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.

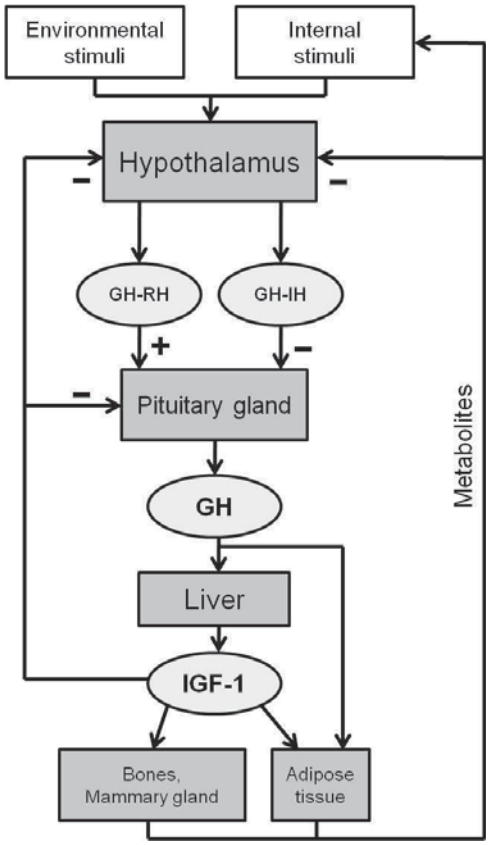

Figure 1: The somatotropic axis: The secretion of growth hormone (GH) from the pituitary is primarily regulated by two hypothalamic neurohormones: a stimulatory hormone, the GH-releasing hormone (GH-RH) and the GH-inhibiting hormone (GH-IH). Different environmental and internal stimuli e.g. physical 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).

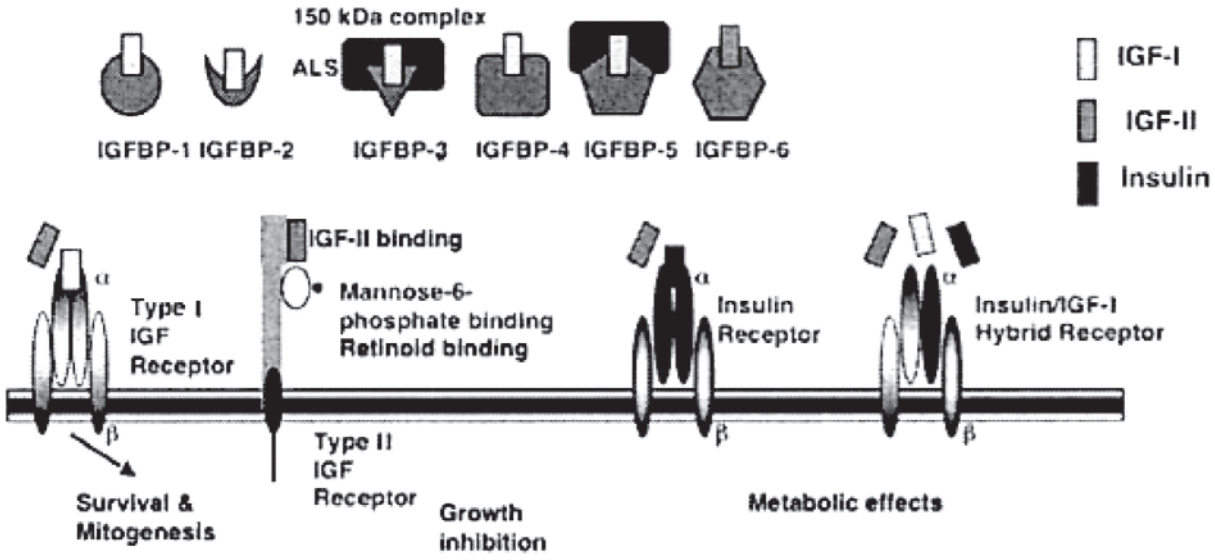

Figure 2: The IGF-System with the Insulin-like growth factors (IGF-1 and IGF-2), their receptors type-1 (IGF-1R) and type 2 (IGF-2R) and their binding proteins (IGFBPs 1-6). ALS= acid labile subunit (modified from Randhawa and Cohen, 2005)

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