, indeed, a study of non-obese, non-diabetic subjects showed that, among others, hypertriacylglycerolaemia and low levels of HDL-C were risk factors for NAFLD[14].

The present study aims to examine both the cross-sectional and the 5-year concurrent associations between carbohydrate quality (GI and intake of sugar, starch and fibre) and the proposed markers of liver function (ALT, GGT, fasting TAG and HDL-C) in an older population.

Materials and methods

Study population

The Blue Mountains Eye Study (BMES) is a population-based cohort study of vision, common eye diseases and other health outcomes in an urban, predominantly Caucasian Australian population aged 49 years and older, which was initiated in 1992 with two 5-year follow-up examinations. A total of 3654 participants took part in the baseline examination (1992–4; BMES-1)[15]. The study was conducted in accordance with the recommendations of the Helsinki Declaration and was approved by the University of Sydney and the Sydney West Area Health Service Human Research Ethics Committees. Written informed consent was obtained from all participants. Details on the study and covariate assessment have been described elsewhere[15].

Study sample

We were interested to examine the cross-sectional and the 5-year concurrent associations of dietary carbohydrate quality with liver enzymes, fasting TAG and HDL-C concentrations. Liver enzymes were first analysed in the first follow-up (BMES-2). Hence, in the present analysis, these data were termed baseline and BMES-3 data (second re-examination) as the respective 5-year follow-up. The analysis was restricted to the participants who had provided a complete and plausible FFQ, as well as a fasting blood specimen at both BMES-2 and -3.

Of the 2335 participants re-examined at BMES-2, 330 had to be excluded due to incomplete (≥12 questions of the FFQ missing, an entire page remaining blank) or implausible dietary data (daily energy intakes were <2500 or >18 000 kJ[16]), and a further 195 because of missing blood samples. Of the remaining 1810 participants, 388 died during the 5-year follow-up period, 161 were lost to follow-up and 218 participated in BMES-3 but did not provide a plausible FFQ and/or a blood sample. A total of 1043 participants had dietary data and blood specimens at both BMES-2 and -3. Another sixty-four participants were excluded because data on BMI, use of lipid-lowering drugs, diabetes mellitus, CHD or smoking status were missing. Moreover, to avoid any confounding due to alcoholic fatty liver disease, we excluded all BMES participants who consumed more than 20 g alcohol per d at both baseline and follow-up (*n* 113), a cut-off point which has commonly used[1,8]. Therefore, the present examination included 866 participants for the analysis of ALT and GGT. Furthermore, at BMES-3, some participants provided only non-fasting blood specimens; hence, fasting TAG and HDL-C concentrations from both visits were available for 755 participants only.

Dietary assessment

Dietary data were collected using a 145-item FFQ modified for the Australian diet and vernacular from an early Willett questionnaire[17]. This FFQ was validated against 4 d weighed food records collected on three occasions during 1 year (*n* 79) and showed moderate-to-good agreement for ranking individuals according to their GI, dietary fibre and total carbohydrate intake[18].

Nutrient intakes were estimated using the Australian Tables of Food Composition (NUTTAB95) and published GI values with the glucose = 100 scale[19]. Additional GI data were obtained from the Sydney University Glycaemic Index Research Service online database (www.glycaemicindex.com). An overall GI value for each participant's diet was calculated by summing the weighted GI of individual foods in the diet with the weighting proportional to the contribution of individual foods to total carbohydrate intake. Additionally, data on total fibre intake and fibre intakes from bread and cereals, vegetables and fruits were extracted from the FFQ.

Liver enzymes and serum lipid levels

Fasting blood specimens were drawn, centrifuged on site and then sent by courier within the same day to the Westmead Hospital, Sydney, for haematological analysis and clinical biochemistry assessment. Fasting serum TAG concentrations were measured on a Reflotron reflectance photometric analyser (Boehringer Mannheim Diagnostics; currently, Roche Diagnostics). CV for repeated measurements of plasma were 1·4 % for TAG and 3·2 % for HDL-C. ALT and GGT were determined using commercial kits performed on an automated analyser (OCD Fusion 5.1; Ortho Clinical), and CV were below 4 % for ALT and below 2·8 % for GGT.

Statistical methods

Statistical analyses were performed using SAS software (version 9.1.3; SAS). Because some of the metabolic and nutritional data were not normally distributed, all continuous data are presented as medians (25th and 75th percentiles).

British Journal of Nutrition

Differences between baseline and the 5-year follow-up in metabolic variables and nutritional intake data were analysed using the Wilcoxon signed-rank test for continuous variables and the Mantel–Haenszel χ^2 test for categorical variables.

We used linear mixed-effect regression models (PROC MIXED in SAS) to construct longitudinal models of trends in ALT, GGT, fasting TAG and HDL-C between baseline and the 5-year follow-up. Because the outcome variables were not normally distributed, all of them were log-transformed before the regression analysis.

In model 1, the following fixed effects were included: sex; time (defined as 1 (baseline) and 2 (5-year follow-up)); the respective dietary variable at baseline; the interaction of this dietary variable with time; the change in the dietary variable, calculated by subtracting baseline values of the respective parameter from the one at follow-up. In this way, the analysis yielded three regression coefficients representing the following: (1) the cross-sectional estimate – an estimate for the regression of carbohydrate quality at baseline on markers of hepatic fat accumulation at baseline; (2) the prospective estimate – the slope of the regression of carbohydrate quality at baseline on the change in the outcomes over 5 years; (3) the concurrent estimate – an estimate for the regression of the change in carbohydrate quality between the 5 years on the concurrent change in the outcomes. The parameters of carbohydrate quality (GI and intake of sugar, starch and fibre; total fibre and fibre from bread and cereals, vegetables and fruits) were energy-adjusted using the multivariate energy density model[20], which required the calculation of fibre densities (g/MJ).

For model 2, the fixed effects of age, diabetes mellitus, any CHD, current or former smoking status, menopausal status, hormone replacement therapy, post-secondary school qualification, good self-rated health and use of cholesterol-lowering as well as other potential influencing medications, e.g. antidiabetic drugs, at baseline were considered as potential influencing factors. BMI and other metabolic and nutritional variables (e.g. total or saturated fat) were additionally examined, including their level at baseline, interaction of baseline level and time, or change in their level during the 5-year period. Only those potential influencing factors that (1) substantially modified the association of the principal dietary variables with ALT, GGT, TAG or HDL-C in the unadjusted models, (2) significantly predicted the outcome variable or (3) improved the fit statistic (Akaike's information criterion) were included in model 2. All analyses were performed with a significance level at $P<0{\cdot}05$.

Results

Among the 866 participants included in the present analysis, more were women and individuals with a younger age, a post-secondary school qualification, a good self-reported health and overweight, but fewer suffered from CHD and smoked compared with the 831 BMES participants who had died or were lost to follow-up. Moreover, those included had lower ALT and fasting glucose concentrations and had consumed slightly more polyunsaturated fat, protein, carbohydrates, sugar, starch and fibre, especially from bread and cereals, and had a lower dietary GI at baseline (data not shown).

Baseline characteristics of the 866 BMES participants included in the present analysis are shown in Table 1. After the 5-year follow-up, the participants' BMI was lower and serum concentrations of ALT, GGT, albumin and bilirubin were higher, whereas concentrations of fasting TAG, glucose, HDL-C and cholesterol improved significantly (Table 2). Regarding 5-year changes in nutritional intake data, energy intake as well as total fat, and saturated fat intake were higher, whereas starch and fibre intake from bread and cereals were lower (Table 2). All of the observed changes were, however, very small.

Carbohydrates and liver enzymes

Carbohydrate quality was not independently associated with ALT, neither in the cross-sectional nor in the concurrent analysis (Table 3, first and second main columns).

The cross-sectional analysis showed inverse associations between fibre intake and GGT in model 1. These were attenuated, but still significant, after adjustment for potentially confounding factors ($P=0{\cdot}02$, model 2; Table 3, third main column). Among the fibre sources, fruit fibre seemed to have the greatest relevance for GGT levels, with higher intakes being related to lower GGT levels. However, this association was attenuated towards a trend after adjustment for confounding factors ($P=0{\cdot}095$, model 2; Table 3, third main column). None of the other parameters of carbohydrate quality was related to GGT – neither in the cross-sectional nor in the concurrent change analyses.

Carbohydrates and TAG and HDL-cholesterol

Higher levels of dietary GI were associated with higher fasting TAG in the cross-sectional analysis, but this association was no longer evident after adjustment for confounding factors. Conversely, associations between fibre intakes and fasting TAG levels in the cross-sectional analysis were maintained ($P=0{\cdot}002$, model 2; Table 4, first main column), with higher

Table 1. Baseline characteristics of the 866 Blue Mountains Eye Study participants

(Percentages, or medians and 25th and 75th percentiles)

	Total (*n*)	Value (%)
Sex		
Female	866	62·7
Age (years)	866	
Median		67·0
25th percentile		62·0
75th percentile		73·0
Diabetes mellitus	866	9·8
Any CHD	866	16·3
Cholesterol-lowering medication	866	9·2
Menopause*	543	94·3
Hormone replacement therapy, ever*	543	38·7
Smoking	866	41·1
Post-secondary school qualification	835	64·2
Good self-reported health at baseline	866	84·2

* Percentage refers to women only.

Table 2. Comparison of the metabolic variables and nutritional intake of the 866 Blue Mountains Eye Study participants at baseline and 5-year follow-up

(Median values and 25th and 75th percentiles)

		Baseline			5-year follow-up			
	n	Median	25th percentile	75th percentile	Median	25th percentile	75th percentile	*P**
Metabolic variables								
BMI (kg/m^2)	866	26·8	24·4	29·9	26·6	24·1	29·8	<0·0001
ALT (U/l)†	866	19	15	26	22	18	28	<0·0001
GGT (U/l)†	866	20	15	29	23	17	32	<0·0001
Albumin (%)	866	42	40	44	43	41	44	<0·0001
Bilirubin, total (μmol/l)	865	10	8	12	11	8	14	<0·0001
Fasting glucose (mmol/l)	737	5·0	4·7	5·5	4·9	4·6	5·3	<0·0001
Fasting TAG (mmol/l)	755	1·34	0·98	1·83	1·28	0·93	1·75	0·0001
Fasting cholesterol (mmol/l)	866	6·0	5·3	6·6	5·4	4·8	6·1	<0·0001
Fasting HDL (mmol/l)	755	1·4	1·2	1·7	1·6	1·3	1·9	<0·0001
Daily nutritional intakes								
Energy (kJ)	866	8201	6869	9750	8360	6890	10 112	0·004
Fat (%)	866	31·6	27·8	35·6	32·5	28·4	36·6	<0·0001
SFA (%)	866	12·1	10·1	14·2	12·5	10·4	14·5	0·02
MUFA (%)	866	11·3	9·9	12·8	11·9	10·2	13·4	<0·0001
PUFA (%)	866	4·9	3·9	6·1	5·1	4·0	6·2	0·05
Protein (%)	866	17·6	15·7	19·6	17·7	15·8	19·7	0·2
Carbohydrates (%)	866	47·8	43·5	52·4	47·1	42·8	51·4	0·002
Sugar (%)	866	25·2	21·2	29·2	25·6	21·6	29·4	0·1
Starch (%)	866	21·7	18·7	24·9	20·5	17·4	23·2	<0·0001
GI	866	56·1	53·2	58·6	56·1	53·4	58·6	0·6
GL	866	132·0	106·1	158·3	132·6	106·1	160·5	0·5
Fibre (g/1000 kJ)	866	3·28	2·71	3·98	3·22	2·65	3·87	0·01
From vegetables (g/1000 kJ)	866	1·14	0·90	1·47	1·17	0·93	1·51	0·05
From fruits (g/1000 kJ)	866	0·78	0·49	1·13	0·83	0·52	1·18	0·02
From bread and cereals (g/1000 kJ)	866	0·83	0·54	1·17	0·69	0·43	0·96	<0·0001
Alcohol (g)	866	1·62	0·21	9·58	1·67	0·17	9·51	0·2

ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; GI, glycaemic index; GL, glycaemic load.
* Wilcoxon signed-rank test.
† To convert U/l to μkat/l, multiply by 0·017.

intakes of fibre from fruits being the relevant source (P=0·003, model 2; Table 4, first main column).

Regarding the associations between carbohydrate quality and HDL-C, a higher dietary GI was cross-sectionally related to lower HDL-C levels (P=0·046, model 2; Table 4, third column). A lower starch intake was also related to lower HDL-C levels, but adjustment for confounding factors attenuated this association (P=0·05, model 2; Table 4, third column). For none of the other aspects of carbohydrate nutrition, a relationship to HDL-C could be observed – neither cross-sectionally nor concurrently.

Sensitivity analyses

Additional adjustment for albumin or bilirubin, metabolic variables sometimes also used to assess liver health, did not alter the results. Furthermore, we repeated the analysis for the entire study sample with data on BMES-2 and -3 including also the 113 participants with a habitually high alcohol intake. The results were comparable with those presented for ALT, TAG and HDL-C, with the observed associations being more pronounced. For GGT, both cross-sectional and 5-year concurrent associations were observed with dietary GI and fibre intake (particularly from fruit sources) in the entire study sample (data not shown). To assess any effect modification by liver marker status (e.g. normal or elevated values), we performed stratified analysis which did, however, yield comparable results (data not shown).

Discussion

To the best of our knowledge, the present study is the first investigating the prospective association of carbohydrate quality with liver enzymes in an older population. The present study confirms previously reported associations between dietary GI and fibre intake with TAG and HDL-C, but suggests that these are not of prospective relevance. Overall, the effect sizes for all observed associations were small. Hence, the present analysis does not support a major role of carbohydrate quality in relation to markers of liver function among the elderly.

Added sugars, particularly from soft drinks, are proposed to be relevant in NAFLD pathophysiology: Kechagias *et al.*[21] found a positive correlation between the intake of simple sugars and the levels of ALT, and case–control studies have indicated a positive association of NAFLD risk and severity with soft drink consumption[4,5]. We did not observe relationships of sugar with any of the analysed markers. This could be due to the fact that soft drinks were rarely consumed by this elderly population. In this context, concern has been expressed that high intakes of fructose primarily derived

Table 3. Mixed models* of the cross-sectional and the 5-year concurrent relationships of markers of carbohydrate quality to log transformed serum alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) levels (U/l) in the 866 Blue Mountains Eye Study (BMES) participants
(β Coefficients and standard errors)

	ALT (*n* 866)						GGT (*n* 866)					
	Cross-sectional estimate			5-year concurrent change estimate			Cross-sectional estimate			5-year concurrent change estimate		
	β	SE	*P*	β	SE	*P*	β	SE	*P*	β	SE	*P*
GI												
Model 1†	0·0018	0·0039	0·6	0·0055	0·0032	0·09	0·0068	0·0045	0·1	0·0063	0·0035	0·07
Model 2‡	0·0007	0·0041	0·9	0·0043	0·0033	0·2	0·0004	0·0048	0·9	0·0053	0·0037	0·2
Sugar intake (%)												
Model 1†	0·0001	0·0025	1·0	0·0025	0·0020	0·2	0·0046	0·0029	0·1	0·0002	0·0022	0·9
Model 2§	0·0016	0·0031	0·6	0·0017	0·0025	0·5	0·0018	0·0036	0·6	0·0014	0·0028	0·6
Starch intake (%)												
Model 1†	0·0039	0·0032	0·2	0·0021	0·0024	0·4	0·0060	0·0037	0·1	0·0028	0·0027	0·3
Model 2‡	0·0008	0·0033	0·8	0·0041	0·0026	0·1	0·0046	0·0038	0·2	0·0033	0·0029	0·3
Fibre intake (g/1000 kJ)												
Model 1†	0·0032	0·0039	0·4	0·0048	0·0034	0·2	0·0149	0·0045	0·0009	0·0034	0·0037	0·4
Model 2‖	0·0004	0·0046	0·9	0·0020	0·0038	0·6	0·0128	0·0054	0·02	0·0020	0·0043	0·6
Fibre intake from bread and cereals (g/1000 kJ)												
Model 1†	0·0042	0·0069	0·5	0·0115	0·0059	0·049	0·0149	0·0080	0·06	0·0042	0·0066	0·5
Model 2‖	0·0020	0·0069	0·8	0·0084	0·0060	0·2	0·0098	0·0080	0·2	0·0052	0·0068	0·4
Fibre intake from vegetables (g/1000 kJ)												
Model 1†	0·0062	0·0078	0·4	0·0052	0·0058	0·4	0·0118	0·0091	0·2	0·0038	0·0065	0·6
Model 2‖	0·0043	0·0076	0·6	0·0032	0·0058	0·6	0·0055	0·0089	0·5	0·0066	0·0066	0·3
Fibre intake from fruits (g/1000 kJ)												
Model 1†	0·0032	0·0064	0·6	0·0035	0·0055	0·5	0·0189	0·0074	0·01	0·0079	0·0060	0·2
Model 2‖	0·0049	0·0081	0·5	0·0039	0·0065	0·6	0·0158	0·0095	0·095	0·0070	0·0073	0·3

GI, glycaemic index.
* Models contain a random statement with an unstructured covariance structure.
† Model 1 contains time defined as 1 (BMES-2) and 2 (BMES-3) and the predictor variable (e.g. dietary GI (per 10 units/d)) as terms at baseline, baseline×time and concurrent change, adjustment for sex and energy (use of the multivariate energy density model).
‡ Model 1 additionally adjusted for BMI as terms at baseline and baseline × time, age, diabetes at baseline, smoking (past and/or concurrent), alcohol consumption (categorical) as terms at baseline, baseline × time and concurrent change, the use of cholesterol-lowering medication and dietary fat (percentage of energy, en%) and fibre intake (g/MJ) as terms at baseline, baseline × time and concurrent change.
§ Same as ‡, but adjustment for fibre intake from fruits (g/MJ) as terms at baseline, baseline × time and concurrent change instead of total fibre intake.
‖ Model 1 additionally adjusted for BMI as terms at baseline and baseline × time, age, diabetes at baseline, smoking (past and/or concurrent), alcohol consumption (categorical) as terms at baseline, baseline × time and concurrent change, the use of cholesterol-lowering medication and dietary fat intake (en%) and dietary GI as terms at baseline, baseline × time and concurrent change.

from high-fructose maize syrup could adversely affect liver function[22]. In contrast to the USA, sucrose is the most commonly used sweetener in Australia, which, however, has been proposed to yield effects comparable with those of high-fructose maize syrup[22]. Unfortunately, additional data that would have allowed separate appraisal of intrinsic and added sugar or fructose intake were not available in the present study.

Dietary fibre, particularly viscous fibre, may exert beneficial effects on blood lipids and inflammatory markers[23]. Indeed, oxidative stress is increasingly recognised as an important parameter in NAFLD pathophysiology and is another possible mechanism linking carbohydrate quality to hepatic steatosis[24,25]. While Valtuena *et al.*[2] did not observe a relationship between total fibre intake and liver steatosis, we saw a favourable association of dietary fibre with GGT and fasting TAG, in particular for fibre from fruit sources.

In contrast to single nutrients such as fructose or fibre, dietary GI gives an estimate of repeated postprandial glycaemic excursions, i.e. it allows us to address the relevance of one particular metabolic response to carbohydrate nutrition. The assumption that a higher dietary GI could be related to NAFLD stems from the observation that a hyperenergetic intake of carbohydrate-rich foods leading to increased postprandial glucose elevations enhances hepatic lipogenesis[26–28]. Valtuena *et al.*[2] showed an association between dietary GI and the degree of hepatic steatosis measured by liver echography. However, in a stratified analysis, they observed a significant impact of dietary GI only for participants, who were insulin-resistant (*n* 60, 24·9 %). They concluded that the combination of hyperglycaemia and hyperinsulinaemia may lead to increased hepatic fat accumulation through elevated lipogenesis, on the one hand, and suppressed β-oxidation, on the other hand[2]. By contrast, we did not observe an interaction with diabetes/impaired fasting glucose status; however, this could reflect insufficient power as the number of participants with these conditions was small (*n* 85, 9·8 %) (data not shown). In the present analysis, dietary GI was not related to liver enzymes. Instead, we observed cross-sectional associations of dietary GI with TAG and HDL-C levels – the latter one also mirrored by a relationship to starch intake, a dietary factor closely related to dietary

Table 4. Mixed models* of the cross-sectional and the 5-year concurrent relationships of markers of carbohydrate quality to log-transformed serum fasting TAG (mmol/l) and HDL-cholesterol levels (mmol/l) in the 755 Blue Mountains Eye Study (BMES) participants

(β Coefficients and standard errors)

	TAG (*n* 755)						HDL (*n* 755)					
	Cross-sectional estimate			5-year concurrent change estimate			Cross-sectional estimate			5-year concurrent change estimate		
	β	SE	*P*	β	SE	*P*	β	SE	*P*	β	SE	*P*
GI												
Model 1†	0·0123	0·0043	0·005	0·0014	0·0035	0·7	0·0053	0·0023	0·02	0·0009	0·0016	0·6
Model 2‡	0·0066	0·0045	0·1	0·0022	0·0037	0·6	0·0048	0·0024	0·046	0·0021	0·0017	0·2
Sugar intake (%)												
Model 1†	0·0005	0·0028	0·9	0·0008	0·0022	0·7	0·0011	0·0015	0·5	0·0001	0·0010	0·9
Model 2§	0·0028	0·0034	0·4	0·0022	0·0028	0·4	0·0002	0·0018	0·9	0·0011	0·0013	0·4
Starch intake (%)												
Model 1†	0·0045	0·0036	0·2	0·0007	0·0027	0·8	0·0046	0·0019	0·02	0·0020	0·0013	0·1
Model 2‡	0·0044	0·0037	0·2	0·0002	0·0029	0·9	0·0038	0·0019	0·05	0·0020	0·0014	0·1
Fibre intake (g/1000 kJ)												
Model 1†	0·0135	0·0043	0·002	0·0030	0·0038	0·4	0·0027	0·0023	0·2	0·0028	0·0018	0·1
Model 2‖	0·0161	0·0051	0·002	0·0038	0·0044	0·4	0·0037	0·0027	0·2	0·0036	0·0020	0·08
Fibre intake from bread and cereals (g/1000 kJ)												
Model 1†	0·0108	0·0076	0·2	0·0103	0·0066	0·1	0·0039	0·0040	0·3	0·0030	0·0031	0·3
Model 2‖	0·0108	0·0076	0·2	0·0117	0·0068	0·09	0·0047	0·0040	0·2	0·0034	0·0032	0·3
Fibre intake from vegetables (g/1000 kJ)												
Model 1†	0·0095	0·0087	0·3	0·0057	0·0066	0·4	0·0023	0·0046	0·6	0·0017	0·0031	0·6
Model 2‖	0·0087	0·0084	0·3	0·0059	0·0066	0·4	0·0033	0·0044	0·5	0·0008	0·0031	0·8
Fibre intake from fruits (g/1000 kJ)												
Model 1†	0·0225	0·0070	0·001	0·0068	0·0061	0·3	0·0043	0·0037	0·3	0·0041	0·0028	0·1
Model 2‖	0·0269	0·0089	0·003	0·0102	0·0072	0·2	0·0046	0·0047	0·3	0·0058	0·0034	0·09

GI, glycaemic index.

* Models contain a random statement with an unstructured covariance structure.

† Model 1 contains time defined as 1 (BMES-2) and 2 (BMES-3) and the predictor variable (e.g. dietary GI per 10 units/d) as terms at baseline, baseline×time and concurrent change, adjustment for sex and energy (use of the multivariate energy density model).

‡ Model 1 additionally adjusted for BMI as terms at baseline and baseline × time, age, diabetes at baseline, smoking (past and/or concurrent), alcohol consumption (categorical) as terms at baseline, baseline × time and concurrent change, the use of cholesterol-lowering medication and dietary fat (percentage of energy, en%) and fibre intake (g/MJ) as terms at baseline, baseline × time and concurrent change.

§ Same as ‡, but adjustment for fibre intake from fruits (g/MJ) as terms at baseline, baseline × time and concurrent change instead of total fibre intake.

‖ Model 1 additionally adjusted for BMI as terms at baseline and baseline × time, age, diabetes at baseline, smoking (past and/or concurrent), alcohol consumption (categorical) as terms at baseline, baseline × time and concurrent change, the use of cholesterol-lowering medication and dietary fat intake (en%) and dietary GI as terms at baseline, baseline × time and concurrent change.

GI values[29]. The present findings are in line with other cross-sectional observational studies[30–36]; however, the absence of a longitudinal relationship questions the clinical relevance of these associations in this age group. Furthermore, evidence from meta-analyses of intervention studies does not support an effect of dietary GI on TAG or HDL-C[37,38].

The main strengths of the present analysis were the prospective study design with repeated measurements of liver enzymes, fasting TAG and HDL-C, as well as dietary intake data in a contemporary sample of older men and women. We could control for repeatedly measured key confounding factors such as BMI, medications taken or health status. However, we cannot preclude residual confounding, resulting from imprecisely measured or unmeasured confounding factors. Additionally, due to the large number of tests, chance findings are a possibility.

Regarding the markers for NAFLD, it has to be considered that raised liver enzymes can only reveal an increased risk, yet no quantitative conclusions about the extent of hepatic fat accumulation can be drawn. Moreover, NAFLD can be present without any elevations in liver enzymes. However, these markers are commonly used as minimally invasive parameters[1,8], and were readily available for the present study sample. Despite the fact that we employed a validated FFQ, misclassification bias can still exist. This may apply to fibre sources in particular, since the FFQ was neither designed nor validated for fibre source-specific analyses. At the 5-year follow-up, participants may have had a greater recall of certain food groups increasingly considered healthy such as fruits and vegetables. However, such a recall bias would have rather translated into an underestimation for the concurrent changes in markers of liver function. Selection bias resulting from the limited number of persons eligible for the longitudinal analysis is a particular concern and may hamper the extent to which the present results can be generalised. Similar characteristics among those lost to follow-up suggest that attrition bias introduced by loss to follow-up was low. However, attrition due to higher natural mortality limits the generalisability of the present findings. Also, selective survival may have occurred because those included in the analysis were healthier at baseline regarding some but not all clinical parameters than those who died during the follow-up.

In conclusion, the absence of longitudinal associations between carbohydrate quality and liver enzymes or serum lipids in this older Australian population does not support a major role of carbohydrate nutrition in liver function among the elderly. A potential impact of carbohydrate nutrition in populations with more adverse dietary habits deserves further investigation, ideally using direct measures of NAFLD status.

Acknowledgements

The BMES was supported by the Australian National Health and Medical Research Council. P. M. and A. E. B. conceived the project; P. M., B. G. and E. R. collected the data; B. G., E. R. and G. C. provided the databases and/or statistical experience, J. G. analysed the data and wrote the manuscript; A. E. B. supervised the project; B. G., E. R., A. W. B., G. C., J. C. B.-M. and P. M. critically revised the manuscript for important intellectual content. All authors read and approved the final version of the paper. Conflicts of interest: J. C. B.-M. is the director of a not-for-profit GI-based food endorsement programme in Australia and manages the Sydney University Glycaemic Index Research Service; A. W. B. is a consultant for Glycaemic Index Limited, a not-for-profit GI-based food endorsement programme in Australia; J. G., A. E. B., B. G., E. R., G. C. and P. M. have no conflicts of interest.

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British Journal of Nutrition