The physiology of marbling: what is it, and why does it develop?

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Abstract. This review describes our current understanding of the factors affecting growth and development of adipocytes in bovine skeletal muscle and discusses alternative hypotheses to explain intramuscular fat deposition: marbling. While genetic predisposition clearly plays a role in the development of this commercially interesting phenotype, animal age, energy nutrition, vitamin nutrition, compensatory growth and previous growth rate may also contribute to the final outcome. The review briefly analyses the data supporting each hypothesis and attempts a mechanistic description based on the development of tissue precursor cells into mature adipocytes. Properties of the mature adipocyte in the context of whole animal and muscle biochemistry are discussed in the companion paper by Pethick *et al.* (2001).

Introduction

The term *marbling* refers to the appearance of white flecks or streaks of fatty tissue between the muscle fibres in meat. It is a beef quality trait that is loaded with contradiction and misunderstanding. On one hand, Australian beef is considered by some in the Japanese market to have too little marbling. On the other, Australian domestic consumers avoid beef that has too much marbling, because they want to limit saturated fats in their diets. In the abattoir, meat graders value carcasses that contain some marbling more highly, based on the understanding that eating quality is improved by marbling. On the other hand, over-fat carcasses are trimmed of fat and are generally considered to be a waste of resources for the producer as well as the processor. These contradictions taken together, explain why Australian cattle producers would like to have better control over marbling in their cattle.

The biology of development of intramuscular fat, as well as the known sources of variation in the marbling trait have recently been reviewed for Meat and Livestock Australia (Harper *et al.* 2001) and the reader is directed to that report for a more complete analysis of all aspects of the science. This essay seeks to provide an update on our thinking around the questions, what is marbling and how does it develop. It is written for the purposes of the Marbling Symposium to be concise and hence we have made some simplifying assumptions and have directed the reader to more complete treatments of the pathways we have discussed only briefly.

In order to keep this work to a reasonable length, some level of cellular and biochemical knowledge will be assumed. The authors are happy to direct the reader to more fundamental texts, should this explanation be inaccessible to them.

What is marbling…

Given the contradictory nature of marbling, it is important to consider the trait from a number of different perspectives and at several levels of complexity.

…from a marketing perspective? As others will discuss during this Symposium, marbling has important implications for the valuation and marketing of beef. It is a contributor to value in the Japanese, USDA and Australian MSA grading systems. In the MSA cuts-based grading system, it is one of several factors which is used to predict the MQ4 score of eating quality.

…at the macroscopic level? Marbling appears as white flecks or veins within the bovine skeletal muscles of commercial interest. The definition does not include fat that forms a connection with any of the subcutaneous or intermuscular fat depots. These "ingressions of fat into the muscle" are not counted as part of the marbling depot during visual assessment, even though this definition is not likely to have validity from a physiological perspective. Marbling can be assessed visually, or measured using image analysis systems, based on the colour difference between fat and lean tissue (<http://msa.une.edu.au/msa>). When visually assessed, the amount of marbling may vary from none in very lean meat to more than 50% of the surface area in highly marbled meat. Meat grading systems (notably the Japanese, US and Australian) employ a subjective assessment of marbling with up to 12 distinct scores; meat having higher visual fat and higher marbling scores being of greater value. There is considerable variation in the distribution of marbling fat between individual animals, even when assessed at one site. At one extreme there is the fine, evenly dispersed flecks or streaks of white fat (shimofori, snow flake marbling), and at the other extreme thick, coarse channels of fat that merge into the intermuscular fat depots.

…in an ultrasound image? Marbling appears as bright regions

within the dark ultasound image of the *longissimus dorsi* muscle, reflecting the different densities of muscle tissue and fat. While ultrasound can be used to quantify intramuscular fat content, the accuracy of the estimate is seriously diminished by thick subcutaneous fat or intramuscular fat contents in excess of 8% w/w. Further, boundaries between connective tissue and muscle also produce an ultrasound reflection (Upton and Wolcott 2001). An ultrasound image may have value in quantification of the distribution of marbling fat if these confounding factors can be overcome.

...at the microscopic level? Marbling fat is a true adipose tissue, in that it is comprised of fat cells (adipocytes), is embedded in a connective tissue matrix, and occurs in close proximity to a blood capillary network. Its location within the interfascicular spaces of muscle is what differentiates it from other adipose depots. Marbling adipocytes are roughly spherical cells with diameters of between 40 and 90mm. They are significantly smaller than adipocytes from other fat depots, of any one animal. It is not clear at this time, if the cellular size difference reflects features of the muscle environment within which these cells develop, or a genetically determined growth potential. Marbling adipocytes normally appear in clusters or "islands" and these islands become visible macroscopically when they contain between 10 and 15 cells. When viewed histologically (Fig. 1), adipocyte islands can be found that contain many hundreds of cells, grouped around well-developed capillary beds.

...in relation to extractable fat? Extremely abundant marbling fat is normally associated with high levels of chemically extractable fat. However at lower levels of marbling, the relationship between intramuscular fat content (IMF%), which is measured chemically, and marbling score, which is assessed visually, is not strong. Variance in the visually assessed marbling scores probably contributes most to the breakdown of the relationship between IMF% and marbling score. Other contributors are likely to be sampling error and the efficiency of chemical extraction. An issue that has not been thoroughly researched, is the effect of distribution and size of marbling fat islands on the assessment of marbling. At the limit, a very low marbling score could be recorded for muscle containing fat islands that are too small to be seen.

...from a physiological perspective? It is not clear that there is any physiological advantage to the animal in developing marbled muscle. Certainly, adipocyte development within muscle is not a common occurrence amongst higher mammals. It is not mechanistically related to the lipid deposition that precedes hibernation in bears, nor is it related to the lipid deposition that precedes migration in birds. There is no evidence to support an hypothesis that marbling fat provides some internal thermal advantage to the animal. Fat development within muscle occurs in higher mammals under circumstances of tissue repair, when damaged muscle is replaced by either scar or fat. Further there are human tumours and diseases that

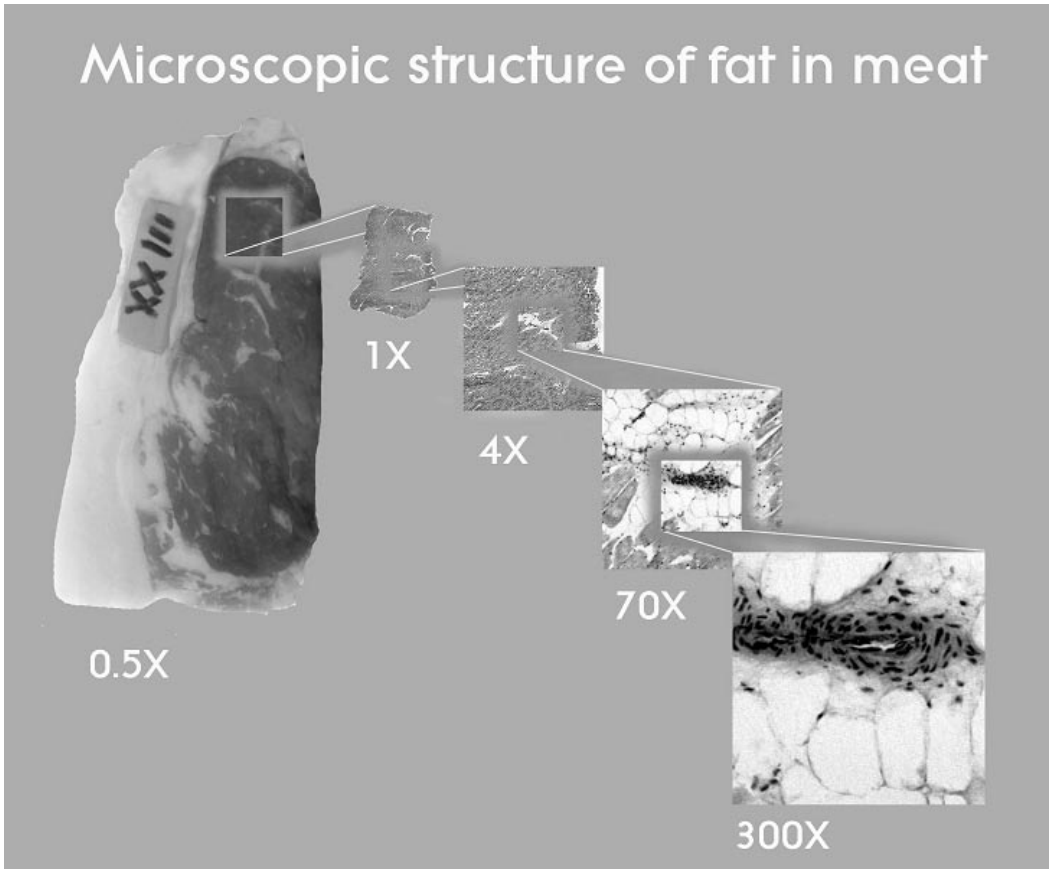

Figure 1. Histological images of adipocytes in meat, under light microscopy. Individual images supplied by P.G. Allingham.

result in the development of fat within muscle, though it is histologically unlike marbling in cattle.

At this time, 'Occam's razor' directs us to accept the simplest explanation for the phenomenon. That is that marbling develops in muscle for no specific function, and because there has been no strong evolutionary pressure on the bovine population, against it's development. In our opinion, marbling develops as a result of some genetic predisposition within particular lines of cattle, and the development is facilitated by highly intensive nutrition over an extended period.

What is marbling from a mechanistic perspective: a cellular hypothesis?

As with any mammalian phenotype, marbling results from both genetic and environmental factors. That genetics plays a major role in marbling development is beyond doubt (see Genetic factors affecting marbling level). Likewise environmental factors are also involved (See Developmental triggers), though biological variation has made these difficult to define or repeat. So, how does marbling develop in those animals that have the capacity to deposit it? In addressing this question, we need to discuss stem cells.

A stem cell is any cell that is capable of self renewal and can undergo assymmetric division to generate differentiated cells. Here 'assymmetric' division refers to cells dividing to form two daughter cells as in mitotic division, but where the two daughters are not identical. The two daughters are destined for different fates. By 'differentiation" we mean, the process by which a cell undergoes a change to an overtly specialised form. In adult bovine muscle, the major differentiated forms of the cells are: muscle cells (myocytes, which perform the contractive work of muscle and form the major textural mass of meat); connective tissue cells (fibroblasts, which form the connective tissue which binds parallel muscle fibre bundles and forms the gristle within meat); blood vessel cells (endothelial cells which surround the blood vessels); and fat cells (adipocytes which store lipid outside the muscle cells but still within the meat). Each of these cells differentiates along an independent developmental program, until they are unified within one material: meat. Development of the muscle cells and endothelial cells is outside the topic of this symposium and so the interested reader is directed to reviews by Buckingham (1998) and Nilius *et al.* (1997) respectively. Connective tissue cells are of interest, only so far as the connective tissue is the matrix within which the marbling adipocytes develop. Once the intramuscular stem cells become fully differentiated fibroblasts, they are of little further interest in this discussion.

In Figure 2, we present a model around which to describe the development of marbling adipocytes in muscle. The model has been developed with reference to an enormous literature on adipocyte development and obesity in rodents and humans (cited in Harper *et al*. 2001). The literature on marbling is considerably more limited and hence some leaps of faith have been taken by the authors in order to facilitate development of this field.

Adult mammalian muscle contains multipotent stem cells (Grounds 1999). These stem cells, represent a large proportion of the cellular population of embryonic muscle, but are a very minor constituent of adult muscle. Nonetheless they can be isolated, propogated and studied in vitro. "Multipotent" refers to the fact that these cells are capable of differentiating into a number of cell types including chondrocytes (cartilage cells), myocytes (muscle cells) or adipocytes (fat cells). Until such time as they adopt a specific phenotype reflecting some of the features of one of these differentiated cells, they are regarded as stem cells. It is these cells that drive on-going growth of the tissue and the tissue's capacity for repair, and it is these cells that we hypothesise develop into marbling adipocytes in certain breeds of cattle.

There are other multipotent cells within muscle, the muscle satellite cells for example. At this time, it is unclear if the multipotent stem cells that become marbling adipocytes are different from the satellite cells, which are responsible for growth and repair of muscle. The difference may only be in the proximity to mature muscle. For the purposes of this discussion, we will assert that some of the satellite cells present in muscle are multipotent stem cells, since they cannot as yet be unequivocally identified under a microscope (Greenwood *et al.* 1999).

The stem cell population in muscle is maintained by "assymmetric" division. For each cell that differentiates into

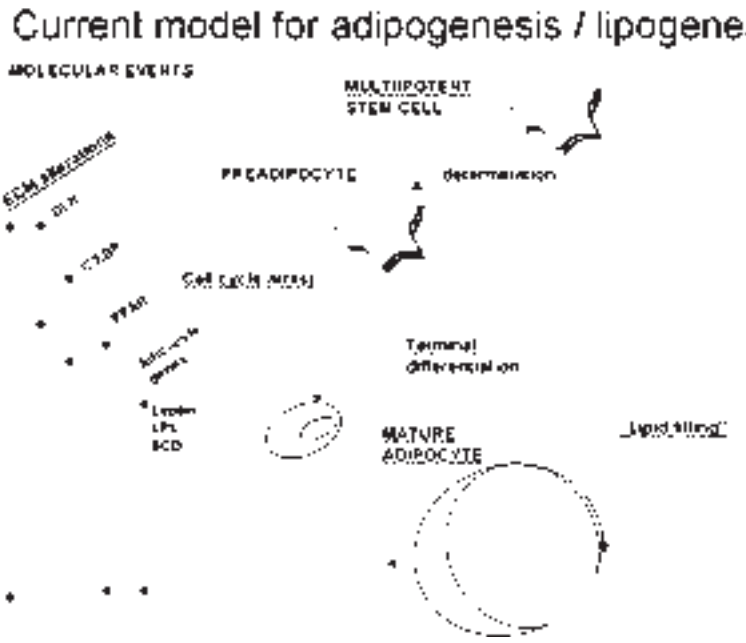

Figure 2. Schematic representing the development of mature adipocytes from multipotent stem cells residing in the tissue

an adipocyte, another daughter is returned to the stem cell pool, where it can be either maintained actively or die. Some stem cells in muscle may also come from the bone marrow, by way of the blood stream (Grounds 1999). The multipotent stem cells lie dormant within the tissue until some external stimulus induces them to progress down a developmental pathway, similar to that shown in Figure 2.

Developmental triggers for stem cells differentiating into marbling preadipocytes

Normal ageing

Unlike muscle and bone, mammalian fat continues to develop through an animal's life. This appears to hold for intramuscular fat as well (Hood and Allen 1973). The microvasculature of muscle continues to develop as the animal ages (Crandall *et al*. 1997). Whether this continues as part of a developmental program or whether development comes in response to localised anoxia is not known. A rich blood supply is a prerequisite of adipogenesis and so the nutrient environment encourages commitment of stem cells to the adipocyte lineage. Adipocytes that are already present at this time, continue to grow in diameter (Hood and Allen 1973), but are joined by new adipocytes (Leibel *et al*. 1989).

Muscles vary in the chronology of intramuscular fat development. Cianzio *et al* (1985) have shown that adipocytes appear earlier in the *longissimus dorsi* muscle than the *pectoralis* for example. More recent studies have suggested that cells of different sizes and locations express different genes and biochemical constituents (Lee *et al*. 1997).

Muscle pathology

One of the authors has previously scanned the literature covering muscle pathology and normal variation amongst the mammals. It is clear that fat accumulation in muscle can occur in mammals other than cattle, but only under relatively extreme pathological conditions. Fat accumulation in Duroc pigs and sheep for example, can get as high as 9% (w/w). The expression of the marbling phenotype in cattle is encouraged through long feeding strategies and high energy diets. Such feeding regimens have not been used in sheep or pigs. It is our opinion therefore that marbling in most cattle does not result from development of a disorder or disease process. This may not be true for the extremes of marbling, such as seen occasionally in the Japanese Black breed of cattle (S. Tsuji, *pers. comm.*).

Vitamin A status.

Low vitamin A levels may stimulate the commitment of multipotent stem cells to the adipocyte lineage. While studies in this area have been difficult due to confounding with breed, there is some support for this view. For example, Oka (1998 a and b) and others (Naruse *et al*. 1994) have shown that reducing vitamin A levels tends to increase the marbling score at slaughter. This was confirmed by showing that vitamin A supplementation at specific ages can reduce the subsequent marbling score. While this work is particularly interesting, its generality has not been shown, since the Japanese Black breed of cattle has a genetic predisposition to marble.

The hypothesis that vitamin A in animal diets might have a role in the initiation of marbling is supported by studies with adipogenesis in other species and in tissue culture systems (Gregoire *et al*. 1998). In these systems, the vitamin A axis was also functionally linked to the thyroid hormone axis (T_3 and T_4) and the insulin-like growth factors (IGFs). Interestingly, Oka *et al* (1998b) investigated T_3, T_4, insulin and IGF-1 levels in Japanese Black cattle and concluded that low vitamin A status significantly changed the normal plasma levels of these hormones. Hence these hormonal axes may work together when initiating and regulating the development of marbling. Furthermore, Oka's work suggested that animals are less responsive to the corrective effects of injected vitamin A after 21 months of age. There are many possible explanations for this age-dependence. Perhaps, 21 months is an age at which the animals undergo a fundamental change in metabolism and response to vitamin A depletion. Alternatively, perhaps 21 months reflects the duration of vitamin A depletion, beyond which irreversible damage is done to the muscle and its cells. Of greatest interest to the cellular hypothesis being presented here however, is the possibility that the population of multipotent stem cells is somehow exhausted by 21 months, and hence the animals' capacity to deposit more marbling is limited.

It is important that limiting vitamin A in feedlot diets not be seen as an industry method for increasing the marbling of beef cattle. Animals fed a low vitamin A diet can easily progress into the hypovitaminosis A state and die. Indeed, hypovitaminosis A or even marginal vitamin A deficiency should be a relatively rare occurrence in the Australian production system, since animals are grass fed up to 200kg hot carcass weight.

Developmental triggers for preadipocytes differentiating into mature adipocytes

In the previous section, we discussed the hypothesis of multipotent stem cells in muscle. Just as there is a small population of these cells in muscle that is destined to marble, there must also be a population of determined cells: cells that are beginning to exhibit characteristics that suggest subsequent differentiation into adipocytes. These are the preadipocytes. As with other predetermined cells (satellite cells, osteoblasts and myofibroblasts) these cells are not easily recognised morphologically. They have been recognised largely through work in tissue culture systems. The 3T3L1 cell line from mice, reproducibly differentiates from preadipocytes to adipocytes in culture, in response to appropriate stimuli (Wang *et al.* 1994). Quite extreme conditions are required to redirect the 3T3L1 cell from its determination to become an adipocyte as opposed to a myocyte or a chondrocyte.

Once differentiation begins, the cells undergo characteristic changes. Early changes include: reduced collagen synthesis; changes in the types of cell-matrix adhesion molecules; changes in the intracellular proteins responsible for cellular size and sharp (Smas and Sul 1995). Synthesis of the Delta-like protein (DLK) is significantly reduced, suggesting that it may play a role in maintenance of the preadipocyte stage, and also providing a technical opportunity to identify the stage (Smas and Sul 1995; Tellam *pers comm*.). Once the cells become fully differentiated adipocytes within muscle, they presumably behave much like adipocytes in other depots, even though they are markedly smaller. The properties of mature adipocytes will be discussed in other contributions to this Symposium (e.g. Pethick and Harper).

Studies in culture have found that cell cycle arrest is a prerequisites of differentiation of adipocytes. Cells that have progressed down the differentiation path towards adipocytes, can no longer divide. Growth from this point is focused on deposition of intracellular fat globules. A great deal of effort has been expended on characterization of the biochemical and hormonal stimuli that drive preadipocytes to differentiate in culture (Smas and Sul 1995). There is almost nothing known about initiators or stimulators of adipogenesis within muscle, but the extent of our knowledge is highlighted below.

Gender effects

Many experiments have examined the marbling levels in the different gender classes of animals: heifers, cows, bulls and steers. Table 1 summarises the results. The general conclusion is that, for a given slaughter weight and time on feed, heifers have higher marbling levels than steers, which in turn have higher marbling levels than bulls. In Charolais heifers, those which had received an ovarian tissue implant had higher marbling levels than controls and other treated animals (Lunt *et al*. 1990). Time of castration also has an effect on marbling level. Meaker *et al*. (1986) found that calves castrated at birth had higher marbling levels than those castrated at six months. Worrell *et al*. (1987) found that castration at 70 days of age, compared with 230 days increased marbling levels also. These observations suggest that the sex hormones influence growth and development of intramuscular adipocytes.

Exogenous growth stimulators

Harvey *et al*. (1993) studied steers which were actively immunized against growth hormone-releasing factor (GRFi) to evaluate the effect of the growth hormone axis on intramuscular fat deposition. Immunised animals had lower marbling scores than controls, suggesting that the growth hormone axis has a positive affect on marbling scores. This may depend on breed also. Although later in life during the fattening stage, exogenous GH tends to decrease im fat (Hunter *et al.* 2001). Gerken *et al*. (1995) used six sets of four genetically identical Brangus steers to study the effects of estradiol and trenbolone acetate on marbling. Neither treatment significantly influenced marbling score even though both implants had increased average daily gains. However, a comparison among implant types showed that steers implanted with the estrogenic implant had significantly lower marbling scores than did steers implanted with the androgenic or combination implants. Corah *et al.* (1995) found that treatment of pairs of genetically identical (cloned) steers with dexamethasone did not enhance intramuscular fat deposition even though previous reports had suggested that administration of exogenous glucocorticoids enhanced deposition of intramuscular fat in cattle.

Weight loss followed by weight gain

As indicated above (Fig. 2), growth arrest is an important prerequisite to adipogenesis at least *in vitro*. It is unclear what the analogous situation to growth arrest might be in the whole animal but growth stasis or weight loss may be a relevant trigger *in vivo*. Whilst some evidence suggests that adiposity is increased by cycles of weight-loss and weight gain (Rodin *et al*. 1990), the extent of increase is not reproducible. A possible explanation for the increased fatness of mammals that have undergone compensatory weight gain involves IGF-1 pulses (Bass 1997).

Protein restriction during prenatal and/or preweaning life appears to have a significant effect on the lipid metabolism of mammals (Harper *et al*. 2001). This has been shown clearly in rats and mice, and there is good epidemiological data to support its occurrence in humans. It is likely that this metabolic effect is mediated by an increase in the number of mature adipocytes. On the contrary, a period of energy or protein restriction at any time during life, reduces the size of the fat depots, and this occurs through reduction in the size of the constituent adipocytes. Realimentation induces a rapid compensatory growth of the adipocytes, as they return to a size similar to animals that had not undergone a

Table 1. Relative performance of sex status on marbling levels in eight published studies: references cited in Harper *et al*. (2001).

Study	Cows	Heifers	Steers	Bulls
A			higher	lower
B			higher	lower
C		higher	lower	
D			higher	lower
E	higher	lower		
F			higher	lower
G		highest	intermediate	lowest
H			higher	lower

restriction. The relatively larger effect of early life nutritional restriction compared with adult life restriction upon the number of preadipocytes, possibly results from a large pool of preadipocytes in the tissue. The link between nutrient restriction and adipocyte hyperplasia has not been thoroughly investigated in ruminants, though it is well established in poultry (Harper *et al*. 2001). In this case, nutrient restriction does indeed lead to a net increase in adipocyte number later in life.

It is difficult to separate the non-specific effects of nutrient deprivation, from the specific effects of particular nutrients. Such experiments have not been done as yet. For example, nutrient deprivation may include vitamin deficiency, and it has already been noted that vitamin A deficiency has a specific effect on expression of marbling (Oka *et al* 1998a). Nutrient deprivation will also result in turnover of adipose tissue that has developed previously, leading to the release of catabolites into the blood stream. Fatty acids are just one product of tissue turnover that are known to influence the development of adipocytes. Intermediaries include the prostaglandins (Reginato *et al*. 1998), and the peroxisome proliferation activation receptor (PPAR) system. This system regulates transcription of a number of genes involved in adipogenesis, and is activated by a variety of lipids and lipid-like compounds *in vitro*, including naturally occurring polyunsaturated fatty acids (Tontonoz *et al*. 1994).

Unlike cell culture models, adipose tissue in living animals has populations of cells at various stages of development. Thus an external trigger (such as reduction in dietary energy intake which affects, for example insulin concentration) would be expected to have different effects on different cells in the population. An increase in lipolysis from mature adipocytes is expected, whereas preadipocytes may undergo cell cycle arrest and commitment to adipogenesis which results in more, mature adipocytes developing sometime in the future. It is therefore difficult to interpret studies which simply look at IMF% especially early in life when there is little intramuscular fat.

Infection or high fever

Animals which have had high fever or significant parasite loads lose considerable weight due to several factors. Diversion of nutrients from the host to the parasite is one explanation for the weight loss. Work by Jewell *et al*. (1988) has also suggested that the animal releases systemic factors that can influence subsequent development of fat. Cachectin is interesting in this regard, since it is believed to mediate muscle wasting in infections such as with HIV, and can influence the number of adipocytes present (Jewell *et al*. 1988). The relevance of this observation is that animals that have recovered from a high fever or significant parasite load can have significantly different fatness than related animals or others in the herd. Whether the animals become fatter or less fat will depend on when infection occurred relative to the animal's growth milestones, and hence the animal's capacity for compensatory growth.

Higher environmental temperatures

Large scale studies performed by the CRC for the Cattle and Beef Industry (Meat Quality) showed that half-sib animals grown in either a temperate region or a subtropical region had markedly different body composition (Harper *et al*. 2001). Animals grown in the hotter climate tended to have lower IMF% and higher subcutaneous fat thickness. Unfortunately, the confounding effects of energy balance were not balanced in the experimental design, and so a clear temperature effect could be defined. Previous studies in pigs have suggested that animals kept at lower ambient temperatures develop more subcutaneous fat and a slightly higher IMF% (Lefaucheur *et al*. 1991). This issue will be dealt with in more detail in other sections of this symposium (Pethick *et al*. 2001)

Factors that influence the growth of mature adipocytes

High energy finishing diet

Growth history appears to be important to the development of fat deposits within muscle. A survey of studies in ruminants (Harper *et al*. 2001), demonstrates that many workers have found that time on high energy feed increases the amount of extractable lipid in muscle in many studies. At the cellular level, this appears to correlate to increases in the size of the adipocytes rather than development of new adipocytes. High energy diets for extended periods are required for many animals to express the marbling phenotype (or high IMF%), though recent studies have suggested that animals will rank the same in either pasture or feedlot finishing systems (D. Johnston and A. Reverter *pers comm.*). Animals without the genetic predisposition to form marbling will deposit subcutaneous fat rather than marbling fat, independently of the duration or intensity of feeding. Further, data presented in a later paper, suggests that the rate of deposition of IMF% is relatively consistent between animals in a feedlot, and hence that the IMF% upon entry to the feedlot, is of great importance to the final marbling outcome.

Genetic factors affecting marbling level

There are significant differences between breeds, and their crosses in the ability of their progeny to marble (D. Johnston and A. Reverter *pers comm*.). There appears to be some consistency amongst breed comparisons, with dairy breeds (e.g. Jersey, Friesian) recording higher marbling scores than British breeds (e.g. Angus, Shorthorn, Hereford) which record higher marbling scores than the European breeds (e.g. Limousin, Simmental, Charolais) which all tend to record higher marbling levels than *Bos indicus* breeds. This is independent of age and time in the feedlot. The Japanese Black breed stands alone for its very high capacity to form abundant marbling.

As mentioned previously (Harper *et al*. 2001), there are approximately 30 estimates of heritability for marbling, the consensus being that the heritability of marbling is moderate to high (see other sections of this Symposium).

Interactions between breed and other factors

Most of the experiments which have been used to estimate genetic parameters for marbling have also examined different finishing systems, gender (heifer, steer or bull) other fixed effects, and included interaction terms in the mathematical models. Lamb *et al.* (1990) found a significant interaction between feeding method and sire line for marbling, but suggested some confounding between these two effects. Other major research studies, for example the Clay Centre studies and the CRC studies have found few significant interactions affecting marbling or IMF% (D. Johnston and A. Reverter, *pers comm*.).

Candidate genes and their relationship to marbling

Most studies support the assertion that multiple genes influence marbling (cf. Morton and Lio 1997). Moreover, the effects of each gene are small relative to total variation in the trait. Nonetheless it is possible to locate regions on a chromosome (quantitative trait loci, QTL) which are associated with differences in the marbling phenotype and perhaps later identify the genes which confer marbling capacity. The search for, and identification of, genes with specific effects on marbling will be discussed by Bill Barendse in his paper for this Symposium.

The only known major gene affecting marbling, is the gene responsible for double muscling in cattle: GDF8 (myostatin). The gene not only affects the size of the muscle that develops, but also the proportion of connective tissue within the muscle and IMF%. Given the site of development of marbling, it seems likely that these three observations are mechanistically linked. The mutations in the GDF8 gene responsible for double muscling have been described by Grobet *et al*. (1997) and Kambadur *et al*. (1997). The GDF8 gene product is a growth regulator for muscle development, and mutations that affect its function generally result in increased muscle mass (McPherron *et al*. 1997). There is an increase in muscle growth, a decrease in the deposition of fat tissues and changes in the conformation of the skeleton as a result of these mutations in cattle (Hanset *et al*. 1982; Shahin and Berg 1985). With respect to development of marbling fat, Wegner *et al*. (1998) demonstrated that GDF8 mutant double muscled animals have: a) fewer islands of adipocytes in their LD muscle, b) slower growth of these islands and c) smaller adipocytes in marbling islands that in non-GDF8 mutant cattle.

For the minor genes affecting marbling there is evidence for at least 5 QTL of moderate effect. Genes underlying two of these confirmed QTL are of most interest in terms of understanding the mechanism of marbling. Barendse *et al*. (1997) have found that polymorphisms near the thyroglobulin gene (TG) on chromosome 14 are associated with marbling capacity. The TG gene spans 300 kb of DNA (Mercken *et al*. 1985) and encodes a protein that indirectly, plays a significant role in regulation of metabolic rate. The polymorphisms that are associated with variation in marbling are not within the coding region of the TG gene. Rather, they lie within the 5' untranslated region of the gene, which may be involved in regulation of the genes activity.

In response to thyroid stimulating hormone, the TG gene is expressed in the thyroid epithelia cell (Salvatore *et al*. 1980). The TG protein is secreted into the lumen of the thyroid gland. At the same time, and under similar hormonal regulation, iodide anion is actively transported into the lumen and activated into a form that can react with the tyrosine amino acid residues of the TG protein. Monoiodotyrosine, diiodotyrosine, T_3 and T_4 are produced through the action of transiodinase (Salvatore *et al*. 1980). The modified TG protein is now taken up by the thyroid epithelial cell by receptor mediated endocytosis, and the iodinated tyrosine residues liberated within the acidic environment of the lysosome (Tietze *et al*. 1989). Monoiodinated and diiodinated tyrosine are recycled within the thyroid gland, because iodine is a relatively rare nutrient. The final active endocrine products, T_3 and T_4 are then transported across the thyroid epithelia cell and released into the blood stream, and on to the sites of action.

It can be seen that the role of thyroglobulin *per se* in regulation of metabolic rate is circumstantial rather than direct. Nontheless, genetic variation in the rate or timing of TG gene expression could subtly influence the release of the thyroid hormones, T_3 and T_4. Evidence in support of this hypothesis comes from a number of angles. Firstly, levels of the thyroid hormones have been implicated in the development of adipocytes in muscle (Salter 1950), the fat percentage of milk (Folley and Malpress 1948), and the differentiation of adipocytes *in vitro* (Ailhaud *et al*. 1992; Smas and Sul 1995). They are also implicated in metabolic rate with high levels of thyroid hormone associated with high metabolic rates. In the context of marbling, it would appear more likely to us, that lower levels of the thyroid hormones would be more consistent with high marbling, since this state would lead to lower metabolic rate.

The second confirmed marbling QTL is on chromosome 5. The closest marker is CSSM34 which is close genetically to the gene RARG (retinoic acid receptor gamma; Barendse 1997). Again, the polymorphism associated with marbling is likely to lie within the non-coding sequence of the RARG gene. The RARG gene product is involved in regulation of transcription of a large family of genes. All-trans retinoic acid, one of the retinoid family of compounds, binds to RARG which binds to specific sequences of the DNA in the nucleus. RARG binding results in an increase in the rate of transcription from the gene to which it bound. The retinoic acid receptors (RAR) and the retinoid-X receptors (RXR) are important regulators of normal development of organs and tissues (Solomin *et al*. 1998). While the detailed mechanism is not known, it is interesting to note the relationship between low vitamin A status and high marbling score that was mentioned earlier.

Although the discovery of these QTL implicates genetic variation in either the thyroid hormone or retinoid receptor axes in individual differences in marbling capacity, the location of QTL near coding sequences is not proof of their involvement. Three facts council us to be cautious about

attributing cause and effect too early. Firstly, the genome is rich in coding sequences, with approximately 30,000 expected in cattle. There may be alternative candidates from other pathways, or as the CRC for Cattle and Beef Quality cross breeding data (Newman and Reverter 2000) suggests, there may be no major genes involved, at least in the British and *Bos indicus* breeds of cattle studied. Moreover, due to the relatively small size of the effects of those QTL identified to date, it is not feasible to use gene transfer experiments to prove that the target sequence actually does cause the effect. Thirdly, if the QTL is not the gene itself, it may be the result of several favourable genes located near each other, in a complex held together by linkage disequilibrium. Such a complex is not stable since linkage disequilibrium would decay through the normal processes of recombination. Hence without specifically recognising that a complex of genes might be involved, it would be difficult to breed specifically for the effect.

Concluding remarks

Irrespective of the mechanisms of marbling, there will continue to be industrial interest in its developmental biology. We doubt that a complete mechanistic understanding of this complex trait will ever be possible. Nonetheless, we confidently expect that research will generate sufficient knowledge for producers to manage marbling in a production setting.

In this context, it seems likely that producers should focus on initiation of more preadipocytes in muscle of animals that have a genetic predisposition to marble. These cells could then go on to produce more mature adipocytes when the animal is both the right age and eating the right diet, to express it's full marbling potential. Given the greater number of multipotent stem cells and preadipocytes in younger animals, focusing nutritional treatments on the young animal (less than 200 kg HCW) would seem to be the right approach. Data presented by Pethick *et al.* (2001), supports the view that cattle enter the feedlot with a certain IMF%, which increases arithmetically during finishing. At the cellular level, we interpret this to mean that there is a certain number of adipocytes in muscle at feedlot entry, and that high energy finishing regimens, simply fill these cells with lipid. Therefore, a nutritional strategy that targets both increased adipocyte numbers and increased adipocyte size, is likely to have most success in increasing marbling score. Despite the indications that vitamin A depletion might initiate more preadipocytes, real animal health and welfare issues should discourage a producer from adopting this strategy.

The most efficient path between our current knowledge and the knowledge we need to manage this phenotype requires constant awareness of developments in other streams of mammalian research. The global chromosomal mapping experiments in humans, mice and rats, coupled with gene function studies, provide a boundless resource for scientists working in the applied fields and profiting from phenotypic variation that has occurred naturally in any one species: in our case, the bovine.

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