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Ontogenetic development of the IGF-system and leptin in offspring of sows fed with gestation diets differing in protein content

The aim of this dissertation was to characterize leptin and the different components of the IGF-system in offspring of sows fed with different protein contents throughout pregnancy and to ascertain long term consequences caused by an inadequate nutritional environment *in utero*. German Landrace gilts (n=78) were fed gestation diets (~15.4 MJ ME/kg) of either low (LP, 6%; n=26), adequate (AP, 12%; n=26), or high (HP, 30%; n=24) protein content. For sampling at day 94 of gestation, 26 gilts with their fetuses were investigated. At parturition, the offspring of the remaining 52 gilts were cross fostered to nursing sows fed a standard lactation diet. Fetal and birth weight of all investigated offspring were recorded and classified into 2 different fetal body weight classes (Fbwc ≤ or > 0.65 kg) or birth weight classes (Bbwc ≤ or > 1.3 kg). In addition, the litter size of any gilt was classified into two groups (Lsgr ≤ or > 12 piglets). Blood and liver samples from 306 piglets in total were collected. In detail, fetuses of day 94 of gestation (dpc), and piglets at their 1^{st}, 28^{th} and 188^{th} day of life (dpn) were sampled. Blood samples were assayed for IGF-1, IGF-2 and leptin by ELISA and for IGF binding proteins via a quantitative, non-radioactive Western ligand blotting protocol established and optimized herein. Liver samples were assayed for IGF-1 receptor by use of ELISA. The concentrations of leptin in offspring were not affected by maternal diet over all postnatal days. With the exception of dpn 1, the IGF-1 concentrations remained unchanged in response to maternal dietary treatment. Piglets born to mothers fed the LP diet had decreased IGF-1 concentrations compared to piglets born to HP mothers ($P < 0.05$). Piglets of lower birth weight also showed lower IGF-1 concentrations; IGF-1 and the body weights on dpn 1 were positively correlated. For IGF-2, an association between its concentration and low fetal body weight was observed. In line with previous studies, nutrient restriction as modulator had more pronounced effects on the circulating IGF-1 concentrations than the ones of IGF-2. The concentrations of the IGFBPs were primarily effected by nutrition *in utero* at the early stages of development. IGFBP-1 was higher in HP fetuses compared to the other two diet groups on dpc 94. For neonatal piglets, IGF-1 and IGFBP-1 were negatively correlated; piglets from LP fed mothers had higher IGFBP-1 values; they also had lower concentrations of IGF-1 than AP and HP piglets. Piglets born to LP fed mothers had lower IGFBP-2 values on dpn 1 and dpn 28; for IGFBP-3 differences related to maternal feeding group were limited to dpn 1: HP piglets had higher IGFBP-3 concentrations than AP piglets. On none of the sampling days, differences between the diet groups were observed for liver IGF-1 receptor content. Neither maternal feeding group nor fetal body weight nor litter size nor sex apparently influenced IGF-1 receptor content on dpc 94 in liver, indicating that different protein diets *in utero* throughout the entire pregnancy might rather act at the level of the ligand than of the receptor, if the IGF-system is concerned at all. Nevertheless the results of this study show clearly, that all ascertained effects of modified protein diets during gestation were limited to prenatal and early postnatal life. Significant effects resulted predominantly from protein restriction *in utero* whereas protein excess exerted no or only few effects on all factors tested. Possibly the inadequate protein supply during gestation could largely be compensated during the suckling period and thereafter when feeding was homogeneous in all groups. The lack of persistent effects by maternal feeding on the endocrine systems indicates adaptive processes; moreover, carcass composition data do not support long lasting effects of subtle early endocrine changes.

Einfluss divergenter Proteinversorgung *in utero* auf die ontogenetische Entwicklung von Leptin und des IGF-Systems in Ferkeln

Das Ziel dieser Studie war es, kurz- und langfristige Reaktionen auf maternales, intrauterines Nahrungsproteindefizit und –überschuss bei Ferkeln unterschiedlichen Alters in Hinblick auf verschiedene Komponenten der IGF-Achse und Leptin zu prüfen und zu charakterisieren.
Jungsauen der Deutschen Landrasse (n=78) wurden während der gesamten Trächtigkeit mit isoenergetischen Diäten (~15,4 MJ ME/kg) unterschiedlichen Proteingehaltes ernährt: Niedrigprotein (NP, 6%; n=26), Adäquatprotein (AP, 12%; n=26) oder Hochprotein (HP, 30%; n=24). Am 94. Gestationstag wurden 26 Jungsauen und deren Föten untersucht. Am Tag der Geburt wurden die Nachkommen der verbliebenen 54 Jungsauen zu Ammensauen umgesetzt, die eine standardisierte Laktationsdiät bekamen. Das Körpergewicht der Föten und der neugeborenen Ferkel wurde festgestellt und in zwei verschiedene fötale Körpergewichtsklassen (fKgk ≤ oder > 0,65 kg) und Geburtsgewichtsklassen (Ggk ≤ oder > 1,35 kg) eingeteilt. Zusätzlich wurden die Wurfgruppengrößen klassifiziert (Wgg ≤ oder > 12). Es wurden Blut- und Leberproben von Föten des 93. Gestationstag (dpc) und Ferkeln des ersten, 28. und 188. Lebenstages (dpn) gesammelt (n=306). Die Blutproben wurden mittels ELISA auf IGF-1, IGF-2 und Leptin untersucht. Desweiteren wurden in den Blutproben die IGF-Bindungsproteine 1-4 (IGFBP 1-4) mit Hilfe eines quantitativen, nicht-radioaktiven Western Liganden Blot Protokolls gemessen, das in dieser Arbeit etabliert und optimiert wurde. Ebenfalls im ELISA wurden Leberproben auf den Gehalt an IGF-1 Rezeptor (IGF-1 R) untersucht.
Die Leptinkonzentration im Blut war über alle postnatalen Tage nicht von der Diät beeinflusst. Mit der Ausnahme von dpn 1, blieb die IGF-1 Konzentration in Bezug auf die maternale Diät *in utero* unverändert. Ferkel von Müttern, denen die NP Diät während der Trächtigkeit gefüttert wurde, hatten eine niedrigere IGF-1-Konzentration im Blut im Vergleich zu den Nachkommen der HP Mütter ($P < 0,05$). Ferkel mit geringerem Geburtsgewicht zeigten ebenfalls geringere IGF-1 Konzentrationen; IGF-1 und die Geburtsgewichte korrelierten an Tag 1 positiv. Für IGF-2 konnte eine Verbindung zwischen dessen Konzentration und niedrigem fötalem Körpergewicht beobachtet werden. In Übereinstimmung mit früheren Untersuchungen, hat Nährstoffrestriktion als Modulator stärkere und profiliertere Einflüsse auf die IGF-1 als auf die IGF-2-Konzentration im Blut. Die Konzentration der IGF-Bindungsproteine wurde hauptsächlich in der frühen Entwicklung der Nachkommen durch die Nährstoffversorgung *in utero* beeinflusst. Am dpc 94 war IGFBP-1 in HP Föten höher als in den Nachkommen der beiden anderen Diätgruppen. Für die neugeborenen Ferkel wurde eine negative Korrelation zwischen IGF-1 und dem IGFBP-1 festgestellt; Nachkommen der NP gefütterten Mütter hatten höhere IGFBP-1 Werte; sie hatten aber auch geringere Konzentrationen an IGF-1 im Blut, als die AP und HP Ferkel. An Tag 1 und Tag 28 hatten NP Ferkel niedrigere IGFBP-2 Werte; für IGFBP-3 waren die Unterschiede aufgrund der maternalen Diät auf Tag 1 limitiert: Die Konzentration an IGFBP-3 war in den HP Ferkeln höher als in den AP Ferkeln. An keinem der Probennahmetage wurden Unterschiede in der hepatischen Konzentration des IGF-1 R in Bezug auf die Diätgruppen festgestellt. Weder die maternale Fütterungsgruppe, noch das fötale Körpergewicht oder das Geschlecht beeinflussten offensichtlich den Gehalt an IGF-1 R in der Leber an dpc 94. Diese Tatsache weist darauf hin, dass unterschiedliche Proteindiäten während der Trächtigkeit eher auf der Ebene der Liganden als auf der Ebene der Rezeptoren wirken, wenn signifikante Effekte überhaupt feststellbar waren. Trotzdem zeigen die Ergebnisse dieser Studie sehr deutlich, dass alle Effekte durch modifizierte Proteindiäten während der Trächtigkeit sich vor allem auf das pränatale und frühe postnatale Leben beschränken. Signifikante Unterschiede resultierten vornehmlich aus der Proteinrestriktion *in utero*, wohingegen Proteinüberschuss keine bis sehr geringe Auswirkungen auf alle getesteten Faktoren hatte. Möglicherweise kann die unzureichende Proteinversorgung während der Trächtigkeit über die Säugezeit und der anschließenden, für alle Gruppen homogenen Fütterung kompensiert werden. Das Fehlen von persistierenden Auswirkungen der maternalen, inadäquaten Ernährung während der Trächtigkeit auf das endokrine System der Nachkommen ist bezeichnend für adaptive Prozesse. Darüber hinaus unterstützen auch die Daten der Körperzusammensetzung keine, durch frühe endokrine Veränderungen hervorgerufene, anhaltende Veränderungen.

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ACTH	adrenocorticotropic hormone
AP	adequate protein
ATP	adenosine-5'-triphosphate
B	body weight
Bbwc	birth body weight class
BMBF	German Federal Ministry of Education and Research
BSA	bovine serum albumin
CNS	central nervous system
D	diet
DM	dry matter
dpc	days post conception
dpn	days post natum
ECL	enhanced chemiluminescence
EDTA	ethylenediaminetetraacetic acid
EGTA	ethyleneglycoltetraacedic acid
ELISA	Enzyme-linked Immunosorbent Assay
FBN	Leibniz Institute for Farm Animal Biology
Fbwc	fetal body weight class
FSH	follicle stimulating hormone
FUGATO	Functional Genome Analysis in Animal Organisms
GfE	Society for nutrition physiology
GH	growth hormone
GH-IH	growth hormone inhibiting hormone
GH-RH	growth hormone releasing hormone
HCW	hot carcass weight
HP	high protein
IGF-1	Insulin-like growth factor 1
IGF-1R	Insulin-like growth factor receptor 1
IGF-2	Insulin-like growth factor 2

IGF-2R	Insulin-like growth factor receptor 2
IGFBP 1-6	Insulin-like growth factor binding protein 1-6
IR	insulin receptor
IUGR	intrauterine growth retardation
Lep	leptin
LH	luteinizing hormone
LP	low protein
LR	leptin receptor
Lsgr	litter size group
M-6-PR	mannose-6-phosphate receptor
n.d.	not defined
N.S.	not significant
NH_4Cl	ammonium chloride
NPY	neuropeptide-Y
PBS	phosphate buffered saline
Prot	protein
PVDF	polyvinylidine fluoride
S	sex
SDS	sodium dodecyl sulfatase
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SE	standard error
SW	slaughter weight
TBST	Tris-buffered Saline Tween-20
TSH	thyroid stimulating hormone
UV	ultra violet radiation

1 Introduction

In domestic farm animal species such as the pig, the number of offspring, their growth rate, feed efficiency and stress resistance are important economic traits. Pig farmers ask for healthy and normal weight piglets preferable with consistent genetics, which will yield carcasses as requested from the market, by efficient feed conversion. Reduced survival and compromised postnatal development due to low birth weight imply economic losses for the pig industry (Litten et al., 2003). Low birth weight piglets have low-grade productivity such as depressed growth and lower carcass and meat quality (Rehfeldt and Kuhn, 2006; Rehfeldt et al., 2008).

For achieving the required traits, it is important to have optimal environmental conditions in pig production and breeding. The literature indicates that a considerable amount of the variation in postnatal growth performance may largely be determined, and essentially be preprogrammed, during the fetal development in uterus (Foxcroft et al., 2006). A lot of intrauterine environmental factors affecting the conceptus such as maternal stress, energy supply, hypoxia and placental insufficiency; and dietary manipulation may alter the expression of the fetal genome with lifelong consequences. The present thesis will focus on the impact of the nutritional environment *in utero* on endocrine hormones, in particular on the insulin-like growth factor system, in the progeny as forwarded by maternal protein supply during gestation.

1.1 Fetal programming

The notion that environmental factors, particularly nutrition, act in early life to program the risk for the early onset of cardiovascular and metabolic disease in adult life and premature death was expressed by David Barker (1990) as the hypothesis of the ‘early’ or ‘fetal’ origins of adult disease. Series of worldwide epidemiological studies in humans and animals were following and they extended the initial observations with the association between pre- and postnatal growth and cardiovascular disease and included associations between early growth patterns and an increased risk for hypertension, impaired glucose tolerance, non-insulin-dependent or type 2 diabetes, insulin resistance, and obesity in adult life (McMillen and Robinson, 2005). To explain the biological basis of the association observed

between birth weight and health outcomes in later life, a number of outlines have been proposed. One of them was the 'Thrifty Phenotype Hypothesis' of Hales and Barker (1992) suggesting that a poor environment *in utero* will initiate an adaptive response which optimizes the growth of key body organs to the disadvantage of others and thereby leads to alterations in postnatal metabolism in order to support postnatal survival under conditions of malnourishment. These adaptations only become adverse in case of abundant postnatal nutrition following the supply experienced *in utero* (Hales and Barker, 1992). The idea that there are embryonic and fetal adaptations to a suboptimal intrauterine environment which result in permanent adverse consequences is in line with the definition of 'programming' by Lucas in 1991 (Lucas, 2007). Programming has been defined as 'the induction, deletion, or impaired development of a permanent somatic structure or the "setting" of a physiological system by an early stimulus or insult operating at a "sensitive" period, resulting in long term consequences for function' (Lucas, 2007). Intrauterine programming can occur at any level within the affected physiological system and may involve structural and functional changes in genes, cell tissues, and even entire organs. These changes may be isolated or widespread events with either discrete or cumulative effects on development depending on the nature and timing of the programming stimulus (McMillen and Robinson, 2005).

1.2 Intrauterine growth restriction (IUGR) and fetal programming in farm animals

Considerable effort has been directed towards defining nutrient requirements of animals over the past 30 years, because suboptimal nutrition during gestation remains a significant problem for many animal species such as cattle, sheep and pigs worldwide (Bell and Ehrhardt, 2002). Overnutrition, as an inadequate form of nutrition, can result from an increased intake of energy and/or protein. Extensive studies have shown that maternal overnutrition retards placental and fetal growth, and increases fetal and neonatal mortality in rats, sheep and pigs (Wallace et al., 2003). In contrast, maternal undernutrition during gestation reduces placental and fetal growth both in domestic farm animals and in humans (Barker and Clark, 1997; Bell and Ehrhardt, 2002). In livestock species, fetal undernutrition frequently occurs worldwide (Wu et al., 2004). Due to the fact that nutritional and developmental

research often involves invasive tissue collections and surgical procedures, it is neither ethical nor practical to conduct such experiments in pregnant women and their children. Therefore, animal models such as mice, rats, sheep and pigs are appropriate for defining the mechanisms of intrauterine growth retardation (IUGR) resulting in lighter birth weight and fetal programming (Wu et al., 2006). The pig model has the advantage that there is a great similarity in organ size, physiology, metabolism and also genetics in comparison to man (Du et al., 2007). Accordingly, it is an appropriate model to design experiments dealing with both birth weight and prenatal as well as neonatal nutrient supply. In sows fed in line with demand, an up to 3-fold difference in body weight among littermates may occur, thus providing a natural form of IUGR (Morise et al., 2008). Low birth weights naturally occur in this polytocous species, in conjunction with intrauterine crowding and increasing litter size (Milligan et al., 2002; Town et al., 2004). Nevertheless, there are factors e.g., limited oxygen and nutrient supply, which mainly lead to IUGR via impaired placental function (Rehfeldt et al., 2010). Induction of IUGR, resulting in lighter birth weight, is a significant problem in pig production (Wu et al., 2006).

Over the past decade, compelling epidemiological studies have linked IUGR with the etiology of many chronic diseases in adult humans and animals (Barker and Clark, 1997). Extensive animal studies motivated the identification of the biochemical basis for nutritional programming of fetal development and its long-term health consequences (Wu et al., 2004). IUGR can be initiated by maternal stress, hypoxia, glucocorticoid administration, dietary manipulation or placental insufficiency and leads to postnatal abnormalities in cardiovascular, metabolic, and endocrine function in diverse animals including primates and pigs (McMillen and Robinson, 2005). In addition to effects of fetal retardation due to poor intrauterine maternal nutrition, it is known that constitution and catch up of nutrition from birth till weaning can exert permanent impacts on performance and health of farm animals (Kim et al., 2004; Foote et al., 2007; Khan et al., 2007). However, it is not entirely clarified how these immediate measurable effects will influence animal performance, health and well-being, and product quality in the long term.

1.3 Programming and related endocrine metabolites and systems

Many metabolic disorders have an endocrine origin and are accompanied by abnormal hormone concentrations. Hormones have an important role in regulating normal growth and development *in utero*, and their concentrations and bioactivity change in response to many of the environmental challenges known to cause intrauterine programming (Fowden and Forhead, 2004). Associated to the hormonal factors, the insulin-like growth factor system seems of major importance (Davis, 1988; Humbel, 1990; Isaksson et al., 1990). This system is a fundamental part of the somatotropic axis as schematically shown in Figure 1.

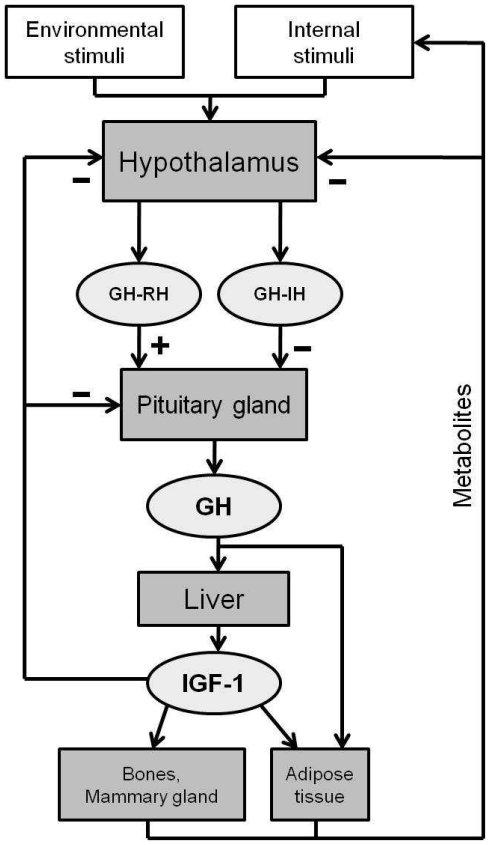

Figure 1: The somatotropic axis: The secretion of growth hormone (GH) from the pituitary is primarily regulated by two hypothalamic neurohormones: a stimulatory hormone, the GH-releasing hormone (GH-RH) and the GH-inhibiting hormone (GH-IH). Different environmental and internal stimuli e.g. physical exertion, fasting, stress, content of nutrients in blood and low plasma glucose concentrations enhance the secretion of GH from the pituitary. The ability of GH to increase the secretion of insulin-like growth factor-1 (IGF-1) from the liver has anabolic consequences on target tissues. Indeed IGF-1 exerts negative feedback control on the secretion of GH by actions both on the pituitary gland and the hypothalamus. In addition to the anabolic growth-stimulating action via IGF-1, GH itself affects lipid metabolism in a catabolic way by stimulating lipolysis in adipose tissue. In states of sufficient nutrient supply the switch from GH to IGF-1 is alleviated whereas in situations of deficient nutrient supply, the stimulatory effect of GH on IGF-1 is compromised, albeit the GH concentrations during fasting are elevated (adapted from Breier and Sauerwein, 1995).

Expression of the insulin-like growth factors 1 and 2 (IGF-1 and IGF-2), IGF binding proteins (IGFBPs) and receptors for the IGFs (IGF1R and IGF2R) is controlled by the nutritional and the hormonal environment *in utero* (Fowden et al., 2005). The concentrations of the IGF proteins in the fetus are positively correlated with birth weight in several species including humans, sheep, rodents and pigs (Daughaday et al., 1982; Gluckman and Butler, 1983; Lee et al., 1993; Ong et al., 2000). Changes in the circulating concentrations of fetal IGFs and IGFBPs may cause alterations in development and growth of various fetal tissues. Thus, if differences in maternal feeding level affect the nutrient supply to the fetus/or the fetal concentration of the IGFs and IGFBPs, overall fetal growth and birth weight will be affected (Rehfeldt et al., 2010). Accordingly, the utilization and partitioning of nutrients are controlled by hormones and growth factors but, contrariwise, the hormonal status can also be influenced by nutrition (Thissen et al., 1994). In the pig, the role and the developmental control of the IGF-system in the fetus is not manifested (Fowden, 2003). Impaired or altered bodily functions or performance in adulthood initiated by inadequate nutrient supply during intra-uterine life suggest the notion of a 'diet memory' inscribing the altered information to be executed at later stages of ontogenesis. For the IGF-system, only few follow-up studies on the effects of intrauterine compromises have been conducted.

Numerous studies in humans and in various animal species have established that intrauterine growth retarded newborns show an increased susceptibility to develop obesity and metabolic syndrome when submitted to high caloric diets in adult life. It has also been shown in several studies that the evolution of leptin, a basic hormone of the lipid metabolism which is mainly produced in adipocytes, is imbalanced in IUGR during late gestation and early postnatal life. Hence it has been suggested that leptin deficiency in IUGR participates in inducing improper programming (Djiane and Attig, 2008). Recent studies showed that plasma leptin concentrations are low in growth-restricted infants at birth (Jaquet et al., 1998). Besides, a negative correlation between body weight at birth and subsequent expression of leptin was shown in pigs (Eckert et al., 2000). In the last decades, several serum proteins and hormones have been strongly related to fetal growth. Albeit the control of growth in the fetus and neonate is complex, the insulin-like growth factors (Giudice et al., 1995; Holmes et al., 1997; Klauwer et al., 1997) and leptin (Harigaya et al., 1997; Ertl et al., 1999) in

particular have been shown to play important roles in mediating fetal and postnatal growth and development as well as being related to nutritional status (Lo et al., 2002). The IGF-system and leptin seem to be reciprocally associated. At the central level, leptin stimulates the secretion of GH; in consequence, GH also appears to have a negative feedback loop with leptin (Quintela et al., 1997; Cocchi et al., 1999). Studies performed *in vivo* in rats and in pigs have clearly demonstrated this stimulatory effect (Tannenbaum et al., 1998; Ramsay et al., 2004). Interestingly, as the IGF-system is known to be deficient in the case of IUGR (Woodall et al., 1996), it can be speculated that leptin's actions on body and organ growth are mediated by stimulation of the IGF-system. Taken together, the growth-promoting effects of leptin appear to be complex, involving at least central and peripheral actions (Djiane and Attig, 2008). Production and excretion of leptin in adipocytes are probably maintained through direct (autocrine and/or paracrine) negative feedback signals (Zhang et al., 1997), as well as by other hormones, many of which are also regulated by leptin (Reidy and Weber, 2000). A major outcome of GH stimulation is the increased production of IGF-I, and several anabolic actions of GH are mediated through IGF-1 (Heiman et al., 1999). Changes in circulating IGF-1 concentrations are positively correlated with changes in blood leptin levels (Heiman et al., 1999). Leptin has a key role in energy homeostasis and there may be a link between leptin and the IGF-system. Experimental models have provided evidence of leptin functioning as a neuromodulator of the GH-IGF axis by connecting this hormonal system with nutritional status (LaPaglia et al., 1998).

1.3.1 The IGF-System

Insulin-like growth factors (IGF-1 and IGF-2) are single chain polypeptides, highly homologous with low molecular weight (7.6 and 7.5 kDa, respectively) that are structurally similar to pro-insulin. Unlike insulin, they are produced by most tissues of the body and are abundant in the circulation. IGF-1 and IGF-2 both act as mitogens and are also involved in differentiation and apoptosis of a multitude of cell types (Cohick and Clemmons, 1993). They regulate cell proliferation in an interconnected action via autocrine, paracrine and endocrine regulatory mechanisms (Pavelic et al., 2007). Complementary to their effects on cell proliferation, the IGFs can also inhibit cell death (Jones and Clemmons, 1995). Their effects are mediated through the IGF-

1 receptor (IGF-1R) which initiates signaling cascades that result in regulation of a number of biological responses (Figure 2).

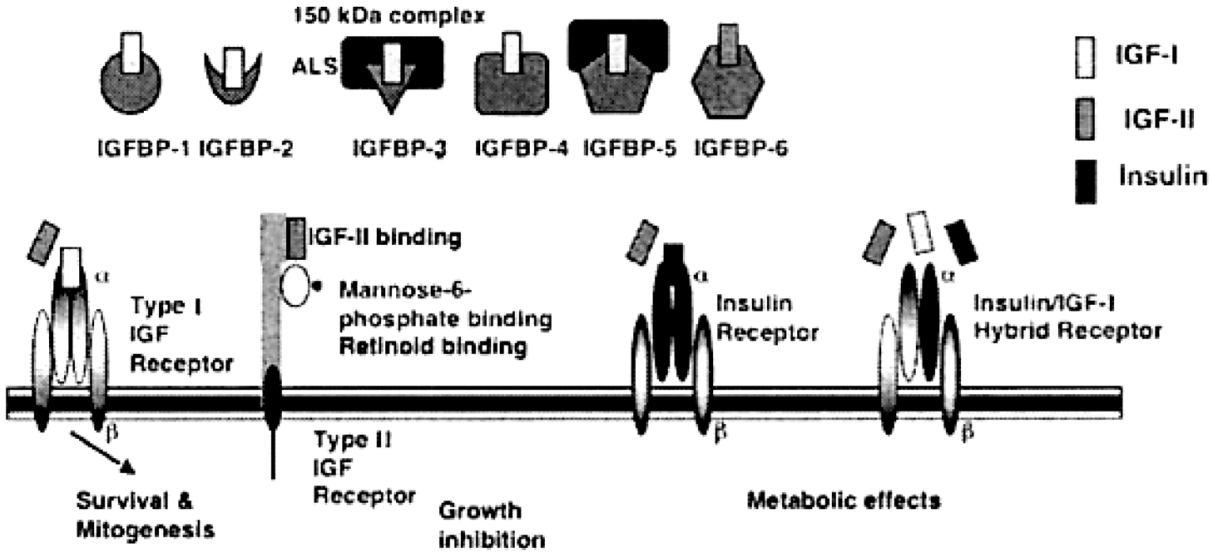

Figure 2: The IGF-System with the Insulin-like growth factors (IGF-1 and IGF-2), their receptors type-1 (IGF-1R) and type 2 (IGF-2R) and their binding proteins (IGFBPs 1-6). ALS= acid labile subunit (modified from Randhawa and Cohen, 2005)

The IGF-1R is very similar to the insulin receptor (IR). They are both classic membrane-bound tyrosine kinase receptors. Structurally, they are heterotetramers, consisting of two identical extracellular α subunits (which bind to the ligand) and two transmembrane β subunits (which trigger an intracellular kinase signaling cascade in response to ligand binding) (Jones and Clemmons, 1995; Pollak et al., 2004). Hybrid receptors formed out of IGF-1R and IR can bind IGF-1, IGF-2 and insulin with differing affinity (Jones and Clemmons, 1995). There is a high similarity in structure between the IR and IGF-1R, and between insulin and the IGFs, which allows for low-affinity binding of IGFs to IR and similar for low-affinity cross-binding of insulin to IGF-1R. The structurally dissimilar type-2 IGF Receptor also known as mannose-6-phosphate receptor (IGF-2R/M6PR) is involved in binding, initialization and degradation of IGF-2 (Pavelic et al., 2007). IGF-2R has no known intracellular kinase signaling domain (Kelley et al., 2002) and achieves no known IGF signaling function (Jones and Clemmons, 1995). The IGFs present in the circulation and throughout the extracellular space are almost entirely bound to members of a family of high affinity IGF binding proteins (IGFBPs). There are six of these binding proteins (IGFBP 1-6)

that specifically bind and modulate the mitogenic/metabolic actions of the IGFs. Four of the IGFBPs (IGFBP 1-4) are found in serum in significant quantities (Zapf et al., 1990). Most of the IGFs in the bloodstream (75-80%) form part of a 150 kDa complex containing IGF-1 or IGF-2, IGFBP-3 and an acid labile subunit (ALS). The remaining 20-25% of IGFs are associated with one of the other IGFBPs in a binary complex. Less than 1% of the IGFs is present in the free form (Gicquel and Le Bouc, 2006). The IGFBPs can have a higher or lower affinity for IGFs than the IGF receptor and, therefore, provide several regulatory functions. These functions include associating IGFs (effectively inhibiting function), prolonging the circulating half life, providing a circulating storage reservoir, and locally concentrating the IGFs, many of which effectively increase the function of the IGFs (Bowman et al., 2010). Stimulatory as well as inhibitory effects of IGFBPs on IGF actions have been described. In addition, IGF-independent effects of several IGFBPs are emerging (Jones and Clemmons 1995). Accumulating evidence indicates important roles for members of the IGFBP family in metabolic homeostasis as recently reviewed by Wheatcroft and Kearney (2009).

1.3.1.1 Interrelation between IGF-1 and nutrition

Under common physiological conditions, the main regulatory factor influencing the synthesis and secretion of IGF-1 is GH (Figure 1) (Buonomo and Baile, 1991). The stimulatory effect of GH on serum concentrations of IGF-1 is mediated by direct effects on IGF-1 synthesis, and through the synthesis of the components of the large 150 kDa ternary complex of IGF-1 and IGFBP-3 with the acid labile subunit (Thissen et al., 1994). Insulin-like growth factor 1 is an endocrine factor, mainly secreted from the liver in response to GH stimulation. It mediates the effects of GH in peripheral tissues (Breier, 1999). The rate of IGF-1 synthesis in tissue and its secretion into the systemic circulation is dependent not only upon pituitary GH but also upon nutrient availability and metabolic hormone status (Buonomo and Baile, 1991; Clemmons and Underwood, 1991). Control of IGF synthesis and secretion therefore may provide an intermediate signaling mechanism that designates to target cells that adequate nutrients have been ingested and are available for cell division and protein synthesis (Clemmons and Underwood, 1991). Acute feed restriction, general nutrient deficiency and periods of insufficient protein intake cause a decline of basal circulating

concentrations of IGF-1 due to reduced IGF-1 gene expression (Buonomo and Baile, 1991; Thissen et al., 1994; Breier, 1999). This explains for the dependency of IGF-1 plasma concentrations of nutritional status (Breier et al., 1986). The IGFs appear to provide an important mechanism linking nutrition and growth (Thissen et al., 1994). To understand the role of the IGFs in mediating the effects of nutrient intake on anabolic responses, it is important to realize that IGFs may function as autocrine/paracrine growth factors as well as classic endocrine hormones (Clemmons and Underwood, 1991). The observation that the IGFs have the potential to fulfill an important role as mediators of tissue anabolism and stimulate both amino acid transport and protein synthesis in skeletal tissue, supports the concept of nutrient-dependent growth signaling (Clemmons and Underwood, 1991). The availability of dietary protein is one main critical regulator of IGF-1. Dietary protein supply seems to be the limiting factor for maximal stimulation of IGF-1 plasma concentration (Breier, 1999). At early stages of development, serum IGF-1 concentrations are critically dependent on dietary protein supply, however this protein dependence decreases with age (Fliesen et al., 1989). Protein deficiency lowers serum IGF-1 concentrations in response to GH and also produces refractoriness to IGF-1 action (Clemmons and Underwood, 1991). Interestingly, protein-deficient rats infused with IGF-1 increased their serum IGFBP-3 concentrations, suggesting that some actions of IGF-1 were not impaired (Hirschberg and Kopple, 1989).

Nutritional status as well as supply of dietary energy and protein is a critical regulator of IGFs and IGFBPs. Diet restriction increases the clearance and degradation of serum IGF-1 through changes in the levels of circulating IGFBPs. More recent observations demonstrate that nutrients may also control the biological action of IGF-1 either directly or indirectly through changes in IGFBPs (Thissen et al., 1994).

1.3.1.2 Interrelation between IGF-2 and nutrition

Both IGF-1 and IGF-2 are expressed in fetal tissues from the earliest stage of pre-implantation to the final phase of tissue maturation before birth. IGF-2 is the primary growth factor supporting embryonic growth, with raising importance of IGF-1 later in gestation (Gicquel and Le Bouc, 2006). IGF-2 is a polypeptide which exhibits 62% homology with IGF-1 and 41% homology with insulin (Rinderknecht and Humbel, 1978). Dependent on the species, serum IGF-2 concentrations are much higher than

IGF-1 concentrations in late gestation (Fowden, 2003). In rats and sheep, IGF-2 levels are high during fetal life and decrease immediately after birth (Moses et al., 1980; Mesiano et al., 1989), whereas in humans, IGF-2 levels increase after birth (Zapf et al., 1981). It is unclear why in spite of evolutionary conservation of this peptide, developmental patterns of serum IGF-2 levels are widely variable among mammalian species (Lee et al., 1991). Research in the area of IGF-2 has concentrated on its role as a fetal growth factor (Moses et al., 1980; Daughaday et al., 1982; Gluckman and Butler, 1983). However, in adult humans serum IGF-2 levels are at least four-fold higher than IGF-1 (Zapf et al., 1980) indicating that IGF-2 may be involved in more than fetal development. In pigs, the preponderance of IGF-2 over IGF-1 in fetal serum and the postnatal increases in serum levels of both IGFs also support the view that IGF-2 is both a fetal and a postnatal growth factor, whereas IGF-1 may be primarily a postnatal growth factor in pigs (Lee et al., 1991). For this reason both IGF-1 and IGF-2 play important but different roles in growth regulation (Owens et al., 1999). Like IGF-1, IGF-2 has a variety of metabolic and growth-promoting actions in vitro that are mediated through IGF-1R (Rechler and Nissley, 1985; Humbel, 1990; Sara and Hall, 1990). Post natum, IGF-2 is cleared via preferential binding to the type-2 receptor (Nielsen, 1992).

Some of the metabolic, growth-promoting and through nutrition initiated actions of IGF-2 are described in the following. During fetal development, IGF-2 stimulates myoblast proliferation and differentiation in a dose dependant manner, as well as IGF-1 does (Florini et al., 1986). Observational research of Owens et al. (1999) suggests that androgens and estrogens may postnataly suppress circulating IGF-2 concentrations in pigs, because the concentrations of plasma IGF-2 were similar in boars and in gilts but were higher in barrows than in intact animals. In addition, Owens et al. (1999) determined a positive association between IGF-2 and backfat depth in pigs. Moreover, plasma IGF-2 concentrations were increased by genetic selection for increased backfat depth in pigs (Buonomo and Klindt, 1993). These findings indicate that IGF-2 may regulate fat deposition (Owens et al., 1999). Lee et al. (1993) hypothesized that adipose growth might be promoted by IGF-2 originating from either liver, muscle, lung, or kidney and indeed, Owens et al. (1999) verified evidence that IGF-2 might be produced by fat tissue, which would result in higher IGF-2 concentrations in plasma from animals with more fat. Nutrient restriction as

modulator has more pronounced effects on circulating concentrations of IGF-1 than IGF-2, irrespective of the cause or nature of the nutrient deficit (Fowden, 2003). This was independently approved by Gluckman and Pinal (2003). They asserted that the *IGF1* gene transcription is more sensitive to changes in nutritional status in late gestation than the *IGF2* gene (Gluckman and Pinal, 2003). Moreover, serum IGF-2 concentrations are reduced in humans following nutritional deprivation (DeHoff et al., 1986; Buonomo et al., 1987). Following an acute fast, IGF-2 plasma concentrations were depressed in mature swine (Buonomo et al., 1988). Changes to maternal protein intake during the first and the second trimester of gestation affected IGF-2 protein and IGF-2 mRNA expression and altered fetal and hence postnatal muscle development (Micke et al., 2011). However, most studies have found no association between serum IGF-2 concentrations and fetal weight (Fowden, 2003).

In conclusion, the role of IGF-2 is not completely understood. Possibly, IGF-2 modulates nutritional effects indirectly. Insulin-like growth factor-2 has the structural requirements that are necessary to stimulate growth. Its concentrations in plasma are much less dependent on GH than are the IGF-1 concentrations, and its role in mediating changes in the anabolic response to nutrient intake is unknown (Clemmons and Underwood, 1991).

1.3.1.3 IGFBPs and nutritional regulation

The insulin-like growth factor binding proteins are soluble carrier proteins that circulate in blood and extracellular fluids and appear to control IGF transport, efflux from the vascular compartment, and association of IGF with cell surface receptors (Martin and Baxter, 1992). All IGFBPs have cysteine-rich regions which are highly conserved and correspond to the IGF binding site. Some IGFBPs, for example IGFBP-3, are extensively glycosilated, whereas others are not. The IGFBPs vary considerable in size, glycosylation and phosphorylation states, and protease sensitivity (Jones and Clemmons, 1995; Hwa et al., 1999). Each of the IGFBPs could have an exclusive role as modulator of IGF action and the different IGFBPs seem to be under independent endocrine and metabolic regulation. Changes in the production of IGFBPs may initiate tissue-specific targeting and distribution of IGFs. Suggesting that they have different, regulated functions in the development of specific tissues, each IGFBP has unique tissue-specific and developmental stage-

specific patterns of expression (Jones and Clemmons, 1995; Hwa et al., 1999). In addition, either systemic or locally produced IGFBPs alter the distribution of circulating IGFs within target tissues by modifying receptor-IGF interactions (Breier, 1999).

The IGFBPs bind to IGF-1 or IGF-2 with high but different affinity: IGFBP-2 has a threefold greater affinity for IGF-2. However, IGFBP-1 binds IGF-1 and -2 with approximately equal affinity (Rajaram et al., 1997). IGFBP-3 is a glycoprotein associating with ALS in serum and with IGFs to form a 150 kDa complex. This complex apparently prevents rapid efflux of IGF-1 and -2 from the vascular compartment and stabilizes the concentrations of IGFs in blood. When associated with the 150 kDa complex, the IGFs do not readily leave the vascular compartment, and their half lives are prolonged to 12-15 h (Guler et al., 1989). This contrasts with the half life of free binding proteins in plasma, which has been estimated to be between 30 and 90 minutes (Zapf et al., 1986) and the half life of free IGF-1, which is estimated at less than 10 min, similar to insulin (Hodgkinson et al., 1989). The primary function of IGFBP-3 may be to act as a carrier of IGFs in the circulation, whereas the primary functions of IGFBP-1 and IGFBP-2 may be to transport IGF across the endothelial surface to specific cell types (Bar et al., 1990). In contrast to IGFBP-1, -2 and -3, IGFBP-4 appears to inhibit IGF actions under most, if not all, experimental conditions. It binds both IGFs with equal affinity and does not appear to adhere to cell surfaces (Jones and Clemmons, 1995). Its main function is to protect cells from overstimulation by IGFs or to allow activation of alternate transmembrane signaling pathways that are inhibited by IGF exposure (Jones and Clemmons, 1995). The amount of IGFBP-5 and -6 in rats or human blood is extremely low and is unlikely to be of physiological significance. Both IGFBPs prefer to bind IGF-2 in comparison to IGF-1 (Rajaram et al., 1997). Similar to IGFBP-3, the cell surface association of IGFBP-5 may also mediate its ability to potentiate IGF actions. The lowering of IGF binding affinity, by association to the extracellular matrix or cell surface association is an important factor in regulating the ability of IGFBP-5 to bind IGF-2 (Jones and Clemmons, 1995). Studies of IGFBP-6 are limited.

The IGFBPs 1 to 4 are the most prevalent IGFBPs in fetal plasma and tissue, although their relative assemblage varies both within and between species. Fetal expression of these IGFBPs is also tissue specific and developmentally regulated

(Donovan et al., 1989; Lee et al., 1993; Carr et al., 1995; Tarantal and Gargosky, 1995). IGFBP expression in the fetus is affected by both the nutritional and endocrine conditions *in utero*. Generally, these conditions have more obvious effects on IGFBP-1, -2 and -4 than on IGFBP-3 (Fowden, 2003).

If the binding proteins serve different functions, it is reasonable to presume that regulation of their secretion may be different. For example, GH and IGF-1 both induce the synthesis and secretion of IGFBP-3, and increase its concentration in plasma (Clemmons et al., 1989). In contrast, IGFBP-1 and -2 are both suppressed by GH (Hardouin et al., 1989), and IGF-1 directly stimulates the secretion of IGFBP-2, whereas it often inhibits IGFBP-1 (Thraikill et al., 1990).

A major regulator of circulating IGFBPs is nutrient intake. Significant reduction in serum IGFBP-3 concentrations is caused through extended fasting and/or protein deficiency (Clemmons et al., 1989), whereas acute changes in nutrient intake do not affect IGFBP3. Its plasma concentrations remain stable throughout the day (Baxter and Martin, 1989). The fact that IGFBP-3 is the major carrier of IGF-1 probably accounts for the stability of IGF-1 in blood (Clemmons and Underwood, 1991).

The growth factors IGF-1 and IGF-2 and their binding proteins appear to be major links between nutrient intake and cellular anabolic response (Clemmons and Underwood, 1991). It is documented that nutrition can markedly affect the relative amounts and ratio of circulating IGF-1 and IGF-2. Such changes would be predicted to influence the serum half lives of the IGFs, the relative partitioning of IGF, IGFBP and IGF: IGFBP complexes between the bloodstream and extra vascular and cellular compartments. In addition, the bioavailability and bioactivity of circulating as well as of locally synthesized IGF and IGFBP would be modified. These alterations may affect overall somatic growth as well as the mitotic activity and differentiation state of target cells in a tissue specific fashion (Simmen et al., 1998).

1.3.1.4 Functions of the IGF-1 and -2 receptors

The biological effects of IGF-1 and IGF-2 on a target cell are mediated by two types of cell surface receptors: IGF receptor type 1 (IGF-1R) and IGF receptor type 2 (IGF-2R), as well as through binding to receptors for insulin (Pavelic et al., 2007). On most cell types IGF-1R and IGF-2R coexist and, on many cells, insulin receptors are also present (Humbel, 1990).

In general, most of the actions of the IGFs are forwarded via the IGF-1R (Pollak et al., 2004) which belongs to the tyrosine-kinase class of growth factor receptors, as well as the insulin receptor. The IGF-1R is a glycosylated heterotetramer, consisting of two α- (ligand binding) subunits (135 kDa), and two transmembrane β-subunits (90 kDa) linked by disulfide bonds, which contain a tyrosine kinase domain activating the receptor by autophosphorylation (Kasuga et al., 1981; Massagué and Czech, 1982). Ligand binding (IGF-1 or IGF-2) to the extracellular part of IGF - 1R instigates cytoplasmatic signal cascades that contain receptor conformational changes which enables them to bind adenosine-5'-triphosphate (ATP) and become autophosphorylated within the β-subunits, resulting in activation of the intrinsic tyrosine kinase activity of IGF-1R and subsequent tyrosine phosphorylation of several substrates. This stimulates downstream signaling through intracellular networks that regulate cell proliferation and survival. The activation of the intracellular networks results in a variety of responses, such as cell proliferation, differentiation, migration and protection from apoptosis (Pavelic et al., 2007).

Recent data have shown that both the insulin and the IGF-1R can mediate metabolic and mitogenic responses to either ligand (Humbel, 1990). In most cells there are different relative binding affinities for insulin receptor: insulin >> IGF-2 > IGF-1 and IGF-1R: IGF-1 > IGF-2 >> insulin (Humbel, 1990). The physiological roles of both insulin and IGF-1 are therefore likely to be determined by the distribution and abundance of their receptors on the cell surface (Cohick and Clemmons, 1993). The fact that insulin at supraphysiological concentrations is mitogenic and that IGF at supraphysiological concentrations shows insulin-like metabolic effects indicates that metabolic effects are mediated via the insulin receptor and mitogenic effects via the IGF-1R, whereas the biological significance of the IGF-2R remains unclear (King et al., 1980).

The IGF-2R is also known as cation-independent mannose-6-phosphate receptor (IGF-2R/M-6-PR) and is structurally as well as functionally different from the IGF-1R. The receptor is a monomeric membrane spanning glycoprotein of 250 kDa, with a large extracellular domain without autokinase activity (Kasuga et al., 1981; Massagué and Czech, 1982), which binds M-6-R, lysosomal enzymes and IGF-2 (Kornfeld, 1992). Finally, the actions of the IGF-2R in IGF-2 signaling are less clear, while it is known that the receptor prefers to bind IGF-2 in comparison to IGF-1 (insulin does

not bind) and that IGF-2R can clearly mediate uptake and degradation of IGF-2 (Humbel, 1990; Jones and Clemmons, 1995). The IGF-2R is present in fetal tissues throughout gestation and seems to be developmentally regulated during bovine fetal life (Pfuender et al., 1995).

Some tissues and cell types express receptors composed of one IGF-1 receptor alpha and beta subunit and one insulin receptor alpha and beta subunit, defined as IGF-1 insulin receptor hybrids (Cohick and Clemmons, 1993). The ligand specificity profiles of these receptors are more comparable to the IGF-1 receptor than to the insulin receptor (Jones and Clemmons, 1995) which leads to a higher affinity for IGF-1 than insulin. Hence, to induce a conformational change in the receptor and to activate autophosphorylation, IGF-1 is more effective than insulin (Moxham et al., 1989; Soos et al., 1990). For most systems, the amounts to which hybrid receptors constitute the total receptor population has not been defined. However, there are some tissues, where 70% of receptors are estimated to be hybrids, e.g. the placenta (Soos and Siddle, 1989). This suggests that in some tissues these receptors may play an important role in coordinating IGF action.

1.3.2 Leptin and its functions of a nutrient sensor

Leptin was the first adipokine identified, i.e. it was the first signaling molecule found to be secreted from white adipose tissue (Zhang et al., 1994). Since then several other cytokines and hormones have been classified as adipokines as recently reviewed by Deng and Scherer (2010). Leptin (from the greek *leptos*, meaning thin) is a 16 kDa polypeptide circulating as a 146 amino acid peptide in serum. This hormone is primarily produced by adipose tissue as a four-helix bundle cytokine. The rate of leptin secretion and its plasma concentration are positively correlated with total fat mass (Hamilton et al., 1995; Houseknecht and Portocarrero, 1998; Wajchenberg, 2000; Fain et al., 2004). Thus leptin circulates as an internal signal indicating the size of body fat stores (Reidy and Weber, 2000). It is a circulating endocrine hormone, capable of communicating information from the periphery to the central nervous system (CNS) (Coleman, 1973) by acting through numerous isoforms of its receptor. Leptin receptors (LRs) are members of the cytokine receptors class I superfamily and are expressed in both the CNS and the periphery (Bjorbaek and Kahn, 2004). The LR family comprises at least 6 receptor isoforms

that arise due to alternative splicing and are found in different tissues throughout the body with different levels of expression (Reidy and Weber, 2000). The isoforms include a long form receptor and several short forms (Tartaglia, 1997). The effects at the hypothalamic level are mediated through the long form of the LR which is predominantly expressed there and exerts a pivotal role in the regulation of food intake. The short form of the LR is the dominant form in the peripheral organs (e.g. ovary, testis, prostate and placenta etc.) and its biological effects concern the modulation of cell proliferation and cell differentiation in adipose tissue, pancreas, stomach, liver, kidney, arteries and immune cells (Margetic et al., 2002; Myers, 2004). The discovery of expression of several functional leptin receptors isoforms in CNS as well as in peripheral tissues indicates a role of leptin in an impressive list of biological functions as for example in lipid and energy metabolism (Tartaglia et al., 1995), nutrient partitioning, food intake and appetite regulation, immunologic processes as well as reproduction and growth (Cunningham et al., 1999; Barb et al., 2001) which are summarized in Figure 3.

Recent studies focused on the fundamental role of leptin as a `lipostat` in regulating body weight. Leptin thus plays a major role in energy balance and lipid metabolism (Reidy and Weber, 2000). It is able to mediate lipid metabolism by central and peripheral ways with many different routes. Leptin regulates lipid storage in adipocytes as well as in skeletal muscle, liver and pancreas and adjusts lipogenesis and fatty acid oxidation (Houseknecht and Spurlock, 2003). The effects of leptin result in decreased triacylglycerol synthesis and increased lipolytic rates (Reidy and Weber, 2000). Leptin also mediates lipid metabolism indirectly by reducing the lipogenic effects of insulin, probably due to the inhibitory effect leptin exerts on the binding of insulin to adipocytes (Walder et al., 1997). Insulin has the exact opposite effect in comparison to leptin, i.e., it is depressing fatty acid oxidation and increasing triacylglycerol synthesis (Muoio et al., 1997). Interestingly, in addition to the inhibition of lipogenesis by leptin, there is reported evidence supporting a role for leptin in the inhibition of adipose cell proliferation (Thomas et al., 1999). In addition leptin seems to reduce adipose tissue mass not only by affecting feed intake via the central nervous system but also by inducing adipocyte apoptosis (Reidy and Weber, 2000).

Besides adipose tissue, the mammary gland is able to produce leptin, particularly during the early phase of lactation. In addition, the mammary gland is involved in the

transport of leptin from the mother to the milk which is in fact an additional source of leptin for the newborn (Uysal et al., 2002).

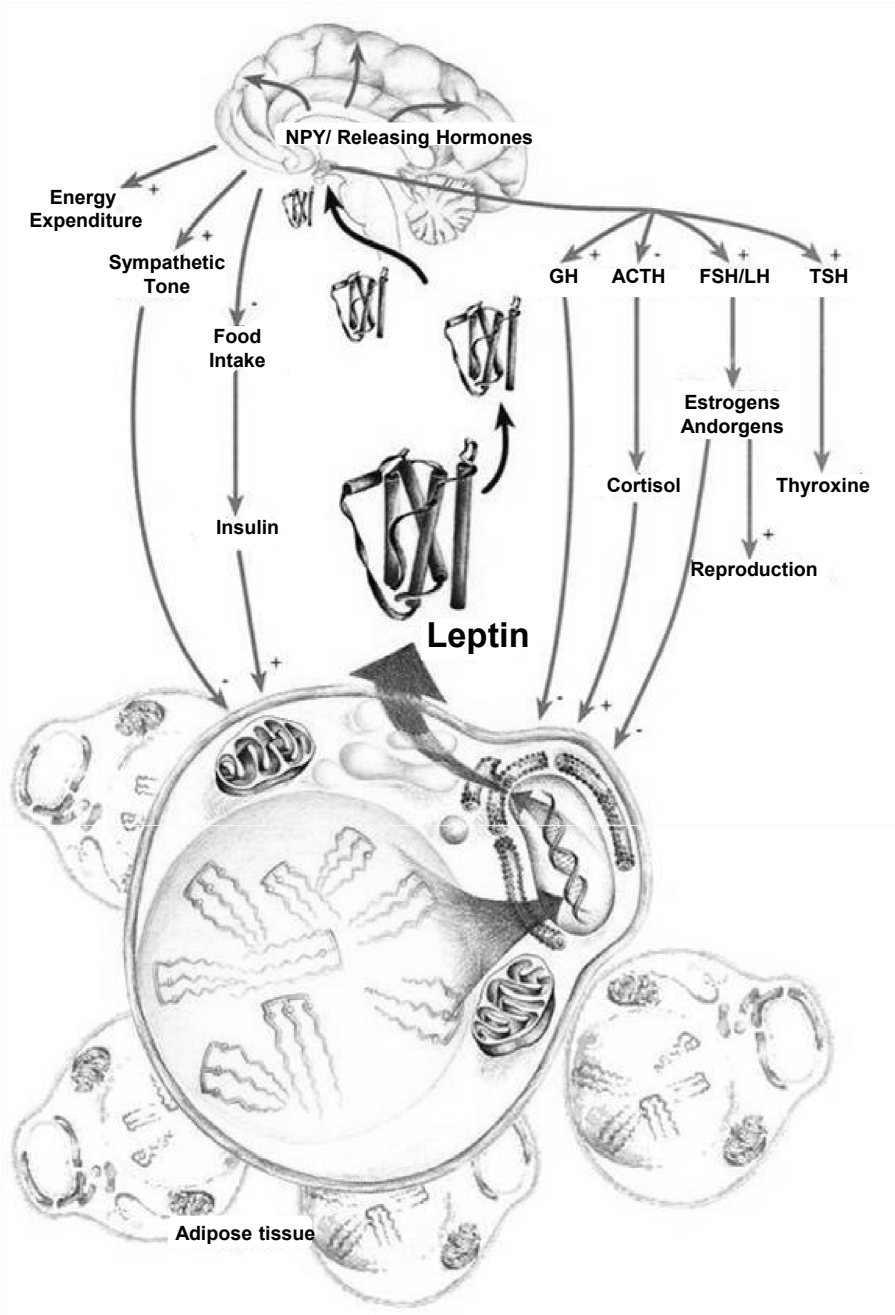

Figure 3: Schematic drawing of leptin coordinating energy homeostasis and neuroendocrine function. Leptin is secreted by adipose tissue and acts on the hypothalamus to control food intake, thermogenesis and insulin action by regulating expression and secretion of multiple neurotransmitters, neuropeptides and hypothalamic hormones including neuropeptide-Y (NPY) and different releasing hormones. In addition, leptin increases energy expenditure and the sympathetic tone as well as it modulates pituitary luteinizing hormone (LH), follicle stimulating hormone (FSH), growth hormone (GH), adrenocorticotropic hormone (ACTH) and thyroid stimulating hormone (TSH; modified from mediagnost).

In early postnatal life as well as prenatal life, leptin has essential functions as an endocrine modulator. One role proposed for leptin *in utero* is the partitioning of nutrients during the development of the feto-placental unit (Thomas et al., 2001). Furthermore, in animals such as the pig, in which fat is deposited before birth, leptin is synthesized in fetal adipose tissue and is present in the fetal circulation throughout late gestation (McMillen et al., 2004). Before birth, in the prenatal environment, leptin also may act as a signal of energy supply and has a 'lipostatic' role (McMillen et al., 2004). As leptin has been implicated as a major sensor of energy and nutrient balance it is not surprising that the leptin axis is regulated by nutritional status (Houseknecht and Spurlock, 2003). In conjunction with nutritional status, leptin has also been implicated in nutritional programming during fetal and neonatal growth with perhaps long-term effects on the susceptibility to obesity and other long time consequences as for example diabetes (Eckert et al., 2000; Houseknecht and Spurlock, 2003). Different investigators kept track on the fact that in most obese mammals circulating leptin concentrations are high, indicating that the organism is resistant to the effects of leptin to inhibit feeding and increase metabolic rate. Based on these observations, the notion of 'leptin resistance' emerged (Houseknecht and Spurlock, 2003). The mechanisms of leptin resistance are only incompletely elucidated (Bjorbaek and Kahn, 2004). One hypothesis suggests that leptin resistance may in fact be programmed during fetal and neonatal life and may result in altered development of neuronal circuitry involved in food intake regulation (Djiane and Attig, 2008).

In addition to its effects on energy homeostasis, leptin regulates neuroendocrine function and traditional endocrine systems (Kershaw and Flier, 2004) including the regulation of immune function, hematopoiesis, angiogenesis, and bone development. Leptin normalizes the suppressed immune functions associated with malnutrition and leptin deficiency (Lord et al., 1998). Leptin also promotes proliferation and differentiation of hematopoietic cells, it alters cytokine production by immune cells, stimulates endothelial cell growth and angiogenesis, and accelerates wound healing (Margetic et al., 2002).

2 Objectives

The present study was undertaken to evaluate the effects of maternal protein deficit and excess throughout gestation on the development of the offspring at different ontogenetic stages. The investigation was focused on potential effects of the different maternal diets on the endocrine system, in particular the IGF-system that is known to play an important role in linking nutrition and growth. Deviant deflections of this system might have long term consequences for the offspring e.g. impaired growth and body composition. In this context, blood and liver samples of fetuses (dpc 94), newborn piglets (dpn 1), weaning piglets (dpn 28) and pigs at market weight (dpn 188) were used to assess several functional components of the IGF-system and to relate these with body growth data. Specifically the following tasks were defined:

- The ligands, i.e. IGF-1 and -2 were quantified in blood plasma samples using commercially available Enzyme-linked Immunosorbent Assays (ELISA) for the human proteins after securing their applicability for porcine samples.
- As the main target receptor, IGF-1 receptor was measured in liver tissue with a commercially available ELISA for human IGF-1 receptor that was also precedingly tested for its applicability in porcine liver samples.
- In view of the important role of the IGF binding proteins within the IGF-system, a semi quantitative nonradioactive Western ligand blot was established and validated to assess their abundance in the plasma samples.
- For leptin, an adipose derived hormone with known interrelation to the IGF-system, the concentrations were determined by an in-house developed ELISA after extensive validation for porcine plasma.
- Identification of factors affecting the respective component of the IGF-system, as well as characterization of relationships between the different target endocrine components.
- Evaluation of potential long-term consequences of maternal diet, endocrine deflections and body growth patterns.

3 Material and Methods

3.1 Animals and treatments

All animal experiments were performed at the Leibniz Institute for Farm Animal Biology (FBN) in Dummerstorf. They were in strict accordance with the German animal protection law and were approved by the relevant authorities (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei, Mecklenburg-Vorpommern, Germany). The samples collected as described below were made available within the scope of the joint project “FeproExpress” (coordinator: Dr. C. Metges, Research Unit Nutritional Physiology, FBN) funded by the German Federal Ministry of Education and Research (BMBF) through the program “FUGATO” **(**Functional Genome Analysis in Animal Organisms).

Primiparous German Landrace gilts (n=78) were randomly allocated to 3 different feeding groups, receiving isoenergetic diets (~15.4 MJ ME/kg DM) with either low (LP, 6 %; n=24), adequate (AP, 12 %; n=26), or high (HP, 30 %; n=28) crude protein contents corresponding to a protein:carbohydrate ratio of 1:10.4, 1:4.8 and 1:1.3, respectively, from mating until farrowing as described by Rehfeldt et al. (2011a). The offspring of these gilts was sampled as shown in Figure 4.

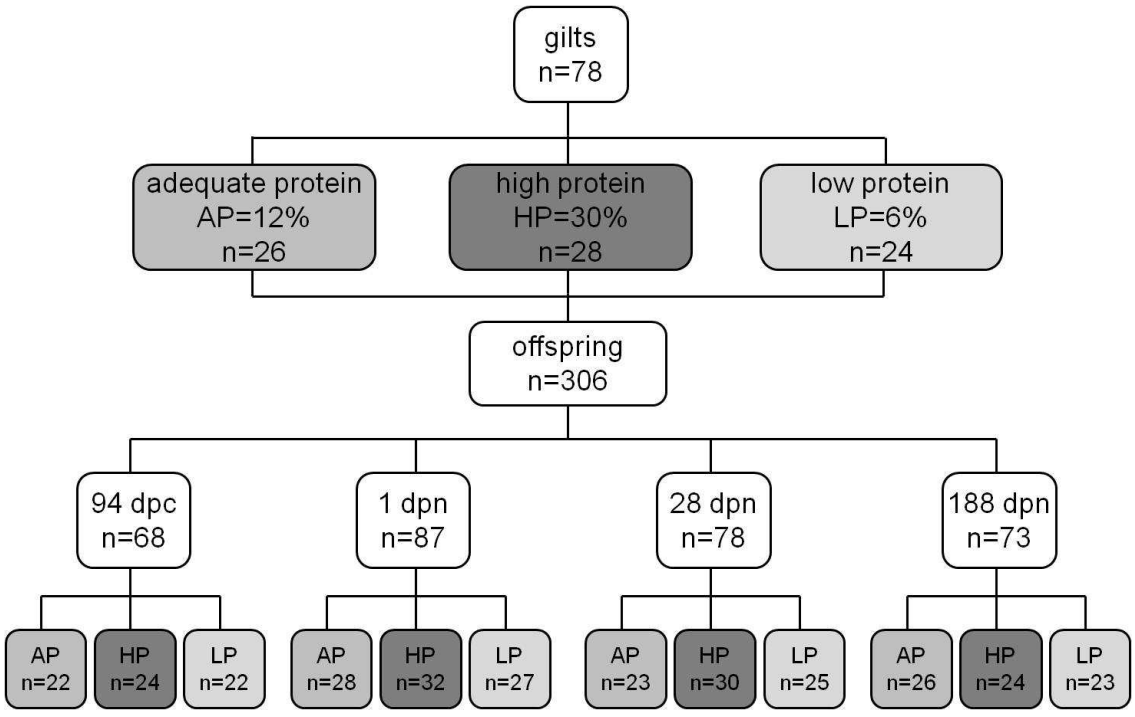

Figure 4: Diagram showing the experimental design and the sampling days.

For the samplings on day 94 of gestation, 26 sows from the 3 different feeding groups (n = 7 to 8 each) were subjected to caesarian section and the fetuses were weighed and euthanized for collecting tissue and blood samples (n=68; AP=22, LP=22, HP=24). For sampling at later postnatal stages, the litter sizes and piglet birth weights were recorded from the remaining 52 gilts and the piglets were then cross fostered within 48 h after birth to nursing sows (2^{nd} to 4^{th} parity). These nursing sows were initially fed a standard gestation diet (11.4 MJ ME/kg, 12.6% crude protein) and then a standard lactation diet (~ 13.0 MJ ME/kg, 15.5% crude protein). Male piglets were castrated at 4 days of age. After weaning at day 28 of age, all piglets received standard fattening diets until slaughter (dpn 188) according to the recommendations of the Society for Nutrition Physiology (GfE, 2006). Tissue and blood samples were collected from the piglets on day 1 post natum (dpn 1; i.e. within 36 h after birth; 4 piglets/litter n = 87; AP: n = 28, LP: n = 27, HP: n = 32), on dpn 28 prior to weaning (2 piglets per litter, n = 78; AP: n = 23, LP: n = 25, HP: n = 30), and on dpn 188 prior to slaughter (2 piglets/litter; n = 73; AP: n = 26, LP: n = 23, HP: n = 24). For the sample collection on dpn 1 and 28, the piglets were euthanized as described earlier (Rehfeldt et al., 2011a); for dpn 188, the animals underwent the routine slaughter procedure.

3.2 Sample collections and analyses

All tissue samples were taken as soon as possible after death. For the present thesis, the focus was on blood and liver samples. Blood was collected from the euthanized fetuses and neonates from *Vena cava cranialis*. In later stages, dpn 28 and 188, blood samples were withdrawn by venipuncture of jugular vein. Liver samples were immediately snap frozen in liqid nitrogen and then stored at -80°C until analysis. Body composition data were assessed and kindly made available from the cooperating partner institute, i.e. by Dr. C. Rehfeld. Research Unit Muscle Biology and Growth, FBN Dummerstorf.

In blood serum, the concentrations of leptin, IGF-1 and IGF-2 were measured by enzyme-linked immune sorbent assays (ELISA). For assessing the IGF binding proteins (IGFBPs), a non-radioactive Western ligand blotting protocol was developed and applied for quantitative comparisons. In liver, IGF-1 receptor was assessed via ELISA. All assay procedures are described below.

3.2.1 ELISA for IGF-1

IGF-1 in plasma was measured by a commercially available Sandwich-ELISA (IGF-1 E-20, Mediagnost®, Reutlingen, Germany). In order to dissociate IGF-1 from the IGFBPs, the samples were diluted in acidic buffer. After neutralization of the samples in the wells, an excess of IGF-2 was used to occupy the IGF-binding sites of the binding proteins. Thereby potential interferences of IGFBPs in the assay can be circumvented, thus allowing for assessment of free IGF-1. Due to the negligible cross-reactivity of the IGF-1 antibody with IGF-2, excess IGF-2 does not disturb the interaction of the first antibody with IGF-1. The commercial assay was developed for use in human samples, however, due to the complete homology of the porcine and the human IGF-1 protein (Bayne et al., 1990), the assay can be used for porcine plasma. In confirmation of this, parallelism of serial dilutions of porcine plasma with the standard curve was demonstrated. The limit of detection was 0.09 ng/mL and the intra- and inter-assay coefficients of variation were 6.8% and 6.7%, respectively.

3.2.2 ELISA for IGF-2

Plasma IGF-2 was measured by a commercial sandwich-ELISA (IGF-2 E30, Mediagnost®, Reutlingen, Germany). The assay was also developed for use in human samples, however, due to the complete homology of the porcine and the human IGF-2 protein (Bayne et al., 1990), the assay can be used for porcine plasma. Similarly as for the IGF-1 assay, the samples were diluted in acidic buffer and the thereby released IGFBPs were blocked by an excess of IGF-1. Due to the negligible cross-reactivity of the IGF-2 antibody with IGF-1, excess IGF-1 does not interfere in the immune reaction. Free IGF-2 was detected with an analytical sensitivity of 0.02 ng/mL and the intra- and inter-assay coefficients of variations were 6.6% and 7.2%, respectively. As for the IGF-1 assay, linearity of serial dilutions of porcine plasma samples was demonstrated thus confirming the applicability of the assay designed for human samples for porcine ones.

3.2.3 Western ligand blots for the IGF-binding proteins

The development of the technique of Western ligand blotting by Hossenlopp et al. (1986) provided a means for separating the various IGFBPs and determining their concentrations semiquantitatively. The IGFBPs in the biologic fluid of interest are separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-

PAGE) and are then transferred to sheets of nitrocellulose. The nitrocellulose sheets are incubated with ^{125}I labeled IGF-1, which serves as a probe for the immobilized IGFBPs. The density of the bands can then be readily determined with use of a densitometer. The sensitivity of this method is sufficient but it employs radioactivity and several days of film exposure are required before results can be obtained. For the quantitative measurement of single IGFBPs, several immunological assays are available, but their use is mostly limited to human and rodent samples for which specific antibodies are available. For livestock species, adequate antibodies are only scarcely available (e.g. for bovine IGFBP-3: Hennies and Sauerwein, 2003). For pigs, antibodies are available for not any of the 6 high affinity IGFBPs. Therefore the Western ligand blot provides indeed a valuable alternative tool to detect the single BPs from any species by using the ligand (IGF-1 or IGF-2) instead of specific antibodies, provided the ligand is available. To overcome the disadvantages of using radioactivity for labeling the ligand, I herein developed a nonradioactive Western ligand protocol using biotinylated IGF-1 as first proposed by (Grulich-Henn et al., 1998). Biotin is detected via its extraordinarily high affinity (K_d = 10^{-15} M) to streptavidin (Bayer and Wilchek, 2006) using horseradish labeled streptavidin to generate a chemiluminescence signal which can then be densitometrically evaluated.

3.2.3.1 Biotinylation of IGF-1

Two hundred micrograms of IGF-1 (Novozymes GroPep, Adelaide, Australia) were dissolved in 200 µL 10 mM HCL and 800 µL 0.1 M $NaHCO_3$ pH 9.0 were then incubated with 65 µL of a biotin (Sigma-Adrich Chemie GmbH, Munich, Germany) stock solution (10 mg/mL dimethylsulfoxide) for 4 h in the dark at room temperature, to give a final molar concentration of biotin:IGF-1 of 50:1. This ratio was based on preliminary studies showing that biotin:IGF-1 ratios of 50:1 or greater gave similar results when used for Western ligand blotting (data not shown). The cross linking reaction was terminated by adding 100 µL 1 M ammonium chloride (NH_4Cl) and by separating the free and bound biotin on a PD-10 column (GE Healthcare, Munich, Germany, formerly Amersham Bioscience, Freiburg, Germany). The column was first allowed to reach room temperature and was then equilibrated with 3 column volumes of phosphate buffered saline (PBS) pH 7.5. The reaction mix was applied and subsequently eluted with PBS and collected in 0.5 mL fractions. One well-separated

peak containing the IGF-biotin esters was identified by UV absorbance at 280 nm. The peak eluate fractions (totally 6 with each 0.5 mL) were pooled and the total protein amount of 64 µg/mL was determined. Bovine serum albumin (BSA; Sigma-Adrich Chemie GmbH, Munich, Germany) and glycerol (Applichem, Darmstadt, Germany) to a final concentration of 1% and 55%, respectively, were added. This solution was stored at -20°C and further used as ligand stock solution.

3.2.3.2 Polyacrylamide gel electrophoresis (PAGE)

Plasma samples as well as the reference standard (a pool of piglet plasma samples) were diluted 1:10 in water and mixed 1:5 with a non reducing 5x electrophoresis buffer (bromophenol blue, glycerol, β-mercaptoethanol, 20 % sodium dodecyl sulfate, 1 M Tris-Hydrochloride, pH 6.8) and heated to 60°C for 20 min. The samples, the reference standard and a biotin labelled molecular weight marker (RPN 2107, GE Healthcare formerly Amersham Bioscience) were loaded onto a 5.6 % stacking gel and electrophoresed through a 12 % polyacrylamide gel. Except for the biotinylated molecular weight marker, the samples were run under non-reducing conditions. SDS-PAGE was performed using a StarPhoresis 2-Gel Mini Vertical System (STARLAB, Ahrensburg, Germany), according to the method of Laemmli (Laemmli, 1970). In each case, two gels were run simultaneously at 150 mA until the dye front had proceeded nearly to the end of the gel.

3.2.3.3 Western blotting

Size-separated proteins were electrotransferred from the gel to Hybond^{TM}-P Membrane (GE Healthcare, Munich, Germany) according to the method of Kyhse-Anderson (1984). After electrophoresis, the gels were fixed in anode-buffer-II containing 25 mM Tris and 10 % Methanol, pH 10.4 for 5 min. The polyvinylidine fluoride (PVDF) membranes were wetted in methanol for 15 sec and then equilibrated in anode-buffer-II for 5 min as well. The transfer of the proteins was completed within 45 min at a constant current of 50 V/membrane using a semi-dry Electro-Blotter (STARLAB). The non-specific binding sites on the membranes were then blocked by immersion in TBS buffer (10 fold: 0.05 M Tris and 0.9 % NaCl pH 7.4) containing 0.1 % Tween-20 (TBST buffer) and 10 % Roti®-Block (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) at 4°C overnight.

3.2.3.4 Enhanced chemiluminescence

Membranes were probed for 2 h with biotin-labelled IGF-1 (100 ng/mL) and washed 5 times (5 x 5 min) in TBST. The PVDF membrane was incubated with streptavidin-peroxidase (Southern Biotech, Eching, Germany) (1:700,000) for 15 min and washed again 5 times. Membranes were covered by a mixture of equal volumes of enhanced chemiluminescence (ECL) detection reagent 1 and 2 (RPN 2135, GE Healthcare) in a final volume of 20 µL/cm² and incubated for 5 min in the dark without agitation. Excess detection reagent was drained off and the membranes were sealed in plastic and placed under a CL-XPosure™ Film (Thermo Scientific, Munich, Germany) in a cassette. The film was exposed for 2 min and developed immediately thereafter. The exposure time was adapted according to the intensity of the bands: exposure time varied from 2 to 10 min. The molecular weight of the developed bands was assessed by comparing with the molecular weight marker.

3.2.3.5 Evaluation of data

The chemiluminescent signals yielded were densitometrically evaluated and the relative quantities of the bands (42 kDa and 39 kDa, 34 kDa, 30 kDa, 24 kDa,) obtained were used for the subsequent statistical comparisons. The optical densities of the bands were matched against the reference standard from which the mean value of IGFBP-2 (34 kDa) was set as 100 % value. The relative amount of each major band was assessed on the basis of its surface area and the mean densitiy was expressed in % relative to the reference standard.

3.2.4 Sandwich ELISA for IGF-1 receptor in liver

For the analysis of IGF-1 receptor in liver, the tissue samples (pieces of 50-100 mg) were transferred into homogenization tubes (Precellys Ceramic Kit 1.4 mm, PEQLAB Biotechnologie GmbH, Erlangen, Germany) containing 800 µL RIPA-buffer (10mM Tris-HCL pH 7.4, 100 mM NaCl, 1 % Triton X-100, 0.5% sodium deoxycholate, 1 mM EDTA, 1 mM EGTA, 1 mM sodium fluoride, 20 mM sodium pyrophosphate, 2 mM sodium orthovanadate, 0.1% SDS, 10% glycerine, protease inhibitor complete® (1 tablet/10 mL; Roche Diagnistics GmbH, Mannheim, Germany). The livers were homogenized two times at 5000 rpm for 15 sec with a 10 sec break between the intervals in a tissue homogenizer (Precellys24, PEQLAB Biotechnologie GmbH). The samples were then centrifuged by 4500 *g* for 10 min at 4°C; supernatants were

transferred into a new tube and again centrifuged with 1500 *g* for 5 min at 4°C. The resulting supernatants were retained and aliquoted. The concentration of total protein in the liver homogenates was measured with the method of Bradford (Bradford, 1976) and the samples were stored at -20°C.

The IGF-receptor was quantified in these homogenates using the UPSTATE® colorimetric STAR (Signal Transduction Assay Reaction) ELISA kit (MILLIPORE GmbH, Schwalbach, Germany). Before measurement, the liver homogenates were diluted with standard dilution buffer (MILLIPORE GmbH) to an end concentration of 2.5 mg protein/mL and 100 µL of this dilution were used in the assay. To evaluate the applicability of this assay which is designed for human samples, for porcine liver, serial dilutions of porcine liver homogenates were tested and parallelism to the (human) IGF-1R standard curve could be confirmed. The inter-assay coefficient of variation was 12.9 % and the intra-assay coefficient of variation was 9.26 %. The limit of sensitivity was 0.13 ng IGF-1R.

3.2.5 Competitive enzyme immunoassay for leptin

Concentrations of plasma leptin were determined in duplicates in all blood samples by EIA as previously described (Sauerwein et al., 2004). Modifications of the assay included the use of a 30-fold molar excess relative to the leptin of biotinamidocaproate N-hydroxysuccinimide ester (Sigma Aldrich) for generating the biotinylated tracer, antiserum dilutions of 1:12,000 with assay buffer containing 1 % goose serum and the use of an incubation chamber at 25°C as well as the use of a horizontal shaker with 150-225 rotations/min for all incubations in microtiter plates. The limit of detection was 0.30 ng/mL and the intra- and inter-assay coefficients of variation were 6.3 % and 13.9 %, respectively.

3.3 Statistical analyses

All statistical analyses were done using the SPSS 17.0 software for Windows (SPSS Inc., Chicago, IL, USA). The final plasma data (IGF-1, IGF-2, leptin, IGFBPs) was tested for Gaussian distribution by the Kolmogorov-Smirnov test and for homogeneity of variance by the Levene's test. If data were normally distributed and variances homogeneous, parametric tests including the linear mixed model with piglets as repeated effects were executed using the following fixed factors:

- maternal feeding group (LP, AP or HP),
- piglet sex (female or (castrated) male),
- fetal or birth body weight class
    - for dpn 94 fetuses: class 1 ≤ 0.65 kg; class 2 > 0.65 kg;
    - for birth weight: class 1 ≤ 1.3kg and class 2 >1.3kg
- litter size group (class 1: < n = 12 and class 2: > 12 piglets/litter)
- The respective interactions, i.e. maternal feeding group x piglet gender, maternal feeding group x litter size group, and maternal feeding group x fetal or birth body weight class.

For group wise comparisons, the post hoc Bonferroni correction was applied. In case of not normally distributed data (and/or inhomogeneous variances), the nonparametric Kruskal-Wallis test was used. The results are displayed as means ± SE and the level of significance was set at $P \leq 0.05$; a trend was defined at $0.05 > P \leq 0.1$.

Nonparametric Spearman rank correlation coefficients (correlations with $P < 0.05$ were considered significant) were calculated. Correlation coefficients (r) between 0.4 and 0.7 were classified as moderate and $r > 0.7$ as strong correlations.

4 Results

The variables of the IGF-system investigated herein were mainly influenced by the maternal diet at dpc 94 and dpn 1. The later postnatal days of sampling (dpn 28 and 188) only scarcely indicated effects of the differential protein supply of the dams. Leptin remained unchanged in response to the different feeding groups over all days. Effects of sex, litter size or body weights on the investigated variables were detectable at the different days of sampling with varying impact. The following detailed presentation of results is limited to significant differences observed for the fixed effects of maternal diet group, fetal or birth body weight class, litter size group, sex and the respective interactions. The particularities of all results are provided in the appendix.

4.1 IGF-1 in plasma

The mean concentrations of IGF-1 across all diet groups in plasma were between 15.9 ng/mL on dpc 94 and 213.4 ng/mL on dpn 188. From dpc 94 to dpn 1 IGF-1 increased to 34.0 ng/mL and to 116.4 ng/mL on dpn 28. As shown in Figure 5 and summarized in Table 1, fetuses of higher fetal body weight (> 0.65 kg) and piglets of higher birth weight (> 1.3 kg) had significantly greater IGF-1 concentrations on dpc 94 and dpn 1, when compared to the lower respective weight classes. At later postnatal days, IGF-1 concentrations were not different between the original birth weight classes. Sex affected IGF-1 values were solely observed on dpn 188 whereby male piglets had lower concentrations than females. With the exception of dpn 1, the IGF-1 concentrations remained unchanged in response to maternal dietary treatments. At this day, the piglets born to LP fed mothers showed a significantly decreased concentration of IGF-1 compared to HP piglets and tended ($P = 0.083$) to have a lower IGF-1 values than AP piglets. Piglets from smaller litters (< 12 piglets) had higher concentrations of IGF-1 on dpn 1 compared to piglets born to greater litters (> 12). In contrast, on dpn 28, piglets from litters > 12 had higher concentrations of IGF-1.

Table 1: Plasma concentrations (means ± SE) of the components of the IGF-system and of leptin and liver content of IGF-1 receptor (IGF-1 R) in offspring of sows fed isoenergetic gestation diets with low (LP), adequate (AP), and high (HP) protein contents at different ages.

		Protein Diets									Effects*						
		AP			HP			LP			D	S	L	B	D x S	D x L	D x B
dpc 94	IGF-1 (ng/mL)	16.6	±	0.73	16.2	±	0.91	14.8	±	0.68	N.S.	N.S.	N.S.	<0.05	N.S.	N.S.	N.S.
	IGF-2 (ng/mL)	28.1	±	0.84 ^{a}	26.1	±	0.63 ^{a}	23.3	±	0.68 ^{b}	<0.001	N.S.	N.S.	<0.05	N.S.	N.S.	N.S.
	43-39 kDa (%)	17.1	±	3.14	15.5	±	1.68	17.2	±	2.64	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	34 kDa (%)	85.2	±	3.55	80.8	±	3.47	92.2	±	5.41	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	30 kDa (%)	28.0	±	3.41 ^{a}	38.4	±	2.88 ^{b}	31.7	±	2.90 ^{ab}	<0.05	N.S.	N.S.	<0.05	N.S.	N.S.	N.S.
	24 kDa (%)	5.03	±	0.75	7.82	±	0.95	6.03	±	0.78	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	IGF-1R (ng/mL)	1.69	±	0.21	1.54	±	0.15	1.62	±	0.13	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
dpn 1	IGF-1 (ng/mL)	35.5	±	3.38 ^{ab}	39.2	±	3.11 ^{a}	27.3	±	3.20 ^{b}	<0.05	N.S.	<0.001	<0.05	N.S.	N.S.	N.S.
	IGF-2 (ng/mL)	28.6	±	1.09	30.1	±	1.21	27.7	±	1.14	N.S.	N.S.	N.S.	<0.05	N.S.	<0.05	N.S.
	43-39 kDa (%)	26.0	±	2.96 ^{a}	43.0	±	5.77 ^{b}	32.0	±	4.23 ^{ab}	<0.05	N.S.	N.S.	<0.05	N.S.	N.S.	N.S.
	34 kDa (%)	109	±	3.92 ^{ab}	116	±	3.50 ^{a}	102	±	4.68 ^{b}	<0.05	N.S.	<0.05	N.S.	N.S.	N.S.	N.S.
	30 kDa (%)	41.8	±	5.63	39.3	±	5.50	58.2	±	7.07	N.S.	N.S.	<0.05	N.S.	N.S.	N.S.	N.S.
	24 kDa (%)	19.9	±	1.87	21.1	±	2.02	18.9	±	1.94	N.S.	N.S.	<0.05	N.S.	N.S.	N.S.	N.S.
	Leptin (ng/mL)	4.05	±	0.38	3.80	±	0.25	3.66	±	0.19	N.S.	N.S.	N.S.	N.S.	N.S.	<0.05	N.S.
dpn 28	IGF-1 (ng/mL)	118	±	9.21	100	±	9.05	130	±	12.3	N.S.	N.S.	<0.05	N.S.	N.S.	N.S.	N.S.
	IGF-2 (ng/mL)	33.6	±	1.30	32.9	±	0.99	33.1	±	0.98	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	43-39 kDa (%)	67.0	±	8.83	63.3	±	8.70	55.4	±	7.22	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	34 kDa (%)	86.7	±	5.58 ^{a}	86.0	±	5.10 ^{ab}	68.1	±	5.61 ^{b}	<0.05	N.S.	N.S.	N.S.	N.S.	N.S.	<0.05
	24 kDa (%)	4.28	±	0.77	4.21	±	0.84	4.32	±	0.45	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Leptin (ng/mL)	3.25	±	0.14	3.04	±	0.11	3.01	±	0.13	N.S.	N.S.	N.S.	<0.05	N.S.	N.S.	N.S.
dpn 188	IGF-1 (ng/mL)	211	±	11.0	201	±	8.72	228	±	11.0	N.S.	<0.05	N.S.	N.S.	N.S.	N.S.	N.S.
	IGF-2 (ng/mL)	40.2	±	0.82	39.1	±	0.87	39.0	±	0.80	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	43-39 kDa (%)	283	±	20.2	249	±	21.4	277	±	17.9	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	34 kDa (%)	71.8	±	4.63	75.8	±	7.48	77.2	±	7.19	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Leptin (ng/mL)	4.56	±	0.25	5.23	±	0.33	4.63	±	0.24	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

* Results of the statistical evaluation testing maternal diet (D), sex (S), litter size group (L, less or more than 12 piglets/litter), and body weight class (B, threshold of 0.65 and 1.3 kg were used for fetal and birth body weight respectively to classify the animals as below or above the respective threshold) as fixed effects and the respective interactions. Different letters designate differences ($P < 0.05$). Abbreviations: dpc: days post conception; dpn: days post natum; IGF-1 and IGF-2: insulin-like growth factor 1 and 2; IGF binding proteins: 30 kDa, 34 kDa, 43-39 kDa and 24 kDa; N.S.: not significant.

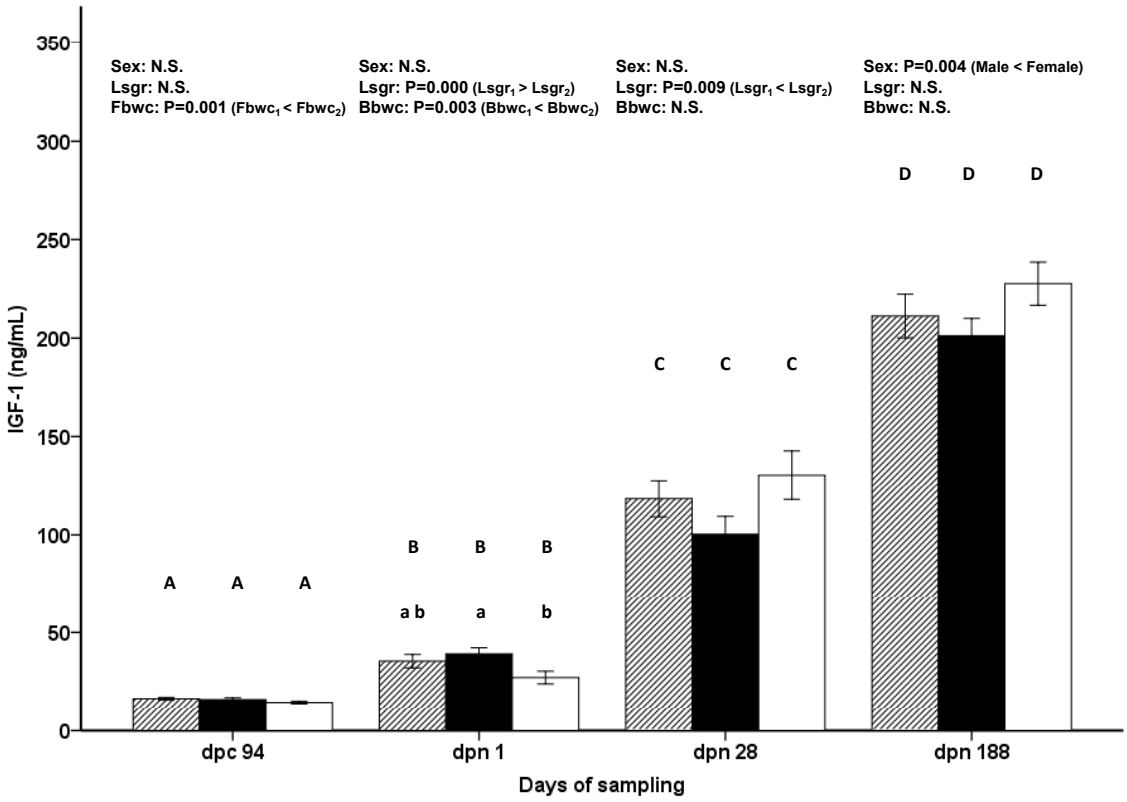

Figure 5: Developmental changes in the plasma concentrations of insulin like growth factor 1 (IGF-1, means ± SE) in offspring of sows fed isoenergetic diets differing in protein content during gestation (▨ adequate protein, i.e. 12%, ■ high protein, i.e. 30% and □ low protein, i.e. 6%); blood samples were collected from fetuses at day 94 of gestation (dpc 94) and from piglets on days 1, 28 and 188 post natum (dpn 1, 28, 188). Statistical results displayed as text insert in the figure comprise the effects of sex (females versus males; males were castrated during the first days of live), fetal body weight class ($Fbwc_1$ ≤ 0.65 kg and $Fbwc_2$ > 0.65 kg), birth weight class ($Bbwc_1$ ≤ 1.3 kg and $Bbwc_2$ > 1.3 kg) and litter size group ($Lsgr_1$ ≤ 12 and $Lsgr_2$ > 12 piglets/litter). Different lower case letters designate significant differences ($P < 0.05$) between diet groups within sampling day; different capital letters designate significant time-related differences between sampling days within the diet groups. N.S.: not significant.

4.2 IGF-2 in plasma

The average IGF-2 concentrations across all maternal diet groups rose from 25.9 ng/mL on dpc 94 to 39.4 ng/mL on day 188. There was an increase in IGF-2 concentrations from dpc 94 to dpn 1 (28.8 ng/mL) and also till dpn 28 (33.2 ng/mL). The IGF-2 concentrations in the offspring of the different maternal diet groups are shown in detail in Figure 6 and Table 1, respectively. At day 94 of gestation, the fetuses from the LP fed dams had lower IGF-2 plasma values than the offspring from AP and HP fed sows. Low body weight on dpc 94 (< 0.65 kg) and big litter size group (n > 12) on dpn 1 were associated with decreased IGF-2 concentrations. In addition,

an interaction (P=0.030) between maternal feeding group and litter size group was observed on dpn 1. At the later postnatal days 28 and 188, no significant differences between feeding group, sex, and litter size group or birth body weight class were ascertainable.

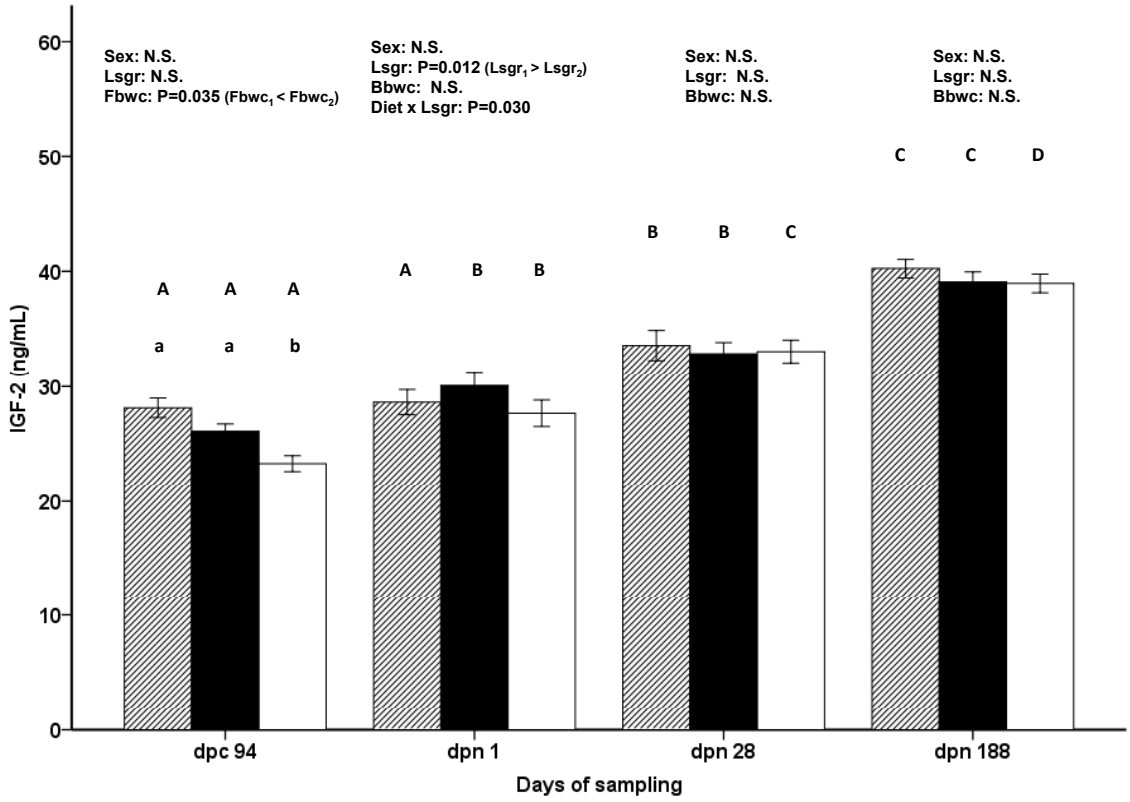

Figure 6: Developmental changes in the plasma concentrations of insulin like growth factor 2 (IGF-2, means ± SE) in offspring of sows fed isoenergetic diets differing in protein content during gestation (▨ adequate protein, i.e. 12%, ■ high protein, i.e. 30% and □ low protein, i.e. 6%); blood samples were collected from fetuses at day 94 of gestation (dpc 94) and from piglets on days 1, 28 and 188 post natum (dpn 1, 28, 188). Statistical results displayed as text insert in the figure comprise the effects of sex (females versus males; males were castrated during the first days of live), fetal body weight class ($Fbwc_1$ ≤ 0.65 kg and $Fbwc_2$ > 0.65 kg), birth weight class ($Bbwc_1$ ≤ 1.3 kg and $Bbwc_2$ > 1.3 kg) and litter size group ($Lsgr_1$ ≤ 12 and $Lsgr_2$ > 12 piglets/litter). Different lower case letters designate significant differences (P < 0.05) between diet groups within sampling day; different capital letters designate significant time-related differences between sampling days within the diet groups. N.S.: not significant.

4.3 IGFBPs in plasma

The Western blots of the IGF-binding proteins using biotin-labeled IGF-1 as ligand showed a distinct double band of 43 to 39 kDa (IGFBP-3), a band of 34 kDa (IGFBP-2), a ~ 30 kDa band (including IGFBP-1, but probably a mix of IGFBPs as for example IGFBP-5, degradation products of IGFBP-3 and/or glycosylated IGFBP-4) and a ~24 kDa band (IGFBP-4), as described for porcine serum (Hossenlopp et al., 1986; Hardouin et al., 1989; McCusker et al., 1989; Yang et al., 1989; Lee et al., 1991). IGFBP-3 was the most abundant binding protein, followed by IGFBP-2, IGFBP-4 and IGFBP-1 (representative example for a Western ligand blot is shown in Figure 7).

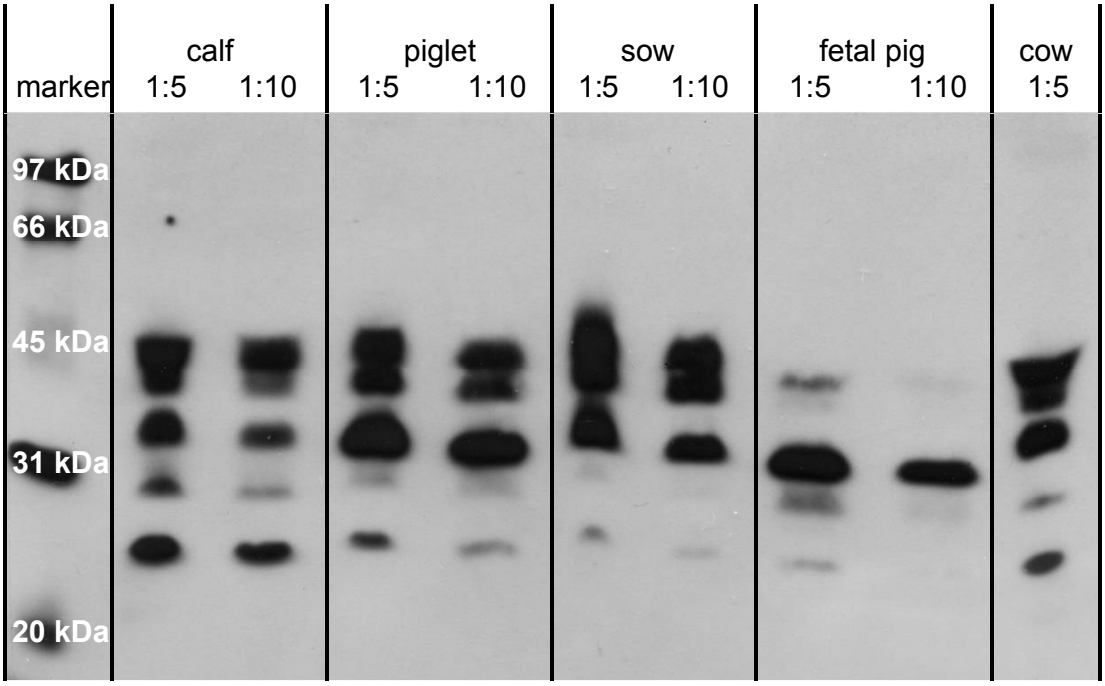

Figure 7: A representative Western ligand blot of plasma pools of varying developmental stages and species in different dilutions pictured on a CL-XPosure^{TM} Film exposed for 5 minutes.

IGFBP-1 could only be detected on dpc 94 and dpn 1 (Figure 8). On dpc 94, IGFBP-1 was affected by maternal feeding: in HP fetuses, the IGFBP-1 concentrations were significantly higher than in AP fetuses and tended to be higher compared to respective LP group. The fetuses of the light fetal body weight class (< 0.65 kg) had higher IGFBP-1 values than the ones of the higher body weight class. At dpn 1,

piglets of the greater litter size group showed higher IGFBP-1 values than piglets of the smaller litter size group. Piglets from the sows fed a LP diet had 1.8 fold higher IGFBP-1 values on dpn 1 than on dpc 94. Piglets from AP sows showed a similar increase (1.5 fold) during this time interval, whereas the IGFBP-1 concentrations in offspring from HP fed sows remained unchanged.

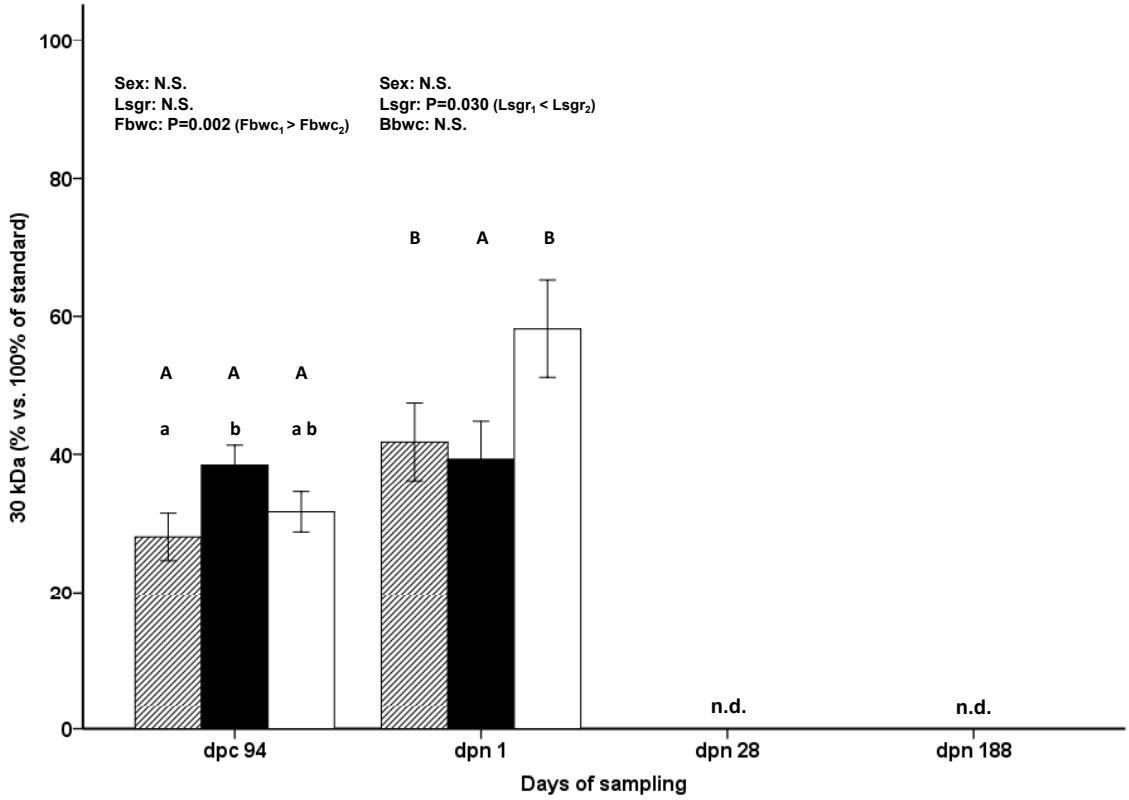

Figure 8: Developmental changes in the plasma concentrations of insulin like growth factor binding protein of 30 kDa (IGFBP-1, means ± SE) in offspring of sows fed isoenergetic diets differing in protein content during gestation (▨ adequate protein, i.e. 12%, ■ high protein, i.e. 30% and □ low protein, i.e. 6%); blood samples were collected from fetuses at day 94 of gestation (dpc 94) and from piglets on days 1, 28 and 188 post natum (dpn 1, 28, 188). Statistical results displayed as text insert in the figure comprise the effects of sex (females versus males; males were castrated during the first days of live), fetal body weight class ($Fbwc_1 \leq 0.65$ kg and $Fbwc_2 > 0.65$ kg), birth weight class ($Bbwc_1 \leq 1.3$ kg and $Bbwc_2 > 1.3$ kg) and litter size group ($Lsgr_1 \leq 12$ and $Lsgr_2 > 12$ piglets/litter). Different lower case letters designate significant differences ($P < 0.05$) between diet groups within sampling day; different capital letters designate significant time-related differences between sampling days within the diet groups. N.S.: not significant, n.d.: not defined.

The **IGFBP-2** concentrations did not differ between sexes, litter size group or birth body weight classes on any day of sampling (Figure 9). However, IGFBP-2 was significantly influenced by maternal diet on dpn 1 and dpn 28. At both days, piglets born to LP fed mothers showed significantly decreased IGFBP-2 values compared

with HP (dpn 1 and 28) and AP piglets (dpn 1). In addition, an interaction (P = 0.035) between maternal feeding group and body weight at birth was detected on dpn 28. In general, the highest IGFBP-2 values were recorded on dpn 1; age related differences were observed for all diet groups but slightly divergent patterns were observed. In LP and AP piglets, the nadir of IGFBP-2 concentrations was reached on dpn 28 and 188, respectively. In HP piglets, the increase from dpc 94 to dpn 1 was apparent, too, but thereafter the values were returned to the level recorded on dpc 94.

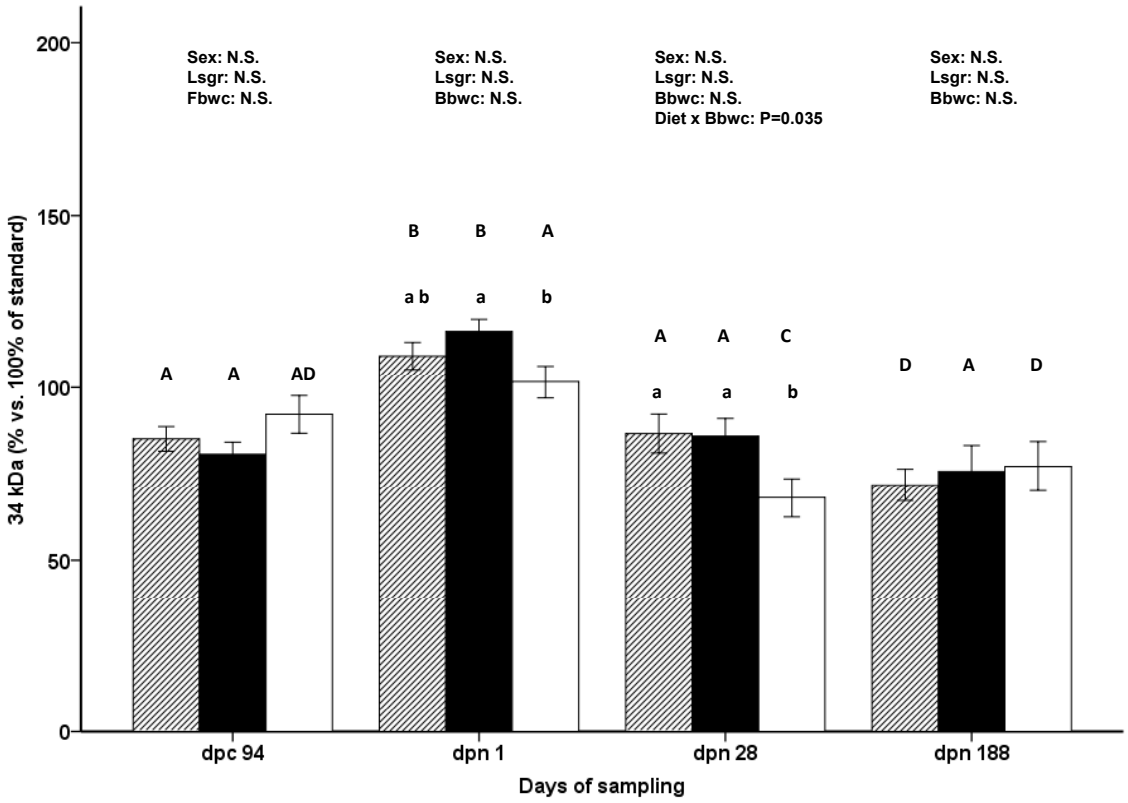

Figure 9: Developmental changes in the plasma concentrations of insulin like growth factor binding protein of 34 kDa (IGFBP-2, means ± SE) in offspring of sows fed isoenergetic diets differing in protein content during gestation (▨ adequate protein, i.e. 12%, ■ high protein, i.e. 30% and □ low protein, i.e. 6%); blood samples were collected from fetuses at day 94 of gestation (dpc 94) and from piglets on days 1, 28 and 188 post natum (dpn 1, 28, 188). Statistical results displayed as text insert in the figure comprise the effects of sex (females versus males; males were castrated during the first days of live), fetal body weight class ($Fbwc_1$ ≤ 0.65 kg and $Fbwc_2$ > 0.65 kg), birth weight class ($Bbwc_1$ ≤ 1.3 kg and $Bbwc_2$ > 1.3 kg) and litter size group ($Lsgr_1$ ≤ 12 and $Lsgr_2$ > 12 piglets/litter). Different lower case letters designate significant differences (P < 0.05) between diet groups within sampling day; different capital letters designate significant time-related differences between sampling days within the diet groups. N.S.: not significant.

For the **IGFBP-3** concentrations, an age related increase was observed in all diet groups (Figure 10) whereby the biggest difference between subsequent sampling

days was from dpn 28 to 188. Comparing dpc 94 and dpn 188, 16-fold higher IGFBP-3 values were seen on dpn 188. Differences related to maternal feeding group were limited to dpn 1 on which the concentration of IGFBP-3 in HP piglets was significantly higher than in AP piglets. For neither sex and body weight nor litter size group differences were detectable; the only exception was dpn 1 on which piglets from litters with less than 12 neonates had higher IGFBP-3 values than those from bigger litters.

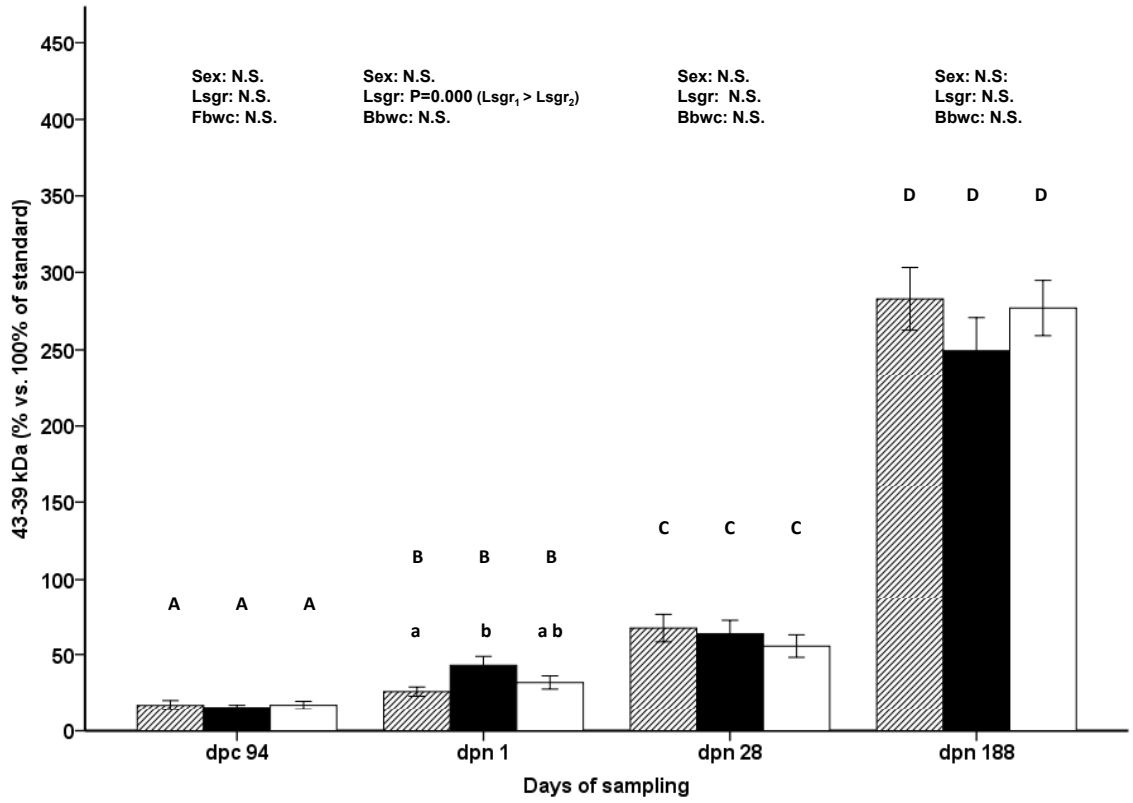

Figure 10: Developmental changes in the plasma concentrations of insulin like growth factor binding protein of 43-39 kDa (IGFBP-3, means ± SE) in offspring of sows fed isoenergetic diets differing in protein content during gestation (▨ adequate protein, i.e. 12%, ■ high protein, i.e. 30% and □ low protein, i.e. 6%); blood samples were collected from fetuses at day 94 of gestation (dpc 94) and from piglets on days 1, 28 and 188 post natum (dpn 1, 28, 188). Statistical results displayed as text insert in the figure comprise the effects of sex (females versus males; males were castrated during the first days of live), fetal body weight class ($Fbwc_1 \leq 0.65$ kg and $Fbwc_2 > 0.65$ kg), birth weight class ($Bbwc_1 \leq 1.3$ kg and $Bbwc_2 > 1.3$ kg) and litter size group ($Lsgr_1 \leq 12$ and $Lsgr_2 > 12$ piglets/litter). Different lower case letters designate significant differences ($P < 0.05$) between diet groups within sampling day; different capital letters designate significant time-related differences between sampling days within the diet groups. N.S.: not significant.

Detection of **IGFBP-4** was limited for dpc 94, dpn 1 and dpn 28 (Figure 11). On dpn 1, the IGFBP-4 concentrations were in average 3.2 and 4.7-fold higher than on

dpc 94 and dpn 28, respectively. The values rose from 6 % on dpc 94 to 20 % on dpn 1 and then decreased at dpn 188 to initial values. On neither of the sampling days, differences between the diet groups occurred. For litter size group, there was an effect on dpn 1 whereby piglets from smaller litters showed higher IGFBP-4 values than those from litters bigger than 12 piglets. Sex and body weight at delivery did not affect IGFBP-4.

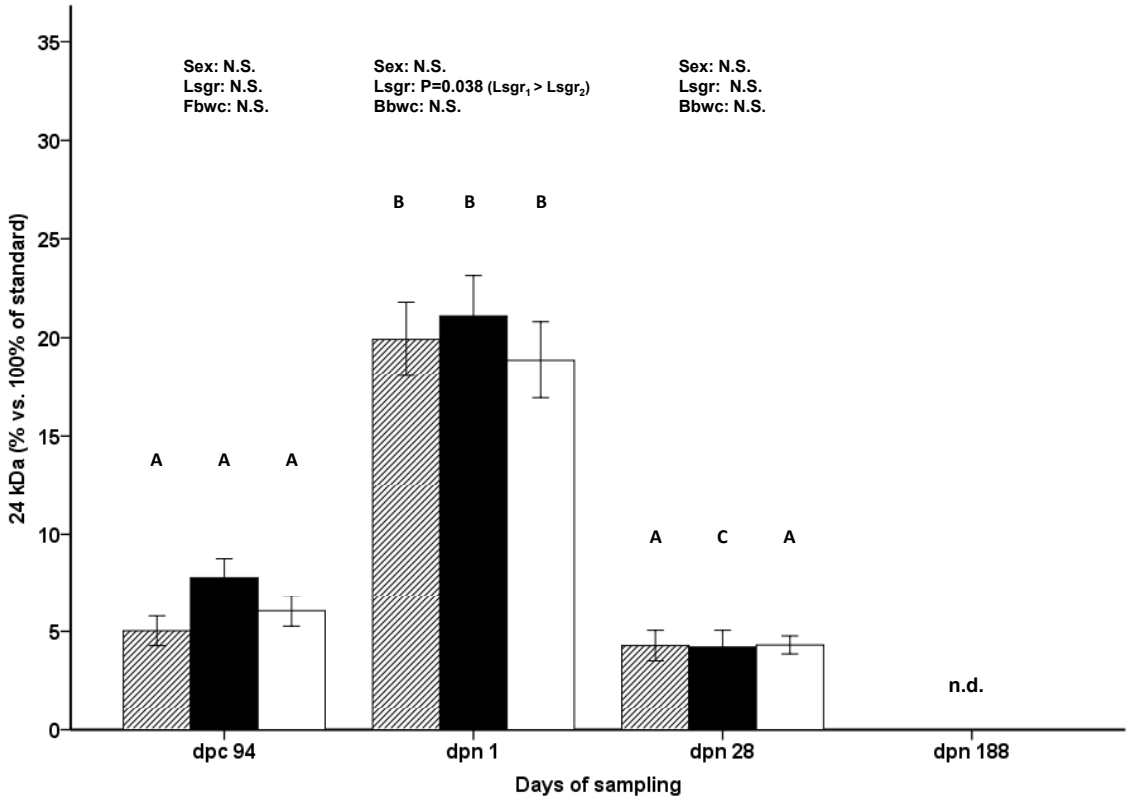

Figure 11: Developmental changes in the plasma concentrations of insulin like growth factor binding protein of 24 kDa (IGFBP-4, means ± SE) in offspring of sows fed isoenergetic diets differing in protein content during gestation (▨ adequate protein, i.e. 12%, ■ high protein, i.e. 30% and □ low protein, i.e. 6%); blood samples were collected from fetuses at day 94 of gestation (dpc 94) and from piglets on days 1, 28 and 188 post natum (dpn 1, 28, 188). Statistical results displayed as text insert in the figure comprise the effects of sex (females versus males; males were castrated during the first days of live), fetal body weight class ($Fbwc_1 \leq 0.65$ kg and $Fbwc_2 > 0.65$ kg), birth weight class ($Bbwc_1 \leq 1.3$ kg and $Bbwc_2 > 1.3$ kg) and litter size group ($Lsgr_1 \leq 12$ and $Lsgr_2 > 12$ piglets/litter). Different lower case letters designate significant differences ($P < 0.05$) between diet groups within sampling day; different capital letters designate significant time-related differences between sampling days within the diet groups. N.S.: not significant; n.d.: not defined.

4.4 IGF-1 receptor in liver tissue

The concentrations of IGF-1 receptor in liver were below the limit of detection of the assay at any of the postnatal days. For the fetal stage assessed on dpc 94, the

average concentration across all diet groups was 1.61 ng/mL (Table 1). Neither maternal feeding group, body weight, litter size nor sex apparently influenced IGF-1 receptor content on dpc 94 in liver.

4.5 Leptin in plasma

On dpc 94, the concentrations of leptin were below the assay limit of detection (0.3 ng/mL). The concentrations assessed postnatal are shown in Figure 12 and Table 1, respectively. The average leptin plasma concentrations of 4.0 ng/mL on dpn 1 correspond to a more than 10-fold increase compared to dpc 94. Comparing dpn 1 and 28, a significant decrease was observed in the HP and LP piglets, and - albeit not reaching the level of significance – in AP piglets. The highest leptin plasma concentrations (average 4.6 ng/mL) were consistently reached in all diet groups by dpn 188 and were about 1 ng/mL higher than on dpn 1. The leptin concentrations were neither affected by sex nor by litter size group. However, on dpn 1 there was an interaction between maternal feeding group and litter size group ($P = 0.014$). Birth weight classes were not different with the exception of dpn 28 where piglets with higher birth body weight exhibited significantly higher leptin concentrations than piglets of lower body weight at birth.

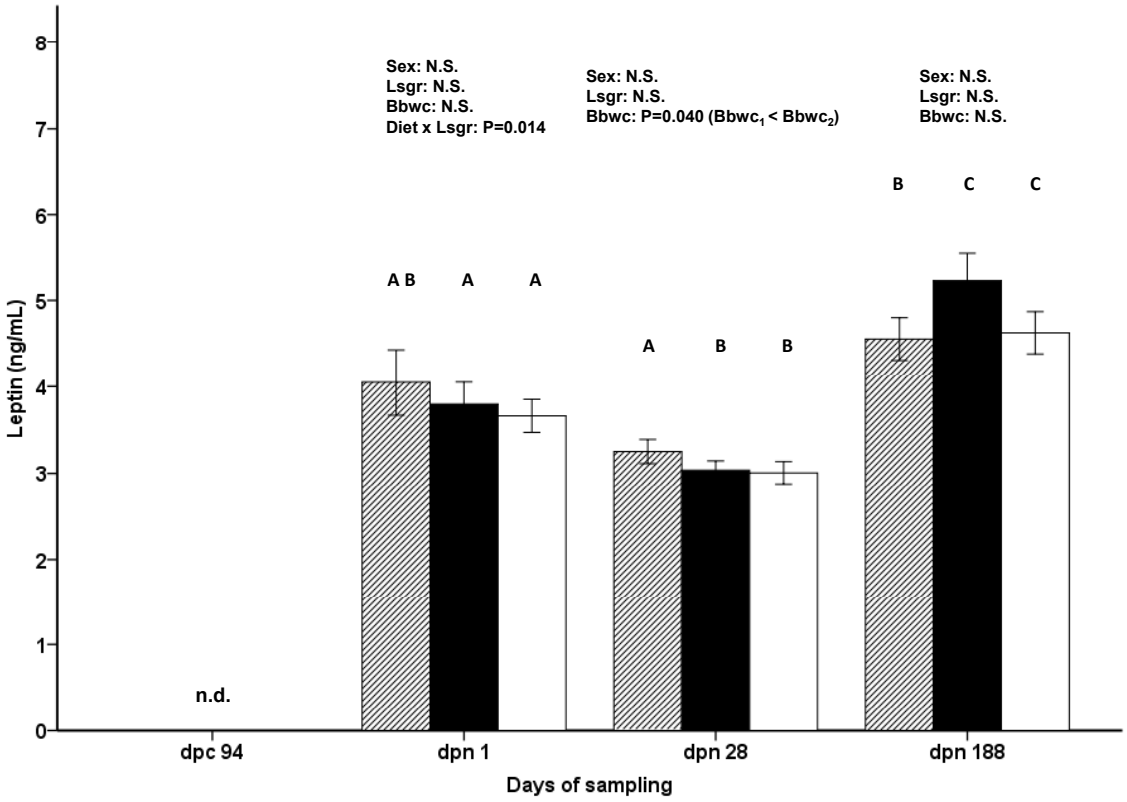

Figure 12: Developmental changes in the plasma concentrations of Leptin (means ± SE) in offspring of sows fed isoenergetic diets differing in protein content during gestation (▨ adequate protein, i.e. 12%, ■ high protein, i.e. 30% and □ low protein, i.e. 6%); blood samples were collected from fetuses at day 94 of gestation (dpc 94) and from piglets on days 1, 28 and 188 post natum (dpn 1, 28, 188). Statistical results displayed as text insert in the figure comprise the effects of sex (females versus males; males were castrated during the first days of live), fetal body weight class ($Fbwc_1 \leq 0.65$ kg and $Fbwc_2 > 0.65$ kg), birth weight class ($Bbwc_1 \leq 1.3$ kg and $Bbwc_2 > 1.3$ kg) and litter size group ($Lsgr_1 \leq 12$ and $Lsgr_2 > 12$ piglets/litter). Different lower case letters designate significant differences ($P < 0.05$) between diet groups within sampling day; different capital letters designate significant time-related differences between sampling days within the diet groups. N.S.: not significant; n.d.: not defined.

4.6 Body composition at the postnatal days

Slaughter weight (SW) and hot carcass weight (HCW) as well as percentages of carcass protein, lipid and dry matter were recorded at dpn 1, 28 and 188 (Table 2).
At their first day of life, piglets exposed to maternal LP diet *in utero* had lower body weights (P = 0.029) and hot carcass weights (P = 0.025) compared to the AP piglets. For both variables, piglets of greater litters showed lower weights and as a matter of course, piglets of the lighter birth weight class showed lower slaughter and hot carcass weights, too (Figure 13 and Figure 14).

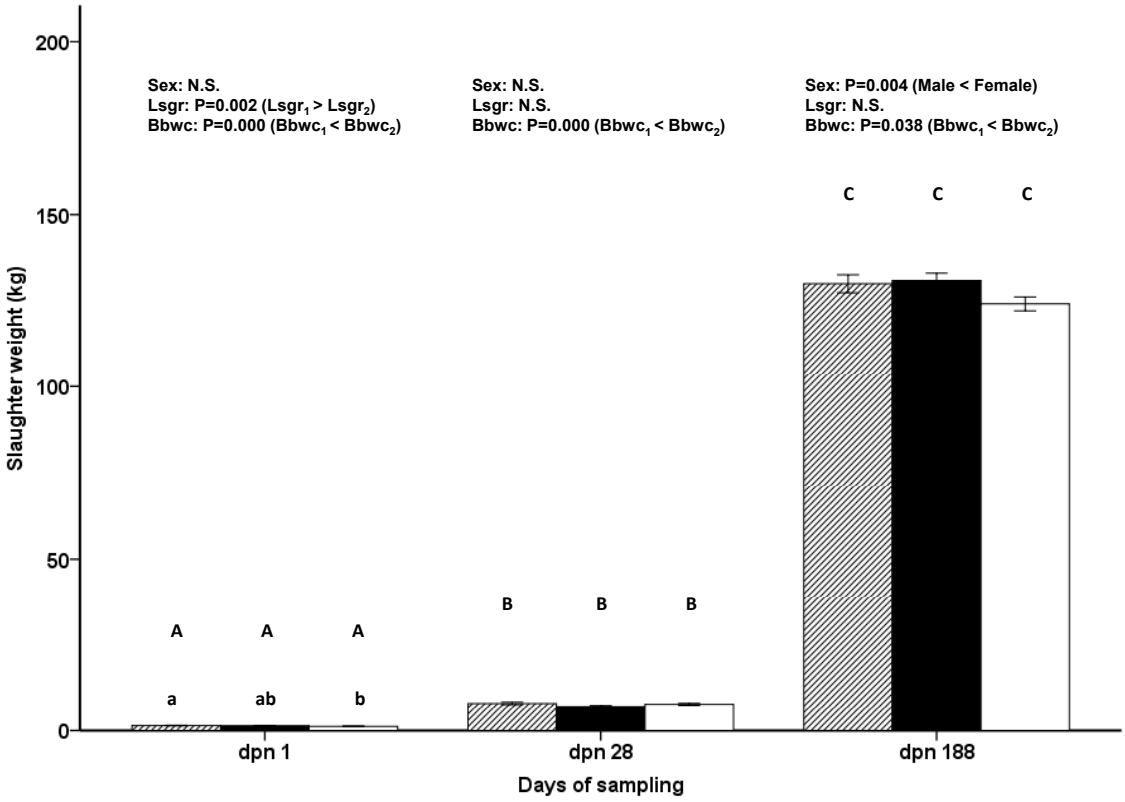

Figure 13: Slaughter weights (means ± SE) of offspring of sows fed isoenergetic diets differing in protein content during gestation (▨ adequate protein, i.e. 12%, ■ high protein, i.e. 30% and □ low protein, i.e. 6%); slaughter weights were determined from piglets on days 1, 28 and 188 post natum (dpn 1, 28, 188). Statistical results displayed as text insert in the figure comprise the effects of sex (females versus males; males were castrated during the first days of live), birth weight class ($Bbwc_1$ ≤ 1.3 kg and $Bbwc_2$ > 1.3 kg) and litter size group ($Lsgr_1$ ≤ 12 and $Lsgr_2$ > 12 piglets/litter). Different lower case letters designate significant differences (P < 0.05) between diet groups within sampling day; different capital letters designate significant time-related differences between sampling days within the diet groups. N.S.: not significant.

Table 2: Carcass characteristics (means ± SE) in offspring of sows fed isoenergetic gestation diets with low (LP), adequate (AP), and high (HP) protein contents at different ages.

		Protein Diets			Effects*						
		AP	HP	LP	D	S	L	B	D x S	D x L	D x B
dpn 1	SW (kg)	1.42 ± 0.07 ^{a}	1.34 ± 0.06 ^{ab}	1.21 ± 0.07 ^{b}	<0.05	N.S.	<0.05	<0.001	N.S.	N.S.	N.S.
	HCW (kg)	1.01 ± 0.05 ^{a}	0.96 ± 0.05 ^{ab}	0.86 ± 0.05 ^{b}	<0.05	N.S.	<0.05	<0.001	N.S.	N.S.	N.S.
	Protein (%)	14.5 ± 0.18	14.4 ± 0.21	14.0 ± 0.17	N.S.	N.S.	N.S.	N.S.	N.S.	<0.05	N.S.
	Fat (%)	1.85 ± 0.11	1.59 ± 0.07	1.68 ± 0.11	N.S.	N.S.	N.S.	<0.05	N.S.	N.S.	N.S.
	DM (%)	20.2 ± 0.34	19.7 ± 0.32	19.4 ± 0.32	N.S.	N.S.	N.S.	N.S.	N.S.	<0.05	N.S.
dpn 28	SW (kg)	7.88 ± 0.40	6.99 ± 0.27	7.63 ± 0.31	N.S.	N.S.	N.S.	<0.001	N.S.	N.S.	N.S.
	HCW (kg)	5.93 ± 0.32	5.23 ± 0.22	5.77 ± 0.26	N.S.	N.S.	N.S.	<0.001	N.S.	N.S.	N.S.
	Protein (%)	16.4 ± 0.13	16.3 ± 0.10	16.5 ± 0.11	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Fat (%)	12.9 ± 0.55 ^{a}	12.7 ± 0.42 ^{ab}	13.8 ± 0.41 ^{b}	<0.05	N.S.	<0.05	<0.05	N.S.	<0.05	N.S.
	DM (%)	32.3 ± 0.54 ^{a}	31.9 ± 0.41 ^{ab}	33.3 ± 0.40 ^{b}	<0.05	N.S.	<0.05	<0.05	N.S.	<0.05	N.S.
dpn 188	SW (kg)	130 ± 2.58	131 ± 2.11	124 ± 2.00	N.S.	N.S.	N.S.	<0.05	N.S.	N.S.	N.S.
	HCW (kg)	105 ± 2.03	106 ± 1.73	100 ± 1.72	N.S.	N.S.	N.S.	<0.05	N.S.	N.S.	N.S.
	Protein (%)	15.9 ± 0.18	15.8 ± 0.18	15.6 ± 0.23	N.S.	<0.001	N.S.	N.S.	N.S.	<0.05	N.S.
	Fat (%)	29.4 ± 0.83	29.7 ± 0.71	30.8 ± 1.01	N.S.	<0.001	N.S.	N.S.	N.S.	<0.05	N.S.
	DM (%)	48.2 ± 0.64	48.3 ± 0.56	49.1 ± 0.74	N.S.	<0.001	N.S.	N.S.	N.S.	N.S.	N.S.

* Results of the statistical evaluation testing maternal diet (D), sex (S), litter size group (L, less or more than 12 piglets/litter), and body weight class (B, threshold of 0.65 and 1.3 kg were used for fetal and birth body weight respectively to classify the animals as below or above the respective threshold) as fixed effects and the respective interactions. Different letters designate differences ($P < 0.05$).
Abbreviations: BW: birth weight, SW: slaughter weight, HCW: hot carcass weight, DM: dry matter, dpn: days post natum; N.S.: not significant.

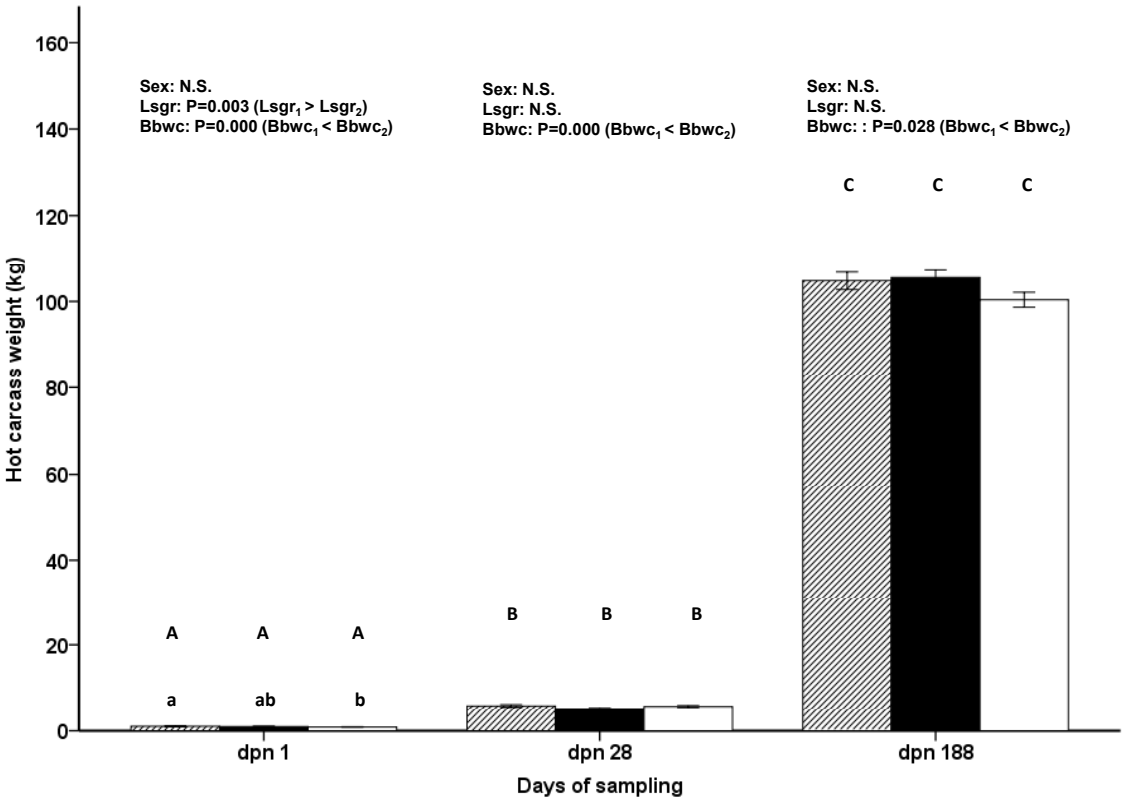

Figure 14: Hot carcass weights (means ± SE) of offspring of sows fed isoenergetic diets differing in protein content during gestation (▨ adequate protein, i.e. 12%, ■ high protein, i.e. 30% and □ low protein, i.e. 6%); hot carcass weights were determined from piglets on days 1, 28 and 188 post natum (dpn 1, 28, 188). Statistical results displayed as text insert in the figure comprise the effects of sex (females versus males; males were castrated during the first days of live), birth weight class ($Bbwc_1 \leq 1.3$ kg and $Bbwc_2 > 1.3$ kg) and litter size group ($Lsgr_1 \leq 12$ and $Lsgr_2 > 12$ piglets/litter). Different lower case letters designate significant differences ($P < 0.05$) between diet groups within sampling day; different capital letters designate significant time-related differences between sampling days within the diet groups. N.S.: not significant.

In addition, piglets of higher birth weights had higher percentages of fat at dpn 1 ($P = 0.020$). For the percentages of protein and dry matter, an interaction between diet and litter size group was detectable ($P = 0.003$ and $P = 0.009$, respectively). For all postnatal days significant differences of SW and HCW between the birth weight classes were observed (dpn 1 = SW: $P < 0.001$, HCW: $P < 0.001$; dpn 28 = SW: $P < 0.001$, HCW: $P < 0.001$; dpn 188= SW: $P = 0.038$, HCW: $P = 0.028$). As a matter of course, piglets with birth weights > 1.3 kg had significantly higher SW and HCW compared to piglets with lower birth weights (< 1.3 kg). On dpn 28, fat and dry matter content were affected ($P < 0.05$) by maternal feeding, litter size and birth weight (Figure 15 and Figure 16). Fat and dry matter content were higher in piglets born to LP fed mothers than in HP and AP piglets. Besides, piglets of low birth weight and

piglets out of small litters exhibited lower percentages of carcass fat and dry matter compared to piglets of heavier birth weights and offspring out of large litters. For both variables, there was an interaction between diet (P = 0.045) and litter size group (P = 0.044).

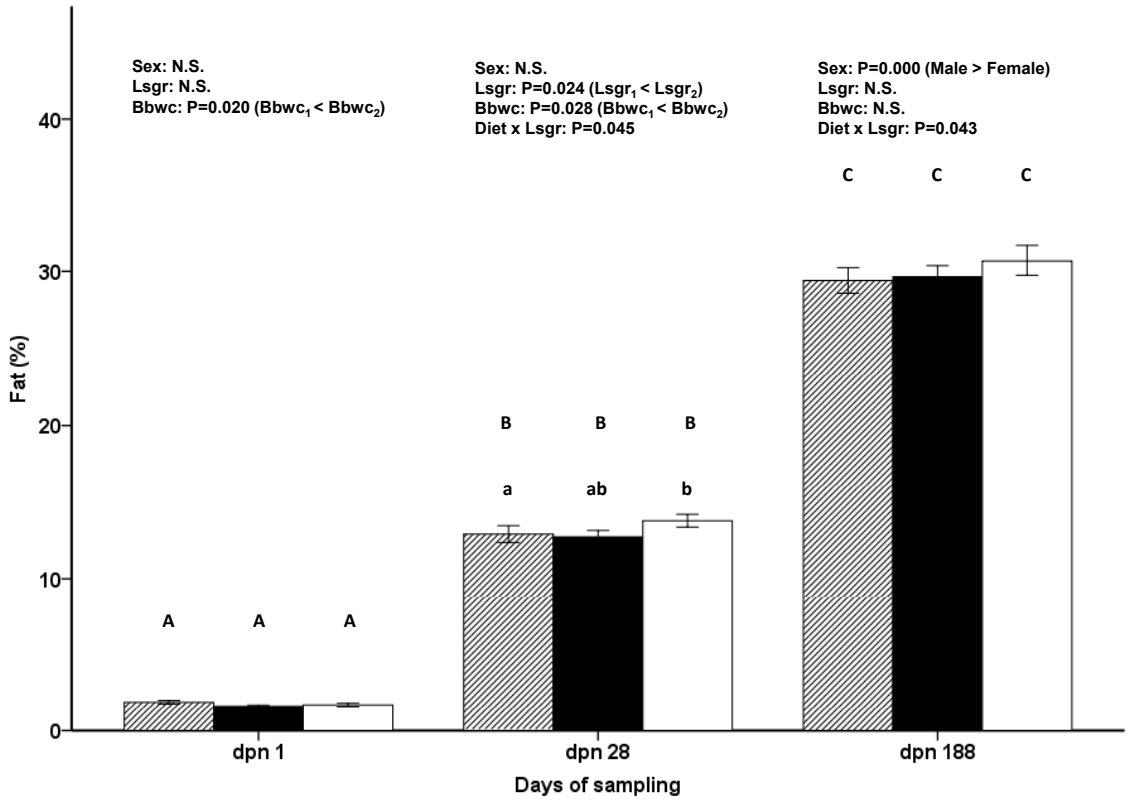

Figure 15: Percentages of fat (means ± SE) in offspring of sows fed isoenergetic diets differing in protein content during gestation (▨ adequate protein, i.e. 12%, ■ high protein, i.e. 30% and □ low protein, i.e. 6%); percentages of fat were determined from piglets on days 1, 28 and 188 post natum (dpn 1, 28, 188). Statistical results displayed as text insert in the figure comprise the effects of sex (females versus males; males were castrated during the first days of live), birth weight class ($Bbwc_1$ ≤ 1.3 kg and $Bbwc_2$ > 1.3 kg) and litter size group ($Lsgr_1$ ≤ 12 and $Lsgr_2$ > 12 piglets/litter). Different lower case letters designate significant differences (P < 0.05) between diet groups within sampling day; different capital letters designate significant time-related differences between sampling days within the diet groups. N.S.: not significant.

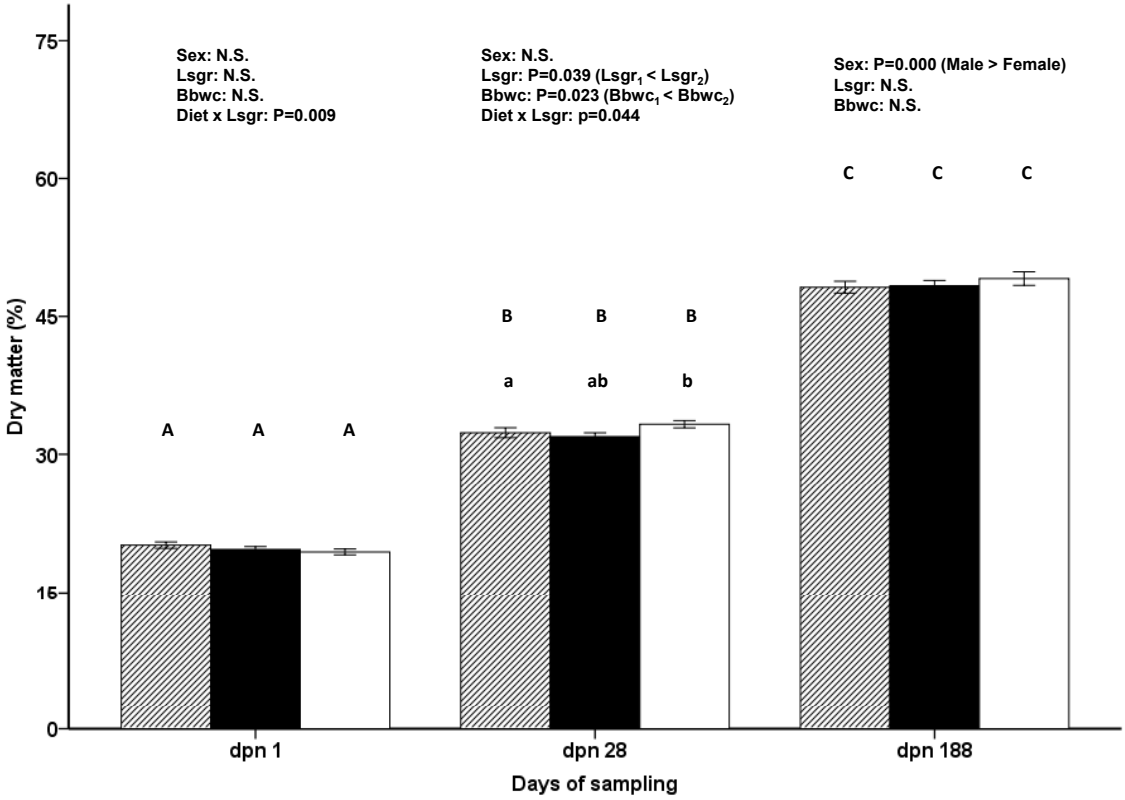

Figure 16: Content of dry matter (means ± SE) in offspring of sows fed isoenergetic diets differing in protein content during gestation (/// adequate protein, i.e. 12%, ■ high protein, i.e. 30% and □ low protein, i.e. 6%); the dry matter content were determined from piglets on days 1, 28 and 188 post natum (dpn 1, 28, 188). Statistical results displayed as text insert in the figure comprise the effects of sex (females versus males; males were castrated during the first days of live), birth weight class ($Bbwc_1$ ≤ 1.3 kg and $Bbwc_2$ > 1.3 kg) and litter size group ($Lsgr_1$ ≤ 12 and $Lsgr_2$ > 12 piglets/litter). Different lower case letters designate significant differences ($P < 0.05$) between diet groups within sampling day; different capital letters designate significant time-related differences between sampling days within the diet groups. N.S.: not significant.

At dpn 188, the percentages of protein, fat and dry matter where affected by sex in that castrated males had lower carcass protein contents ($P < 0.001$) compared to females, but higher fat ($P < 0.001$) and dry matter percentages ($P < 0.001$, Figure 17, Figure 15 and Figure 16). An interaction between diet and litter size group was observed for carcass protein and fat content ($P = 0.018$ and $P = 0.043$, respectively). Expectedly, SW, HCW, lipid and dry matter percentages increased from dpn 1 to dpn 188 significantly.

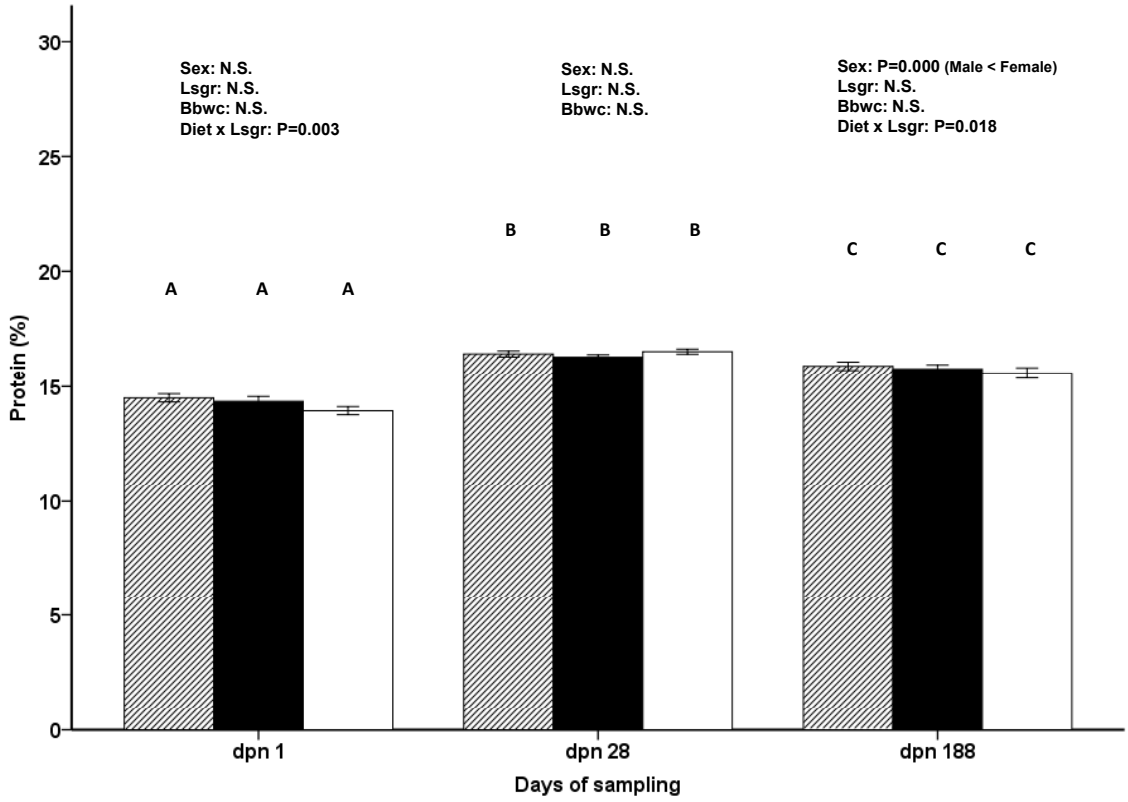

Figure 17: Percentages of protein (means ± SE) in offspring of sows fed isoenergetic diets differing in protein content during gestation (▨ adequate protein, i.e. 12%, ■ high protein, i.e. 30% and □ low protein, i.e. 6%); the percentages of protein were determined from piglets on days 1, 28 and 188 post natum (dpn 1, 28, 188). Statistical results displayed as text insert in the figure comprise the effects of sex (females versus males; males were castrated during the first days of live), birth weight class ($Bbwc_1$ ≤ 1.3 kg and $Bbwc_2$ > 1.3 kg) and litter size group ($Lsgr_1$ ≤ 12 and $Lsgr_2$ > 12 piglets/litter). Different lower case letters designate significant differences ($P < 0.05$) between diet groups within sampling day; different capital letters designate significant time-related differences between sampling days within the diet groups. N.S.: not significant.

4.7 Correlations

4.7.1 Correlations on each sampling day within the three different maternal feeding groups

Correlations between the various variables assessed (plasma concentrations of IGF-1, IGF-2, IGFBPs 1-4, and leptin, liver IGF-1R content, body weight at birth, slaughter weight, hot carcass weight as well as dry matter, protein and fat portion of the carcass) were calculated for dpn 1, 28 and 188 within the 3 different maternal feeding groups AP, HP and LP and are depicted in Figure 18.

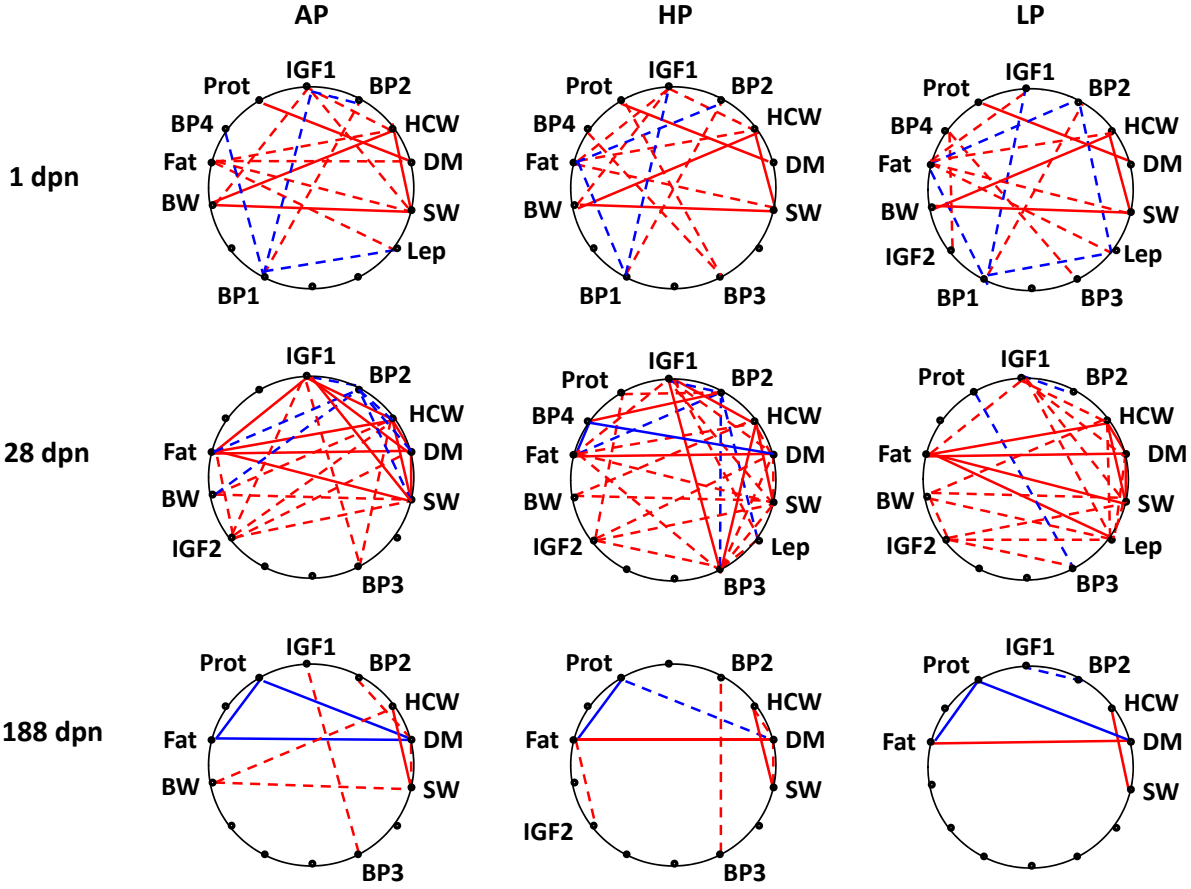

Figure 18: Spearman correlation coefficients between the components of the IGF-system and leptin measured in plasma and of various carcass characteristics assessed on day 1, 28 an 188 of postnatal life (dpn) in offspring from sows fed isoenergetic diets with low (LP), adequate (AP), and high (HP) protein contents. Positive significant correlations are in red, negative significant correlations are displayed in blue ($P \leq 0.05$). The presentation is limited to correlations with $r \geq 0.4$; moderate correlations ($0.4 < r \leq 0.70$) are indicated by dashed lines and strong correlations ($r > 0.7$) by unbroken lines. Variables: birth weight (BW), slaughter weight (SW), hot carcass weight (HCW), carcass dry matter content (DM), carcass fat content (FAT), carcass protein content (Prot); Concentrations: insulin-like growth factor 1 (IGF-1), insulin-like growth factor 2 (IGF-2), insulin-like growth factor binding proteins 1-4 (BP-1, BP2, BP 3, and BP 4), leptin (Lep).

The display is limited to significant correlations with r ≥ 0.4. Negative and positive correlations of moderate (r = 0.4 – 0.7) as well as of strong intensities (r > 0.7) were identified. Apparently dpn 28 had the most correlations in all three diets in comparison to dpn 1 and dpn 188, whereas dpn 188 showed only a few correlations between the variables assessed. For the variables of carcass composition, correlation of dpn 188 were mostly observed between these variables whereas, correlations between carcass composition data and the IGF-system disappeared as compared to earlier ages. In both the LP and the HP group, the correlation between fat and dry matter content was negative, but was positive in the AP group.

4.7.2 Correlations within dpc 94, dpn 1, dpn 28 and dpn 188 irrespective of feeding group

Taking all data from the different feeding groups together for a pooled assessment of correlations within the different days provided the results presented in Table 3. The significant correlations detected and displayed in this table ranged between r = 0.18 and r = 0.889. Positive correlations of IGF-1 were found with IGF-2 on dpc 94, and dpn 28 (Table 3).

Negative associations between IGF-1 and the IGFBPs were apparent for IGFBP-1 on dpc 94 and dpn 1, and for IGFBP-2 on dpn 28. The only positive association of IGF-1 with the IGFBPs was limited to IGFBP3 on dpn 28. The correlations of IGF-2 with the IGFBPs showed a similar pattern, i.e. positive correlations were limited to IGFBP-3 on dpc 94 and dpn 28, and negative correlations were evident for IGFBP-2 (dpn 28) and IGFBP-1 (dpc 94, dpn 1). Interrelationships between the different IGFBPs were mostly limited to the fetal stage and were consistently positive, i.e. on dpc 94; IGFBP-3 was correlated with IGFBP-2, -1 and -4. Further associations between the other IGFBPs were also exclusively positive and occurred between IGFBP-2 and -1 on dpc 94 and dpn 1 and between IGFBP-2 and -4 on dpn 28. IGFBP-1 was also correlated with IGFBP-4 on dpc 94.

Postnatal associations between the components of the IGF-system and leptin were exclusively positive for IGF-1 and IGF-2. Correlations between leptin and the IGFBPs were either positive (IGFBP-3 on dpc 94 and IGFBP-4 on dpn 1) or negative in case of IGFBP-1 (dpn 1).

Table 3: Spearman correlation coefficients between components of the IGF-system and leptin measured in plasma and various carcass characteristics assessed on day 94 of gestation (dpc 94) and days 1 and 28 of postnatal life (dpn) as well as over all sampling days (dpc 94, dpn 1, 28, 188) in offspring from sows fed isoenergetic diets with low (LP), adequate (AP), and high (HP) protein contents.

		IGF-2	43-39 kDa	34 kDa	30 kDa	24 kDa	Lep	BW	SW	HCW	Prot	Fat	DM
IGF-1	dpc 94	0.454			-0.409				0.575				
	dpn 1				-0.632		0.305	0.432	0.547	0.534		0.411	
	dpn 28	0.397	0.541	-0.477			0.525		0.637	0.682		0.655	0.596
	all dpn	0.610	0.770	-0.588	-0.776	-0.828	0.334		0.887	0.889	0.402	0.883	0.869
IGF-2	dpc 94		0.261		-0.248				0.370				
	dpn 1				-0.296								
	dpn 28		0.378	-0.325			0.169		0.394	0.340		0.346	0.364
	all dpn		0.613	-0.325	-0.522	-0.624	0.259		0.625	0.618	0.291	0.625	0.631
43-39 kDa	dpc 94			0.322	0.246	0.358			0.311				
	dpn 1					0.449		0.261	0.225	0.244			
	dpn 28						0.400	0.260	0.409	0.455			
	all dpn			-0.343	-0.570	-0.695	0.428	0.201	0.784	0.787	0.247	0.754	0.755
34 kDa	dpc 94				0.472								
	dpn 1				0.407							-0.338	
	dpn 28					0.300			-0.313	-0.330		-0.476	-0.411
	all dpn				0.565	0.557	-0.167		-0.538	-0.536	-0.330	-0.550	-0.509
30 kDa	dpc 94					0.352			-0.450				
	dpn 1						-0.289	-0.229	-0.375	-0.343		-0.498	
	dpn 28												
	all dpn					0.795			-0.829	-0.827	-0.671	-0.836	-0.816
24 kDa	dpc 94												
	dpn 1						0.250						
	dpn 28												
	all dpn						-0.244		-0.858	-0.858	-0.492	-0.868	-0.867
Lep	dpc 94												
	dpn 1								0.279	0.250		0.423	
	dpn 28							0.339	0.330	0.386	0.243	0.329	0.243
	all dpn							0.207	0.329	0.329	-0.180	0.346	0.275

Variables: fetal or birth body weight (BW), slaughter weight (SW), hot carcass weight (HCW), carcass dry matter content (DM), carcass fat content (Fat), carcass protein content (Prot); Concentrations: insulin-like growth factor 1 (IGF-1), insulin-like growth factor 2 (IGF-2), insulin-like growth factor binding proteins 1-4 (30 kDa, 34 kDa, 43-39 kDa, 24 kDa), leptin (Lep).

Considering potential relationships between the IGF-system and carcass characteristics, exclusively positive correlations were observed for IGF-1, IGF-2 and IGFBP-3, whereas occasional correlations between the other IGFBPs with carcass traits were consistently negative. Leptin was positively correlated to most carcass traits but the correlation was relatively weak: the r values ranged between 0.243 (leptin x protein portion on dpn 28) and 0.423 (leptin x fat portion on dpn 1).
Interestingly for dpn 188 there were only a few and weak correlations detectable: IGF-1 and IGFBP-3 as well as IGFBP-3 and IGFBP-2 were positively correlated (r =0.256 and r = 0.299, respectively. Between IGF-1 and IGFBP-2 were a negative correlation observed (r= -0.381). In favor of a better general view of Table 3, the few correlations of dpn 188 are not presented.

4.7.3 Correlations over all days irrespective of feeding group

IGF-1 was highly correlated in a positive or negative way to nearly all parameters tested (Table 3). Correlations between IGF-1 and IGFBP-1, -2 and -4 were negative. All other significant correlations of IGF-1 were positive. The same pattern was found for IGF-2, but the correlation was weaker as indicated by generally lower r values (Table 3). Correlations of IGFBP-1 and IGFBP-4 with slaughter weight, hot carcass weight as well as percentages of fat and dry matter over all days of sampling were high and consistently negative (r values between -0.816 and -0.868). IGFBP-1 and -4 were also negatively correlated with carcass fat content but these correlations were only moderate (r = -0.671 and -0.492, respectively). A highly positive correlation was found between IGFBP-1 and IGFBP-4. For leptin weak positive correlations with all carcass traits were confirmed with the exception of carcass protein content for which r = -0.18 was observed.

5 Discussion

5.1 Leptin

In the current study, the observation of slightly decreasing leptin concentrations from early postnatal life until day 28 post natum and a further increase to later ages agrees with previous studies (Qian et al., 1999; Morise et al., 2009). Leptin secretion and consequently its plasma concentration are positively correlated with total fat mass (Hamilton et al., 1995; Houseknecht and Portocarrero, 1998; Wajchenberg, 2000; Fain et al., 2004) which normally increases with age in growing individuals. The three different protein levels in the maternal diets tested in the present study had no effect on the concentrations of leptin in the offspring over all postnatal days. Due to the fact that all piglets after birth were cross fostered to sows fed an adequate protein diet during gestation and lactation, these findings suggest that potential prenatal differences in leptin plasma concentrations which may have resulted from intra uterine protein deficit or excess, are possibly compensated by the homogenous and adequate protein supply through milk from the foster sows in the early postnatal days.

In samples obtained during fetal life, i.e. on dpc 94, leptin could not be assessed since the concentrations were mostly below the assay`s limit of detection at 0.3 ng/mL. From previous studies reporting leptin blood concentrations of 0.1 to 0.8 ng/mL in unsuckled neonatal piglets (Tatara et al., 2007; Szymeczko et al., 2009), relatively low concentrations had to be expected and thus it was not surprising that the prenatal concentrations could not be quantified. However, in animals such as the pig, in which fat is deposited before birth, leptin is synthesized in fetal adipose tissue and is present in the fetal circulation throughout late gestation albeit at low concentrations (McMillen et al., 2004).

The new born piglets had mean plasma leptin concentrations of 4.1 ng/mL. When comparing these values with the prenatal concentrations being below the limit of detection of the assay used and also when comparing with literature data (see Table 4), we obtained considerably lower concentrations. The most probable explanation for this rapid increase is intestinal absorption of leptin from colostrum: The mammary gland is able to produce leptin, particularly during the early phase of lactation (Smith-Kirwin et al., 1998; Smith and Sheffield, 2002) and leptin is secreted into milk and is

thus potentially available for the newborn (Uysal et al., 2002). In rats, leptin is transferred from the maternal circulation to the milk then to the stomach of the suckling pup, and afterwards to the neonatal circulation (Casabiell et al., 1997). The piglets in the present study were already suckled before blood samples could be collected on dpn 1 and thus the relatively high plasma concentrations of leptin probably resulted from the absorption of leptin contained in colostrum. In support of this notion, Szymeczko et al. (2009) showed a significant increase due to suckling in blood leptin concentrations from newborn piglets within 24 h; see also Table 4.

Table 4: Plasma concentrations of leptin, IGF-1 and IGF-2 in fetuses and piglets of different developmental stages

	day of sampling				
author, year	dpc	dpn	leptin (ng/mL)	IGF-1 (ng/mL)	IGF-2 (ng/mL)
Simmen et al., 1988		1		16.3	
		2		12.5	
		7		22.5	
		28		21.0	
Hausmann et al., 1991	70			7.5	135
	90			13.0	210
	110			20.0	200
Lee et al., 1991	90			17.9	200
		1		50.0	230
		30		150.0	370
Lee et al., 1993	90			10.1	88
		21		90.6	220
		170		182.3	326
Buonomo et al., 1993		1		200.0-320.0	290
		28		250.0	180
		188		80.0	120
Peng et al., 1996	90			70.0	430
		1		135.0	460
		21		165.0	490
		180		240.0	720
Bauer and Parvizi, 199	98-113			250.0	
		300-360		150.0-190.0	
Qian et al., 1999		42 - 49	0.60		
		105 - 112	2.70		
		140 - 154	3.00		
Carroll et al., 2000		1		29.0	80
		14		35.0	60
Clapper et al., 2000		70		98.0-125.0	
		90		148.0	
Weiler et al., 2002		19	1.24		
Mostyn et al., 2005		4	2.90	34.3	
		7	2.90	46.5	
		14	2.90	44.1	
Litten et al., 2005		3 - 10	2.50 - 2.90		
Tatara et al., 2007		1*	0.10	60.2	
Szymeczko et al., 200		1*	0.78		
		1	1.87		
		7	1.87		
Morise et al., 2009		7	2.50	26.0	
		28	1.60	21.5	

Abbreviations: dpc: days post conception, dpn: days post natum, *: before suckling.

In our study, piglets of the higher birth weight class had significantly higher leptin concentration compared to piglets of the lower birth weight class on dpn 28. Different previous studies reported a positive correlation between leptin plasma concentration with total fat mass as mentioned before. Thus leptin circulates as an internal signal indicating the size of body fat stores (Reidy and Weber, 2000). Indeed, the body composition analyses showed that the piglets with higher birth weights had higher lipid contents in empty body (13.5 %) and higher slaughter weights (8.2 kg) on dpn 28 in comparison to the piglets of lower birth weights (12.8 % and 6.8 kg, respectively; Table 5). We also found a positive correlation between leptin and body fat content for the piglets on dpn 1 and dpn 28, and also a positive correlation between leptin and slaughter weight at these two postnatal days. In addition, leptin and slaughter weight were correlated over all postnatal days.

Table 5: Amounts and concentrations of slaughter weight, leptin and fat (means ± SE) between piglets of the low and high birth body weight class at different age.

		Birth body weight class					
		<1.3 kg			>1.3 kg		
		mean		SE	mean		SE
dpn 1	Slaughter weight (kg)	1.03	±	0.03	1.67	±	0.03
	Leptin (ng/mL)	3.76	±	0.24	3.93	±	0.22
	Fat (%)	1.56	±	0.07	1.87	±	0.09
dpn 28	Slaughter weight (kg)	6.76	±	0.23	8.20	±	0.25
	Leptin (ng/mL)	2.92	±	0.11	3.27	±	0.09
	Fat (%)	12.76	±	0.38	13.50	±	0.37
dpn 188	Slaughter weight (kg)	124.8	±	1.52	131.80	±	2.12
	Leptin (ng/mL)	4.63	±	0.25	4.87	±	0.20
	Fat (%)	30.2	±	0.72	29.66	±	0.69

Abbreviations: SE: standard error, dpn: days post natum, dpc: days post conception.

Interestingly, on dpn 188, the leptin concentrations of high versus low birth weight piglets were not different, although the difference between the two birth weight classes in slaughter weights was maintained at this age. Houseknecht and Spurlock (2003) perceived leptin as a major sensor of energy and nutrient balance and speculated about its regulation by the nutritional status. In this context, leptin has also been associated with nutritional programming during fetal and neonatal growth which may lead to long-lasting effects (Eckert et al., 2000; Houseknecht and

Spurlock, 2003). Our findings indicate that there was a short time effect of the different intrauterine environments on fat content and body weights of the offspring, but these effects were not maintained until later adolescent stages. Furthermore our results show that plasma leptin was not affected by nutritional status in terms of intrauterine protein deficit or excess. In our study, leptin was not as highly correlated to body composition traits, in particular fat percentage of the whole empty body, as might have been expected. Analogous findings are reported for the sows of the present study by Metges et al. (under revision) and Rehfeldt et al., (2011a): although the increase of back fat thickness in the HP diet group was least from insemination until 109 dpc; this difference in comparison to the AP and LP diet group was not reflected by concordant differences in leptin concentrations. However, when verifying correlations of plasma leptin with carcass characteristics, the associations reported in the literature for pigs (Berg et al., 2003), were mostly not as close as for humans or laboratory rodents (Frederich et al., 1995; Maffei et al., 1995). This might be due to the fact that most model studies in which close relations could be established included a relatively wide range of different body fat contents, i.e. from lean to morbidly obese individuals. In contrast, studies in livestock species usually consider less variation in relatively homogenous groups. Taken together, the suitability of leptin as strong indicator of body composition in pigs at marketing age is not supported.

In prenatal as well as in early postnatal life, leptin has essential functions as an endocrine modulator just as the IGFs for the IGF-system at these stages of development. Leptin has a key role in energy homeostasis and there may be a link between leptin and the IGF-system. Experimental models have provided evidence of leptin functioning as a neuromodulator of the GH-IGF axis by connecting this hormonal system with nutritional status (LaPaglia et al., 1998). Interestingly, as the IGF-system is known to be deficient in the case of IUGR (Woodall et al., 1996), it can be speculated that the actions of leptin on body and organ growth are at least partially mediated by stimulation of the IGF-system. Anyway, changes in circulating IGF-1 concentrations are positively correlated with changes in leptin blood levels (Heiman et al., 1999), and indeed we also observed positive correlations between IGF-1 and leptin on dpn 1 and dpn 28 as well as over all postnatal days.

5.2 IGF-1 and -2

In contrast to leptin, the IGF-1 concentrations were well detectable in fetal samples. The concentrations of IGF-1 at the different sampling days and their age-related changes were in compliance with previous studies in pigs (Hausman et al., 1991; Lee et al., 1991; Lee et al., 1993; Peng et al., 1996; Carroll et al., 2000; Mostyn et al., 2006) as exemplified in Table 4. With the exception of dpn 1, the IGF-1 concentrations remained unchanged in response to maternal dietary treatments. At dpn 1, the piglets born to mothers fed the LP diet with only 6% protein during gestation had significantly decreased IGF-1 concentrations compared to piglets born to mothers fed the HP diet (30 % protein) and tended to have lower IGF-1 values than piglets born to mothers fed an adequate protein diet during gestation. In a study by Davis et al. (1997), an analogous finding for decreasing IGF-1 concentrations in newborn pigs from dams fed protein restricted diets (0.5% protein) throughout gestation was obtained. In adult humans, and also in other species, serum IGF-1 concentrations are markedly lowered by protein deprivation (Thissen et al., 1994; Breier and Sauerwein, 1995). Due to the fact that the availability of dietary protein is one main critical regulator of IGF-1, dietary protein supply seems to be the limiting factor for maximal stimulation of IGF-1 plasma concentrations (Breier, 1999).

In parallel with the results of the IGF-1 concentrations in LP piglets on dpn 1, the present investigation also revealed significantly lower IGF-1 concentrations in piglets of lower birth weight, when compared to the higher birth weight class and thus confirmed earlier studies (Davis et al., 1997; Ritacco et al., 1997). As expected, the piglets born to mothers fed the low protein diet had lower slaughter weights than the AP and HP piglets. Consequently, we could demonstrate a positive correlation between the IGF-1 concentrations and the body weights of the piglets at dpn 1. These findings are consistent with recent investigations in which positive correlations between the IGF concentrations in the fetus and birth weight were established in several species including humans, sheep, rodents and pigs (Daughaday et al., 1982; Gluckman and Butler, 1983; Lee et al., 1993; Ong et al., 2000). Moreover, we showed that piglets from smaller litters had higher concentrations of IGF-1 on dpn 1 as compared to piglets born to greater litters. This observation is in line with the fact that the piglets from smaller litters also had higher slaughter weights than piglets out of greater litters (Table 6).

In contrast, on dpn 28, piglets from greater litters had higher concentrations of IGF-1 and exhibited higher slaughter weights than compared to piglets from smaller litters. For both days positive correlations between IGF-1 concentration and slaughter weight were obtained. The reversals of the observations of dpn 1 versus dpn 28 suggest a trend towards a compensatory response to the maternal protein restriction. Compensatory growth is a well-known phenomenon (Lawrence and Fowler, 1997), for piglets, the work of Schoknecht et al. (1993) exemplarily shows, that once solid food was offered to weight reduced suckling piglets, these piglets gained weight and were the same weight compared to the other piglets after some weeks. At later postnatal days, the IGF-1 concentrations were not different between the original birth weight classes. These results are in line with the statement of Fliesen et al. (1989) whereupon the serum IGF-1 concentrations in rats at early stages of development (first weeks of age) critically depend on dietary protein supply, however this protein dependency decreases with age. Condensed, our results suggest that there is an effect of protein restriction *in utero* which generates a high correlation between the IGF concentrations and body weight at pre- and early postnatal days, in parts in parallel and/or in consequence of litter size. However, in no case these correlations were maintained until dpn 188.

Table 6: Slaughter weight and concentrations of IGF-1 and IGF-2 (means ± SE) of piglets from smaller and bigger litter size group at different age.

		Litter size group					
		1-12			> 12		
		mean		SE	mean		SE
dpc 94	Slaughter weight (kg)	0.620	±	0.03	0.670	±	0.02
	IGF-1 (ng/mL)	16.02	±	0.76	15.90	±	0.57
	IGF-2 (ng/mL)	25.66	±	0.73	26.08	±	0.64
dpn 1	Slaughter weight (kg)	1.406	±	0.06	1.264	±	0.05
	IGF-1 (ng/mL)	41.08	±	3.13	28.43	±	1.79
	IGF-2 (ng/mL)	30.26	±	0.90	27.78	±	0.95
dpn 28	Slaughter weight (kg)	7.113	±	0.26	7.702	±	0.26
	IGF-1 (ng/mL)	96.01	±	7.00	128.7	±	8.49
	IGF-2 (ng/mL)	32.54	±	0.92	33.60	±	0.83
dpn 188	Slaughter weight (kg)	129.1	±	2.14	127.6	±	1.77
	IGF-1 (ng/mL)	219.0	±	10.3	209.1	±	7.43
	IGF-2 (ng/mL)	39.85	±	0.70	39.21	±	0.66

Abbreviations: SE: standard error, dpn: days post natum, dpc: days post conception.

Further correlations were observed for IGF-1 as well as for IGF-2: Both IGFs showed correlations with their binding proteins in a similar manner, probably based on their similar age-related changes; IGF-1 and IGF-2 were also intercorrelated on dpc 94 and dpn 28, thus indicating a high association between both IGFs.

Both IGF-1 and IGF-2 are expressed in fetal tissues, but our results support IGF-2 as the primary growth factor supporting fetal growth, as indicated in recent studies (Gicquel and Le Bouc, 2006). At day 94 of gestation, protein restricted fetuses had lower IGF-2 values and showed lower weights compared to the offspring from AP fed dams. While most studies have found no association between serum IGF-2 concentrations and fetal weight (Fowden, 2003), we could demonstrate an association between low fetal body weight and decreased IGF-2 concentration. Consequential our findings suggest that there is the same association between IGF-2, protein restriction *in utero* and body weight at dpc 94 as mentioned before for IGF-1 at dpn 1. In agreement with our findings, IGF-2 is considered as being most important as fetal growth regulator (DeChiara et al., 1991) and it is thus plausible why research in the area of IGF-2 was focused on this aspect (Moses et al., 1980; Daughaday et al., 1982; Gluckman and Butler, 1983).

The average IGF-2 concentrations investigated in our study across all maternal diet groups steadily increased from dpc 94 until day 188. The absolute concentrations we measured herein markedly differ from those reported in the recent literature about IGF-2 concentrations in piglets of approximately the same age (Table 4). This divergence likely reflects differences in the assays used in the respective studies. In our investigation herein we used a commercial human IGF-2 ELISA (IGF-2 E30, Mediagnost®, Reutlingen, Germany) for analysing porcine IGF-2, whereas most of the recent investigations used radioimmunoassay for determining IGF-1 and -2 values in porcine blood (Lee et al., 1991; Lee et al., 1993; Peng et al., 1996). Based on the fact that the amino acid sequence of human and porcine IGF-1 is completely homologous, and the one of human and porcine IGF-2 differs in only one position, i.e. 98.5 % homology (Humbel, 1990), we used these human ELISAs for quantifying each IGF in porcine blood. Moreover the parallelism was documented between human standard and porcine sample. Thus we can only speculate about the reasons of the 2 to 20-fold lower IGF-2 concentrations we assessed in our study as compared to the ones from the literature. Noticeably, this divergence was limited to IGF-2

whereas the IGF-1 concentrations measured herein were well within the range of concentrations reported earlier for pigs at comparable ages. Potentially the binding between the human IGF-2 antibodies out of the commercial ELISA and the IGF-2 molecules in the plasma samples of fetuses and piglets were not as cross-reactive as assumed. The binding sites of the antibodies might have been not specific enough for recognizing the porcine IGF-2 molecules, but such a difference in binding affinity would require differences in the molecular structure of human versus porcine IGF-2, albeit this is not probable based on the sequence. What reason really caused this difference in absolute concentrations for porcine IGF-2 in all our samples is presently not clear. Due to this divergence, our results are not comparable to recent findings and we could not attest the preponderance of IGF-2 over IGF-1 in fetal serum in pigs as described by Lee et al. (Lee et al., 1991). Nevertheless the validity of our data in terms of relative comparisons has been proven in the pilot experiments (3.2.1 page 22 and 3.2.2 page 22).

In line with previous studies, we found that protein restriction as modulator has more pronounced effects on circulating concentrations of IGF-1 than on IGF-2 (Fowden, 2003). Besides, IGF-1 and -2 were more affected by protein restriction than by protein excess, which is in agreement with the study of Nissen et al. (2005) demonstrating that the fetal concentrations of IGF-1 and IGF-2 are not affected by maternal feeding above requirements In this context, the findings of Rehfeldt et al. (2004) whereupon increased maternal feed does not necessarily result in increased nutrient supply to the fetuses, are of relevance. In the present study, primiparous sows were used for the experiment of protein excess- and deficit during gestation. Schoknecht et al. (1993) showed differences between primiparous and multiparous sows for such experiments. In primiparous gilts, maternal growth is continued during gestation, whereas in multiparous sows adult size is probably already reached. The progeny of primiparous pigs might be expected to be more vulnerable to maternal protein restriction than progeny of multiparous dams, given the consequences of fetal-maternal competition for nutrients (Schoknecht et al., 1993). Concerning our study, the effect of protein deficit during gestation might thus have been amplified for the progeny caused on the competing situation for protein supply between the fetus and the continuously growing primiparous sows.

Schoknecht et al. (1993) focused on the effects of maternal protein deficiency using diets of only 0.5% protein throughout the entire pregnancy, on piglet growth. In their study growth was consistently affected suggesting that gene expression in the progeny controlling long-term postnatal somatic growth is affected adversely only if maternal protein insufficiency is extended over all or most phases of prenatal life (Schoknecht et al., 1993). However, the protein restriction to 0.5% of the diet is very drastic and the components of the IGF-system would probably be significantly affected but were not investigated in the study. Nevertheless, in view of the huge differences in protein restriction of our study and the one by Schoknecht et al. (1993), comparisons with our results are of limited use only. However, the concentrations of the IGFs in the fetus with birth weight are reportedly correlated in pigs (Lee et al., 1993), the latter being knowingly correlated with postnatal growth.

Sex differences in plasma IGF-1 values were solely observed on dpn 188; for IGF-2 there were no differences between sexes throughout the study. Owens et al. (1999) hypothesized that sex differences in plasma IGF-1 probably reflect those in GH secretion and contribute to the sexually dimorphic growth, because IGF-1 promotes growth and is GH-responsive (Owens et al., 1999). Metz and Claus (2003) showed that barrows in comparison to boars had less GH and significantly lower IGF-1 concentrations. Consequently, the current observations that male pigs had lower IGF-1 concentrations than females albeit the slaughter weights of male and females were not significantly different might be attributed to the fact that the male pigs in our study were castrated as commonly done to avoid boar taint in porc. Due to this they displayed comparatively lower IGF-1 values. No distinct differences between life weight at slaughter and hot carcass weight between barrows and sows were concordantly reported (Cisneros et al., 1996).

5.3 IGFBPs

In adults, decreased nutritional intake and in particular protein restriction reduce the plasma concentrations of IGF-1 and also changes the relative concentrations of the IGFBPs in plasma (Breier, 1999). For the IGFBPs, indeed nutrient intake has been identified as major regulator of their plasma concentrations (Thissen et al., 1994). The IGFBPs 1 to 4 are the most prevalent IGFBPs in plasma and tissue, although their relative portions vary (Zapf et al., 1990). In response to dietary protein

restriction, the concentrations of the various IGFBPs change divergently and not in a consistent direction and extent as for example shown in rats (Lemozy et al., 1994).

In our study **IGFBP-1** was affected by maternal protein feeding during gestation on dpc 94. We observed an association between maternal protein feeding, IGFBP-1 values and fetal body weight class. Fetuses from dams fed the excess protein diets (HP) had significantly higher IGFBP-1 concentrations and lower fetal body weights compared to the other two diet groups. In addition, fetuses of the light fetal body weight class, irrespective of the maternal diet, had higher IGFBP-1 values than the ones of the higher body weight class. These findings of dpc 94 are not in line with results from later postnatal stages: Lemozy et al. (1994) reported that protein restriction in young rats increased their IGFBP-1 values , and in newborn pigs fasted for 24 h, higher IGFPB-1 concentrations than in fed controls are reported (McCusker et al., 1989). Also for long-term dietary restriction, increased IGFBP-1 values are reported for human patients (Busby et al., 1988). The latter findings are more comparable to the situation we found for neonatal piglets. At this point of development we observed a negative correlation between IGF-1 and IGFBP-1; piglets from LP fed mothers had higher IGFBP-1 values, while they had significantly lower concentrations of IGF-1 than the AP and HP piglets. This negative correlation agrees with previous findings in human serum (Rajaram et al., 1997).

In this investigation piglets from the sows fed a LP diet showed a similarly intensive increase of IGFBP-1 values from dpc 94 to dpn 1 as compared to AP piglets, whereas the IGFBP-1 concentrations in offspring from HP fed sows remained unchanged during this time interval. In view of the mostly inhibitory effects of IGF-1 on IGFBP-1 reported from cell culture experiments (Thraikill et al., 1990), our results do not support such a relation. However, the transition from intra to extra uterine life were not addressed in previous studies about the regulation of IGFBP-1 and considering the complex and dynamic changes occurring in this phase, the situation might well be different. In addition, the possibility that the IGFBP-1 quantified herein from the 30 kDa band in the Western Ligand blot system, might actually not be entirely specific for IGFBP-1 (McCusker et al., 1989), as mentioned before in 4.3.

Consistent with a previous study of Peng et al. (1996), we found the highest IGFBP-1 concentrations at the first day of life. In fact, detection of IGFBP-1 was limited to dpc 94 and dpn 1 and the respective band was not displayed at later ages. Lee et al.

(1991) detected IGFBP-1 only in postnatal sera, representing only a minor portion of total IGF-binding activity in porcine serum. In general, decreasing IGFBP-1 concentrations from early to later postnatal stages were demonstrated (Lee et al., 1991; Lee et al., 1993; Peng et al., 1996). Such low concentrations are probably the reason why we could not detect IGFBP-1 at days 28 and 188 post natum since the sensitivity of the Western ligand blot system we used was obviously not sensitive enough.

The **IGFBP-2** concentrations did not differ between sexes, litter size group or birth body weight classes on any day of sampling. Based on the fact that the binding affinity between IGFBP-2 and IGF-2 is about 3-fold higher than that of IGFBP-2 and IGF-1 (Clemmons and Underwood, 1991), and the use of IGF-1 as labeled ligand in the Western ligand blot might have resulted in a less sensitive detection of IGFBP-2 and thereby detection of potential differences for these aforementioned fixed factors might have been compromised.

Nevertheless, the IGFBP-2 values obtained on dpn 1 and dpn 28 of piglets born to mothers fed the protein restricted diet during gestation are consistent with a previous study in rats, in which IGFBP-2 was decreased during protein restriction (Lemozy et al., 1994). Rajaram et al. (1997) hypothesized that nutrition-induced changes in serum IGFBP-2 represent the direct effect of dietary protein on IGFBP-2 expression in liver, however, this was not assessed in our study and needs further investigation.

Thraikill et al. (1990) suggested that IGF-1 directly stimulates the secretion of IGFBP-2, and thus the increased values of IGFBP-2 in HP piglets might be explained, since they also had increased concentrations of IGF-1 on dpn 1. The adjustment to similar concentrations of IGFBP-2 in all piglets regardless of maternal diet until day 188, might have resulted from the postnatal feeding being equal in all groups: in undernourished children, protein refeeding normalized the serum IGFBP-2 levels, and high-protein intake was required to achieve complete normalization (Pucilowska et al., 1993).

The interaction between body weight at birth and maternal feeding detected on dpn 28, showed that piglets born to mothers fed the LP diet with lower birth weights had lower IGFBP-2 values on dpn 28 compared to piglets with higher birth weights but adequate protein supply during gestation. Piglets born to mothers fed the AP or HP diet during gestation and with higher birth weights had reduced IGFBP-2

concentrations compared to piglets with lower weight at birth but the same nutrient environment *in utero*. In this case, it is obvious that protein restriction during fetal development has a greater impact on the IGFBPs of the offspring than protein excess.

The ontogenetic changes of IGFBP-2 we observed are consistent with reports by Lee et al. (1993). In general, we recorded the highest IGFBP-2 values on dpn 1; age related differences were observed for all diet groups. The concentration of IGFBP-2 tended to lowest concentrations on dpn 28 and dpn 188, respectively. Similarly, IGFBP-2 increased during the latter half of fetal life in pigs and then gradually declined during postnatal development (Lee et al., 1993).

Our finding that IGFBP-2 represents a major form of IGFBPs in fetal and early postnatal porcine plasma, agrees with the one of McCusker et al. (1989). In their study, IGFBP-3 was also shown to predominate in later postnatal ages and also in maternal porcine plasma, respectively.

For the **IGFBP-3** concentrations, an age related increase was observed in all diet groups (Figure 5), whereby the biggest difference between subsequent sampling days was from dpn 28 to 188. Comparing dpc 94 and dpn 188, 16-fold higher IGFBP-3 values were seen on dpn 188. Similar changes, i.e. a 10-fold increase from dpc 90 to dpn 169 have been demonstrated earlier for IGFBP-3 (Lee et al., 1991; Lee et al., 1993; Peng et al., 1996). In conclusion, our data do confirm the notion that IGFBP-3 blood concentrations are low during fetal development and increase thereafter in postnatal life.

Focusing on IGFBP expression in the fetus, the effects of nutritional and endocrine conditions *in utero* have been demonstrated to concern mainly IGFBP-1, -2 and -4 rather than IGFBP-3 (Fowden, 2003). The lack of maternal diet effects on IGFBP-3 at dpc 94 in our study is thus in line with this study. Verified differences related to maternal feeding group were limited to dpn 1 when the concentrations of IGFBP-3 in HP piglets were significantly higher than in AP piglets. Again, this result agrees with the fact that on dpn 1, piglets from litters with less than 12 neonates had higher IGFBP-3 values than those from bigger litters, because the piglets out of the smaller litters were primarily born to mothers fed the HP diet during gestation.

In general, serum IGFBP-3 levels are regulated by IGF-1 and GH (Rajaram et al., 1997), i.e. GH and IGF-1 both induce the synthesis and secretion of IGFBP-3 and

thus increase its concentrations in plasma (Clemmons et al., 1989). Our observation of higher IGF-1 values in the HP piglets together with higher IGFBP-3 concentrations on dpn 1, supports the findings of Rajaram et al. (1997) and Clemmons (1998). The apparent coherence between IGF-1 and IGFBP-3 pictured in our findings supports the fact that IGFBP-3 is the major carrier of IGF-1 and probably accounts for the stability of IGF-1 in blood, as already hypothesized by Clemmons and Underwood in 1991.

In contrast, our results could not support a significant reduction in serum IGFBP-3 concentrations in response to extended fasting and/or protein deficiency as reported for adult stages (Clemmons et al., 1989; Lemozy et al., 1994; Smith et al., 1995). Possibly this relationship is not or only partially applicable for fetal and neonatal life. Consequently, further investigations should clarify this.

Likewise, refeeding either adequate energy or protein intake will increase the concentration of IGFBP-3 (Clemmons et al., 1989). Pertaining to the statement of Clemmons et al., our findings of the IGFBP-3 concentrations in HP piglets on dpn 1 indicate that the preceding high protein supply during fetal life has articulately stimulated IGFBP-3 concentrations. Our results definitely support the actuality of increased IGFBP-3 concentrations after refeeding as shown in the ontogeny of IGFBP-3 in LP piglets. The animals were thus able to compensate their decreased IGFBP-3 values in comparison with the other two protein diets when fed adequate protein levels in postnatal life, as shown for dpn 28 and dpn 188.

Detection of **IGFBP-4** was limited to dpc 94, dpn 1 and dpn 28. The highest IGFBP-4 concentrations were detectable on dpn 1, in accordance to reports by Peng et al. (1996). In their study, the IGFBP-4 concentrations were increased from dpc 90 to highest values on dpn 1 and then decreased again to dpn 21, 90 and 180 reaching similar concentrations as during fetal life. In our study, IGFBP-4 was even undetectable on dpn 188.

On none of the sampling days, differences between the diet groups occurred, although the plasma concentrations of IGFBP-4, an inhibitor of IGF action, are reportedly decreased with undernutrition both in serum and in tissues at the transcriptional level (Breier, 1999). IGFBP-4 appears to inhibit IGF actions under most, if not all, experimental conditions (Jones and Clemmons, 1995). This actuality

is supported by our results: We showed a highly negative correlation between IGFBP-4 and IGF-1 over all experimental postnatal days.

Correlations between the IGFs and the IGFBPs 1-4 on all postnatal days agree with previous findings in human serum (Rajaram et al., 1997), where positive correlations were limited to the IGF-s and IGFBP-3. For IGFBP-1, IGFBP-2 and IGFBP-4 negative correlations were detectable. These data suggest that different mechanisms may regulate the secretion of the various IGFBPs in humans and also in pigs. Nevertheless, the observed interrelationships between the different IGFBPs showed a remarkable association between the IGFBPs and thus support their main function as principal carrier of the IGFs (Thissen et al., 1994).

Even though the fetal IGF-1 concentrations were increased in the fetuses of the heavier body weight class, the abundance of fetal IGFBPs remained largely unaffected, implying that the half life and transport of the IGFs to peripheral tissues by IGFBPs might not or only marginally be concerned.

5.4 IGF-1 receptor

The availability and thus action of the IGFs are modulated through the high affinity binding proteins, whereas the cellular response towards the IGFs is mediated through their receptors, which are present on virtually all cell types (Thissen et al., 1994). The IGF-1 receptor mediates most of the effects of IGFs (Collett-Solberg and Cohen, 2000). We limited our measurements of the IGF-1 receptor in the fetuses and piglets from our study to liver tissue since the liver is considered as the principal source of circulating IGFs (Yakar et al., 2002). However, the concentrations of IGF-1 receptor in liver at any of the postnatal samples were below the limit of detection of the assay available. For the fetal stage, the concentrations across all diet groups were within the measuring range of the assay. For the mRNA expression of the IGF-1 receptor, Lee et al. (1993) reported that the hepatic mRNA abundance in pigs did not change during the second half of fetal life, then declined by day 21 post natum, and further to nearly undetectable levels by day 170. Our results seem to support their mRNA findings of the IGF-1 receptor ontogenesis, coincident with results previously found in rats (Alexandrides et al., 1989; Werner et al., 1989). Neither maternal feeding group nor body weight nor litter size nor sex apparently influenced IGF-1 receptor content on dpc 94 in liver, which indicates that different protein diets

in utero throughout the entire pregnancy might rather act at the level of the ligand than of the receptor, if the IGF-system is concerned at all.

5.5 Body composition at the postnatal days

Body weight and components of body composition are highly associated with the IGF-system and leptin, as modulators of growth and development (Fowden and Forhead, 2004). In pigs, body weight and composition are influenced due to inadequate protein supply *in utero* (Rehfeldt et al., 2011b). At their first day of life, piglets exposed to maternal LP diet *in utero* had lower body and hot carcass weights compared to the AP piglets. In line with this, piglets of the lighter birth weight class, mainly born to LP fed gilts, showed lower slaughter and hot carcass weights, too. Likewise Rehfeldt et al. (2011b) showed significantly reduced body and hot carcass weights on dpn 1 in LP piglets in an experiment with the same dietary treatments as in this study. These results are in line with further previous studies, in which body weight at birth was significantly lower in progeny of dams fed a protein-deficit diet with 0.5% protein throughout pregnancy compared to progeny of control dams (Atinmo et al., 1974; Pond et al., 1991; Schoknecht et al., 1993). As potential reason for impaired fetal growth resulting in significantly reduced birth body weights the deficit mainly of essential amino acids in response to LP feeding *in utero* is considered. Beside essential amino acids, aberrant concentrations of other hormones such as insulin, leptin and IGF-1 might form additional causal factors for the fetal growth retardation in LP offspring (Rehfeldt et al., 2011a). Our data at least partially support this hypothesis indicating a relationship between reduced birth weight and decreased leptin and IGF-1 concentrations on dpn 1 in piglets born to LP fed gilts during gestation. In addition, we detected positive correlations between IGF-1 and all three weight parameters (birth weight, slaughter weight and hot carcass weight) as well as between leptin and all three weight variables on dpn 1.

The highly positive correlation between body weight, hot carcass weight and slaughter weight, observed for all three diet groups on dpn 1 explains for the logic coherence, why the low birth weight piglets exhibited lower slaughter and hot carcass weights on dpn 1. Moreover, the reduced slaughter and hot carcass weights of piglets born to gilts fed the low protein diet were to be expected according to previous studies (Pond et al., 1992; Davis et al., 1997) in which piglets of protein restricted

dams exhibited reduced birth weights, too. In the current report, for both variables, slaughter and hot carcass weights, piglets of greater litters showed lower weights. These results can be interpreted as consequences of reduced birth weights of large litters which are in turn attributable to the reduced space per fetus *in utero* (Foxcroft et al., 2006).

The composition of the whole body in terms of protein, lipid and ash contents obtained by chemical analysis was not significantly changed by dietary treatment in a similar study (Rehfeldt et al., 2011b), whereas we observed significantly lower lipid percentages for piglets of lower birth weights at dpn 1. Interestingly, there was no evidence for a correlation between birth weight and analytical lipid percentage observed on dpn 1.

For the percentages of protein and dry matter, an interaction between diet and litter size group was detectable. The greater litters showed lower values of protein and dry matter in response to the HP diet *in utero*, whereas the AP and LP diet did not affect the protein or dry matter percentage in different litter size groups. The reasons for these interrelations are not clear.

Grippingly, for all postnatal days significant differences of slaughter weights and hot carcass weights between the birth weight classes were observed. As a matter of course, pigs with higher weights at birth had significantly higher slaughter weights and hot carcass weights on dpn 28 and dpn 188 compared to piglets with lower weights at birth. The maternal low protein diet did not affect slaughter or hot carcass weight of the offspring on dpn 28 or 188, showing that the decrease in birth weight, found in LP pigs was compensated during postnatal life. Nevertheless, our results show an indirect relation between reduced birth body weights due to the LP diet *in utero* and the reduced slaughter and hot carcass weights on dpn 28 and 188, because verifiably piglets with low birth weights were mainly from the dams fed the LP diet during gestation. Regarding these findings we hypothesize that porcine fetuses that are exposed to a protein restriction *in utero* will have significantly reduced birth weight and will not be able to entirely compensate this protein deficit thus remaining in a growth retarded state in their later life. The stunting effect of maternal protein restriction throughout gestation on postnatal development of progeny was expected, based on previous observations (Pond et al., 1969; Atinmo et al., 1974; Atinmo et al., 1976). In addition Rehfeldt et al. (2008) reported that low

birth weight piglets grew distinctly slower than middle and heavy birth weight piglets and exhibited the lowest postnatal growth performance.

On dpn 28, exclusively fat and dry matter content were affected by maternal feeding, litter size and birth weight. Lipid and dry matter content were higher in piglets born to LP fed mothers than in HP and AP piglets. These results are again consistent with the findings of Rehfeldt et al (2011b) where the carcasses of LP pigs had increased lipid percentages in whole empty body on dpn 28. These recent findings of Rehfeldt et al. support our findings albeit they were reported in pigs at market weight. In this study, pigs of low birth weight exhibited the lowest lean mass and the highest degree of fatness and thus lower carcass quality compared with pigs of higher birth weights (Rehfeldt et al., 2008). To confirm this hypothesis, more body composition traits, especially lean mass, perirenal fat and back fat thickness should be evaluated.

For lipid and dry matter percentage, there was an interaction between diet and litter size group on dpn 28: HP piglets showed higher lipid percentages in smaller litters, whereas LP and AP piglets showed lower lipid percentages in smaller litters. The same interaction between diet and litter size group was observed for carcass protein and fat content on dpn 188. In this case, AP pigs differed from HP and LP pigs. AP pigs exhibited higher chemical lipid percentages in smaller litters as compared to HP and LP pigs that displayed lower lipid portions in smaller litters. The interaction between diet and litter size group for carcass protein was displayed as follows: AP pigs had lower carcass protein values corresponding to smaller litters, whereas HP and LP pigs displayed higher protein percentages in smaller litters. What in detail causes this interaction between diet and litter size for different analytical components of the whole empty body is not known yet.

The effects of sex on the percentages of protein, fat and dry matter of the whole empty body on dpn 188 are in compliance with findings of Schoknecht et al. (1993) and Rehfeldt et al. (2008). They reported that females had higher lean percentages than male castrates (Rehfeldt et al., 2008) and female carcasses were significantly lighter and leaner, with less back fat at the ribs, greater trimmed ham and loin, lower belly weight compared to carcasses of castrated males (Schoknecht et al., 1993). Again, more detailed data on body composition and carcass quality as for example provided by Rehfeldt et al. (2011b) might help to detect interrelationships not visible so far.

5.6 Conclusion

In the present study we tested the effects of protein deprivation and protein excess throughout pregnancy on the offspring. As a contribution to basic research, this approach is relevant for pig production even though such large variations in protein content are not targeted during routine procedures of pig farming. Nevertheless the effects of such deflections contribute to our understanding of nutrient regulation during fetal life. Moreover, the animal experiment provides an interesting model for humans. As obvious from recent studies, using the pig model for studying biological interrelationships and pathways in humans provides several advantages.

The notion of a sort of 'diet memory' initiated by inadequate nutrient supply during intra-uterine life resulting in impaired or altered endocrine or bodily functions or performance in adulthood is not comprehensively supported by the present study. All statistically sound effects of inadequate protein diets during gestation were limited to prenatal and early postnatal days. Furthermore, the provable effects were predominantly arising from protein restriction *in utero*. Protein excess did not or only scarcely exert short or long term effects on all factors tested in our investigation. The reasons for this are not entirely clear. One can assume that the observed effects of protein restriction on the variables investigated herein reflect the competing situation of protein supply between fetuses and the primiparous sow. It should be investigated in further studies if the results of this study are repeatable in multiparous sows under the same conditions. Alternatively, the extent of protein restriction might be a reason for the marginal effects. Due to the fact that a protein restriction of 0.5% during gestation showed multiple consequences on the offspring, as shown by Schoknecht et al., 1993, it could be speculated that perhaps a protein restriction of 6% was not severe enough to influence the fetal nutrient environment as extensive as it was expected. Moreover, in recent studies in which protein restriction was realized throughout gestation and where clear effects of this restriction were observed (Schoknecht et al., 1993), the offspring was not cross fostered after birth as in our study, but rather suckled by their dams. Although these piglets had free excess to a standard preweaning starter diet, the colostrums of their dams could probably amplify the effects of the protein restriction of their dams during gestation. The reason of our cross-fostering-model was to focus exclusively on the long term effects of protein excess- and deficit throughout pregnancy, but exclude effects of potentially divergent

nutrient supply during the weaning period. The sows serving as foster mothers had received adequate protein supply before and during lactation. This situation seemed to cause a sort of catch up possibility for the *in utero* impaired offspring, immediately after birth. Based on our cross-fostering-model it seems obvious that the possible long term effects of the modified nutritional environment *in utero* could be attenuated by the possibility of catching up directly after birth.

In summary, the present work showed that the IGF-system and leptin in progeny were sparsely swayed by inadequate protein supply during gestation at different stages of ontogeny. Leptin seemed to be insignificantly regulated by the protein content in the maternal diets and accordingly we could not detect long term consequences on leptin concentrations. For the IGFs, nutrient restriction as modulator had more pronounced effects on circulating concentrations of IGF-1 than on IGF-2. The present study revealed an apparent connection between reduced birth weights and aberrant respectively decreased concentrations of IGF-1 and leptin in piglets born to mothers fed LP diet throughout pregnancy. Based on our findings, it seems that leptin and IGF-1 were additional causal factors for fetal growth retardation, at least in LP offspring. For the IGFBPs, only short time effects of protein restriction were detectable; IGF-1 receptors in liver were not even affected during fetal age. The result of marginal alterations that were mainly restricted to early ages and were apparently compensated at later ages, is valid only for the ages studied. It remains open whether alterations might emerge at later ages and whether other regulative systems might have been affected. In consequence, even though we did not observe major and persistent deviations, general recommendations should adhere to aim for providing all prerequisites for an optimal intrauterine environment.

The present study within this thesis was limited to endocrine regulatory changes, in particular within the IGF-system. However, the multifaceted approach of the present animal study in which epigenetic, metabolic and transcriptomic techniques were used in other related projects beyond the scope of this thesis and will yield additional information once a circumspect and comprehensive analysis of all data can be done.

6 Summary

Healthy and normal weight piglets preferable with consistent genetics, which yield carcasses by efficient feed conversion, are requested from the pig industry. Reduced survival and compromised postnatal development due to low birth weight imply economic losses for the pig farmers. Size at birth, reflecting fetal growth and development, is an important predictor of perinatal survival and adult health in pigs. Fetal growth is a complex process that depends on the genotype and epigenotype of the fetus, maternal nutrition, the availability of nutrients and oxygen to the fetus. The IGF-system as well as leptin are knowingly affected by intrauterine growth retardation. In consequence some of these alterations may lead to permanent pathological programming of the IGF-system and/or energy homeostasis with energy balance and lipid metabolism, in which leptin plays a key role.

The aim of this dissertation was to characterize leptin and the different components of the IGF-system in offspring of sows fed diets with different protein contents throughout pregnancy. Furthermore we wanted to identify relationships between the different target endocrine components and to evaluate potential long-term consequences of maternal diet, endocrine deflections and body growth patterns caused by this nutritional fetal programming.

For this purpose, gilts of the German Landrace were fed gestation diets containing a low (LP, 6%), an adequate (AP, 12%), or a high (HP, 30%) protein level. Twenty six gilts and their offspring were investigated for sampling at day 94 of gestation. At parturition the offspring of the remaining 52 gilts were cross fostered to nursing sows fed a 12% protein gestation diet and then a standard lactation diet. Fetal and birth weights of the whole investigated offspring were recorded and classified into two different fetal body weight classes and two birth body weight classes (Fbwc ≤ or > 0.65; Bbwc ≤ or > 1.3 kg). In addition, the litter size of any gilt was classified into two groups (Lsgr ≤ or > 12 piglets). Blood and liver samples of overall 306 piglets were collected. In detail, samples from fetuses of day 94 of gestation, from the 1st day of life, 28th day of life (weaning piglets) and the 188th day of life (pigs at marked weight) were collected.

At first, blood samples were assessed by an in house developed ELISA for **leptin**, that has extensively validated for porcine plasma. In samples obtained during fetal

life, i.e. on dpc 94, leptin could not be assessed since the concentrations were mostly below the assay`s limit of detection. In the offspring over all postnatal days the three different protein levels in the maternal diets had no effect on the concentrations of leptin. These findings suggest that potential prenatal differences in leptin plasma concentrations which may have resulted from intra uterine protein deficit or excess, are possibly compensated by the homogenous and adequate protein supply through milk from the foster sows in the early postnatal days. However, we observed a positive correlation between leptin and body fat content for the piglets on dpn 1 and dpn 28, and also a positive correlation between leptin and slaughter weight at these two postnatal days. These findings indicate that there was a short time effect of the different intrauterine environments on fat content and body weights of the offspring, but these effects were not maintained until later adolescent stages. In this study, leptin was not as highly correlated to body composition traits, in particular fat percentage of the whole empty body, as might have been expected.

The main components of the IGF-system are the ligands, i.e. **IGF-1 and -2**. They were quantified in the blood plasma samples using commercially available ELISA for the human proteins, after securing their applicability for porcine samples. In contrast to leptin, the IGF-1 concentrations were well detectable in fetal samples. With the exception of dpn 1, the IGF-1 concentrations remained unchanged in response to maternal dietary treatments. At dpn 1, the piglets born to mothers fed the LP diet with only 6% protein during gestation had significantly decreased IGF-1 concentrations compared to piglets born to mothers fed the 30% protein diet and tended to have lower IGF-1 values than piglets born to mothers fed an adequate protein diet during gestation. In parallel with the results of the IGF-1 concentrations in LP piglets on dpn 1, the present investigation also revealed significantly lower IGF-1 concentrations in piglets of lower birth weight, when compared to the higher birth weight. As expected, the piglets born to mothers fed the low protein diet had lower slaughter weights than the AP and HP piglets. Consequently, we could demonstrate a positive correlation between the IGF-1 concentrations and the body weights of the piglets at dpn 1. Moreover, we showed that piglets from smaller litters had higher concentrations of IGF-1 on dpn 1 as compared to piglets born to greater litters. This observation is in line with the fact that the piglets from smaller litters also had higher slaughter weights than piglets out of greater litters. Condensed, our results suggest

that there is an effect of protein restriction *in utero* which generates a high correlation between the IGF concentrations and body weight at pre- and early postnatal days, in parts in parallel and/or in consequence of litter size. However, in no case these correlations were maintained until dpn 188.

Further correlations were observed for IGF-1 as well as for IGF-2: Both IGFs showed correlations with their binding proteins in a similar manner, probably based on their similar age-related changes; IGF-1 and IGF-2 were also correlated on dpc 94 and dpn 28, thus indicating a high association between both IGFs.

The IGF-1 and IGF-2 are equally expressed in fetal tissues, but our results support IGF-2 as the primary growth factor supporting embryonic growth. We could demonstrate an association between low fetal body weight and decreased IGF-2 concentration. In concequence, our results suggest that there is the same association between IGF-2, protein restriction *in utero* and body weight at dpc 94 as mentioned before for IGF-1 at dpn 1. The IGF-2 is considered as being most important for fetal growth regulation. Nevertheless we found that protein restriction as modulator has more pronounced effects on circulating concentrations of IGF-1 than on IGF-2. Besides, IGF-1 and -2 were more affected by protein restriction than by protein excess.

In view of the important role of the **IGF binding proteins** within the IGF-system, a semi-quantitative, nonradioactive Western ligand blot was established and validated to assess their abundance in the plasma samples.

In our study **IGFBP-1** was affected by maternal protein feeding during gestation on dpc 94. We observed an association between maternal protein feeding, IGFBP-1 values and fetal body weight class. Fetus from dams fed the excess protein diets (HP) had significantly higher IGFBP-1 concentrations and lower fetal body weights compared to the other two diet groups. In addition, fetuses of the light fetal body weight class, irrespective of the maternal diet, had higher IGFBP-1 values than the ones of the higher body weight class. Piglets from the sows fed a LP diet showed a similarly intensive increase of IGFBP-1 values from dpc 94 to dpn 1 as compared to AP piglets, whereas the IGFBP-1 concentrations in offspring from HP fed sows remained unchanged during this time interval. We found the highest IGFBP-1 concentrations at the first day of life. In fact, detection of IGFBP-1 was limited to dpc 94 and dpn 1 and the respective band was not displayed at later ages.

The secretion of **IGFBP-2** is directly stimulated by IGF-1, and thus the increased values of IGFBP-2 in HP piglets might be explained, since they also had increased concentrations of IGF-1 on dpn 1. The adjustment to similar concentrations of IGFBP-2 in all piglets regardless of maternal diet until day 188, might have resulted from the postnatal feeding being equal in all groups. The interaction between body weight at birth and maternal feeding detected on dpn 28, showed that piglets born to mothers fed the LP diet with lower birth weights had lower IGFBP-2 values on dpn 28 compared to piglets with higher birth weights but adequate protein supply during gestation. Piglets born to mothers fed the AP or HP diet during gestation and with higher birth weights had reduced IGFBP-2 concentrations compared to piglets with lower weight at birth but the same nutrient environment *in utero*. In this case, it is obvious that protein restriction during fetal development has a greater impact on the IGFBPs of the offspring than protein excess.

Verified differences related to maternal feeding group were limited to dpn 1 when the concentrations of **IGFBP-3** in HP piglets were significantly higher than in AP piglets. This result agrees with the fact that on dpn 1, piglets from litters with less than 12 neonates had higher IGFBP-3 values than those from bigger litters, because the piglets out of the smaller litters were primarily born to mothers fed the HP diet during gestation. In general, serum IGFBP-3 levels are regulated by IGF-1 and GH, which both induce the synthesis and secretion of IGFBP-3 and thus increase its concentrations in plasma. In compliance we observed higher IGF-1 values in the HP piglets together with higher IGFBP-3 concentrations on dpn 1. This apparent coherence between IGF-1 and IGFBP-3 supports the fact that IGFBP-3 is the major carrier of IGF-1 and probably accounts for the stability of IGF-1 in blood.

On none of the sampling days, differences between the diet groups occurred related to **IGFBP-4** concentrations. IGFBP-4 appears to inhibit IGF actions, which we could support by our results: We showed a highly negative correlation between IGFBP-4 and IGF-1 over all experimental postnatal days.

As the last part of the IGF-system and as the main target receptor, **IGF-1 receptor** was measured in liver tissue with a commercially available ELISA for human IGF-1 receptor that was also preliminarily tested for its applicability in porcine liver samples. Neither maternal feeding group nor fetal body weight nor litter size nor sex apparently influenced IGF-1 receptor content on dpc 94 in liver, which was the only point of

development were we could verify the IGF-1R. This ascertainment indicates that different protein diets *in utero* throughout the entire pregnancy might rather act at the level of the ligand than of the receptor, if the IGF-system is at all concerned.

In addition, **body weight** and also **components of body composition** are influenced due to inadequate protein supply *in utero*. At their first day of life, piglets exposed to maternal LP diet *in utero* had lower body and hot carcass weights compared to the AP piglets. In line with this, piglets of the lighter birth weight class, mainly born to LP fed gilts, showed lower slaughter and hot carcass weights, too. As potential reason for impaired fetal growth resulting in significantly reduced birth body weights the deficit mainly of essential amino acids in response to LP feeding *in utero* is considered. Beside essential amino acids, aberrant concentrations of other hormones such as insulin, leptin and IGF-1 might form additional causal factors for the fetal growth retardation in LP offspring. Our data at least partially support this hypothesis indicating a relationship between reduced birth weight and decreased leptin and IGF-1 concentrations on dpn 1 in piglets born to LP fed gilts during gestation. The maternal low protein diet did not affect slaughter or hot carcass weight of the offspring on dpn 28 or 188, showing that the decrease in birth weight, found in LP pigs was compensated during postnatal life.

In conclusion, the results of this study show clearly, that all ascertained effects of modified protein content in diets fed during gestation were limited to prenatal and early postnatal days. Furthermore, the provable effects were predominantly arising from protein restriction *in utero*. Protein excess did not or only scarcely affect short or long term effects on all factors tested in our investigation. It could be speculated that the absent, probably expected, effects are caused on the cross fostering of the *in utero* impaired piglets, directly after birth. This situation caused a sort of catch up possibility for the offspring, which probably resulted in adjustment of the impaired endocrine and metabolic situation during prenatal and early postnatall stages.

7 Zusammenfassung

In der Schweinefleischproduktion werden gesunde Ferkel mit möglichst einheitlicher Genetik nachgefragt, die problemlos, bei guter Futterverwertung, die vom Markt gewünschten Schlachtkörpergewichte und -qualitäten erreichen. Ferkelsterben und beeinträchtigte postnatale Entwicklung, zurückzuführen auf ein zu geringes Geburtsgewicht, bedeuten für den Ferkelerzeuger und auch den Schweinemäster wirtschaftliche Einbußen und Verluste. Das Geburtsgewicht, das das fötale Wachstum und die fötale Entwicklung wiederspiegelt, spielt eine wichtige Rolle zur Vorhersage der Überlebenswahrscheinlichkeit der neugeborenen Ferkel und für die Gesundheit der adulten Schweine. Fötales Wachstum ist ein komplexer Prozess, der vom Genotypen und Epigenotypen des Fötus, der maternalen Ernährung und der Verfügbarkeit von Nährstoffen und Sauerstoff für den Fötus abhängig ist. Man weiß bereits, dass das IGF-System genau wie auch Leptin durch die intrauterine Wachstumsretardierung beeinträchtigt wird. Einige dieser Veränderungen könnten somit eine permanente pathologische Programmierung des IGF-Systems und/oder der Energiehomöostase mit Energiebilanz und Fettmobilisierung hervorrufen, in der Leptin eine Schlüsselrolle spielt.

Das Ziel dieser Arbeit lag darin, Leptin und die verschiedenen Komponenten des IGF-Systems in den Nachkommen von Sauen zu charakterisieren, die während der Trächtigkeit mit Rationen unterschiedlichen Proteingehaltes gefüttert worden waren. Außerdem wollten wir Beziehungen zwischen den verschiedenen endokrinen Zielparametern bestimmen, genauso wie eventuell auftretende Langzeitkonsequenzen und endokrine Veränderungen und Abweichungen in der Wachstumsstruktur der Nachkommen untersuchen.

Zu diesem Zweck wurden Jungsauen der Deutschen Landrasse während ihrer Trächtigkeit mit Diäten unterschiedlichen Proteingehaltes gefüttert. Die Proteindiäten unterschieden sich durch 6% Protein (Niedrigproteindiät), 30% Protein (Hochproteindiät) und 12% Protein (Adäquatproteindiät). Es wurden Proben der Nachkommen von 26 Jungsauen am 94. Trächtigkeitstag genommen und untersucht. Am Tag der Geburt wurden die Nachkommen der verbliebenen 52 Jungsauen zu Ammensauen umgesetzt, die während ihrer Trächtigkeit eine Diät mit 12% Protein

bekommen hatten und von da ab eine standardisierte Laktationsdiät bekamen. Das Fötal- und Geburtsgewicht aller Nachkommen wurde festgehalten und in zwei fötale Körpergewichtsklassen (≤ 0,65 kg >) und zwei Geburtsgewichtsklassen (≤ 1,3 kg >) eingeteilt. Außerdem wurde die Wurfgruppengröße jeder Jungsau in zwei Gruppen klassifiziert (≤ 12 >). Blut- und Leberproben der insgesamt 306 Ferkel wurden gesammelt. Im Einzelnen wurden Proben von Föten des 94. Gestationstages (dpc 94), des ersten Lebenstages (dpn 1), des 28. Lebenstages (dpn 28) und des 188. Lebenstages (dpn 188) untersucht.

Zuerst wurden die Blutproben in einem Leptin ELISA gemessen, der bereits hausintern entwickelt war und für diese Arbeit intensiv für porcines Plasma validiert wurde. In den fötalen Blutproben von dpc 94 konnte die Leptinkonzentration nicht bestimmt werden, da diese unterhalb der Nachweisgrenze des Testsystems lag. An den postnatalen Tagen war kein Einfluss durch die drei verschiedenen Proteingehalte in der Fütterung der Jungsauen während der Trächtigkeit auf die Leptinkonzentration im Blut der Nachkommen erkennbar. Die Ergebnisse deuten darauf hin, dass potentielle pränatale Unterschiede in der Plasmaleptinkonzentration, bedingt durch die intrauterine Unter- bzw Überversorgung mit Protein, möglicherweise durch die homogene und adäquate Proteinversorgung nach der Geburt über die Milch der Ammenmutter kompensiert werden konnte. Trotzdem wurde an dpn 1 und 28 eine positive Korrelation zwischen Leptin und dem Fettgehalt des Schlachtkörpers ermittelt. Außerdem gab es an diesen beiden Tagen eine positive Korrelation zwischen der Leptinkonzentration und dem Schlachtgewicht der Ferkel. Diese Ergebnisse belegen eine kurzzeitige Beeinflussung des Fettgehaltes und des Körpergewichtes der Nachkommen durch die unterschiedliche, intrauterine Nährstoffumgebung. Diese Effekte blieben nicht bis zum adulten Entwicklungsstadium bestehen. Desweiteren zeigen unsere Ergebnisse, dass Leptin nicht so deutlich mit der Körperzusammensetzung, insbesondere dem Fettgehalt des vollständig leeren Schlachtkörpers korreliert ist, wie es erwartet wurde.

Die Hauptkomponenten des IGF-Systems, die Liganden IGF-1 und IGF-2 wurden im Plasma mit Hilfe eines kommerziell erhältlichen, humanen ELISA quantifiziert. Durch eine fundierte Evaluierung wurde sichergestellt, dass dieser humane ELISA auch für

den Nachweis von porcinem IGF-1 und IGF-2 im Plasma geeignet ist. Im Gegensatz zu Leptin waren beide Liganden auch im fötalen Plasma nachweisbar. Mit Ausnahme von dpn 1 blieb die Konzentration des IGF-1 im Plasma in Bezug auf die maternalen Diätgruppen unverändert. An dpn 1 hatten die Nachkommen der Niedrigproteinsauen signifikant geringere IGF-1 Konzentrationen im Vergleich zu den Ferkeln der Hochproteinsauen und auch tendenziell niedrigere IGF-1 Konzentrationen im Vergleich zu den Ferkeln von Müttern die während der Trächtigkeit adäquate Mengen an Protein gefüttert bekommen hatten. Gleichzeitig mit den Ergebnissen für die IGF-1 Konzentration in den Niedrigproteinferkeln an dpn 1, konnte die vorliegende Untersuchung signifikant niedrigere IGF-1 Konzentrationen in den Ferkeln mit geringerem Geburtsgewicht, im Vergleich zu den Ferkeln mit höheren Geburtsgewicht, zeigen. Wie erwartet, hatten die Ferkel der Niedrigproteinsauen auch geringere Schlachtgewichte als die Nachkommen den Hochproteinsauen und der Mütter mit adäquater Proteinversorgung während der Trächtigkeit. Folglich konnten wir einen positiven Zusammenhang zwischen den IGF-1 Konzentrationen und den Körpergewichten der Ferkel an dpn 1 offenlegen. Darüber hinaus konnten wir an dpn 1 zeigen, dass Ferkel aus kleineren Würfen (≤ 12) höhere Konzentrationen an IGF-1 im Vergleich zu Ferkeln aus größeren Würfen aufwiesen. Diese Feststellung stimmt mit der Tatsache überein, dass die Ferkel aus kleineren Würfen ebenso größere Schlachtgewichte aufzeigten als Ferkel aus größeren Würfen. Zusammengefasst suggerieren unsere Resultate einen Einfluss der intrauterinen Proteinrestriktion auf die Nachkommen, der sich deutlich in der hohen Korrelation zwischen der IGF-1 Konzentration und den Körpergewichten an den pränatalen und frühen postnatalen Tagen zeigt und in Teilen auch parallel dazu in Konsequenz der Wurfgruppengröße deutlich wird. Allerdings war keine dieser Korrelationen bis zum Tag 188 beständig. Für beide Liganden des IGF-Systems, IGF-1 und IGF-2, konnten wir weitere Korrelationen ermitteln: Beide IGFs zeigten dieselben Korrelationen mit ihren Bindungsproteinen, was sich wahrscheinlich durch dieselben, altersbezogenen Veränderungen begründen lässt. Außerdem waren IGF-1 und IGF-2 an dpc 94 und an dpn 28 miteinander korreliert, was eine sehr hohe Assoziation zwischen den beiden Molekülen bezeichnet. Im fötalen Gewebe werden IGF-1 und IGF-2 gleichmäßig exprimiert, aber unsere Ergebnisse bestätigen wiederum IGF-2 als hauptsächlichen Wachstumsfaktor des embryonalen

Wachstums. Wir konnten außerdem eine Assoziation zwischen niedrigem fötalen Körpergewicht und reduzierter IGF-2 Konzentration beweisen. Daraus folgend ergibt sich an dpc 94 der gleiche Zusammenhang zwischen der intrauterinen Proteinrestriktion, der IGF-2 Konzentration und dem fötalen Körpergewicht, wie wir vorher schon für IGF-1 an dpn 1 anführen konnten. Auch wenn IGF-2 als wichtigster fötaler Wachstumsfaktor betrachtet wird, konnten wir beweisen, dass bei regulatorischer Proteinrestriktion IGF-1 mehr als IGF-2 beeinflusst wird. Überdies wurden beide Liganden mehr durch die intrauterine Proteinrestriktion als durch den intrauterinen Proteinüberschuss beeinflusst.

Begründet durch die ebenso wichtige Rolle der IGF Bindungsproteine im IGF-System, wurde ein semiquantitativer, nichtradioaktiver Western Liganden Blot etabliert und validiert. Mit diesem Western Liganden Blot wurde die Menge der einzelnen Bindungsproteine im Plasma bestimmt.

Das IGFBP-1 im Plasma der fötalen Nachkommen war durch die maternale, intrauterine Fütterung beeinflusst. Wir konnten einen Zusammenhang zwischen der maternalen Fütterungsgruppe, dem Gehalt an IGFBP-1 und der fötalen Körpergewichtsklasse feststellen. Föten von Müttern, die während der Trächtigkeit einen Überschuss an Protein bekommen hatten, hatten signifikant höhere IGFBP-1 Konzentrationen und ein geringeres Körpergewicht im Vergleich zu den Nachkommen der anderen beiden Diätgruppen. Zusätzlich wiesen die Föten der leichteren Körpergewichtsklasse, unabhängig von der maternalen Fütterungsgruppe, höhere Mengen an IGFBP-1 im Plasma auf. Ferkel der Niedrigproteinsauen zeigten im Vergleich zu den Nachkommen der Mütter, die die adäquate Proteindiät während der Trächtigkeit bekommen hatten, einen gleichstarken Anstieg an IGFBP-1 von dpc 94 bis dpn 1, wohingegen die Konzentration an IGFBP-1 in den Nachkommen der Hochproteinsauen in diesem Zeitraum nahezu unverändert blieb. Wir konnten die höchsten Konzentrationen an IGFBP-1 an dpn 1 feststellen. Tatsächlich war die Messung im Plasma nur an dpc 94 und dpn 1 möglich, da zu den späteren Probentagen keine dementsprechende Bande im Western Liganden Blot detektiert werden konnte.

Die Sekretion des IGFBP-2 wird direkt durch IGF-1 stimuliert, wodurch die erhöhten Mengen an IGFBP-2 in den Nachkommen der Hochproteinsauen am ersten

Lebenstag erklärt werden könnten, da diese zu diesem Zeitpunkt auch erhöhte IGF-1 Konzentrationen aufwiesen. Die Adaption bis zum dpn 188 an gleiche IGFBP-2 Konzentrationen aller Nachkommen, ungeachtet welche Proteindiät die Mutter in der Trächtigkeit erhalten hatte, könnte durch die gleiche Fütterung aller Nachkommen ab der Geburt begründet sein. Die an dpn 28 festgestellte Wechselbeziehung zwischen dem Geburtsgewicht und der maternalen Fütterungsgruppe verdeutlichte, dass die Nachkommen der Niedrigproteinsauen mit niedrigeren Geburtsgewichten niedrigere Gehalte an IGFBP-2 aufwiesen als die Nachkommen mit höherem Geburtsgewicht und adäquater intrauteriner Proteinversorgung. Schwerere Nachkommen der mit Protein adäquat- und überversorgten Sauen hatten reduzierte Konzentrationen an IGFBP-2 im Vergleich zu Ferkeln mit geringerem Geburtsgewicht aber der gleichen intrauterinen Nährstoffumgebung. Durch diese Ergebnisse ist offensichtlich, dass Proteinrestriktion einen größeren Einfluss auf die IGF Bindungsproteine hat als intrauteriner Proteinüberschuss.

Die offensichtlichen Einflüsse der maternalen Diät auf das IGFBP-3 waren auf dpn 1 reduziert. Hier war die Konzentration an IGFBP-3 in den Nachkommen der Hochproteinsauen signifikant höher als in den Nachkommen der Adäquatproteinsauen. Das wiederum stimmt mit der Tatsache überein, dass an dpn 1 Ferkel aus kleineren Würfen höhere IGFBP-3 Konzentrationen im Blut aufwiesen als die Ferkel aus größeren Würfen, da die Ferkel aus den kleineren Würfen hauptsächlich Nachkommen der Hochproteinsauen waren. Im Allgemeinen wird die Konzentration an IGFBP-3 im Blut durch IGF-1 und GH reguliert, die wiederum beide die Synthese und Sekretion von IGFBP-3 induzieren und dadurch die Konzentration erhöhen. Übereinstimmend mit diesen Fakten konnten wir erhöhte IGF-1 Konzentrationen in den Nachkommen der Hochproteinsauen ermitteln, die auch eine erhöhte IGFBP-3 Konzentration an dpn 1 aufzeigten. Dieser offensichtliche Zusammenhang zwischen IGF-1 und IGFBP-3 unterstützt die Tatsache, dass das IGFBP-3 das bedeutendste Bindungsprotein für IGF-1 darstellt und somit die Stabilität von IGF-1 im Blut bedingt.

An keinem der Probennahmetage wurden Unterschiede für das IGF-Bindungsprotein-4 im Zusammenhang mit der intrauterinen Proteindiät verifiziert. Dass das IGFBP-4 die Aktivität des IGF-1 inhibiert, können wir durch unsere

Ergebnisse allerdings unterstützen, da wir eine sehr hohe negative Korrelation zwischen IGFBP-4 und IGF-1 an allen Probennahmetagen zeigen konnten.

Als letzter Teil des IGF-Systems und als wichtigster Zielrezeptor, wurde der IGF-1 Rezeptor (IGF-1R) in Lebergewebe mit Hilfe eines kommerziell erhältlichen ELISA für den Nachweis von humanem IGF-1R herangezogen, wobei dieser vorher ebenfalls ausführlich auf seine Funktionalität für porcine Leberproben geprüft wurde. Der IGF-1R konnte nur in den Leberproben der Föten (dpc 94) nachgewiesen werden. Weder die maternale Fütterungsgruppe, noch das fötale Geburtsgewicht, die Wurfgruppengröße oder das Geschlecht beeinflussten offensichtlich den IGF-1 Rezeptor zu diesem Zeitpunkt. Diese Feststellung deutet darauf hin, dass unterschiedliche Proteindiäten während der ganzen Trächtigkeit, wenn überhaupt, dann wahrscheinlich hauptsächlich auf der Ebene der Liganden und nicht auf der Ebene der Rezeptoren wirken, vorausgesetzt das IGF-System ist überhaupt betroffen.

Zusätzlich wurden in dieser Arbeit auch die Körpergewichte ermittelt und einzelne Komponenten der Körperzusammensetzung der Nachkommen durch eine chemische Analyse des Schlachtkörpers untersucht. Beide wurden durch die inadäquate Proteinversorgung während der Trächtigkeit beeinflusst. An dpn 1 wiesen die Ferkel der Niedrigproteinsauen niedrigere Körper- und Schlachtgewichte im Vergleich zu den Nachkommen der Adäquatproteinsauen auf. In Übereinstimmung damit zeigten Ferkel der leichteren Geburtsgewichtsklasse, die hauptsächlich Nachkommen der Niedrigproteinsauen waren, ebenso niedrigere Schlachtgewichte. Als potentiellen Grund für das beeinträchtigte fötale Wachstum, resultierend in reduzierten Geburtsgewichten, wird das Defizit an vor allem essentiellen Aminosäuren, begründet durch die Unterversorgung mit Protein während der Trächtigkeit, angesehen. Neben essentiellen Aminosäuren könnten auch veränderte Konzentrationen anderer Hormone, wie zum Beispiel Insulin, Leptin und IGF-1 eine zusätzliche Begründung für die fötale Wachstumsretardierung in den Nachkommen der Sauen, die während ihrer Trächtigkeit proteinrestriktiv ernährt wurden, darstellen. Diese Hypothese wird teilweise durch die Andeutung einer Beziehung zwischen dem reduzierten Geburtsgewicht und den sinkenden Leptin- und IGF-1-Konzentration an

dpn 1 in den Nachkommen der Niedrigproteinsauen unterstützt. Die maternale Niedrigproteindiät hatte jedoch keinen Einfluss auf das Schlachtgewicht der Tiere an dpn 28 oder 188, was wiederum zeigt, dass ein reduziertes Geburtsgewicht, wie in den Nachkommen der Niedrigproteinsauen, im postnatalen Leben kompensiert werden konnte.

Zusammenfassend zeigen die Ergebnisse unserer Studie deutlich, dass alle durch die modifizierten Proteindiäten begründeten, erkennbaren Effekte ausschließlich auf die pränatalen und die frühen postnatalen Entwicklungsstadien der Nachkommen limitiert waren. Desweiteren lassen sich die nachweisbaren Effekte weitestgehend auf die intrauterine Proteinrestriktion zurückführen. Intrauteriner Proteinüberschuss hatte hingegen keinen bzw. kaum Einfluss, weder kurzzeitig noch längerfristig, auf alle in unserer Studie getesteten Faktoren. Es kann darüber spekuliert werden, ob die nicht vorhandenen, aber möglicherweise erwarteten Effekte durch das Umsetzen der intrauterin beeinträchtigten Ferkel zu Ammensauen direkt nach der Geburt begründbar sind. Diese Hypothese würde in einer Art Nachholvermögen gründen, die es den Nachkommen ermöglicht, die vorher beeinträchtigte endokrine und körperliche Situation durch eine Adaption an eine veränderte adäquate Nährstoffumgebung während ihrer postnatalen Entwicklung zu kompensieren.

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