

Muscle fiber and adipocytes in relation to meat quality in cattle and pigs

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In the Muscle Biology and Growth Unit, at the Research Institute for the Biology of Farm Animals in Dummerstorf, Germany, we investigate growth and development of skeletal muscle and intramuscular (i.m.) adipose tissue at the cellular level. Emphasis is placed on factors important for muscular growth and meat quality. These include endogenous factors (genetics, sex) as well as hormonal regulation and environmental conditions (quality and quantity of nutrition). The characterization of those factors controlling differentiation, hyperplasia and hypertrophy of muscle cells and adipocytes is prerequisite in modulating growth processes, maintaining physiological balance during growth, and ultimately in achieving higher quality meat.

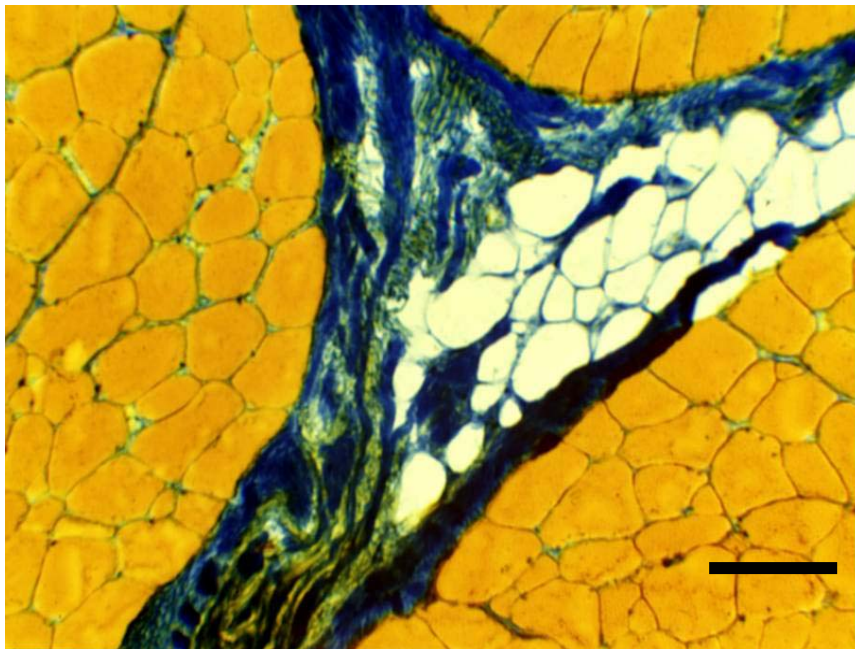


Fig. 1: Muscle fibres (orange), connective tissue (blue), and adipocytes (white) are the main tissues of muscle. Bar shows 100 μ m.

Muscle fibres and PSE (Pale, Soft, and Exudative Meat)

Attempts have been made for some time to associate the microstructure of meat, which consists of muscle and fat cells, with meat deposit and quality. The processes of taking several samples from the live animal and semi-automatic microscopic evaluation, as described by Wegner et al. (1990), are effective methods of examining growing muscle. They allow insight to the growth of muscle and fatty tissue at the cellular level. They also provide opportunities for the early recognition of meat performance and quality in pigs (pale, soft, exudative meat) that can be used in the selection process. By taking several muscle samples from the same animal at different

ages, using a total of 170 Landrace boars, it was possible to establish characteristic growth patterns for the diameter of white, intermediate and red muscle fibres of *M. longissimus dorsi* and for the fat cells of the interior and exterior layers of backfat (Wegner et al., 1990).

The objective of a study with cattle was to investigate the growth and breed related changes of muscle fibre characteristics in cattle and their importance to meat quality (Wegner et al., 2000). Cattle of four breeds with different growth impetus and muscularity were reared and slaughtered under experimental conditions. German Angus as a beef type, Galloway as a hardy type, Holstein Friesian as a dairy type, and double-muscling Belgian Blue as an extreme type for muscle growth were used. Between five and 17 bulls of each breed were slaughtered at 0, 2, 4, 6, 12, 18, and 24 months of age. Muscle fibre traits were determined and classified by computerized image analysis. Several measures of meat quality were also determined, including shear force value, meat color, and i.m. fat content. The postnatal growth of *M. semitendinosus* in cattle was characterized by a nearly 10-fold increase of muscle fibre area from birth to 24 months of age. In the first few months after birth, a transformation of type IIA fibres into IIB fibres was observed, whereas type I fibres were nearly unaffected by age. The apparent total muscle fibre number of *M. semitendinosus* did not increase during postnatal life. These results confirm that fibre number is determined during fetal development. Throughout the study, the double-muscling Belgian Blue bulls had almost twice the fibre number compared with the other breeds, emphasizing a more extensive hyperplasia of muscle fibres during fetal development in Belgian Blue. The apparent number of type I fibres was, however, not affected by breed, which suggests that the additional fibres found in Belgian Blue postnatally were type IIB and IIA fibres. We did not find significant differences in muscle fibre total number, muscle fibre type frequencies, and meat quality characteristics between breeds with the exception of Belgian Blue. Having pooled the four breeds, we found that paler meat was related to a higher frequency of type IIB fibres, a lower area of type IIA and type I fibre, and a higher total muscle fibre number. These findings, based on information from double muscling, give insight into the biological causes of variation in meat quality. Further investigation, in particular within each breed, is necessary to identify the superior fibre traits for bovine meat production.

Rehfeldt et al. (1999) reviewed the importance of muscle fibre number in muscle growth, and the influence of selected genetic and environmental factors on muscle fibre number. The total number of muscle fibres is mainly determined prenatally, when multinucleated myofibres form from myoblasts. In general, muscle fibre number remains almost unchanged during postnatal growth, and is inversely correlated with the size of the individual muscle fibre. Species-specific differences in muscle mass are primarily due to differences in the number of muscle fibres. Differences in muscle mass obtained by breeding and selection are due to changes in both muscle fibre number and muscle fibre size. Genetic variability and heritability are sufficiently high to use fibre number and fibre size in farm animal selection. Moderate postnatal feed restriction does not influence muscle fibre number, whereas strong feed restriction is able to induce fibre loss. The prenatal period of muscle development is more sensitive to nutritional deficiencies in reducing fibre number. Physical activity has been shown to influence postnatal muscle fibre number. Activity stimuli are able to induce increases, whereas disuse of muscles may be followed by decreases in muscle fibre number. The postnatal application of growth promoters induces no changes in muscle fibre number, whereas the prenatal period seems to be more sensitive to hormonal factors. It has been concluded that the number of muscle fibres formed during prenatal myogenesis and the degree of postnatal fibre hypertrophy are significant in the determination of lean growth and ultimate meat quality after slaughter. Achieving an optimum balance of sufficiently

large numbers and sizes of muscle fibres may be an important step in producing both high quantity and quality of meat in farm animals.

Adipocytes and marbling in beef

A technique developed for objective evaluation of marbling using automatic image analysis (Albrecht et al., 1996) makes it possible to measure parameters which represent essential characteristics of the i.m. fat deposits in meat. This permits detailed comparisons of different samples, which provides much more information than marbling points which were previously established subjectively, or fat content determined by chemical analysis. As an initial area of application, this technique was used to examine the i.m. fat deposits in samples of beef from different breeds of cattle.

The accumulation of subcutaneous fat and i.m. fat storage (marbling of meat) are the results of both hypertrophy and hyperplasia of fat cells. The growth related changes in fat cell diameter under the influence of endogenous and exogenous factors are presented by Wegner et al.(1998). Sixty bulls representing the breeds Belgian Blue, German Angus, Galloway and German Holstein were grown to the age of 24 months and slaughtered. Ten bulls of each breed were slaughtered at the age of 2, 4, 6, 12 and 24 months. Shot biopsy samples and post mortem muscle were used to measure the adipocyte diameter and perform computer image analysis of the i.m. fat islands. The hypertrophy of the subcutaneous fat cells shows characteristic growth curves, which are influenced by breed and nutrition. The increase in number and the enlargement of the i.m. fat islands may have been caused by proliferation and differentiation processes of adipocytes contributing to the breed specific differences in marbling. Future examinations should explore the regulation of differentiation of adipocytes at the cellular and molecular level as a basis for innovating methods for farm animal breeding and improvement of product quality.

Identifying the genetic and physiological background of phenotypic variability between animals of different nutrient turnover is a well recognised prerequisite for efficient breeding strategies and improving meat quality. We recently initiated a project on properties of nutrient transformation in cattle with respect to secretion type and accretion type. For deeper insight into the genetic and physiological background of both types, an experiment has been initiated using segregating F₂ offspring of crosses between Charolais bulls and German Holstein cows. With respect to their phenotypic and physiological properties, these two breeds are especially suitable for a study of the accretion and secretion type. The basis of the experimental design and the intended investigations are described by Kühn et al. (2002).

Ruminants transform feed components preferentially in body mass or milk. The accretion type of cattle are apt in accreting feed as meat, while the secretion type of cattle secrete metabolised feed as milk. In growing bulls, body weight, fat mass, and marbling vary distinctly between German Holstein and Charolais. We show the growth and type related differences in muscle fibres, adipocytes, and hormones in the two metabolic types of cattle (Bellmann, et al., 2003, in press). Biopsy samples of *M. semitendinosus* and blood were taken at 6, 8, 10, 13, and 16 months of age from 13 bulls of each metabolic type. Postnatal growth was characterized by a nearly twofold increase in muscle fibre area while a constant fibre type frequency was observed. Clear differences in growth potential between Charolais and German Holstein bulls were found. Charolais bulls exhibited higher daily weight gain or higher weight, but also greater muscle fibre growth. The higher muscle growth potential of Charolais was

accompanied by lower fat accretion and metabolically linked with lower plasma concentrations of insulin, glucagon, and leptin. The amount of subcutaneous adipose tissue was directly correlated with leptin in Charolais and with insulin and glucagon in German Holstein bulls. The results suggest that different utilization of nutrients leads to a more pronounced protein synthesis in Charolais and elevated fat synthesis in German Holstein to meet the episodic energetic demands during lactation in this breed.

A study on adipogenesis related gene transcripts (Ren et al., 2002) was performed to determine whether the expression of the obese (Ob) gene and lipoprotein lipase (LPL) gene in fat tissues and expression of the long isoform leptin receptor (Ob-Rb) gene in the hypothalamus were different between these two cattle breeds. Obese mRNA levels in subcutaneous and perirenal fat depots, but not in the omental fat depot, were significantly higher in German Holstein than in Charolais. Lipoprotein lipase mRNA expression in the perirenal fat depot of German Holstein was greater than that of Charolais. No significant differences in LPL mRNA levels were found in subcutaneous and omental fat depots, and Ob-Rb mRNA levels in the hypothalamus between the two breeds. Both Ob and LPL expression were greater in perirenal and omental fat depots than in the subcutaneous fat depot. Data indicated that, in bovine, the Ob and LPL gene expression levels in perirenal fat are an important index that is associated with body fat content, while Ob-Rb in hypothalamus is not.

At the cellular level, we hope to find a marker for preadipocytes to clarify at which age, or by which factors hyperplasia in adipose tissue occurs. We developed an immunohistochemical technique to locate and quantify preadipocytes in bovine muscle tissue by Preadipocyte factor-1 (pref-1). This transmembrane protein is part of the family of EGF-like repeat-containing proteins that are involved in cell fate determination. Although highly expressed in preadipocytes, pref-1 expression is completely abolished during differentiation into an adipocyte. Reverse transcription polymerase chain reaction demonstrated the bovine i.m. adipose tissue contains the three splice forms of pref-1 (A, C2, and E) previously demonstrated to be expressed in bovine adipose tissue. Furthermore, Western blots confirmed that the protein for pref-1 was expressed in i.m. adipose tissue. Polyclonal antibodies against pref-1 were tested against a cell culture of bovine preadipocytes from an embryo source to confirm that the antibody would function at immunolocating bovine preadipocytes. The antibody was tested in sections of *M. longissimus dorsi* from Charolais and German Holstein cattle. Immunohistochemical results showed that pref-1 is expressed in the perimycium near mature adipocytes and blood vessels. The pool size of preadipocytes appeared to be very low. Previous reports, however, demonstrated that preadipocytes are known to divide, providing an endless source of adipogenic precursor cells. Additionally, some stem cells in muscle are derived from a bone marrow source via the blood stream.

Future research may include *in situ* hybridisation analysis of muscle tissue to characterize the mRNA expression of pref-1. To include Japanese Black (Wagyu) cattle should be a good choice for future research in intramuscular fat deposition.

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