

Preface

The phenomenon of nutrition in early life having lifetime effects on health, growth, and metabolism is well recognized. It has been termed “nutritional programming” and is defined as a long-term change in the structure or function of an organism resulting from a stimulus acting at a critical period of development in early life. Nutrients (energy, macro- and micronutrients) might be critical signals acting directly or via “receptors” in sensitive tissues, and thus program later function.

The relation between pre- and early postnatal nutrition with growth, later health and performance and the underlying mechanisms are not satisfactorily understood. This is especially true in the case of farm animals. Therefore, the purpose of the International Dummerstorf Workshop ‘Early Nutrition, Growth and Metabolism’, September 12-13, 2003, held in Rostock-Warnemünde, Germany, is to discuss present knowledge and identify research opportunities.

The scientific program includes three sessions each introduced by a main talk entitled

1. **The effect of nutrient deficiency on fetal development, pregnancy outcome and adult metabolism**
 2. **Nutritional effects on the regulation prenatal and postnatal growth**
- and,
3. **Nutritional and hormonal control of muscle growth and fat deposition,**

given by internationally renowned expert scientists. The main talks are supplemented by poster presentations. This volume of the Archive of Animal Breeding contains the proceedings of the workshop comprising the full papers of the main talks and abstracts of the presented posters.

We as the organizers at the Research Institute for the Biology of Farm Animals in Dummerstorf, are pleased to welcome colleague scientists and doctoral students from more than 10 countries in Europe and overseas. Our meeting, held in one of the most beautiful areas of Germany at the coast of the Baltic Sea, will offer an excellent opportunity to network, to meet professional colleagues, to establish new contacts with scientists, and, last but not least, to discuss the latest developments in the field.

We hope that this workshop will be an inspiring one and aids to strengthen the international scientific exchange and supports collaborative ties.

We are grateful to the Deutsche Forschungsgemeinschaft and the Bayer AG helping us to make this meeting possible.

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The effect of nutrient deficiency on fetal development, pregnancy outcome and adult metabolism

Summary

During pregnancy, the developing embryo or fetus is totally reliant on the mother for nutrients and for removal of wastes. Any imbalance of delivery, therefore, can result in serious consequences for the fetus. These can occur not only in the perinatal period, but can also persist into adulthood or indeed develop only when adult. It has been proposed that birthweight, or some other aspect of size at birth, is a good indicator for problems in adulthood. The hypothesis, known as the Barker hypothesis, is that lower birthweight, though still within the normal distribution, reflects inappropriate nutrition in utero. In this review, we examine the evidence in both human and animal studies. We also summarise the data identifying possible mechanisms underlying the observations and conclude that these may be different while still generating a common phenotype. In summary, we conclude that there is good evidence to support the Barker hypothesis, though the cause or causes may be more complex than simple malnutrition.

Key Words: Barker hypothesis, thrifty phenotype, pregnancy, cardiovascular disease, Type 2 diabetes

Zusammenfassung

Titel der Arbeit: **Einfluss von Nährstoffdefiziten auf die Fötalentwicklung, Graviditätsergebnis und Stoffwechsel im Erwachsenenalter**

Der sich entwickelnde Embryo oder Fötus ist während der Gravidität vollkommen abhängig von der Versorgung mit Nährstoffen und der Entfernung von Stoffwechselendprodukten durch die Mutter. Jede unangepasste Zufuhr von Nährstoffen kann deshalb schwerwiegende Konsequenzen für den Fötus nicht nur in der perinatalen Phase haben. Diese Veränderungen können auch bis ins Erwachsenenalter hinein bestehen oder sich sogar erst im Erwachsenenalter entwickeln. Es wurde vorgeschlagen, dass das Geburtsgewicht oder verschiedene Aspekte der Körpergröße bei der Geburt ein guter Indikator für Stoffwechsel- oder Gesundheitsprobleme im Erwachsenenalter seien. Es wurde die Hypothese, bekannt als die Barker-Hypothese, aufgestellt, dass ein vergleichsweise niedriges Geburtsgewicht, das sich jedoch noch in der physiologischen Spannweite bewegt, ein Symptom für eine unangepasste in utero-Nährstoffversorgung ist. In dieser Übersicht, werden die Belege für diese Hypothese aus Human- und Versuchstierstudien beleuchtet. Mögliche Ursachen und Regulationsmechanismen für diese Beobachtungen werden zusammengefasst. Es wird geschlussfolgert, dass trotz gleichen Phänotyps die möglichen Ursachen für diese Phänomene unterschiedlich sein können. Zusammenfassend wird festgestellt, dass es vielfältige Belege für die Barker-Hypothese gibt, dass aber die Ursache oder die Ursachen komplexer sind als einfach Mangelernährung.

Schlüsselwörter: Barker's Hypothese, Gravidität, Herz-Kreislauf-Erkrankungen, Typ 2-Diabetes

Introduction

It seems almost axiomatic that, since the developing fetus is totally dependent on the mother for nutritional requirements, inappropriate nutrition in utero will result in serious consequences for the offspring. Indeed, it has been known for many years that alterations in nutrition, both pre- and post-conception, changes pregnancy outcome. This work has been carried into the industrial arena, with, for example, diets being designed for specific breeding periods (AHERNE et al., 1992), (FREETLY et al., 1998). These consequences can occur not only in the immediate post-natal period, but

can also carry through into adulthood. This phenomenon has been termed “programming” since the fetus is “programmed” to show effects long after the stressor is no longer present. In the original papers, it was suggested that programming was associated with changes in birthweight. These changes occur within the normal weight range and are not evidenced as consequences of intrauterine growth retardation (IUGR). The changes associated with IUGR may mimic, but are not necessarily associated with, the changes underpinning the programming effect. There is a considerable literature addressing the problem of IUGR and the long-term consequences. We have decided it is beyond the remit of this review to address the problem, but refer the reader to excellent reviews by (DIAMOND, 2001; BOTERO et al., 1999; HENRIKSEN, 1999).

The Barker hypothesis and the thrifty phenotype

Barker hypothesis

The Barker hypothesis, or the “fetal origins hypothesis”, suggests that fetal adaptations to under-nutrition lead to permanent alterations in the body’s structure, function and metabolism. These alterations then render the individual to an increased risk of disease in adulthood. Several studies suggested that fetal or prenatal influences may modulate death from heart disease in adulthood (FORSDAHL, 1977), (MARMOT et al., 1984). The relationship was first explicitly demonstrated by Barker’s group, (OSMOND et al., 1993), who studied a large cohort of men and women from Hertfordshire who were, at the time of the study, aged about 70. There was a 2-fold decrease in death from coronary heart disease between those at the lower and upper ends of the birthweight distribution. In a second study in Sheffield, the data suggested that the incidence of heart disease increased among those who were smaller at birth because they failed to grow, rather than being premature (BARKER et al., 1993).

The data was then re-analysed more carefully, since birth weight is a very crude parameter, and it was determined that the ponderal index (birthweight/length³) may be a better indicator of risk. In a Finnish study, the relationship between size and the development of heart disease was strongest when the placenta was small (FORSEN et al., 1997). In the Sheffield study, however, the relationship was U-shaped, with highest mortality rates being at either end of the distribution (BARKER et al., 1993).

Since these studies, there have been many more that support the argument that size, or some other related aspect of size at birth and at one year of age, especially when the placental:fetal ratio is taken into account, is a strong risk factor for the development of later disease (RICH-EDWARDS et al., 1997). These have not only been in the UK, but have also taken place in countries as diverse as India (MOORE et al., 1996), (STEIN et al., 1996) and the Gambia (MOORE et al., 1997)

The four principles underlying programming of growth have been enunciated by Barker and his colleagues (DENNISON et al., 1997). They propose that

Undernutrition during early life has permanent effects

Undernutrition has different effects at different times

Rapidly growing fetuses are more sensitive to the effects of undernutrition

Permanent effects of undernutrition include reduced cell number, altered organ structure and resetting of hormonal axes.

For example, Barker’s original hypothesis proposed that undernutrition in early stages of development tended to produce proportionally small individuals, who still had

increased susceptibility to hypertension and stroke, but not coronary heart disease (BARKER et al., 1989). Those who were undernourished later in gestation also had increased risk of coronary heart disease and stroke. However, more recent data (KWONG et al., 2000) in rat embryos, and data from domestic animals, such as the large offspring syndrome (McEVOY et al., 2000), suggest that alterations to the embryonic environment may be more widespread and complex. The critical window suggests too that, since different tissues and organs are undergoing differentiation and proliferation at different times, stress will produce different effects if applied during each of the trimesters (BERTRAM et al., 2001).

Furthermore, there have also been recent suggestions that programming can induce changes in adult health and well-being without necessarily altering birthweight (HOET et al., 1999). This is an important observation, since it separates the two aspects and means that decreased birthweight *per se* is not likely to cause increased blood pressure. Clearly there is still much to learn.

The concept of undernutrition may be an over-simplification, however. Imbalances or deficiencies in specific nutrients can have important and dramatic effects. These are most clearly seen in animal models and some will be described in more detail below, but there are also data that imply that deficiencies in certain micronutrients, notably zinc, can have serious effects on pregnancy outcome in humans, as reviewed in (CAULFIELD et al., 1998), (OSENDARP et al., 2003). Data also show that maternal iron status can be a strong predictor for adult disease (GODFREY et al., 1991)

Thrifty phenotype

The thrifty phenotype hypothesis has been developed as an extension of the Barker hypothesis (HALES et al., 1992). In essence, the theory suggests the specific nutrient regime is not a problem rather that alterations in the nutrient intake that causes difficulty. There are several pieces of information that support the concept.

In Nauran islanders, the incidence of non-insulin-dependent diabetes mellitus (NIDDM) rose markedly after the second world war (ZIMMET et al., 1984). During the war, they suffered from severe nutritional deficiency. After the war, their nutrition improved dramatically as a result of phosphate mining and its associated affluence. The children exposed to this change in diet had been poorly nourished in utero and were, therefore, poorly equipped to deal with the improvement in nutrition. In contrast, the nutritional input to children from the same population born after the war remained at the same level pre- and post-natally. As would be predicted by the thrifty phenotype hypothesis, while obesity, exercise and other risk factors had not changed, the epidemic of diabetes was markedly reduced.

Other studies also support the theory. For example, Cohen and co-workers have studied Ethiopian Jews who migrated from Ethiopia to Israel during a severe famine. After only 4 years in Israel, as many as 9 % of the population under 30 years of age developed NIDDM. This was an extremely high figure, and could not be explained easily (COHEN et al., 1988). Desai and colleagues suggested that the data could be explained if overnutrition in later life uncovered a defect in β -cell secretion of insulin that was not observed when the plane of nutrition remained constant (DESAI et al., 1995).

This extension of the Barker hypothesis clearly has important implications, since it suggests that the changing level of nutrition is more important than the absolute

amount. It is also very significant that the programming effect may remain dormant and may only be exposed if there is precipitating event.

Diet, genetics, or both?

The data discussed above suggest that small birth weight, or an associated parameter; ponderal index, placental:fetal ratio, length etc are associated with incidence of adult disease. The fetal origins hypothesis states that this is associated with malnutrition, of whatever cause, in utero. However, other workers have suggested that this excludes the possibility of a genetic cause as well as, or instead of, a nutritional imbalance and that the genotype may have an important role to play. The most obvious way to test this hypothesis is to examine incidence of disease in cohorts of twins.

Several studies have now examined the relationship between size at birth and development of disease in twins, both monozygotic and dizygotic, to test whether there is a genetic component to development of disease. Unfortunately, the data are inconclusive. The most recent published study (JOHANSSON-KARK et al., 2002) suggests there is little support for the fetal origins hypothesis, but does point out wide confidence limits, which may be a problem with using twins for this kind of study. Further, there appears to be a negative relationship between weight and blood pressure in monozygotes, and a positive relationship in dizygotes. These relationships are also found when examining within twin pairs.

In another study, looking at adolescent twins, the authors concluded that, while there was evidence for an intrauterine effect, there was also support for a genetic effect, since the relationship between size and blood pressure decreased when genetic factors were taken into account (CHRISTENSEN et al., 2001).

Poulsen and colleagues, in contrast, tested the hypothesis in the opposite direction (POULSEN et al., 1997). They determined whether impaired glucose tolerance was associated with lower birth weight in twins, and also whether NIDDM was associated with a decrease in birth weight. They found a clear relationship, which does support the hypothesis, although they also conclude that the data does not preclude a coincident genotype contributing to both low birth weight and impaired glucose tolerance or NIDDM.

In summary, these studies do not provide unequivocal evidence one way or the other. It is also clear that the way the study is designed will produce different results. Poulsen's approach, also used by BO et al (2000), who found similar results, cannot really test for a genetic factor, since they are already looking at a defined subset of twins. The open ended studies do not find such a strong relationship. We conclude, therefore, that there are more factors than simple intra-uterine nutrition, but it is also clear that nutrition can, and does, have a significant role in the development of adult disease. This conclusion is also strongly supported by the animal experiments carried out by many groups throughout the world and outlined below. Parenthetically, a good meta-analysis would also be very useful!

Animal studies - Hypertension

Most of our understanding of mechanisms of programming comes from animal work. A variety of different species and nutritional stresses have been used. Each gives

slightly different results, but when taken together, provide good information about how programming may occur.

Early studies by Winick and Nobel, using global nutritional restriction, showed that malnutrition during pregnancy led to changes in cell number in tissues such as the pancreas (WINICK et al., 1966). Snoeck and co-workers demonstrated that maternal protein deprivation causes reduced β cell proliferation in the offspring (SNOECK et al., 1990). Both approaches have now been extended; most especially the global restriction by Hanson's and Gluckman's groups (WOODALL et al., 1996), (OZAKI et al., 2001) and isocaloric low protein restriction by Langley-Evans, Jackson and co-workers (LANGLEY-EVANS et al., 1994). Additionally, alterations in fat content (KOUKKOU et al., 1998), calcium levels (BERGEL et al., 2002), iron deficiency (CROWE et al., 1995), (LEWIS et al., 2001) and overnutrition of the adolescent sheep have all also been used (WALLACE, 2000).

The low protein diet in rats has generated considerable amounts of information. The effects are complex, and can be different depending on the level, source and timing of maternal nutrition before and during pregnancy. For example, if the mother has been given a low protein diet prior to conception, the pups grow more rapidly from about day 14 to day 20, so that they are larger than controls. From then to term, however, their rate of increase falls and at term they are smaller than the normal counterpart (LANGLEY-EVANS et al., 1996) (REES et al., 1999). In contrast, if the dam is fed the low protein diet from conception onwards, the pups are symmetrically smaller, having grown at a faster rate between day 0 and day 14. The growth rate then falls and they are smaller, not larger, at day 20 (LANGLEY-EVANS et al., 1996).

The low protein diet causes, at least in some strains of rat, an increase in blood pressure. This can be seen by 3-4 weeks of age, and is maintained into adulthood. The changes in blood pressure were associated with increases in angiotensin converting enzyme (ACE) activity and in the renin-angiotensin system generally (MANNING et al., 2001). In a series of experiments testing the possible role of ACE and the renin-angiotensin system, Langley-Evans and colleagues studied the effect of manipulations on the system on blood pressure in the offspring of protein deprived dams. They showed that captopril treatment (an ACE inhibitor) decreased blood pressure in the hypertensive animals (LANGLEY-EVANS et al., 1995). Interestingly, if they started the treatment in young animals, the decrease in blood pressure was maintained into adult in the absence of captopril (SHERMAN et al., 1998). This argued that the generation of angiotensin II (ANGII) was a critical step in the programming of hypertension. They have used other, more specific blockers of the pathways such as losartan, an antagonist of the ANGII receptor, and obtained similar results (SHERMAN et al., 2000).

Other nutritional restriction models can also generate increases in blood pressure. Hanson and co-workers, Hales and colleagues and our own group (GAMBLING et al., 2002) have all used maternal iron deficiency anemia in a rat model (CROWE et al., 1995), (LEWIS et al., 2001), (LEWIS et al., 2002). Blood pressure was increased in offspring of anaemic mothers, and this was a specifically pre-natal event (GAMBLING et al., 2002). However, there was no change in the renin-angiotensin system (LEWIS et al., 2002).

Poston and colleagues have used a high fat diet to examine programming in the rat. They have shown that a diet including 20 % fat during pregnancy changes blood

pressure in the offspring (KHAN et al., 2003). In contrast to the renin-angiotensin hypothesis, she has tested whether there are alterations in vascular function. She has also tested whether the high fat diet changes the fatty acid composition of the vessel membranes. In essence, while noradrenaline-induced constriction was not altered, acetyl choline-induced relaxation was decreased in the femoral arteries of offspring born to high fat mothers. The changes appear to be associated with alterations in the fatty acid profiles of the membranes of the aorta (GHOSH et al., 2001). Importantly, there were no alterations in responses to mediators of nitric oxide, suggesting endothelium-derived relaxing factor processes may not be involved. This was supported by a recent study from the same group. Using telemetry and a higher fat diet, they showed that male offspring had not change in blood pressure. Females, in contrast, had an increase of between 5 and 10 mm Hg. Despite this, there was no difference between males and females in arterial response to acetylcholine (KHAN et al., 2003). The group conclude that there is a clear dissociation between endothelial dysfunction and elevation of blood pressure. This may not be the case in protein restriction. In a recent paper, the authors propose that there may be changes in endothelium-dependent relaxation, implicating nitric oxide and associated signalling pathways in vascular adaptations to programming (BRAWLEY et al., 2003).

The change in blood pressure is often also associated with changes in kidney size and, possibly, function. In one study, albeit using a rather more severe protein restriction (6 % vs 9 % on other studies) Vehaskari and colleagues (VEHASKARI et al., 2001), in addition to changes in the renin-angiotensin system, demonstrated a decrease in the number of glomeruli in kidneys of offspring of deficient rats. This was quite marked; 28 % in males and 29 % in females, but at this stage they have not presented data to show how function is affected.

The fact that different dietary manipulations can produce the same phenotype implies that there may be mechanisms operating in common. One possibility relates to alterations in glucocorticoid metabolism. This hypothesis was advanced by Benediktsson and co-workers in 1993 (BENEDIKTSSON et al., 1993). Fetal protection against maternal corticosteroids is usually mediated by placental 11 β -hydroxysteroid dehydrogenase (11 β HSD). This group showed that placental activity of 11 β HSD correlated positively with term fetal weight and negatively with placental weight. Further, they found that the offspring of rats treated with dexamethasone had lower birth weights and higher blood pressure when adults than did their control counterparts.

This hypothesis was taken further by Langley-Evans. In a careful and comprehensive approach, he inhibited the enzyme with carbenoxolone during pregnancy (LANGLEY-EVANS, 1997). Further, he performed both pharmacological and surgical adrenalectomies on animals with control or low protein diets (LANGLEY-EVANS, 1997; GARDNER et al., 1997). All of these treatments gave the predicted effect, showing that loss of protection against maternal glucocorticoids was the critical factor in development of high blood pressure in the offspring. His group then showed that the mid to late gestation window was the critical period when the fetus was most sensitive to the programming effect of maternal steroids (LANGLEY-EVANS, 1997)

More recently, Bertram and co-workers have also studied the expression of the glucocorticoid receptor in the low protein rat model of pregnancy (BERTRAM et al., 2001). In common with Langley-Evans, they show a marked decrease in 11 β HSD

expression in the placenta of the low protein fed animals (LANGLEY-EVANS et al., 1996). At the same time, there is a rise in the glucocorticoid receptor (GR) mRNA levels in kidney and in lung. To test whether this had functional consequences, they measured mRNA levels of the Na⁺/K⁺ ATPase α and β subunit mRNAs. They found a very significant rise in the kidney of offspring of dams eating a low protein diet. Importantly, these changes are maintained and, indeed, increased, in the offspring.

These papers provide a persuasive model for the mechanism of low protein induced hypertension. However, there are several questions that remain unanswered. Firstly, what is the mechanism(s) for other models, such as Fe deficiency, or high fat? Secondly, is there a common mechanism regulating the GR and 11 β HSD expression, or is there a preliminary step. Thirdly, why are the effects maintained and reinforced into adulthood? Fourthly, how is the alteration generated during fetal life?

This last question has been addressed by Rees' group, who initially showed that feeding low protein diets resulted in changes in only a few amino acid levels in the mother and her fetus. Their initial studies implicated threonine as being particularly important (REES et al., 1999), but supplementing the diet with extra threonine did not reverse the decrease in fetal size (REES et al., 2000). Instead, the data suggest that there are changes in the pathway regulating synthesis of homocysteine (REES et al., 2000). This means that more attention has to be paid to the amino acids involved in DNA methylation. In essence, the argument is that the high levels of methionine result in an increase in homocysteine levels. This then alters levels of S-adenosyl methionine (SAM) and methylene tetrafolate. SAM is the methyl donor for newly synthesised DNA, and thus the increase may result in rises in DNA methylation. In support of this, there is a rise in global DNA methylation in the liver from offspring of low protein dams (see (REES, 2002) for a more comprehensive review).

Animal studies – Glucose metabolism.

Initial studies by Snoeck et al showed that maternal protein deficiency led to a reduction in β -cell proliferation and decrease in islet size in the pancreas of neonatal offspring (SNOECK et al., 1990). This was associated with a marked decrease in vascularisation in both the head and the tail of the pancreas. Langley and colleagues followed this with a comprehensive study of glucose tolerance in animals whose mothers were fed differing loads of protein (LANGLEY et al., 1994).

They found, in 9 week rats, that those fed the 9% (second lowest) protein concentrations had a lower peak glucose concentration and the area under the curve was decreased by 28 %. Those whose mothers were fed 6 % protein had a more rapid clearance and the area under the glucose tolerance curve was decreased by 40 % compared to controls. Interestingly, when the experiment was repeated in animals aged 44 weeks, no differences were seen. These results would appear to contradict the morphological data.

The critical difference, however, may relate to the period of deficiency. The post-natal return to normal protein in the mother may make the model similar to the recuperated animals in work by Hales and colleagues, where cross-fostering onto mothers fed a normal diet reverses the reduced activity of glucokinase in the pancreas of the offspring. Curiously, however, cross-fostering normal offspring onto deficient dams does not decrease GK activity (DESAI et al., 1997).

The differences in glucose tolerance seen by Hales and co-workers were not as apparent as those by Langley-Evans. However, one interesting observation was that the animals from low protein mothers had a greater deterioration in tolerance than controls.

There are also important changes in liver metabolism of glucose. There appears to be a reduction in response to glucagon in 3-month-old offspring, related to a marked decrease in glucagon receptors. At the same time, there appears to be a significant increase in insulin receptor expression, with an increase in insulin turnover (DESAI, 1997).

In contrast to the pancreas, GK levels in the liver, which are reduced in the offspring of low protein mothers, do not recover in the recuperated animals (DESAI et al., 1997). Further, they are still low 11 months later, despite the fact that the animals have been on a normal diet all their post-natal lives. PEPCK levels, the enzyme which is rate limiting for gluconeogenesis, show the opposite trend, being increased in the neonate and still higher 11 months later. Desai and colleagues hypothesise that the changes occur as a result of differential cell proliferation. They suggest that the population of perivenous hepatocytes is increased with a concomitant decrease in periportal cells, where the two enzymes are predominantly located. In support of this hypothesis, they show that two other enzymes, located in these liver compartments, show similar changes.

Conclusions

In this review, we have examined the relationship between intra-uterine nutrition and the development of adult disease. We have not included a large body of work, in humans and animals, (SIBLEY et al., 1997), (GAMBLING et al, 2002b) (WALLACE et al., 2000), examining the relationship between the placenta and placental function and fetal growth. Nor have we considered in any detail how the pre-conception environment alters pregnancy outcome. Other important aspects of this research, such as the large offspring syndrome, which implicates the role of imprinted genes and alteration of their expression by external factors (SINCLAIR et al., 2000), (YOUNG, 2001) have also not been considered. This is not because they are not important, but rather that the area is too large to discuss in one review.

It is clear, taken as a whole, that the maternal environment has a critical effect on pregnancy outcome and development of disease. It is also clear, however, that we still have an enormous amount to learn. Some of the questions have been outlined above, but we are sure the reader will also find many of their own. We are also sure that most of these will not yet have been answered and that this will be a valuable and fruitful area of research for many years to come, not only providing information on which to base therapy and therapeutics, but also providing answers to questions of fundamental biological importance.

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BERNHARD H. BREIER

Nutritional effects on the regulation of prenatal and postnatal growth

(Nahrungsabhängige Beeinflussung der Regulation von pränatalem und postnatalem Wachstum)

Prenatal growth. Prenatal growth is dependent on the ability of the mother to provide adequate nutritional support to the placenta and the fetus and the ability of the fetus to manage the maternal resources appropriately. The term ‘maternal constraint’ describes the limitation of fetal growth by the maternal environment and the mother’s capacity to supply nutrients to the fetus. Prenatal growth is therefore regulated by endocrine factors which influence the partitioning of nutrients between mother, placenta and fetus and regulate the utilisation of available substrate by the fetus.

Transition from prenatal to postnatal growth regulation. Prenatal growth and development are adapted to conditions of maternal and placental substrate limitation. After birth the neonatal animal does not need this acute link to the maternal system. The major rise in fetal glucocorticoids during the immediate pre-partum period initiates a switch from a fetal to a postnatal state of growth regulation. The essential role of this rise in fetal glucocorticoids on lung maturation is well known. However, perinatal glucocorticoids have a far more general role in switching fetal growth and metabolism to a postnatal state. For example, perinatal glucocorticoids play a major role in cardiac development, induction of hepatic gluconeogenic enzymes and activation of a variety of endocrine systems. The developmental switch to postnatal growth regulation allows growth to be directly linked to environmental factors and nutrition.

Influence of early life nutrition on postnatal growth and metabolism. The biological phenomenon of lifetime consequences of nutrition (and other environmental factors) during prenatal life on postnatal growth and metabolism is increasingly recognised. This concept has been termed the ‘fetal origins hypothesis’ and the process which underlies this concept has been termed ‘programming’. One general thesis is that the fetus adapts to adverse environmental cues *in utero* with permanent readjustments of homeostatic systems to maximise its chances for survival. These adaptations may include resetting of metabolic and endocrine systems and a change of growth trajectory.

Recent studies have raised the possibility that changes in maternal nutrition and consequently altered materno-placental supply of nutrients may alter fetal metabolism and endocrine status with major postnatal health consequences compatible with the ‘fetal origins hypothesis’. In addition, there is increasing interest in the lifetime consequences of nutrition during infancy and its role on growth and metabolic health in later life.

Scientific progress in these areas will be discussed to identify further opportunities for research in farm animals.

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Nutritional and hormonal control of muscle growth and fat deposition

Summary

This review will consider the processes of skeletal muscle (myogenesis) and fat (adipogenesis) development and the factors involved in regulating them in a variety of species. In particular, the effects of hormones and maternal nutrition during different stages of pregnancy on numbers and types of skeletal muscle fibres will be considered, along with possible underlying mechanisms. Similarly, effects of maternal nutrition on adiposity will also be considered and the relationship between skeletal muscle (fibre number and type) and adiposity. The implications for both agriculture and medicine will be considered.

Key Words: Myogenesis, adipogenesis, nutrition, pregnancy, hormones, growth factors

Zusammenfassung

Titel der Arbeit: **Nährstoffe und Hormone regulieren Muskelwachstum und Fettansatz**

Diese Übersicht beschäftigt sich mit der Regulation von Vorgängen der Skelettmuskel- und Fettgewebsentwicklung in verschiedenen Species. Es werden insbesondere Effekte von Hormonen und der maternalen Ernährung und deren mögliche Ursachen während verschiedener Stadien der Trächtigkeit auf die Anzahl und Typen von Skelettmuskelfasern behandelt. Ferner werden Einflüsse maternaler Ernährung auf die Fettgewebseinlagerung und das Verhältnis zwischen Fasernzahl und -typ und Fetteinlagerung untersucht. Mögliche Schlussfolgerungen für Landwirtschaft und Medizin werden erörtert.

Schlüsselwörter: Myogenese, Fettgewebsentwicklung, Ernährung, Gravidität, Hormone, Wachstumsfaktoren

Introduction

One of the most striking results indicating the developmental link between skeletal muscle and adipose tissue comes from gene knockout studies to identify the roles of the myogenic regulatory factors (MRF), MyoD and myf-5 (RUDNICKI et al., 1993). Mice carrying null mutations for both these genes are born alive, but are immobile and die soon after birth. Immunohistochemical analysis indicated a complete lack of muscle (both myoblast precursor cells and muscle fibres), thus illustrating the role of these two transcription factors in committing embryonic mesenchymal cells to become muscle cells. Interestingly, “the spaces normally occupied by skeletal muscle contained either amorphous loose connective tissues or expanded areas of adipose tissue. Other organs, for example, the heart, lungs, liver, and bowel, appeared completely normal” (RUDNICKI et al., 1993). Hence, it appears that commitment to become an adipose cell may be one of the defaults, in the event of mesenchymal cells not being exposed to factors inducing them to become muscle cells or other cell types (e.g. bone).

This review will be split into three parts. The first will look at the processes and regulation of myogenesis and in particular skeletal muscle fibre formation. The second part will cover the processes and regulation of adipogenesis and the link between maternal nutrition and adiposity. Finally, the link between skeletal muscle and adiposity will be discussed and the implications for agriculture and medicine.

Skeletal Muscle Development (Myogenesis)

Skeletal muscle starts to develop at a very early stage of embryonic development (see reviews by BASS et al., 2000; BUTTERY et al., 2000; MALTIN et al., 2001; PICARD et al., 2002), with all muscle cells initially starting off in the myotome and dermomyotome regions of the somite. Commitment to the muscle cell lineage appears to be initiated by wnt and shh signalling peptides (WIGMORE and EVANS, 2002), which result in the switching on of gene expression for one of the two muscle specific transcription factors, MyoD and myf-5. The expression of one or other of these is enough to induce the cell to commit to the muscle-lineage, resulting in the cell becoming a myoblast (see Figure). At this stage the cells are still mononuclear and are able to migrate to other sites within the embryo and proliferate in response to various growth factors (BRAMELD et al., 1998; BUTTERY et al., 2000). Initiation of gene expression for the third MRF, myogenin, results in the alignment and fusion of the myoblasts and their differentiation into myotubes and later muscle fibres (Figure). The latter stages of differentiation also involve the fourth MRF (MRF4), require some degree of innervation and result in the formation of large multinuclear cells. Hence, myogenesis involves combinations of four muscle specific transcription factors regulating commitment to become a myoblast and later differentiation into muscle fibres.

Muscle Fibre Formation

The numbers of muscle fibres within a specific muscle are set at around the time of birth in most mammals (rodents being the exception). Postnatal growth of muscle therefore involves increases in fibre size (hypertrophy) rather than numbers of fibres. Hence the processes of myogenesis predominantly take place *in utero*. The formation of muscle fibres takes place in 2 (or 3) waves, with primary fibres being formed first and secondary fibres developing around the primaries (MALTIN et al., 2001; PICARD et al., 2002; WIGMORE and EVANS, 2002). It also appears that there are specific myoblast precursors for these different populations of fibres. Thus, embryonic myoblasts form primary muscle fibres in early-mid gestation; while fetal myoblasts form secondary muscle fibres in mid-late gestation. In general, the primary fibres tend to become slow oxidative (type I) muscle fibres, while the secondary fibres tend to become faster fibre types (types IIA, IIB, etc). However, some degree of plasticity is seen postnatally, such that primary fibres can become fast fibres in “fast” muscles and secondary fibres can become slow fibres in “slow” muscles (MALTIN et al., 2001; PICARD et al., 2002). In some species, tertiary muscle fibre formation has been described either during mid-to-late gestation or the early postnatal period. Initially, these fibres are closely associated with secondary fibres and appear to form both fast and slow fibre types. The relative timings of initial formation of the different generations of fibres in various species are shown in the Table.

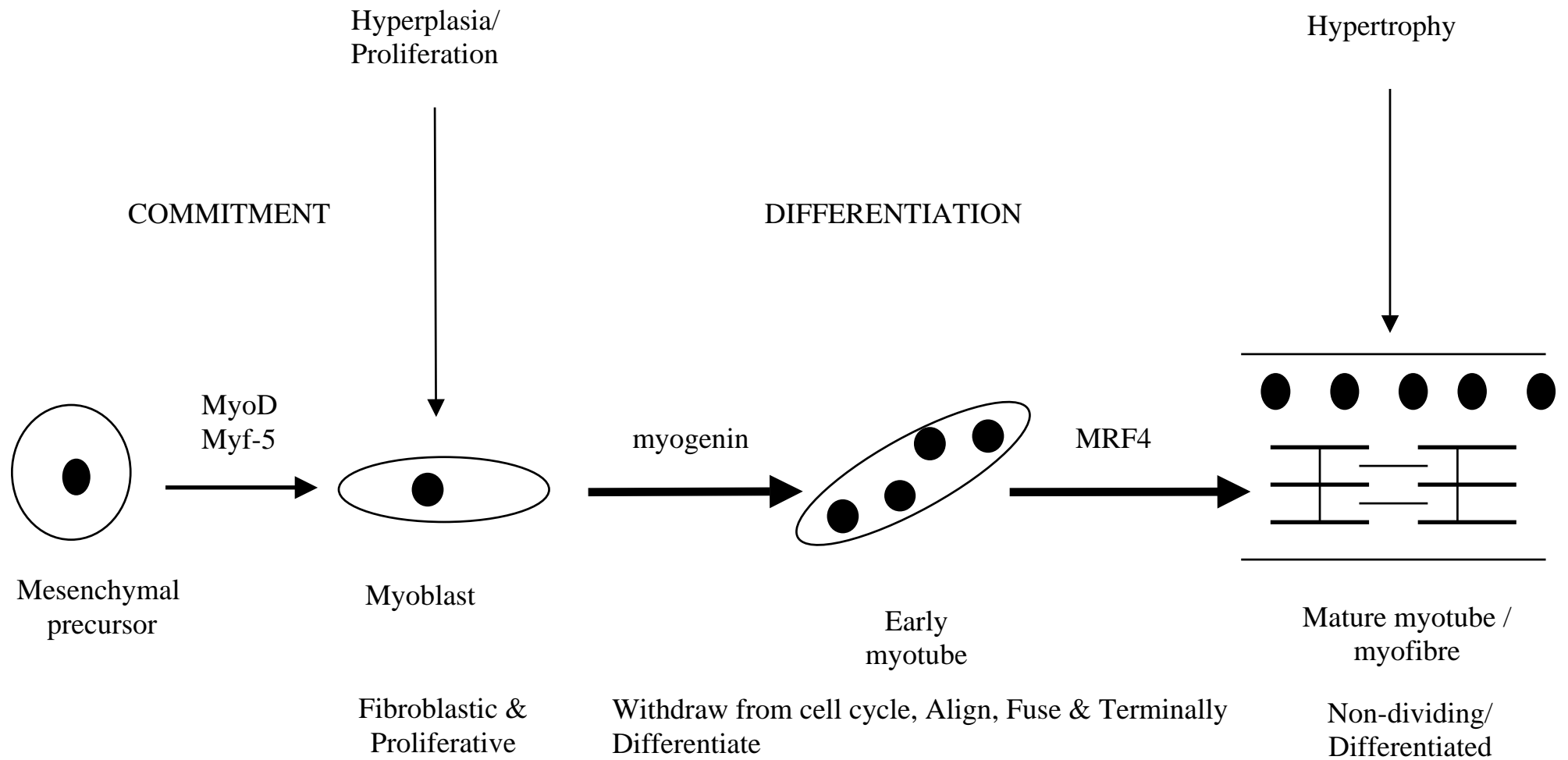


Fig.: Processes of myogenesis, including the myogenic regulatory factors (MRF transcription factors), MyoD, myf-5, myogenin and MRF4 and the timing of their expression

Table

Stage of appearance of the different generations of muscle fibres in various species (Adapted from PICARD et al., 2002)

	Primary	Secondary	Tertiary	Length of Gestation	Reference
Poultry	3-7 df	8-16 df	-	21 days	Bandman & Rosser (2000)
Rat	14-16 df	17-19 df	-	22 days	Wilson et al. (1988)
Guinea pig	30 df	30-35 df	-	68 days	Dwyer et al. (1995)
Pig	35 df	55 df	0-15 dpn	114 days	Lefaucher et al. (1995)
Sheep	32 df	38 df	62-76 df	145 days	Wilson et al. (1992)
Bovine	60 df	90 df	110 df	278-283 days	Gagniere et al. (1999)
Human	56 df	90 df	110-120 df	280 days	Draeger et al. (1997)

df: days of fetal life, dpn: days of postnatal life

A population of myoblasts (satellite cells) survive into adulthood and are able to proliferate and fuse with existing fibres during postnatal growth (fibre hypertrophy) or in response to muscle damage. These satellite cells may also relate to the myoblast population that forms the tertiary fibres. The numbers present in the basement membrane of adult muscle fibres gradually decrease with age, so that the capacity for muscle growth/regeneration decreases with age.

Embryo culture effects on muscle fibre number

As already stated, the number of muscle fibres within a muscle is fixed around the time of birth in most mammals. Hence, any regulation of the numbers of fibres takes place in utero.

Studies involving embryo culture and transfer techniques in sheep indicate that this can be as early as the pre-implantation period. An increase in both primary and secondary fibre size and in the secondary-to-primary fibre ratio is seen in muscles from large sheep fetuses obtained from *in vitro* embryo culture (MALTIN et al., 2001). Embryo culture in serum-supplemented media appears to significantly stimulate growth and this growth advantage is retained throughout gestation. Interestingly, the effects are only on the numbers of secondary muscle fibres and not primary fibres. This turns out to be a recurrent finding in a number of the studies (see below) indicating that the numbers of primary fibres are determined genetically and appear not to be susceptible to environmental factors.

Genetic regulation of muscle fibre number

One of the most striking indications for the regulation of muscle fibre number is the phenomenon of double muscling in cattle (BASS et al., 2000; BRAMELD et al., 1998; BUTTERY et al., 2000). The Belgian blue is a classic example and studies have demonstrated that the increase in numbers of muscle fibres is related to increased levels of mitogenic growth factors in the fetal circulation, resulting in increased rates of myoblast proliferation. The time period during gestation when myoblast proliferation is taking place is also expanded, along with a delay in the timing of differentiation. The delay in differentiation is associated with a delay in local expression of insulin-like growth factor-II (IGF-II, GERRARD and GRANT, 1994), an important local regulator of myoblast differentiation via its positive effects on myogenin gene expression. Disruption in the gene for myostatin, a member of the transforming growth factor beta family has been demonstrated in these cattle (BASS et al., 2000). Myostatin (GDF-8) was originally identified in mice (McPHERSON et al., 1997) and like other TGF β family members has been shown to decrease both

proliferation and differentiation of myoblasts *in vitro*. Hence, the gene mutation results in loss of functional myostatin protein, eliminating a local inhibitor of myoblast proliferation and resulting in increased myoblast proliferation and thereby increased numbers of muscle fibres. Interestingly, a number of gene mutations have been described in cattle and more recently pigs, with varying effects on muscling, including no effect on muscle fibre number in some of the cattle (SMITH *et al.*, 2000) and all the pig studies to date (JIANG *et al.*, 2002a, 2002b). This suggests that the lack of myostatin is not the only factor responsible for double muscling.

Hormonal regulation of muscle fibre number

It is ten years since REHFELDT *et al.* (1993) first demonstrated that administration of growth hormone (GH) to pregnant sows during early gestation (days 10-24) resulted in increases in the total number of muscle fibres in the semitendinosus (ST) muscle of offspring. The same group have recently published a similar study (REHFELDT *et al.*, 2001b), investigating in greater detail the effects of GH administration to pregnant sows during early gestation (days 10-27). They demonstrated an increase in the numbers of both primary and secondary muscle fibres in 2 muscles (ST and psoas major, PM) of offspring from the GH-treated sows, but only in piglets below the 75th quartile for (within-litter) weight at birth. Hence, GH increases the numbers of fibres in the medium-to-small offspring, but not in the large offspring. Another study (KELLEY *et al.*, 1995) investigated the effects of administering GH to pregnant sows during early-to-mid gestation (days 28-40). This resulted in increased ST muscle weight and longissimus dorsi (LD) cross-sectional area in neonatal offspring (KELLEY *et al.*, 1995). However, the authors did not measure numbers or sizes of the different fibre types and so the mechanism is unclear. Administration of GH during mid (days 50-64) gestation actually reduced ST muscle weight, with no effect on the total numbers of fibres (REHFELDT *et al.*, 1993); whereas GH treatment during late gestation (days 80-94) resulted in increased birth and organ (lung, stomach, liver, etc) weights, a more mature muscle morphology and a degree of muscle fibre hypertrophy (REHFELDT *et al.*, 1993). Hence early pregnancy is the critical period for exogenous GH effects on numbers of fibres, the period when myoblast proliferation is taking place prior to differentiation into fibres.

Interestingly, administration of the beta-agonist clenbuterol to pregnant rats during early-to-late gestation (days 4-17) resulted in reduced muscle DNA, while administration throughout pregnancy and lactation reduced the total numbers of fibres and increased cross-sectional area of both fast and slow fibres in soleus of weanling offspring (MALTIN *et al.*, 1990). The pregnant mothers administered clenbuterol had increased muscle weights, suggesting that clenbuterol directed nutrients towards the mother's muscles and away from the developing fetuses or lactating mammary gland.

Nutritional regulation of muscle fibre number

A number of studies have demonstrated effects of maternal nutrition on the numbers of muscle fibres in the resulting offspring. In rats, dietary restriction (to 30% of control animals) throughout pregnancy results in reduced numbers of fibres in both soleus and lumbrical muscles (WILSON *et al.*, 1988). Refeeding to the levels of control at birth and during lactation had no effect on numbers of fibres in soleus, but increased it in lumbrical muscles. This suggests that the number of fibres is set at birth in soleus

muscle, but slightly later in lumbrical muscle. The differences in numbers of fibres are due to differences in secondary fibres, with no effects on numbers of primary fibres observed.

Similar studies in guinea pigs indicate that dietary restriction (to 60% of ad lib controls) during early (0-25 days gestation), mid-to-late (25-term) or throughout pregnancy resulted in similar decreases in total numbers of fibres and secondary-to-primary fibre ratio in biceps brachii muscle at birth (DWYER et al., 1995). Dietary restriction during very early pregnancy (0-15 days gestation) had no effect, while there were no effects of any dietary restriction on soleus muscle fibre number. Supplementation of the restricted diet with either protein alone or carbohydrate alone (but not fat), prevented the reduction in biceps muscle fibre number with effects again shown to be on secondary fibres rather than primary fibres (DWYER & STICKLAND, 1994).

In pigs, reduced nutrient supply to the developing fetus results in runting and runt piglets have reduced muscle weights associated with increased fibre diameters (HEGARTY & ALLEN, 1978). This is due to reduced numbers of secondary fibres (HANDEL & STICKLAND, 1987; WIGMORE & STICKLAND, 1983) and is associated with reduced growth rates and increased adiposity (HEGARTY & ALLEN, 1978). Litters of pigs show a wide distribution of birth weights, growth rates and numbers of muscle fibres, indicating that some fetuses have sufficient nutrients to meet their genetic potential, while others are undernourished. In an attempt to overcome runting within litters, DWYER et al (1994) doubled the dietary intake of pregnant sows during early (25-50 days), late (50-80 days) or early-to-late (25-80 days) gestation. Increased nutrient supply at any stage resulted in an increase in secondary-to-primary fibre ratio, due to an increase in the number of secondary fibres, but no effect on primary fibres. The spread of numbers of fibres within a litter was compressed towards the high end, indicating that more of the litter were approaching their genetic potential.

Maternal undernutrition (70% of control group) in sheep from 30d prior to breeding until 100d gestation tended to reduce numbers of muscle fibres (at 140 days gestation), but didn't reach statistical significance (NORDBY et al., 1987). A study comparing muscles of single or twin lambs and also lambs born in the spring or autumn indicated possible effects of nutrition (McCOARD et al., 1997). Single lambs born in the autumn (after the winter period for the southern hemisphere) had reduced numbers and cross-sectional area of fibres in semitendinosus muscle compared with spring born lambs. However, no effect was seen in plantaris muscle. The comparison of singles and twins indicated no effects on numbers of fibres, but reduced cross-sectional area of fibres (in semitendinosus, plantaris and gastrocnemius) in twins. This may relate to a greater degree of undernutrition in the twins during late gestation, after muscle fibre formation, but when fibre hypertrophy is taking place. Two sheep studies were recently carried out at Nottingham. The first study was to identify the critical timing for muscle development, and involved monitoring changes in gene expression for myogenin and various growth factors in developing sheep muscle (FAHEY et al., 2003a). This indicated a peak for myogenin expression (and myogenesis) around 85 days gestation. In the second study, maternal nutrition was reduced (to 50% of controls) during three periods of gestation – before (30-70 days), during (55-95 days) and after (85-115 days) this peak for myogenesis. Nutrient restriction immediately

prior to myogenesis (days 30-70) resulted in an increased proportion of slow fibres and a decreased proportion of fast fibres in neonatal (14 days) lambs, as measured via both western blotting (FAHEY et al., 2003b) and histochemical (FAHEY et al., 2003c) techniques. The reduced numbers of fast fibres were associated with increased fast fibre diameters (FAHEY et al., 2003c), with no effects of maternal nutrition on either muscle weights or slow fibre diameters. This was interpreted as indicating reduced numbers of fast fibres, which are suggested as being predominantly secondary or possibly tertiary fibres (see Table 1). Whether the decrease in numbers of fibres is associated with reduced growth rates and/or increased adiposity is to be determined.

Mechanisms for the regulation of muscle fibre number

Proliferation v differentiation

One of the critical aspects of the effects of exogenous hormones or nutrition on muscle fibre numbers is the timing of the insult, particularly in relation to the normal timing for myogenesis and the formation of different generations of fibres (see Table 1). We therefore suggest that for an insult to affect numbers of muscle fibres, it must be during the period when myoblast (or precursor cell) proliferation is taking place and immediately prior to the onset of differentiation. Any insult that increases the rate or time of proliferation (or delays the onset of differentiation) will result in increased numbers of fibres that subsequently develop. Conversely, an insult that decreases the rate or time of proliferation (or induces an early differentiation) will result in decreased numbers of fibres that subsequently develop. Hence, in our sheep studies, we identified the period when myogenesis was mainly taking place (via a peak in myogenin expression) and demonstrated that reduced nutrient supply immediately before this time, resulted in decreased numbers of secondary (or tertiary) fibres.

Molecular regulators

In both the fetal sheep ontogeny study (FAHEY et al., 2003a) and previous in vitro studies of primary fetal sheep myoblasts (BRAMELD et al., 1999), we have shown increases in local expression of both IGF-I and IGF-II genes with differentiation. In both cases, the increase and timing of the peak for IGF-II mRNA was identical to that used as a measure of differentiation (myogenin mRNA in vivo, creatine kinase activity in vitro). However, IGF-I expression was delayed and peaked after the measure of differentiation. Similar patterns have been observed in pigs (GERRARD et al., 1998), with the peak of IGF-II expression being at the same time (59 days) as the appearance of secondary muscle fibres (Table 1). This suggests that local IGF-II expression may play a key role in the regulation of myogenesis, whereas local IGF-I expression probably changes as a result of myogenesis. Indeed, local IGF-II expression has been shown to be essential for spontaneous differentiation of myoblasts in culture using both antibody (YOSHIKO et al., 2002) and antisense (FLORINI et al., 1991; YOSHIKO et al., 2002) technologies.

We have previously shown increased muscle IGF-II mRNA at 80 days gestation (BRAMELD et al., 2000), but reduced muscle IGF-II mRNA at 140 days gestation, in response to nutrient restriction of pregnant ewes during early-to-mid gestation (40-80 days). IGF-I expression in the liver was reduced at the time of the nutrient restriction (80 days) and increased above that of controls after refeeding. Other studies in sheep indicate that the effects of dietary restriction on both the maternal and fetal GH-IGF

systems are similar (BAUER et al., 1995). Maternal undernutrition resulted in increased GH concentrations, decreased IGF-I concentrations and no effect on IGF-II concentrations in plasma samples from both mother and fetus (BAUER et al., 1995). Similarly, fasting of late gestation ewes significantly reduced fetal serum IGF-I concentrations (THOMAS et al., 1997), with no effect on IGF-II.

One possibility is that increasing nutrition (or exogenous GH) results in increased circulating IGF-I concentrations via increased hepatic IGF-I expression in the fetus. The increased IGF-I may then stimulate myoblast proliferation and delay local IGF-II expression and muscle differentiation, thus resulting in increased numbers of fibres. Conversely, nutrient restriction results in decreased circulating IGF-I concentrations via decreased hepatic IGF-I expression. The decreased circulating IGF-I then fails to stimulate myoblast proliferation and leads to early local IGF-II expression and early muscle differentiation, thus resulting in decreased numbers of fibres.

In the case of GH administration, it is known that the response in circulating IGF-I in both mother and fetus is quite slow, but it is not known how long the increased IGF-I persists for after cessation of treatment. If the decline is as slow as the increase, then the effects of GH may persist for much longer than any diet effect.

The IGF binding proteins have also been linked to muscle growth and development (see BRAMELD et al., 1998). We have seen increased secretion of IGFBP-3 into the culture media by clones of fetal sheep myoblasts found to be fusion-positive (i.e. differentiate well) compared with those that are fusion-negative (i.e. don't fuse at all – GUAN, BRAMELD, HARPER & BUTTERY, unpublished data). Similarly, increased IGFBP-3 mRNA has been described in well-differentiated porcine embryonic muscle cells (JOHNSON et al., 2003). Whether IGFBP-3 alters IGF-mediated effects or has direct (IGF-independent) effects on muscle cells is still to be identified. Muscle cells (both primary cultures and cell lines) have also been shown to express IGFBP-2, -4 and -5, to different degrees and with differing changes with differentiation (particularly comparing cell lines with primary cultures – BRAMELD et al., 1998). Hence the role of the IGFBP's in the regulation of myogenesis is still to be determined.

Adipose Tissue Development (Adipogenesis)

Fat cells (adipocytes) share a common mesenchymal cell origin with skeletal muscle cells (BRUN et al., 1996; GREGOIRE et al., 1998; HAUSMAN et al., 2001; SMAS & SUL, 1995). However, unlike muscle cells, a lack of exposure to signalling factors (e.g. wnts – ROSS et al., 2000) appears to result in that cell becoming a fat cell. Hence it might be postulated that the adipocyte lineage is the default, if the cell isn't committed to some other cell type. Adipogenesis involves determination of the stem-like precursor cell to become an adipoblast, followed by sequential differentiation steps to become a preadipocyte and then an adipocyte. The adipoblast and preadipocyte stages are associated with the capacity to proliferate and therefore increase in number. Terminal differentiation into an adipocyte, on the other hand, is associated with exit from the cell cycle and therefore loss of the capacity to increase in number. Adipocytes, once formed, tend not to be lost and are able to increase (or decrease) in size according to energy intake and requirements, but with limits to the degree of hypertrophy possible. The molecular regulation of adipogenesis appears to be via a cascade of transcription factors, switching on gene expression of each other

and the various genes involved in nutrient uptake, lipogenesis and lipolysis. The most important amongst these, in terms of differentiation of preadipocyte into adipocyte, are believed to be two CCAAT enhancer binding proteins (CEBP alpha and beta) and PPAR gamma (BRUN et al., 1996; GREGOIRE et al., 1998; HAUSMAN et al., 1993; SMAS & SUL, 1995). Unlike muscle, where the number of muscle fibres is set at birth, the capacity for preadipocytes to proliferate and form adipocytes persists throughout life. Hence there appears to be a limitless capacity to store fat. The effects of hormones, growth factors and nutrients in regulating adipogenesis have been reviewed previously (BRUN et al., 1996; GREGOIRE et al., 1998; HAUSMAN et al., 1993; 2001; SMAS & SUL, 1995).

Maternal Nutrition and adiposity

There is epidemiological evidence in humans, indicating a link between maternal nutrition during pregnancy and adiposity of the offspring (JACKSON et al., 1996; MARTORELL et al., 2001; METGES, 2001). Under- or over-nutrition during pregnancy, resulting in small or large babies, have both been associated with increased risk of fatness in adults. Data from the Dutch famine suggests that undernutrition during the first 2 trimesters increases the prevalence of obesity, whereas undernutrition during the last trimester decreases the prevalence of obesity. These effects may be countered to some degree depending on whether the offspring are breastfed or not, since breastfeeding is associated with reduced risk.

Studies in laboratory animals have shown similar results, with either undernutrition throughout pregnancy or overnutrition during late pregnancy resulting in increased adiposity of resulting offspring compared to ad libitum controls (FIOROTTO et al., 1995). Malnutrition (30% of controls) throughout pregnancy in rats, followed by adequate nourishment before weaning, has also been shown to result in increased adiposity of offspring, particularly when exposed to a hypercaloric diet (VICKERS et al., 2000). This is at least partly due to the malnourished offspring being hyperphagic.

The protein content of the maternal diet (or more probably the balance of nutrients) appears to be important, since prenatal exposure to high protein (40%) diets (compared to adequate (20%) protein) throughout pregnancy results in increased adiposity of young rat offspring (DAENZER et al., 2002). This was associated with a reduction in energy expenditure and not food intake, since the animals were pair-fed. We have recently noted that rats subject to protein restriction (9% protein vs 18% protein control) throughout gestation exhibit increased central fat deposition as young adults (BELLINGER et al., 2003). The degree of adiposity may be related to the timing of protein restriction, with early gestation as a critical period.

There appear to be few studies in farm animals investigating the effects of maternal nutrition on adiposity of progeny. A few studies have been carried out in sheep. Undernutrition (70% of control) from 30 days before breeding until 100 days gestation had no effect on any of the measures of fat used at slaughter (58.5kg) of the progeny (NORDBY et al., 1987). A greater degree of undernutrition (50% of control) throughout pregnancy increased perirenal (PR) adipose tissue weights at 110days gestation (GOPALAKRISHNAN et al., 2001), with no effect on fetal or placental weights. There appears to be a marked difference relating to the timing of the insult. Undernutrition (50% of control) during early-to-mid gestation (28-80 days gestation) results in increased PR adipose tissue weights (BISPHAM et al., 2002), whereas a

similar degree of undernutrition (50% of control) during late gestation (from 115 days gestation) results in decreased PR adipose tissue weights (BUDGE et al., 2002), all at 145 days gestation. This matches the epidemiological data in humans (see above). The increased PR weights due to undernutrition in early pregnancy is associated with increased leptin expression and decreased IGF-I and GH-receptor expression (BISPHAM et al., 2002). Whether the greater degree of undernutrition (50% of control) at specific time points during pregnancy results in altered levels of adiposity in adult animals is still to be determined.

Studies in pigs relate to runting (intra uterine growth retardation). Runted pigs not only have fewer muscle fibres (see above), they also have reduced post-natal growth rates and increased amounts of intramuscular fat and PR adipose tissue in later life, compared with their high birth weight littermates (HEGARTY & ALLEN, 1978; POWELL & ABERLE, 1981). The increased adiposity in runted pigs is due to increased numbers of small diameter adipocytes in the various fat depots (POWELL & ABERLE, 1981). Hence, undernutrition during pregnancy in pigs is also associated with increased adiposity of progeny in later life, but the sensitive time for the effect is not known.

Hormone treatment during pregnancy and effects on adiposity

A recent study in rats suggests that prenatal exposure (8-12 days gestation) to leptin results in reduced adiposity in adult offspring (NILSSON et al., 2003), associated with a tendency for increased muscle weights. The dams reduced their food intake the day after the first injection but no differences were observed thereafter. There were also no differences in food intake of the offspring, suggesting that the reduced adiposity was due to increased energy expenditure.

GH administration to pregnant sows during early gestation (days 10-27) resulted in increased subcutaneous fat weights of neonatal offspring, particularly in middle and large birth weight littermates (REHFELDT et al., 2001a). In contrast, GH administration to pregnant sows during early-to-mid gestation (days 28-40) resulted in reduced backfat thickness in 20kg offspring (KELLEY et al., 1995), with no differences at market weight (102kg). Hence, there may be differences associated with both the timing of the GH administration during pregnancy and also the age at which the offspring are studied.

Links between muscle fibre number/type and adiposity

A number of studies indicate possible links between skeletal muscle and adiposity. In humans the percentage of type I (slow oxidative) fibres is negatively correlated with percentage body fat (HELGE et al., 1999). Similarly, rats prone to developing obesity on a high fat diet have a higher percentage of faster type II fibres than obesity-resistant rats (MRAD et al., 1992). In contrast, rats bred to have an increased proportion of fast fibre types have been shown to be resistant to developing obesity even when fed a high fat diet (SUWA et al., 2002). The discrepancy has been suggested as indicating that the oxidative capacity of the muscles is more important than the fibre type, since oxidative capacity in different fibre types differs between humans and rats (MRAD et al., 2002). Another postulated reason for the discrepancy relates to differences in circulating hormone concentrations.

In farm animals, increased numbers of muscle fibres are generally associated with increased growth rates and reduced adiposity (e.g. runted pigs). The secondary fibres are the ones that appear to be susceptible to changes in maternal nutrition and these initially form fast fibre types. However, the plasticity in the system means that the fibre types can change with age and relate to post-natal nutrition and circulating hormone concentrations (e.g. thyroid and growth hormones). Hence, we still do not understand what the relationship is between muscle fibre development during fetal life and adiposity in later life. The data available suggests that the timings for effects during pregnancy are similar. One possibility is that reduced myoblast proliferation, resulting in fewer muscle fibres being formed, also leads to more preadipocytes being formed. Alternatively, since a large proportion of energy expenditure is due to skeletal muscle, then perhaps a combination of reduced numbers of fibres and altered fibre type composition results in reduced energy expenditure. Any excess energy will then be deposited in adipose tissue and a build up will result in increased adiposity.

Implications for Agriculture and Medicine

This area obviously requires further work, but a greater understanding of the mechanisms relating to myogenesis and adipogenesis and the links between them may lead to benefits for both agriculture and medicine. The agricultural benefits relate to improved efficiency in animal production and the capability to manipulate body composition leading to improvements in meat and carcass quality. In particular, ensuring that animals receive adequate nutrition during critical times of their life may not only relate directly to improved lean deposition and reduced fat deposition directly, but rather to the reduction in the number of animals sent to slaughter either underweight or overfat. The medical benefits relate to the identification of possible new risk factors relating to human obesity and the various co-morbidities associated with it.

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Rape products in dairy cow rations - digestibility and animal health parameters

(Rapsprodukte in Rationen von Milchkühen – Verdaulichkeit und Parameter der Tiergesundheit)

After calving within one week, a total of 18 cows were divided into two groups so that the trial could start on the 9th and end on the 301st day of lactation. Both groups were fed protein-equivalent amounts of extracted rape grit (2.01 kg d.m.) and rapeseed cake (2.29 kg d.m.) in an otherwise identical ration (5.48 kg d.m. maize silage, 3.29 kg d.m. wilted grass silage, 0.86 kg d.m. hay, 8.01 kg d.m. barley, 0.80 kg d.m. wheat bran, 1.78 kg d.m. dried pulp). The feed ration was administered as a "total mixture" using group feeding during the first and last third of the lactation period, and single feeding during the second third of the lactation period. Milk quantities, ingredients and selected blood parameters were measured every four weeks. In addition, ration digestibility was determined for five cows from each group during the single feeding period, followed by drawing and analyzing ruminal fluid. Rapeseed cake, while stimulating milk yield, reduced the milk's fat and protein content. It did not affect feed intake but significantly reduced crude fiber digestibility and the molar acetate content in the total acid while increasing that of propionic acid. This, and the lower crude fiber digestibility found, may be interpreted as indicating an inhibition of activity, particularly as regards the species of cellulolytic bacteria, in the presence of rapeseed fat. The ingestion of rapeseed cake considerably increased the amount of monounsaturated fatty acids in the milk fat at the expense of saturated ones, while at the same time slightly reducing polyunsaturated fatty acids. The n6-fatty acid content was slightly higher, that of n3-fatty acids was unchanged. It was remarkable that the milk fat in the group given rapeseed cake showed a higher content of trans fatty acids. Particularly noteworthy was the much greater activity of gamma glutamyl transferase and glutamate dehydrogenase in the blood serum which indicated liver damage, and a lower tetraiodine thyronine content in the serum of the group given rapeseed cake. The lower milk fat content measured for that latter group was due to the inhibition, particularly of cellulolytic bacteria, in the rumen which in turn was caused by the hydrogenation of unsaturated fatty acids and the resulting conversion products which had a toxic effect on these acids. In addition, fat synthesis in the udder was hampered by trans and conjugate diene fatty acids produced by intraruminal fat hydrogenation. The lower protein content should primarily be the result of reduced bacterial protein synthesis in this group. Feeding rape products to dairy cows had no effect on colostrum quality, birth weight, and calf development.

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Influence of maternal nutrition on post-natal skeletal muscle growth (Einfluss der maternalen Ernährung auf das postnatale Wachstum der Skelettmuskulatur)

Research has shown that the early stages of pregnancy may play a major influence on the post-natal growth rate and health status of an individual. Epidemiological studies have shown a direct correlation between fetal undernutrition and increased cardiovascular disease and diabetes in adult life. Several studies have shown in mammals that maternal undernutrition during gestation can significantly reduce the number of muscle nuclei and/or fibres which form in the offspring. This often leads to impaired post-natal growth, but the cellular and molecular mechanisms are poorly understood. In this study, we examined the effects of two levels of maternal nutritional restriction (50% and 40% of ad lib) on specific skeletal muscles in the offspring at weaning. The growth rate from birth to weaning was significantly greater for the offspring of 50% ad lib mothers than the other groups. Results at weaning showed that the number of nuclei was significantly reduced in the rats born to mothers fed the 40% restricted diet whereas this number was similar to controls in the rats from the 50% group. There was no apparent influence on muscle fibre number. Quantitative real time PCR analyses showed that lower amounts of nuclei in the 40% group correlated with lower IGF-1 mRNA levels whereas the levels of all other transcripts examined were similar to controls. In contrast, the pups from the 50% group had elevated IGF-1, IGF-1R, IGF-1BP4 and -5 but not IGF-1R mRNAs when compared to controls. Levels of PCNA and M-cadherin transcripts were also increased in the 50% group whereas those for MyoD and Myostatin were similar to controls. This study suggests that pups from the 50% group may have caught up with controls in terms of muscle nuclei number due to elevated IGF-1 levels and increased satellite cell activity. The results also imply, in this rat model at least, that maternal nutrition of 50% of ad lib (rather than ad lib) may lead to better postnatal growth.

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Quality of the newly hatched chick as a hinge between hatching and breeding: effect of composition of prestarter

(Qualität von Brutküken als Schnittstelle zwischen Brut und Zucht: Einfluss der Zusammensetzung der Prästarter-Diät)

The benefits of using a prestarter have well been stated. It has been proven that a delay in first feeding, as occurs in practice, leads to a reduced performance of the chicks with respect to growth, immune system activation, enzyme stimulation, organ development, whereas the use of an appropriate prestarter would better meet the specific needs of the newly hatched chick. Today's broiler feeding programmes provide a starter feed from placement to 15 days of age. However, the chicks' requirements change during the first weeks of their life and this should be taken into account while feeding the chicks. In the present study, the effect of the composition of the first feed was investigated. Broiler chicks were hatched and reared under a commercial lighting and temperature regime. Chicks from the middle of the hatching curve were randomly allocated to three treatment groups and were given three different feeds with isocaloric substitutions between fat, carbohydrates and proteins during the first five days (low-protein diet: 12.6% CP, 10.6% CF, 51.4% CHO; low-fat diet: 24.2% CP, 4.3% CF, 50.4% CHO; low-carbohydrates diet: 23.1% CP, 11% RF, 39.1% CHO). Thereafter, chicks were reared until slaughter age using commercial starter and grower feeds. Feed intake and body weights of the animals were recorded and blood samples were taken regularly. In addition, a representative number of chickens were sacrificed for dissection. Body weight, growth rate, feed intake and feed conversion of the animals that were fed a low protein diet, i.e. with a high energy/protein ratio, during the first five days were negatively influenced from d3 until slaughter age. The analysis of hormones and metabolites showed that these animals had a higher metabolism until several days after the switch to the commercial feed. The allometric analysis of the body organs and tissues indicated that a deficiency of protein can be covered by the use of the yolk sac. After depletion, the development of all organs is slowed down and sometimes this delay can not be caught up at slaughter age. The main function of the residual yolk seems to overcome exogenous of feed deficiencies. The residual yolk is only able to fulfill this function during the first few days after hatch. This study shows that the main focus for the requirements of the newly hatched chick should be on proteins, necessary for the early development of the digestive organs. Since carbohydrates are the main component of most feeds and since the digestion of lipids from feed only becomes fully active after a few days, these two macronutrients demand less attention in formulating prestarters.

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Quality of the newly hatched chick as a hinge between hatching and breeding: effect of time of first feeding

(Qualität von Brutküken als Schnittstelle zwischen Brut und Zucht: Einfluss der Zeit der ersten Fütterung)

Recently, much research is conducted to elucidate the beneficiary effects of early feeding of broiler chicks, both from economical point of view as out of concern for animal welfare. In the current study, the effect of early feeding was investigated. Broiler chicks were hatched and reared under a commercial lighting regime using a commercial starter and grower feed. A 4x4 random block design experiment was set up. Chicks from the middle of the hatching curve were randomly allocated to the four treatment groups. Feed was administered immediately after hatch (treatment 1), whereas chicks from treatments 2, 3, 4 only had access to food respectively 16, 35, 48 h post hatch. Feed intake and body weights of the chickens were recorded regularly. At regular intervals blood samples for posterior analysis of hormones and metabolites, were taken and animals were sacrificed for dissection. A significant ($P < 0.05$) rise in body weight until day 8 was observed when food was already available during the first 16 hours of life. Animals who had access to feed immediately after hatch, had a significantly higher body weight until week 4 of age compared to animals who had no feed access until 48 hours after hatch. From week 4 to 6, this difference was not significant, although the animals didn't fully recover from this initial depression. This finding corroborates the data of earlier studies. When chicks were fasted until 35 or 48h, their development was retarded for 2 days. This is also reflected in the plasma levels of T3 and several metabolites. The allometric analysis of the body organs and tissues indicated that early feeding had potential economic benefits, although at organ level, no significant effects were seen at day 42, only trends. Our hypothesis is that feeding triggers maturation and that birds fed early are physiologically more mature than their unfed counterparts, even though they are exactly of the same age. This differential maturation is reflected in the differences in organ weights, especially during the first 3 days. The early feeding is beneficiary because it eliminates the use of immunoglobulins in the yolk sac as an energy source and the residual yolk can be used for supplementation of the deficiencies in the exogenous feed so that this feed can be utilized more efficiently.

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Fetal programming by high protein diet in rats (Fetale Programmierung durch Hochprotein-Diät bei Ratten)

There is increasing evidence that nutritional programming during fetal development might be involved in the world wide increasing obesity epidemic. In the rat model it has been shown that nutritional programming by low maternal protein intake during gestation results in intra-uterine growth restriction and subsequent development of metabolic disturbances in adult life such as hypertension, impaired glucose tolerance and insulin resistance. Although there is some epidemiological evidence that high protein intake during early development might also influence later adiposity, so far there is little direct experimental evidence. We have recently shown that a high (40%) protein intake of rats during gestation resulted in a decreased birth weight, a fast catch up growth during suckling period, and increased adiposity and decreased energy expenditure at 8 weeks of age compared to the group fed adequate (20%) protein (Daenzer et al., J Nutr. 132: 142-144, 2002). Here we investigated parameters of protein metabolism of pregnant mothers and their fetuses during feeding of different protein concentrations. Rats were time-mated and fed isocaloric diets containing either low (LP, 10% w/w), adequate (AP, 20%) or high protein (HP, 40%) concentrations. Plasma amino acid (aa) concentrations during gestation were measured by HPLC. Additionally we studied the incorporation of orally applied ¹³C-labelled lysine (lys) and leucine (leu) into maternal plasma protein and into fetal and placental protein. Placental and fetal weights were not different in LP, NP or HP on gestation day 14 and 19. Urea concentration in maternal plasma was highest in the HP group at all time points, indicating an increased aa oxidation. Maternal plasma concentrations of branched chain aa were also highest in the HP group. Interestingly, taurine concentrations were increased during gestation in LP and AP, but not in HP. The incorporation of ¹³C-lys and ¹³C-leu into maternal plasma protein was lower with increasing protein intake (LP>NP>HP). The incorporation of ¹³C-leu and ¹³C-lys into both placental and fetal protein was decreased in HP as compared to AP or LP. These results are indicative of higher oxidation of dietary aa after HP feeding with the consequence of lower aa availability for fetal or placental protein synthesis. We conclude that feeding HP diets during pregnancy might have consequences for fetal development, especially during time of rapid tissue growth at gestation days 19-22.

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Influence of feeding on meat quality and fatty acid composition in beef*

(Einfluss der Fütterung auf die Fleischqualität und Fettsäure-Zusammensetzung bei Rindfleisch)

The meat quality and fatty acid composition of beef muscle can be affected by different factors including diet, breed and age. The aim was to enhance the content of beneficial fatty acids in beef and improving meat quality for the consumer. The experiment included sixty four beef cattle of two different breeds (German Holstein bulls and German Simmental bulls). One group of each breed was kept on pasture with finishing and the other group was maintained on concentrate indoor to investigate the influence of feeding on meat quality and fatty acid composition in muscle. The German Simmental bulls are growing faster in both feeding groups (concentrate and pasture with finishing) compared to German Holstein bulls. The intramuscular fat of longissimus muscle of German Holstein bulls showed significantly higher values for both feeding systems. The bulls of the groups fed concentrate accumulated higher intramuscular fat in the muscle of both breeds compared to pasture feeding groups. The colour investigations of both muscles (longissimus and semitendinosus) showed that the beef produced by pasture feeding is darker. The shear force values of pasture fed bulls were higher compared to the concentrate bulls. Pasture feeding increased significantly the contents of total n-3 fatty acid in muscles of both breeds. The total n-3 fatty acid concentrations increased three times in muscles and two times in subcutaneous fat in pasture fed bulls compared to concentrate fed bulls. Pasture feeding increased the absolute contents of all investigated n-3 fatty acids (C18:3 n-3, C20:5 n-3, C22:5 n-3, C22:6 n-3) in both muscles and in subcutaneous fat of German Holstein and German Simmental bulls. There was an significant influence of feeding on the contents of n-6 fatty acids (C18:2 n-6, C20:4 n-6) in both muscles and subcutaneous fat. Pasture feeding decreased the absolute concentrations of n-6 fatty acids of both breeds. Finally, the ratio of n-6/n-3 fatty acids is beneficially low on pasture groups and ranged from 1.68 to 2.03 in both muscles and from 0.74 to 0.83 in subcutaneous fat. In the case of saturated fatty acids (C14:0, C16:0) a significantly decrease was observed in both muscles by pasture feeding, but the saturated fatty acids are increased in subcutaneous fat by pasture feeding. Beef enriched with n-3 fatty acids by pasture feeding represents an important source for n-3 fatty acid intake for humans.

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In vivo investigation of the placental transfer of ¹³C-labeled fatty acids in humans

(In vivo-Untersuchung des plazentalen Transfers von ¹³C-markierten Fettsäuren beim Menschen)

Cord blood shows higher percentages of long-chain polyunsaturated fatty acids than maternal blood, which agrees with the assumed importance of long-chain polyunsaturated fatty acids for pre- and postnatal infantile development. As the mechanism for the enrichment of these fatty acids on the fetal side of the placenta are not fully understood yet, we compared placental transfer of different fatty acids in humans in vivo using stable isotopes. *Subjects and Methods:* Four pregnant women undergoing cesarean section received four hours before delivery an oral dose of uniformly labelled ¹³C-palmitic acid (PA), ¹³C-oleic acid (OA), ¹³C-linoleic acid (LA) and ¹³C-docosahexaenoic acid (DHA). Maternal blood was collected at -4 (basal), -3, -2, -1, 0 and +1 hour relative to the time of the cesarean section. At the time of birth, venous cord blood and placental tissue were collected. From tissue and plasma samples lipids were extracted and phospholipids, triglycerides and non esterified fatty acids (NEFA) were isolated. Fatty acid composition was determined by gas-liquid chromatography and isotopic enrichment by gas chromatography-combustion-isotope ratio mass spectrometry. *Results:* ¹³C-enrichment in NEFA of cord plasma tended to be higher than in NEFA of placenta with statistically significant differences for the nonesterified OA and DHA (PA: 0.024±0.011 APE vs. 0.001±0.001 APE; OA: 0.042±0.008 APE vs. 0.005±0.003 APE; LA: 0.038±0.010 APE vs. 0.008±0.002 APE; DHA: 0.059±0.009 APE vs. 0.010±0.003 APE). The ratio of tracer fatty acid concentrations of placenta to maternal plasma was significantly higher for ¹³C-DHA than for the other fatty acids (¹³C-PA: 7.1±1%; ¹³C-OA: 3.8±0.4%; ¹³C-LA: 9.2±1.3%; ¹³C-DHA: 25.9±3.4%). *Conclusion:* These results suggest that only a part of the placental NEFA participated in fatty acid transfer and that the placenta showed a preferential accretion of DHA relative to the other fatty acids.

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Effects of growth hormone-releasing factor (GRF) given in early pregnancy on postnatal growth and muscle characteristics of Meishan-derived dam line and Large White pigs

(Effekte einer Verabreichung von GRF (growth hormone-releasing factor) während der frühen Trächtigkeit auf das postnatale Wachstum und Muskeleigenschaften bei einer Meishan-Sauenlinie und Large White-Schweinen)

This study was done to determine the effects of GRF given in early gestation on postnatal performance of two breeds of pigs. Large White (LW; n = 11) and Meishan-derived dam line (M, with 50% Meishan genes; n = 17) pregnant gilts were assigned to two groups: 1) saline injections, and 2) injections of 1 mg of a GRF analog, given thrice daily from days 18 to 33 of gestation. Jugular blood samples were collected from gilts on days 17 and 34 of gestation and were assayed for IGF-I. Three pigs per litter (12.5, 50 and 87.5 percentile ranking in weight) were slaughtered on day 28 postpartum and 4 (2 castrated males and 2 females) were slaughtered at 108.2 ± 2.5 kg BW. Weights of the left and right longissimus dorsi (LD) and BW were obtained in piglets slaughtered on day 28. Pigs slaughtered at 108.2 kg were weighed on days 56, 78 and 118 and their backfat thickness was measured at slaughter. Feed intake was recorded daily in those pigs and feed efficiency was calculated. At both slaughter ages, samples from the right LD were taken for the determination of number and size (cross-sectional area) of muscle fibers. Exogenous GRF increased IGF-I concentrations (554.0 vs 127.2 ng/mL, SEM = 21.5, P < 0.001) in gilts from both breeds on day 34 of gestation but did not affect (P > 0.1) any of the muscle characteristics measured at both slaughter ages. Feed efficiency was not altered by GRF (P > 0.1) but was greater in M than LW pigs (P < 0.001). Weight of pigs did not differ between treatments on day 28 of lactation (7.2 vs 6.9 kg for control and GRF, respectively, SEM = 0.5; P > 0.1), yet, for pigs slaughtered at 108.2 kg, control animals were heavier than GRF-treated (P < 0.05) on days 56, 78 and 118 (76.7 vs 73.9 kg, SEM = 0.8). Comparison of fiber characteristics both at day 28 and at 108.2 kg indicated a faster growth rate in LW than M pigs. In market weight pigs, there was a tendency (P = 0.07) for mean cross-sectional area of the LD to be larger in LW than in M pigs (5316 µm² vs 4509 µm², SEM = 192). LW pigs also had larger loin eye areas than M (56.9 vs 42.9 cm², SEM = 1.3, P < 0.001) indicating a greater total fiber number. Backfat thickness was greater (28.8 vs 15.4 mm, SEM = 1.4, P < 0.001) in M than LW pigs at 108.2 kg. In conclusion, GRF given in early lactation did not improve postnatal growth or muscle characteristics of pigs from either breed and M pigs had poorer carcass quality than LW.

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Influence of feed quality on the expression of PPAR γ ₂ in skeletal muscles on calves

(Einfluss der Futterqualität auf die Expression von PPAR γ ₂ in der Skelettmuskulatur von Kälbern)

Peroxisome proliferator-activated receptor gamma2 (PPAR γ ₂) is one of the nuclear receptors and it is known to be a main regulator of adipogenesis. Although PPAR γ ₂ mRNA is expressed at early stages in the course of adipocyte differentiation *in vitro*, no study has examined the influences of nutritional differences in the early periods of growth on the expression of PPAR γ ₂ mRNA in skeletal muscles in cattle. The purpose of this experiment was to investigate the influence of feed quality on the expression of PPAR γ ₂ mRNA in skeletal muscles of calves. Nine Holstein and six Japanese Black calves were used. All trial calves were nursed artificially until they were 2 months of age. They were divided into two groups at 2 months of age: groups R and C. In group R, the calves were fed only roughage. In group C, they were fed a considerable amount of concentrate (over 2.5% of their body weight) and given *ad libitum* access to Italian ryegrass hay (roughage). Muscle samples were taken by biopsy from *M. longissimus thoracis* (LT) and *M. biceps femoris* (BF) at 2 and 5 months of age in Holstein and at 5 months of age in Japanese Black calves. Total RNA was isolated from these tissues with ISOGEN. Semi-quantitative analysis of reverse transcription-polymerase chain reaction (RT-PCR) was used to measure the expression of PPAR γ ₂ mRNA. For amplification of PPAR γ ₂ the following primer pair was used: forward 5'-GTG GTG GCA AAT CCC TGT TC-3' and reverse 5'-CGG AAG AAA CCC TTG CAT CC-3'. The ribosomal protein L7 was used as a standard for each PCR reaction. There were some significant differences of the expression of PPAR γ ₂ mRNA between groups, ages, and muscles in Holstein. At 5 months of age in LT, the expression of PPAR γ ₂ mRNA in group C was significantly higher than in group R. In group C, the expression of PPAR γ ₂ in LT was significantly higher at 5 months than at 2 months of age. In group C, the expression of PPAR γ ₂ was observed to be significantly higher in LT than in BF. At 5 months of age, no significant differences were observed between both muscles of Holstein and Japanese Black calves. This suggests that the level of adipocyte differentiation in skeletal muscles is not significantly different between the two breeds at 5 months of age. In this experiment, feed quality influenced the expression of PPAR γ ₂ in skeletal muscles during 2-5 months. These data suggest that feed quality might influence adipocyte differentiation in bovine skeletal muscles during the early growth period.

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Influence of feed quality on growth regulating factors in skeletal muscles on calves

(Einfluss der Futterqualität auf wachstumsregulierende Faktoren in der Skelettmuskulatur von Kälbern)

Muscle mass is regulated not only by growth factors such as Insulin-like growth factor I (IGF-I) but also by inhibiting factors. Myostatin (GDF-8) is a member of the TGF- β superfamily of secreted growth and differentiation factors that play important roles in regulating embryonic development and tissue homeostasis in adults. Early growth is an important period in the development of skeletal muscle in cattle, although it is not known whether different qualities of feed influence the expression of myostatin mRNA in skeletal muscle *in vivo* or not. The purpose of this study was to evaluate the influence of feed quality on calves by investigating the expression of myostatin mRNA in skeletal muscle and IGF-I concentration in blood plasma. All Holstein cows were fed artificial milk until 2 months of age. After that, these animals were divided into two groups: one was fed only roughage (group R, n=4). The other group was fed concentrate (group C, n=5). At 2 and 5 months of age, muscle samples were taken from the *M. longissimus thoracis* (LT) and *M. biceps femoris* (BF) by biopsy. The frozen muscle materials were crushed and dissolved using ISOGEN, after which the total RNA was extracted from each sample. Semi-quantitative analysis of reverse transcriptase-polymerase chain reaction (RT-PCR) to measure the expression of myostatin mRNA was performed on each sample, and the ribosomal protein L7 (RPL7) was used as an internal standard. At 3 and 5 months of age, blood samples were taken from the jugular vein of cattle. IGF-I concentration was measured. At both 2 and 5 months of age, there were no significant differences in the expression of myostatin mRNA between groups R and C. However, the expression of myostatin mRNA seemed to be different in LT and BF. In particular, group R at 5 months of age had a two fold greater expression of myostatin mRNA in LT than in BF ($P < 0.05$), and in group C, the expression of myostatin mRNA wasn't significantly different between LT and BF. At both 3 and 5 months of age, IGF-I concentration was higher in group C ($P < 0.05$). Above all, at 5 months, IGF-I concentration in group C was 13.5-fold greater than that of group R. When comparing calves at 3 and 5 months of age, the IGF-I concentration in group R did not significantly change during the 2 months period, and increased 4.9-fold in group C ($P < 0.01$). From this study, it was clear that the expression of myostatin mRNA was not influenced by feed quality. During the early growth period, growth factors such as IGF-I affected by nutritional levels were more important than myostatin and regulated the growth of the whole body. Under normal conditions of nutrition, we found that myostatin was not a limiter of growth.

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ANKE HEYER¹, KRISTINA ANDERSSON² and KERSTIN LUNDSTRÖM¹

Piglet performance and production and meat quality of growing/finishing pigs in response to increased maternal feed allowance during early gestation

(Wachstum und Fleischqualität von Mastschweinen unter den Bedingungen einer erhöhten mütterlichen Futteraufnahme während der frühen Trächtigkeit)

The objective of this study was to verify the hypothesis of an increased muscle fibre genesis (hyperplasia) in foetal development in growing/finishing pigs from gilts/sows given additional feed during gestation. This might lead to a higher growth rate, higher proportion of valuable parts in the carcass (ham, loin, side fat), higher lean meat content and more tender meat. 20 gilts were inseminated and divided in four groups with different allowances of conventional feed during gestation day 25-80. The control group received 2.3 kg à 12.4 MJ ME/kg, the other groups received additionally +35%, +70%, +100% of the control diet. The same feeding regimen was repeated the following year with the same females. The offspring was raised conventionally and slaughtered at 105 kg live weight. Production traits (born/weaned piglets per gilt/sow, daily weight gain), carcass measurements (lean meat content, size of valuable parts, back fat thickness) and technological meat quality measurements (pH, internal and surface reflectance, water holding capacity) were registered. The hypothesis of improved daily weight gain, lean meat content, carcass quality and meat quality of growing/finishing pigs could not be confirmed in this study. The number of weaned piglets increased with increased feed allowance for the sows (2nd year), but not for the gilts (1st year). Total litter weight and daily weight gain of the piglets were not affected by the maternal nutrition. Offspring from sows fed the highest feed allowance (+100%) had significantly inferior daily weight gain compared to those from sows fed the control diet. Higher feed allowance during gestation did not affect carcass quality and technological meat quality traits of the growing/finishing pigs. The feed supplementation was positive in an economical point of view, as the higher number of weaned piglets per sow compensated the higher feeding costs. Table 1. Effect of sows maternal nutrition on selected pig production data Control +35% +70% +100% p-value No. weaned piglets 9.5±0.68a 11.4±0.61ab 11.8±0.68b 12.4±0.61b 0.043 Litter weight, kg 17.3±1.78 21.0±1.59 20.4±1.78 20.5±1.59 0.437 Daily weight gain, g 880±17.2a 848±14.9ab 814±16.7b 815±14.8b 0.040 Lean meat, % 57.4±0.56 58.4±0.48 58.9±0.54 58.4±0.47 0.275.

Different letters within the rows indicate significant differences ($p < 0.05$) between the values.

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Postnatal development of intestinal phosphate transport in goats (Postnatale Entwicklung des intestinalen Phosphat-Transportes bei Ziegen)

Introduction: In growing goats a well established bone mineralisation as well as an adequate energy metabolism has to be developed postnatally. An essential prerequisite is phosphate (Pi) and therefore, an adequate dietary phosphate (Pi) intake as well as a high intestinal absorption of Pi is necessary. Pi is transported in the small intestines of goats by a sodium-dependent transport process, which is molecularly modulated by an apically located NaPi type IIb transporter. Aim of the study was to determine the expression pattern of sodium dependent Pi transport in the jejunum during postnatal development of goats. **Material and Methods:** Young goats, aged from one day up to 150 days, were slaughtered and in segments of jejunum amounts of NaPi IIb mRNA were determined semi-quantitatively using northern analysis. Brush border membranes (BBM) of jejunal epithelia were prepared with a modified Mg²⁺-EGTA precipitation method for functional and structural measurement of NaPi transport. Data were recorded in the milk-fed period (non-ruminating) and in the onset of weaning up to ruminating status (ruminating period). Data of each period were correlated with age by linear regression analysis. **Results and Discussion:** Na⁺-dependent Pi transport was expressed on a high level at birth, increased during non-ruminating period and decreased after onset of ruminating status. NaPi IIb mRNA as well as specific protein levels increased continuously from a very low level during non-ruminating period, indicating a transcriptionally regulated gene expression. In the ruminating period, mRNA levels declined, while NaPi IIb protein level was unaffected. Low amounts of NaPi IIb in the first few days after birth despite a high Na⁺/Pi transport capacity like in 150 day old goats indicated that sodium dependent Pi transport might have been mediated by another transport system in these newborn goats. Later on, NaPi IIb transport system matured and seemed to be responsible for the peak in Pi transport capacity at onset of weaning. At this ontogenic stage, a higher demand of Pi rose in the young ruminants since salivary Pi secretion started due to the mechanically stimulated salivary flow. Closing the endogenous Pi cycle by increasing salivary Pi secretion, the flow of Pi from the plasma pool into the saliva was enhanced and has to be balanced by an adequate intestinal Pi uptake. In ruminating goats, Pi transport capacity declined due to a reduction of transporter affinity. NaPi IIb protein level was high despite reduced NaPi IIb mRNA amounts. With adequate dietary Pi uptake according to the respective ontogenic stage young goats could adapt their intestinal Pi transport capacity due to the needs of their metabolism.

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Transient and persistent effects on growth and growth-related metabolism in young growing pigs fed soy protein isolate or casein diet

(Transiente und persistente Effekte auf das Wachstum und den wachstumsbezogenen Stoffwechsel nach Fütterung einer Sojaproteinisolat- oder Kasein-Diät bei jungen wachsenden Schweinen)

We aimed to explore transient and persisting dietary effects on growth, metabolism and gene expression in young growing pigs. Two feeding experiments (E1 and E2) were carried out with castrated male pigs weighing between 10 and 30 kg. Pigs were fed isoenergetic and isonitrogenous diets at 50% protein requirement (9 % protein in the diet) with either soy protein isolate (SPI) or casein (CAS) as the sole protein source. Intake of digestible protein and metabolizable energy amounted to $7.1 \text{ g} \cdot \text{kg BW}^{-0.62} \cdot \text{d}^{-1}$ and $1800 \text{ kJ} \cdot \text{kg BW}^{-0.62} \cdot \text{d}^{-1}$ in E1, and $5.4 \text{ g} \cdot \text{kg BW}^{-0.62} \cdot \text{d}^{-1}$ and $1430 \text{ kJ} \cdot \text{kg BW}^{-0.62} \cdot \text{d}^{-1}$, respectively, in E2. In E1, 12 animals received the CAS diet for 24 days during the first period; 6 animals were then switched to a SPI diet whereas 6 animals continued on the CAS diet for another 31 days (second period). In E2, a third period of 31 days was added in which the SPI group was switched back to CAS diet. The control group was fed on the CAS diet throughout E 2 (86 days). In E1 SPI compared to CAS feeding (31 days) resulted in a lower live weight, protein retention and protein synthesis. Furthermore, SPI feeding resulted in increased liver weight and decreased thyroid weight. Content of hepatic carbohydrate was increased, but hepatic glutathione was decreased. After the SPI diet was changed back to CAS (E2) decrease in live and thyroid weight persisted whereas the other metabolic parameters approached to values of the CAS group. At the molecular level, in E1 mRNA abundance of genes (decreased level of methyl transferase and eucaryotic releasing factor 1) were significantly altered in SPI group as compared to CAS. In E2 these SPI-induced alteration of gene expressions persisted. We conclude that the depleted methionine content in the SPI diet causes both significant changes of transcription and translation.

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A low threonine diet down-regulates circulating level of IGF-I by altering plasma IGFBP profile without affecting hepatic IGF-I mRNA expression*

(Eine Threonin-Mangel-Diät führt zu einer Down-Regulation von zirkulierendem IGF-I durch Veränderung des IGFBP-Profiles ohne Effekte auf die hepatische IGF-I mRNA Expression)

We have previously reported that a low lysine diet reduces plasma IGF-I and IGFBP3 levels whereas it does not affect IGF-I mRNA expression in the liver in pigs aged 6-9 wk (KATSUMATA et al., 2002). From these observations, we concluded that the reduction in plasma IGF-I caused by a low lysine diet might have been due to suppression of the post-transcriptional events; increased clearance rates of circulating IGF-I due to lower IGFBP3 in pigs fed a low lysine diet might have been involved in the response. In order to examine whether this finding could be extended to the other essential amino acids, we conducted a study to elucidate influence of a low threonine diet on plasma IGF-I, hepatic IGF-I mRNA, and plasma IGFBP profile. Two male 6-wk-old pigs from each of seven litters were used. Each littermate was assigned to one of two diets, control or low threonine (LT), provided 14.3 MJ DE/kg for both diets, 170 g protein/kg for the control diet and 167 g protein/kg for the LT diet. The control diet contained all essential amino acids in the recommended amounts, including 8.2 g threonine/kg. The LT diet was similar but contained only 5.1 g threonine/kg. Pigs were pair-fed these diets for 3 wk. Growth rate and feed efficiency of pigs fed the LT diet were significantly lower than those of pigs fed the control diet ($P < 0.01$). Plasma IGF-I level of pigs fed the LT diet was 44% lower than that of pigs fed the control diet ($P < 0.01$). Plasma free threonine concentration of pigs fed the LT diet was lower than that of the pigs fed the control diet ($P < 0.001$). Interestingly, plasma concentrations of free valine, isoleucine, phenylalanine, lysine, and histidine were significantly higher in the pigs fed the LT diet compared with those of the pigs fed the control diet ($P < 0.01$). Plasma IGFBP2 level of pigs fed the LT diet was significantly higher than that of pigs fed the control diet ($P < 0.05$), while pigs fed the LT diet had significantly lower plasma IGFBP3 level compared with their littermates fed the control diet ($P < 0.05$), suggesting clearance rate of circulating IGF-I was increased by the LT diet. Dietary threonine level did not affect IGF-I mRNA abundance in the liver. We conclude that lower plasma IGF-I level caused by reduced dietary threonine may have been partly due to increased clearance rate of circulating IGF-I but not due to IGF-I gene expression in the liver.

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Birth weight affects postnatal growth, body composition, and meat quality in pigs

(Das Geburtsgewicht beeinflusst das postnatale Wachstum, die Körperzusammensetzung und Fleischqualität bei Schweinen)

The birth weight of piglets is very important for their postnatal development. Pigs of low birth weight have lower survival rates and slower growth rates than pigs of high birth weight (KISNER et al., 1995; WOLTERS et al., 2001). In addition, human medical research has shown that low birth weight is accompanied by higher risk for cardiovascular diseases, diabetes mellitus, obesity, and neurobiological problems in later life (LI et al., 2001; CHEUNG 2002). The aim of Exp. 1 was to investigate the relation between birth weight of piglets and their body composition. From 16 litters three piglets per litter (lightest, heaviest, middle-weight) were selected for body analysis. The lightest piglets (1.06 ± 0.06 kg) exhibited the smallest percentages of meat, total protein, total chemical fat, and the lowest total muscle fibre numbers, whereas the percentages of internal organs, skin, bone, and total water were highest. The results of the heaviest piglets (1.72 ± 0.06 kg) were opposite to that of the lightest weight group, and that of the middle-weight piglets (1.44 ± 0.06) were intermediate. The aim of Exp. 2 was to study the influence of birth weight on postnatal growth and carcass quality. The remaining piglets from Exp. 1 were allowed to suckle on sow until 28 days of age. During the subsequent growing and finishing periods, the pigs were fed ad libitum and housed in groups until slaughter on day 182 of age. One group of pigs ($n=58$) was randomly selected for detailed carcass analysis. These pigs were assigned to one of three birth weight classes (25% low weight, 50% middle weight, 25% heavy weight) with limits for low < 1.20 kg; middle 1.20-1.62 kg; heavy >1.62 kg. According to the differences in birth weight, the weights of pigs at 28 and 182 days of age were significantly different. Lower daily gains were observed with decreasing birth weights ($P=0.07$). Pigs of low birth weight had smaller muscle meat percentages and loin muscle areas compared to pigs of high birth weight ($P=0.09$; $P=0.08$, respectively). The percentage of internal adipose tissue increased with decreasing birth weight ($P=0.11$). Pigs of low birth weight also exhibited lower relative heart weights than pigs of high birth weight ($P=0.02$). The drip loss of M. long. dorsi tended to be increased in pigs with light birth weight ($P=0.08$). In addition, the pigs of low birth weight exhibited the lowest muscle fibre number, the largest fibre size with highest myonuclear number per fibre, and the highest percentages of abnormal giant fibres ($P<0.05$). The results show that birth weight is important in determining postnatal growth, carcass composition, and meat quality of growing/finishing pigs.

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SIMON C. LANGLEY-EVANS, ANGELA FAHEY and PETER J. BUTTERY

Early gestation is a critical period in the nutritional programming of nephron number in the sheep

(Die frühe Trächtigkeit ist eine kritische Periode in der nutritiven Programmierung der Nephronenzahl bei Schafen)

Studies of human populations with a high risk of hypertension and related diseases indicate that nephron number at birth may be a determinant of poor health in later life. In humans and other species that are relatively mature at birth, maximum nephron number is achieved before the end of gestation. Individuals born with lower nephron number are more prone to developing a cycle of progressive nephron loss and rising blood pressure in order to maintain renal filtration during adult life. Reduction of nephron number in humans appears to be related to overall fetal growth and studies of rodent models indicate that renal development is vulnerable to the programming effects of maternal undernutrition. In this study we evaluate the impact of undernutrition during specific phases of sheep pregnancy upon the fetal kidney. 32 twin bearing ewes (North Country Mules) were allocated to four groups. Control animals (C) were fed 100% of daily maintenance requirements throughout pregnancy using a pelleted diet of straw nuts and soya. This was initially 8.6 MJ/day fed as two equal rations at 0800 and 1600hr daily. Nutrient restricted ewes (NR) were fed 50% of maintenance requirements from either day 30-70 (NR30), day 55 to 95 (NR55) or day 85 to 115 (NR85). Lambs were weighed at birth and slaughtered at 2 weeks old. Nephron number was determined in a single kidney using a maceration method. Group n Nephrons/kidney Control 15 419513 \pm 33572 NR30 10 295434 \pm 29546 * NR55 15 406655 \pm 29815 NR85 15 394040 \pm 23421 All data are mean \pm SEM. ANOVA indicated a significant effect (P=0.043). * indicates significantly different to all other groups, (P<0.01). Birth weight was lower in the NR85 group than in controls. Nephron number was positively related to weight at birth (r=0.33, P=0.011) and was significantly reduced in lambs subject to NR in the early phase of gestation. This study indicates that, as in rodents, the fetal ovine kidney is sensitive to maternal nutritional status. Unlike in rodents, the critical period for programming the ovine kidney is in early rather than late gestation.

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PIA M. NISSEN and NIELS OKSBJERG

Effects of increased maternal nutrition of the sow during gestation on muscle fibre characteristics, postnatal growth and meat quality of the offspring

(Effekte einer erhöhten maternalen Ernährung während der Trächtigkeit von Sauen auf Muskelfasereigenschaften, postnatales Wachstum und Fleischqualität der Nachkommen)

The objective of this study was to examine the effect of increased maternal feed intake during early to mid gestation, on muscle fibre characteristics, performance and technological meat quality (pH₂₄, Minolta colour, drip loss and pigment content) of the offspring in pigs. Thirty-nine pregnant sows were either fed restrictively (15 MJ NE/day from d 1 to 90, then 24 MJ NE/d from d 91 to 112 and again 15 MJ NE/d from d 113 to 115) throughout gestation (C) or fed ad libitum from d 25-50 (A25-50) or from d 25-70 (A25-70) of gestation and as C in the remaining periods. Offspring were slaughtered litterwise at an average live body weight of 104 ± 14 kg. The lightest, middle and heaviest weight pig of each sex per litter, selected by carcass weight after slaughter, was used for analysis. Estimates for total-, primary and secondary muscle fibre number, muscle fibre area and DNA and RNA content were analysed in muscle samples from *M. semitendinosus*. Technological meat quality traits were analysed in *M. longissimus dorsi*. Total-, primary and secondary muscle fibre number, fibre area and content of DNA and RNA were not significantly affected by increased maternal nutrition. The weight of *M. semitendinosus* was lower in A25-50 pigs compared to C pigs ($P = 0.019$). Also the average daily gain, carcass weight and muscle deposition rate was numerically lower for A25-50 pigs. An interaction between treatment and pig weight was found for muscle deposition rate ($P = 0.006$), where the lightest pigs from treatment A25-50 had a lower muscle deposition rate than the lightest pigs from treatment C and A25-70. Although not significant, middle and heavy weight pigs from treatment A25-70 also had a higher muscle deposition rate than C and A25-50 pigs. These results can not be explained by data on muscle fibre characteristics. We found no effect of increased maternal nutrition on the measured meat quality traits in the offspring. Thus, ad libitum feeding of pregnant sows from d 25 to 50 or from d 25 to 70 of gestation does not have any beneficial effect on muscle fibre number, area or postnatal growth of the offspring. It seems that maternal ad libitum feeding from d 25 to 50 in gestation has a negative effect on postnatal muscle growth, where especially the lightest weight pigs within a litter are affected.

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PIA M. NISSEN and NIELS OKSBJERG

Intra-litter variation in muscle fibre characteristics, growth performance and meat quality traits of pigs

(Wurfbezogene Variation von Muskelfasereigenschaften, Wachstum und Fleischqualität von Schweinen)

The objective of this study was to examine the intra-litter variation in postnatal growth performance, technological meat quality and muscle fibre characteristics, when littermates were selected by carcass weight. Thirty-nine litters from Danish Landrace x Large White sows and Danish Landrace or Large White boars were used in this study. Litters were fed ad libitum from 2 weeks of age until slaughter. They were slaughtered litterwise at an average live body weight of 104 ± 14 kg. The lightest (LW), middle (MW) and heaviest (HW) weight pig of each sex per litter, selected by carcass weight after slaughter, was used for analysis. M. semitendinosus was analysed for muscle fibre characteristics and DNA and RNA content and M. longissimus dorsi was analysed for technological meat quality traits (pH₂₄, Minolta colour, drip loss and pigment content). Categorising littermates in LW, MW and HW pigs at the same age reflects the differences in postnatal growth rate within a litter. Thus, average daily gain, carcass weight, muscle mass and muscle deposition rate differed across pig weight groups ($P < 0.0001$), and also the total amount of DNA and RNA differed among pig weight groups ($P < 0.0001$). This large intra-litter variation in growth performance can be explained by variation in both the number of muscle fibres and the growth rate of the fibres. Thus, HW pigs had a higher number of total muscle fibres than MW and LW pigs ($P < 0.0001$), which were not different from each other. Both primary (P-) and secondary (S-) fibre number were higher in HW pigs ($P < 0.0001$). Even though the number of P- and S fibres were similar in LW and MW pigs, the S/P-ratio was lower for MW compared with LW pigs ($P = 0.001$). This can be explained by a numerically higher number of P-fibres and a lower number of S-fibres in MW pigs. The fibre area of both type I ($P = 0.012$) and II fibres ($P = 0.004$), and therefore also the MFA ($P = 0.004$), were higher in MW and HW compared with LW pigs. In agreement with this, we found that the DNA and RNA content per muscle fibre ($P = 0.02$ and 0.002 , respectively) were lower for LW compared with MW and HW pigs. Pigment content was significantly higher in MW and HW compared with LW pigs, but no other measured meat quality traits were significantly different across pig weight groups. Thus, intra-litter variation in postnatal growth performance can be explained by variation in both muscle fibre number and muscle fibre growth rate. The different growth rate between LW and MW pigs could partly be explained by a larger MFA in MW pigs, whereas the number of muscle fibres was the same. Growth rate differences between MW and HW pigs could be subscribed to a higher number of equal sized muscle fibres in HW pigs.

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Administration of somatotropin (pST) to early-pregnant sows changes the maternal metabolic and hormonal status and results in more balanced litters in terms of lean growth

(Eine Behandlung von Sauen mit Somatotropin (pST) während der Frühträchtigkeit verändert den maternalen metabolischen und hormonellen Status und resultiert in ausgeglicheneren Würfen)

To investigate the effects of elevated circulating concentrations of maternal somatotropin during early gestation, crossbred gilts received daily i.m. injections of either 6 mg of recombinant porcine somatotropin (pST) or of a placebo (control) from day 10 to 27 of gestation (EXP1) or with gradual withdrawal from d 28 to 37 (EXP2). A series of changes was found with regard to maternal circulating hormones and growth factors as for example increases in insulin-like growth factor (IGF)- I and decreases in thyroxin and progesterone concentrations. Nutrient availability was elevated in terms of glucose and free fatty acids. Changes in placental regulatory proteins of the ST- IGF axis were found, and placental and fetal growth were stimulated by pST. Skeletal muscle development was markedly influenced as seen by increases in muscle fibre number (primary and secondary fibres) in pigs of low and middle weight littermates. The expression of Myf5 and MyoD transcripts was increased in fetuses at d 62 of gestation. At birth, increases in muscular creatine phosphokinase activity, DNA and protein concentrations were found. Treatment with pST from d 10 to d 37 of gestation (EXP2) had minimal effects on postnatal growth in total as well as on carcass and meat quality. However, an increased balance between littermates in lean growth and muscle structure was apparent. In conclusion, elevated ST concentrations during early gestation cause considerable endocrine and metabolic changes and are capable to alter fetal and postnatal growth.

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MANFRED SCHWERIN¹, MANFRED BEYER², HERMANN SWALVE³,
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Temporary consumption of soy protein diet results in prolonged postabsorptive changes of hepatic gene expression as compared to casein diet in growing pigs

(Eine zeitlich begrenzte Aufnahme einer Sojaprotein-Diät führt zu anhaltenden postabsorptiven Veränderungen der hepatischen Genexpression im Vergleich zu einer Kasein-Diät bei wachsenden Schweinen)

We studied diet-associated postabsorptive hepatic gene expression in growing pigs at living day 83. fed protein restricted diet based on casein (CAS). In the test group casein was replaced temporary in the period from day 29 to day 56 by soy protein isolate (SPI). Transcription analysis by Real-Time-RT-PCR of genes exhibited SPI-associated modification of their expression shows that temporary feeding of SPI diet results in prolonged changes of hepatic gene expression in growing pigs as compared to CAS persisting at least 4 weeks after the end of the dietary challenge. Although amplitude and level of mRNA abundance is much lower increased compared to pigs chronically fed soy protein isolate significant higher transcription levels ($P < .05$) of two genes involved in the metabolism of oxidative stress response (glutathione-S-transferase, peptide methionine sulfoxide reductase) indicate an prolonged oxidative stress response in pigs fed temporary SPI diet. Additionally, significantly higher expression levels ($P < .01$) of genes involved in replication, transcription and translation processes (DNA methyltransferase, TLS (translocated in liposarcoma)-associated protein, replication factor C, eukaryotic release factor 1) point to an increased metabolic activity in pigs temporary fed with SPI diet as compared to CAS diet. Comparison of hierarchical clusters of postabsorptive gene expression data across pigs chronically fed with the two different protein diets and across pigs fed the CAS diet with a temporary replacement of CAS by SPI in half of the group, respectively, indicates a direct SPI-associated co-regulation of genes related to oxidative stress response with genes related to regulation of gene expression and to neuronal signaling and an strong autonomous co-regulation of the genes related to regulation of gene expression in animals temporary fed with SPI after the end of the dietary challenge.

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NIGEL SCOLLAN¹, MEWA DHANOA¹, JANET DAWSON² and PETER BUTTERY²**Effect of diet and stage of development on adipocyte size in Hereford-Friesian steers**

(Einfluss von Ernährung und Entwicklungsstadium auf die Adipozytengröße bei Hereford-Friesen-Ochsen)

As part of a large study on animal growth and nutrient partitioning, this paper reports the effects of diet and stage of development on adipocyte size in subcutaneous fat. Eighty four Hereford x Friesian steers (140 kg) were allocated on liveweight to one of 4 dietary treatments; grass silage fed either alone (diet S) or supplemented with fishmeal (diet FM; 150 g/kg silage dry matter intake fed at equal ME intake to silage) or forage-concentrate diets of silage and a barley/soya concentrate (80:20) at ratios of 70:30 or 30:70 (on a DM basis). These diets were selected to investigate the effects of supplying additional protein at constant ME (FM diet) or extra energy and protein (70:30 diet), the latter such that it was non-limiting for growth (30:70 F:C diet). Of the 21 animals/treatment, 3 were slaughtered at liveweights ranging between 250-550 kg, at 50 kg intervals). At slaughter, samples of subcutaneous adipose tissue were taken and fixed in osmium tetroxide for subsequent determination of adipocyte size by image analysis. For each animal, diameter distributions of 1000 randomly selected cells were described by fitting a normal distribution 2 component model. Final body fat (kg) and subcutaneous fat depth (mm) were 121, 122, 149, 175 (s.e. 0.055 log scale) and 10.9, 9.1, 12.9 and 17.2 (s.e. 0.18 log scale) for S, FM, 70:30 and 30:70 F:C respectively, averaged across slaughter weights. The distribution of adipocyte cell diameters was found to be bi-model, reflecting 2 distinct populations of adipocytes. The main large adipocyte population had a mean diameter of around 100 µm at 250 kg liveweight which increased to around 140-150 µm by 550 kg liveweight on all diets. The second smaller population of cells had a mean diameter of 40-70 µm. In silage-fed animals, the size of these adipocytes increased with increasing maturity while this declined in animals fed on the protein and energy supplemented diets. A constant population of adipocytes (< 50 µm diameter) was evident at all stages of development for all diets. The number of cells in the diameter range 100-150 µm declined as liveweight increased while those diameters >150µm increased indicating cell hypertrophy. The results indicate that, at least in the animal weight range studied, there was a continual recruitment/filling of adipocytes. No evidence for a distinct phase of secondary hyperplasia during the final fattening phase was obtained, at least in subcutaneous adipose tissue.

Adipocyte diameters of large (LA) and small (SM) populations (µm)

kg	S		FM		70:30		30:70		S.e.d	
	LA	SM	LA	SM	LA	SM	LA	SM	LA	SM
250	101	46	100	65	106	69	110	58	6.7	8.3
300	106	46	106	60	114	65	114	57	5.2	6.5
350	112	50	113	54	122	62	119	55	4.1	5.1
400	118	54	120	49	123	58	123	53	3.7	4.6
450	124	58	126	44	138	55	128	52	4.2	5.3
500	130	62	133	38	146	51	133	50	5.4	6.7
550	136	66	139	33	153	48	137	48	6.9	8.6

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Diacylglycerol acyltransferase activity in the muscle tissue of two metabolically different breeds of cattle

(Diacylglycerol Acyltransferase Aktivität im Muskelgewebe bei zwei metabolisch unterschiedlichen Rinderrassen)

Intramuscular (i.m.) fat in beef results in marbling and, ultimately, enhanced meat quality. Diacylglycerol acyltransferase (DGAT) (EC 2.3.1.20) catalyses the acyl-CoA dependent acylation of sn-1,2-diacylglycerol to generate triacylglycerol (TAG). Previous studies have shown that the mammalian enzyme substantially affects the flow of carbon into storage lipid. Increased insight into the role of DGAT in i.m. fat deposition may lead to new diagnostic tools for selecting cattle with high marbling potential. The objective of this study was to elucidate the relationship between DGAT activity and other factors pertaining to i.m. fat deposition. Samples of m. longissimus dorsi from German Holstein (n = 17), a dairy breed, and Charolais (n= 16), a beef breed, were obtained at slaughter after 18 months, and microsomal fractions were prepared via differential centrifugation. Microsomal DGAT activity was determined by measuring the incorporation of [1-¹⁴C]oleoyl CoA into TAG. The Bradford method was used to determine the protein content of microsomes and DGAT activity was expressed as either total activity (pmol TAG min⁻¹ [g wet tissue weight]⁻¹) or specific activity (pmol TAG min⁻¹ [mg protein]⁻¹). Both total DGAT activity and specific activity were highly correlated (r = 0.95, P < 0.001) and therefore only specific activity was reported. The ANOVA model used determined the influence of breed and sire. Charolais had a higher body mass (738 vs 665 kg, P < 0.001) and greater area of m. longissimus dorsi (125.0 vs. 89.1 cm², P < 0.001) than German Holstein, which had a greater percentage of total body fat (24.4 vs 19.4, P < 0.001) and i.m. fat in the m. longissimus dorsi (4.1 vs. 2.7, P = 0.015) along with a greater marbling score (2.8 vs. 2.1, P = 0.002). German Holstein had a higher specific DGAT activity than did Charolais (66.7 vs. 47.6 pmol TAG min⁻¹ [mg protein]⁻¹), although this was not significant (P = 0.34). There was no influence of sire on DGAT activity. In German Holstein, there was significant negative correlation, however, between specific DGAT activity and body mass (r = - 0.72, P = 0.001), and between DGAT activity and area of m. longissimus dorsi (r = - 0.53, P = 0.03), while no similar correlation was seen in Charolais. In German Holstein, there was also a significant negative correlation between DGAT activity and the level of peroxisome proliferator-activated receptor γ , a transcription factor regulating fat specific genes, in subcutaneous fat (r = - 0.59, P = 0.07). A positive correlation, however, was seen in Charolais (r = 0.75, P = 0.02). These results suggest that DGAT is involved in i.m. fat deposition, although further investigation must be performed using other muscles and fat depots.

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Heat production in mink kits during development of homeothermy (Wärmeproduktion bei Nachkommen von Nerzen während der Entwicklung der Homoiothermie)

The mink is a polytocous strict carnivore giving birth to altricial young. Neonatal mink kits are devoid of thermoregulatory capacity, blind, almost hairless and with limited locomotor abilities. They are totally dependent on their mother for nourishment for about 4 weeks, during which period they are kept warm in the nest-box by the mother and by huddling together with their littermates. Homeothermy development is still poorly described, and measurements have been based on rectal and surface temperature whereas no data on heat production exist. Estimates of age when homeothermy has been reached have varied between 25 and 45 days. The objective of this study was to describe the development of homeothermy in mink kits by means of measurement of their heat production. Measurements of heat production (HE) were performed on single kits by means of a Micro-Oxymax respirometer. Each set of measurements lasted 3 hours. Measurements were performed at 30 °C (H) and 15 °C (L) with different kits, the first representing a temperature to be likely to prevail in a nest-box with dam and kits and the second being clearly below normal conditions. The kits were weighed before entering the calorimeter and after measurements. Measurements were performed with neonatal, 1, 8, 14, 20, 22, 24, 29, 32, 35, 39, 42, 48 and 54 days old kits. Heat production was strongly affected by kit age and temperature ($P < 0.001$) and kits kept at the different temperatures responded differently as indicated by significant interaction effects: in H kits HE increased from 2.4 to 16.7 $\text{kJ} \cdot \text{kg}^{-0.75} \cdot \text{h}^{-1}$ from birth until 14 days of age whereas HE of L kits varied from 0.8 (day 14) to 3.1 (day 8) kJ during the corresponding period. From 18 to 39 days of age HE in H kits was relatively stable about 20 – 25 $\text{kJ} \cdot \text{kg}^{-0.75} \cdot \text{h}^{-1}$, and finally during the period 42 to 54 days of age HE ranged from 32 to 38.1 kJ . L kits had a HE below 10 $\text{kJ} \cdot \text{kg}^{-0.75} \cdot \text{h}^{-1}$ until 24 days of age, then increasing from 24.1 kJ day 29 to 38.1 kJ on day 39. From 42 until 54 days of age HE values of L kits ranged between 43.6 and 47.5 $\text{kJ} \cdot \text{kg}^{-0.75} \cdot \text{h}^{-1}$, hence clearly higher than H kits. When results were evaluated for each hour during the 3 h measurements it was clear that H kits of all ages maintained a stable HE throughout the period. In contrast, in the L kits HE declined profoundly hour by hour until an age of 29 days and from 32 to 39 days of age there was only a moderate decline. From 42 to 54 days of age the L kits increased HE as measurements progressed. Collectively these data suggest that homeothermy develops late in mink kits, and that the ability to respond to an ambient temperature challenge is not fully developed until an age of about 42 days.

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Whole body and hindlimb protein degradation is differentially altered by feeding in 10 and 28-d-old piglets

(Der Proteinabbau wird in Ganzkörper und Keule bei 10 und 28 Tage alten Ferkeln durch die Fütterung unterschiedlich beeinflusst)

The neonatal period is characterized by a high rate of muscle protein accretion, which is partly due to an elevated rate of skeletal muscle protein synthesis in response to feeding. However, little is known about the regulation of muscle protein accretion by protein breakdown in response to feeding during the neonatal period. To determine the feeding-induced response of protein breakdown at the whole body level and across the hindlimb in neonatal piglets, overnight-fasted 10- and 28-day-old piglets (n=6/age group) were infused for 7 h with [1-¹³C]phenylalanine and [ring-D₄]tyrosine during an initial 4 h fasting period, followed by a 3 h refeeding period. Refeeding was achieved by a continuous intra-duodenal infusion of an elemental diet. Plasma samples were obtained simultaneously from the carotid artery and the vena cava; blood flow of the caudal aorta was recorded using ultrasonic flow probes. Whole body phenylalanine kinetics showed that younger piglets have a higher protein turnover rate than older piglets. This was suggested by a higher whole body phenylalanine flux (tendency, $P = 0.09$), an increased utilization of phenylalanine for protein synthesis ($P = 0.01$), and a higher rate of phenylalanine appearing from whole body protein breakdown (tendency, $P = 0.09$) in 10-d-old piglets in comparison to 28-d-old piglets. Furthermore, 10-d-old piglets were more responsive to feeding for whole body protein synthesis ($P = 0.03$), and numerically more responsive to feeding for whole body protein breakdown ($P = 0.12$) than 28-d-old piglets. Hindlimb phenylalanine kinetics demonstrated that blood flow was markedly increased by feeding in 28-d-old piglets when compared to 10-d-old piglets ($P < 0.01$). This increase in hindlimb blood flow in response to feeding resulted in a greater response of muscle protein synthesis to feeding in older piglets ($P = 0.04$), without differential response to feeding between groups for hindlimb muscle protein degradation ($P = 0.87$). However, fractional protein synthesis rates in hindlimb skeletal muscle measured following feeding period, were greater in 10- than in 28-d-old piglets ($P < 0.05$). Results suggest that the high anabolic capacity of neonates is sustained by elevated protein turnover rates in response to feeding. The reduction in whole body proteolysis associated with feeding is due to a reduction in protein degradation in tissues other than the hindlimb in young animals.

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Metabolic challenge of ewes during late gestation – ewe performance and leptin

(Metabolische Herausforderung bei Schafen während der späten Trächtigkeit – Leistungsmerkmale und Leptin)

The nutritional status of the dam during gestation may influence foetal growth, viability and the milk yield of the dam. The physiological role of leptin in pregnancy and in the transition from gestation to lactation remains to be clarified: it has been proposed to be involved in control of maternal nutrient availability and foetal energy homeostasis. The objective of the present study was to challenge ewes metabolically in late gestation. Hypotheses were that ewe performance, lamb birth weights, colostrum and milk yield, and leptin concentrations in ewe and lamb plasma as well as milk would be affected by the experimental treatment. A low (NS: artificially dried grass *ad lib*; n=5) or a high (S: artificially dried grass + supplement; n=8) plane of nutrition was given 6 weeks *pre partum* to yearling ewes pregnant with a single lamb (n=6) or twins (n=7). All ewes were kept on the same feeding level (S) 4 weeks after lambing. Balance and respiration experiments were performed about 2 weeks before parturition. Blood samples for leptin concentrations were taken from ewes and lambs. Milk intake during the first week of life was measured by means of deuterium dilution technique. Milk samples were analysed for chemical composition and leptin. Total ME intake increased during the experimental period and during late gestation S ewes tended to have a higher total ME intake ($P = 0.08$) than NS ewes (11.0 vs 12.2 MJ ME/d). The average heat production (HE) was 550 kJ/kg^{0.75} and not affected by feeding treatment. The oxidation of fat provided a larger part of HE in NS than in S ewes (57.4 vs. 46.6 %; $P < 0.01$). Reversibly, oxidation of carbohydrate contributed a significantly higher part of HE in S ewes (47.8 vs. 36.6 %; $P < 0.01$). Only ~6% of HE was made up by protein oxidation. Animals in both groups were in negative energy balance (-58 and -108 kJ/kg^{0.75}). This in combination with positive values for energy retained in protein resulted in an average body fat mobilisation of 51 g/d. Supplemented ewes generally had higher plasma leptin concentrations than NS ewes (0.84 vs. 0.69 ng/ml) ($P = 0.05$) in late gestation, but *post partum* plasma leptin concentrations equalled out. Colostrum yield was lower in NS (66.0 g) than in S ewes (181g) ($P = 0.002$) and ewes with a single lamb (88.5 g) produced less than ewes with twins (158 g) ($P = 0.03$). Lambs from S ewes had a higher average milk intake (996 g/d) than lambs from NS ewes (960 g/d) during the 1st week of lactation ($P = 0.03$) and single lambs had a higher average milk intake than twins (1043 vs. 946 g/d) ($P = 0.05$). Leptin in colostrum was higher in NS ewes with one lamb than S ewes with one lamb ($P = 0.007$), however, the ewes' feeding regimen or litter size did not affect the leptin concentration in mature milk. Leptin concentrations in milk did not correlate to plasma leptin concentrations in either ewes or lambs. Plasma leptin concentrations at birth did not correlate with birth weights ($P = 0.54$), but they increased with progressing age of the lambs and a low but significant correlation with the lambs' live weight ($r = 0.18$, $P = 0.03$) was found. The lambs' average leptin intake on day 7 was neither affected by litter size nor maternal feeding in late gestation. Lambs born to S ewes generally had higher plasma leptin concentrations than lambs born to NS ewes, and plasma leptin in single lambs was higher than in twin lambs.

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