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Biological determinants of intramuscular fat deposition in beef cattle:

Current mechanistic knowledge and sources of variation

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Contents

1. INTRODUCTION

The term *marbling* refers to the appearance of white flecks or streaks of fatty tissue between the muscle fibres in meat. Marbling 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 don't want 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.

This review will encompass the biology of development of intramuscular fat, as well as the known sources of variation in the marbling trait. It seeks to provide answers to the questions: what is marbling; how does marbling develop; what influences its development enough to be of industrial significance; what realistically can a cattle producer do to manipulate marbling; and what research questions need to be addressed from this point in our knowledge. The Australian beef production system will be the frame of reference for discussion of the applicability of some of the conclusions.

2. DESCRIPTION OF THE TRAIT

Marbling appears as white flecks or streaks in the muscle. It does not include subcutaneous or intermuscular fat or any ingression of intermuscular fat into the muscle. Marbling fat is generally termed intramuscular fat but is distinct from fat or lipid present within the muscle cells themselves (the myocytes). Intramuscular fat is a true adipose tissue, comprising fat cells (adipocytes) located in the interfascicular spaces, embedded in a connective tissue matrix in close proximity to a rich blood capillary network. Individual cell diameters are normally between 40 and 90μm, which is significantly smaller than adipocytes from other fat locations. Intramuscular adipocytes normally appear in clusters or "islands" and these islands need to contain between 10 and 15 cells before they become visible as marbling (i.e. approx. 1 mm across). When viewed histologically (Fig. 1), adipocyte islands can be found that contain many hundreds of cells, and islands have developed around well-developed capillary beds.

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 resulting in improved grading scores.

Although the quantity of marbling fat is generally the determinant of the overall quality grade, the appearance characteristics of marbling can significantly affect the value of the meat. Fine, evenly dispersed flecks or streaks of white fat (shimofori or snow flake) are preferred by Japanese consumers over thick, coarse channels of fat. Where fat is finely and evenly distributed fat throughout the meat the total fat content of the meat is generally higher than anticipated by visual marbling score (Albrecht *et al.,* 1996).

3. VISUAL ASSESSMENT OF MARBLING

Carcase sides are usually assessed for marbling in the chiller between 18 and 60 h post-mortem. The temperature of meat at the time of assessment is commonly between 4 and

12°C. Where carcases have been effectively electrically stimulated, ribbing may proceed after 8 h provided the temperature of the *M. longissimus dorsi* is below 12°C. The carcase sides are quartered by cutting (ribbing) between the ribs (Japanese use 5/6th rib, Australian and US 10 to13th rib, although actual site will depend on market requirements). The cut meat surface is allowed to *bloom* for a minimum of 20 mins to regenerate oxymyoglobin, giving the meat a bright red colour. Against this red background and using a standard light source, marbling is subjectively assessed in the *M. longissimus dorsi* by comparison with marbling standards (Japan, Meat Grading Association (1988), AUS-MEAT (1998)). Fat intrusions originating from intermuscular (seam) fat are excluded from the consideration of marbling score. Where marbling fat is attached to the edge of the muscle it may be considered to be marbling, where it narrows to 1 mm in width. Thick inclusions are not considered as part of the marbling score. This is a relatively recent change to the scoring system, which now brings the AUS-MEAT system into line with our major competitors, who for some time have included these intrusions in their scoring.

There are many anecdotal reports which imply that marbling score is sometimes underestimated during chiller assessment measurements. When vacuum-packed loins are opened and re-assessed after storage at 0-1°C for several days or up to perhaps 6 weeks, it has been observed that marbling scores were about 1 score higher than that obtained initially. There are several factors that may account for these observations. Firstly, and most importantly, the temperature of the marbling fat may be up to 12°C lower than when measured initially. Secondly, with extended time of storage, the fatty acids of the tri-acylglycerols may have undergone polymorphic changes (Hernqvist, 1988) into more stable crystalline structures having higher melting points and thus appear more opaque.

The importance of temperature on visual marbling score is illustrated by the work of Pethick *et al.,* (1997) where 107 carcasses were initially assessed under commercial conditions (day after slaughter, carcass temperature 11-12°C) and then the removed *M. longissimus dorsi* was re-assessed 24 h later at 5°C. At initial assessment, only 41 carcasses achieved a marbling score of >2 whereas after a further 24 h with chilling to 5°C, 51 were >2.

An investigation of thermal properties of bovine fat by differential scanning calorimetry revealed that major phase transitions occurred at about 8 -15°C and at about 35 -40°C. Depending on the unsaturation of the fat, the enthalpy associated with each transition was markedly different with the more unsaturated samples showing higher enthalpy changes in the lower temperature ranges (Yang *et al.,* 1999). Thus, at chiller temperatures of around 10°C the appearance of the fat (or opaqueness) will depend on the proportion of the fat that has undergone the phase transition. In meat where the marbling fat is more unsaturated and there is incomplete phase transition, the fat will be partly translucent (less white) and will not appear boldly against the red muscle background. Under these conditions it would then be expected that chiller assessment of marbling would result in a lower marbling score.

It has been observed that chiller assessment of marbling is influenced by melting point of the fat. In animals fed diets with saturated triglyceride protected from rumen metabolism, higher visual marbling scores have been observed at the same intramuscular fat content (Tume *et al*., 1996).

Where marbling fat appears as large channels of fat (not inclusions) visual marbling score is commonly overestimated compared with those where marbling consists of small evenly distributed clusters of fat cells. Some of these difficulties in visually evaluating marbling have been overcome experimentally (Albrecht *et al.,* 1996) using objective image analysis techniques on stained sections of meat. The procedure involved a rapid automated measurement of parameters such as area of muscle, total area of fat, number of areas of fat, proportion of round and long fat, which was used to give an overall assessment of marbling without being subject to visual error. Their work also showed that the fat content based on

proportion of areas was greater than the actual intramuscular fat content (r = 0.82) presumably resulting from the presence of other components such as water and protein in those spots usually assessed to be fat. These factors need to be considered in any use of image analysis for estimation of fat content.

4. ASSESSMENT OF MARBLING IN LIVE ANIMALS AND INTACT CARCASSES

Various techniques have been used to determine fat content or marbling in living beef cattle; many based on medical methodologies such as real-time ultrasound scanning (RTUS). However, even though many different devices are available and quite large numbers of cattle have been assessed by RTUS, the accuracy obtained has not been suitable for estimating fat content or marbling score over the full range of marbling likely to be encountered. The accuracy is however satisfactory within limited ranges of IMF% and is useful for genetic evaluation of cattle for breeding programs (Graser *et al*., 1998), or prediction of final marbling score in feedlot cattle (Brethour, 2000; Oddy *et al*., 2000).

An evaluation of four commercially available RTUS systems was recently performed using 81 crossbred steers (Herring *et al.,* 1998). Whilst there were differences in precision between individual systems and between technicians, the correlations between the RTUS predictions and objective measurements for fat content and marbling score were modest, being 0.20 to 0.61 and 0.30 to 0.75, respectively.

Earlier work has shown that there is an effect on response caused by differences in eye muscle area and fat thickness (Herring *et al.,* 1994) and a trend for the RTUS systems to be more precise when measuring leaner animals (Herring *et al.,* 1998). However others have found a bias in the opposite direction which may imply that these effects result from limitations in the prediction formula (Wilson *et al.,* 1998).

5. RELATIONSHIP BETWEEN RIB POSITION AND MARBLING SCORE

The cross-sectional shape and size (rib eye area) of the *longissimus* muscle varies along its length, being significantly smaller in the thoracic compared with the lumbar region (Ozutsumi and Okada, 1982). There is compelling evidence that intramuscular fat content also varies along the length making the site of measurement particularly important (Lawrie, 1961). Winkel and Thornton (1990) measured intramuscular fat and marbling score on successive slices of cube roll (*M. longissimus thoracis*) between the 5/6th and the 12/13th rib and reported that there was a linear decrease in both fat content (9.7 to 6.9%) and marbling score (3.6 to 2.9). By scoring each cut end of 240 cube rolls they also found that there was a difference of approximately one marble score unit with the anterior end being higher. This was confirmed by Zembayashi and Lunt (1995) who determined the intramuscular fat contents of slices taken from near the 6th, 9th and 12th thoracic vertebra and from the 2nd and 5th lumbar vertebra of *longissimus dorsi* for seven pure breeds and cross-breeds including Japanese Black cattle. Intramuscular fat content decreased from the 6th to the 12th rib but increased again in the lumbar regions of the *longissimus*. Similar finding have been reported by Ozutsumi and Okada (1982) and Wello *et al.* (1988) for various Japanese breeds. Mitsumoto *et al*. (1993) have found that muscle cross-sectional area and fat content are inversely related when sampled along the length of the longissimus muscle.

6. RELATIONSHIP BETWEEN INTRAMUSCULAR FAT CONTENT AND MARBLING SCORE

The relationship between visual marbling score and intramuscular fat content has been investigated by a number of groups in Australia, the US and Japan. Generally, marbling scores do not correlate well with actual fat contents although several groups have found a moderate relationship based on a descriptive scoring system (Berg *et al.,* 1985; Ozutsumi *et al.,* 1985; Savell *et al.,* 1986). Taylor and Johnson (1992) measured the ether-extractable fat from *M. longissimus* of four breeds of cattle (grain-fed 120-204 days) at both the 5th and 10th ribs. The mean AUSMEAT marbling scores at the 5th and 10th rib were 2.6 $\pm$ 0.9 and 2.4 $\pm$ 0.7 (n=48) respectively. Although there was a trend for marbling score and fat contents to be higher at the 5th rib position, overall, the correlation with marbling score at both sites was low (0.57 and 0.32 respectively).

Correlations between intramuscular fat content and visual marbling score were also found to be weak in a group of Angus steers fed on various grain-based rations for 154 days (Pethick *et al.,* 1997). In this work, measurements were made separately by two accredited assessors on carcases at 12°C, quartered at the 10/11th ribs. Carcases had marbling scores ranging from 0 to 4 (AUS-MEAT, 1998). For samples where there was no visible marbling, the intramuscular fat content ranged from about 0.7 to nearly 8% and for marbling score 2, ranged from about 1% to more than 13%. Only some 30-40% of the variation in fat content was explained by the visual marbling score (r^2 = 0.306 and 0.386 for each assessor). In a recent study (Pethick unpublished), correlations of between 0.46 and 0.52 were obtained. The higher correlations have been attributed to the use of heavier carcasses and lower temperatures of assessment.

In a recent study by Oddy *et al.* (2000; FLOT 210), the correlation (r^2)between IMF% and MSA marbling score was 0.43, in groups of animals that had MSA marbling scores between 1.2 and 3.6, and IMF% between 5.1 and 17.9%.

In a study by Cameron *et al.* (1994), Japanese Black and American Wagyu cattle were assessed at the 6th-7th rib interface for marbling score and for ether-extractable fat content with visual assessments being made in Japan and the US respectively. This enabled coverage of the whole range of marbling scores from JAP 1 (no visible marbling) to JAP 12 (heavily marbled). Marbling score was linearly related to fat content (r^2 = 0.7619, n=155) but the variation in fat content was very great at each marbling score. In those samples where there was no visible fat (marbling score 1), values of fat contents ranged from 3.0 to 7.4% (n=6) and for a marbling score of 3, the range was 5.6 to 15.5% (n=38). Clearly, for a smaller range of marbling scores and larger steps between grades, as available in the Australian studies, the correlation coefficient would be markedly lower.

The major lipids present in meat are the neutral lipids, comprising mainly the triacylglycerols, cholesterol and free fatty acids and the polar phospholipids, present mainly as structural and signalling elements in cell membranes. The content of phospholipids is relatively constant (0.5-0.7g/100g muscle) irrespective of the total fat content. As fat content of muscle increases, it is primarily the result of the accumulation of triacylglycerols (Sinclair and O'Dea, 1987). Phospholipids are generally in close association with membrane proteins and are not readily removed from tissues by use of non-polar solvents such as di-ethyl ether or petroleum ether. However, polar solvents such as chloroform/methanol readily extract both the neutral and the majority of the polar lipids.

A clear distinction must be made between the terms *total fat content and extractable fat* by a particular solvent. Generally, most of the published data on the fat content of meat or intramuscular fat is based on Sohxlet extraction methods with non-polar solvents (AOAC, 1984) and measures the content of neutral lipids. The content of neutral lipids, in particular the tri-acylglycerols, is what is actually required and it is this class of lipid that is associated

with positive sensory characteristics of meat (Arneth, 1998). In most cases, this non-polar extraction method is adequate for determining fat content as long as it is understood that the actual total fat content will be somewhat higher especially in very lean meat where the ratio of phosholipids to total lipids is high.

Sahasrabudhe and Smallbone (1983) evaluated 7 solvent extraction methods for determining the neutral and polar lipid contents of minced beef ranging in fat contents from less than 3% to more than 20%. As expected, methods employing chloroform/methanol were the most effective in removing both neutral and polar lipids from meat irrespective of the total fat contents. However, those methods using petroleum ether or di-ethylether were capable of only removing 89% of the triacylglycerols from low fat meat (<3%). This would indicate that there may be a 10% underestimation of fat content in lean meat samples. However, at the higher fat contents the discrepancy was essentially within experimental error, due to such factors as between animal variation.

Brackebusch et al. (1991) found that the fat contents of all muscles of the carcass were linearly related (P less than 0.001) to that of the longissimus muscle (r^2 0.67-0.84) and hence that the fat contents of all muscles would be proportionally less in carcasses with low marbling scores when compared to carcasses with higher marbling scores (Fig. 2)

7. EFFECT OF FATTY ACID COMPOSITION AND TEMPERATURE ON MARBLING APPEARANCE

If a hot, well-marbled carcass side is quartered soon after slaughter and inspected for marbling it is likely that the marbling score will be reduced. At these high temperatures the marbling fat is in a liquid phase and is quite translucent. As the temperature of the cut surface begins to fall marbling will appear and continue to develop until it reaches a stabilised crystalline structure.

The lipid composition of marbling fat can have a significant effect on the visual appearance of marbling at a given temperature, particularly within the range of temperatures commonly used during chiller assessment. This results from differences in melting point of various mixtures of lipids as would be found in a marbling fat island. For example, Tume *et al*. (1996) observed that meat from animals with more saturated fat had higher marbling scores in the chiller, despite similar IMF%. Interestingly, when the samples were reassessed after a period at constant temperature in the laboratory, the samples with more saturated fat had the same visually assessed marbling score as did the samples with less saturated fat. Data from Pethick *et al*. (MRC report, UMUR.004 also reflect on this point (Fig. 3).

8. OTHER TISSUE CHARACTERISTICS THAT MAY AFFECT VISUAL MARBLING SCORE OR TOTAL FAT CONTENT

Visual assessment of marbling is obviously dependent upon fat being visible and here lies its weakness. Where fat clusters are very small and dispersed, perhaps each site comprising fewer than 10 adipocytes, marbling may not be visible but the extractable fat could be significant. In addition to lipids stored in adipocytes, muscle cells can also contain lipid. This intracellular lipid is not visible to the naked eye, however. Muscle cells are an important site for the uptake, metabolism and storage of lipids. Lipid stored as triglyceride is utilised as an energy source for muscle at rest and particularly during and following exercise (see Cortright *et al.,* 1997). Whilst these lipids contribute to the total fat content they are not visible in quartered carcasses and are therefore not included in scoring for marbling. Likewise, muscle lipid is likely to be a minor contributor to total meat lipid content.

The amount of intracellular lipid within muscle cells is dependent upon the muscle's physiological function which in turn relates to the complement of different muscle fibre types. Muscles are generally composed of three types of muscle fibres (types I, IIA, and IIB) and the proportion of these will differ depending on the metabolic and contractile properties of that muscle. Bovine *M. longissimus thoracis et lumborum*, the muscle in which marbling is usually assessed, consists mainly of the fast twitch, glycolytic, type IIB fibres (42- 56%) with similar proportions of slow tonic, oxidative fibres, type I (27-29%) and fast tonic glycolytic fibres, type IIA (16-30%) (Hunt and Hedrick, 1977; Morita *et al.,* 1995). The cross-sectional area of IIB fibres is considerably larger than that of other fibre types and they comprise up to 65% of the *longissimus* muscle area.

The intracellular lipid content of muscle fibre-types varies considerably. The oxidative muscles (predominantly type I) have higher lipid contents than the glycolytic fibres. Various histological stains have been used to identify those fibres rich in lipid containing droplets: lipid droplets are found in close proximity to mitochondria (Gauthier, 1970). Rabbit soleus muscle is composed of almost entirely of oxidative type I fibres and has a total lipid content of 4.8g/100g whereas the *Psoas major*, being highly glycolytic (about 80% type II fibres) has a lipid content of about 1.2g/100g (Alasnier *et al.,* 1996).

Although an attempt has been made to relate muscle fibre type with visually assessed marbling (Morita *et al.,* 1995), the results are not convincing. They found a relationship between content of type IIB fibres and marbling score (r=+0.63) for biopsied samples but not for carcase muscle samples.

If one is trying to understand the development of intramuscular fat, one needs to realise that the structure of muscle changes through the animal's life as a function of age, physical work and genetic predisposition. In the context of normal development, Brandstetter *et al*. (1998) have found that muscle fibre develop towards being more glycolytic as the animal ages and that the act of castration has significant effects on the muscle fibre type profile. Furthermore, physical exertion or endurance training also induce significant remodelling of a mammals muscles (Harper, 1999). The direction of this remodelling depends on the form of the physical exertion; being designed to either increase oxidative capacity or glycolytic capacity. This plasticity in muscle phenotype is likely to have an effect on the total amount of fat present in the muscle as well as its distribution.

In addition to the location of lipid within muscle, the composition of lipid may also affect visually assessed marbling. Fatty acid composition of adipose tissue generally (Yang *et al.,* 1999), including marbling fat, varies between cattle from different breeds (Perry *et al.,* 1993; May *et al.,* 1993), feeding regimens (Marmer *et al.,* 1984; Tume and Yang, 1995) and climatic backgrounds (Lazlo, 1970). In general, fatty acid composition becomes less saturated with increasing animal age (Waldman *et al.,* 1968) and is less saturated in the outer (cooler) adipose locations in the body (Wood *et al.,* 1986). Different types of feed (eg. forage with and without added fat or formulated rumen-bypass fat) and rumen modifiers, can alter the composition of fatty acids absorbed from the intestine. The lipid metabolism of rumen bacteria and protozoa is sensitive to type of feed and affected by breed type resulting in different lipids being made available for absorption in the intestines (O'Kelly and Spiers, 1989; 1991).

In cattle, the largest changes in fat composition occur in the contents of stearic acid (C18:0) and the mono-unsaturated fatty acids oleic (C18:1) and palmitoleic acids (C16:1). Palmitic acid (C16:0), a major component (about 25% of total acids) remains reasonably constant (24-28%) but stearic acid may vary from 5 to 30%, being replaced by the mono-unsaturated acids. Stearic acid has a melting point of 70°C whereas the mono-unsaturated fatty acids melt at <15°C. Thus, as the fatty acid composition changes, the overall melting properties of the fat (and thus softness and appearance) will change markedly. Slip points (indicators of melting properties) of bovine fat were found to vary between 22.8 and 45.1°C for various groups of cattle (Smith *et al.,* 1998), indicating the extent of the differences that may occur.

Thus for meat of the same fat content, but different fatty acid composition, there may be large differences in the visual appearance of marbling and thus the melting properties of the fat. As stated above, this point was clearly demonstrated by Tume *et al.* (1996). Cattle were fed a dietary formulation that resulted in change fatty acid composition in the fat. Dissected marbling fat had an unsaturation ratio (sum of all unsaturated fatty acids/saturated fatty acids) of about 1.00 in treated animals and 1.30 for the untreated controls. The slip points of fat from treated and untreated animals were 36.4°C and 31.5°C respectively. AUS-MEAT chiller assessment indicated that the feed treatment resulted in a marked improvement of marbling score with 48 carcasses achieving a score of >3 compared with only 22 carcasses from the control group. However, analysis of meat samples showed that the fat content of the treatment group at each marbling score was actually lower than controls. This example reinforces the point that more saturated fat composition can affect visual appearance under commercial conditions. However, when the samples from these groups were compared again at the same temperature, there was no difference.

9. ONTOGENY OF FAT DEPOSITION IN DIFFERENT TISSUES OF CATTLE

It is useful to know the pattern of fat deposition (within and between tissues) over time. Although there are good general descriptions in terms of dissected fat (Berg and Butterfield, 1976), the evidence in terms of chemical fat content is less clear. A common conclusion from animal developmental studies is that intramuscular fat is late developing (Vernon, 1981). Indeed the usually quoted developmental order is abdominal, then intermuscular, then subcutaneous, then finally intramuscular. However, because fat is deposited at a greater rate than lean tissues later in life the concentration of fat in lean will inevitably increase later in an animal's life. Thus it can be said that the trait (% fat) is late maturing, but this should not be interpreted as intramuscular adipocytes or the intramuscular fat pool itself is late maturing. To determine if there is a difference in fat deposition over time, it is more informative to express the data as proportions of total carcass fat that develop within various depots, because changes in these proportions would indicate if intramuscular fat develops at a different rate from other fat depots. When fat deposition have been described in this way (Johnson *et al.,* 1972), the proportional distribution of fat between carcass pools is found to be constant over a wide range of carcass fat contents (in the range from 5 to over 150 kg total fat; Fig. 4). Other data (Cianzio *et al.,* 1982 &1985, Trenkle *et al.,* 1978) are also consistent with this observation.

Given that the fat depots within the carcass develop at a constant rate, what is the pattern of muscle and intramuscualr fat growth as the animal grows to near maturity? The available data (Fig. 5) shows that the intramuscular fat content, expressed as % fat, increases in a linear fashion up to a carcass weight of about 400kg for British (Duckett *et al*. 1993) or Japanese Black x Holstein (Aoki *et al*., 1999) type cattle undergoing prolonged feedlotting. In the study of Aoki et al. (1999) where the cattle where fed to heavier weights there is a suggestion that the intramuscular fat content does not increase beyond about a carcass weight of about 420 kg and this was a consistent finding for 4 different muscles.

The biology behind these changes in lipid content of muscle remain unknown but are likely a result of differing rates of muscle and fat accretion as the carcass weight increases. The results of Pethick *et al*. (2000; FLOT 209) suggest that the increase in intramuscular fat, expressed as % fat, relies on continued fat synthesis within muscle combined with a decreasing rate of muscle growth (Table 8). In addition the study confirms the pattern of fat accretion described in Fig. 6 such that fat accretion in the carcass depots (subcutaneous and intermuscular) and within muscle occur at the same rate through the 304 – 417kg carcass range. That is biologically, intramuscular fat is not late maturing BUT the expression of marbling (% fat) is late maturing. These conclusions have important implications for

backgrounding and feedlotting. In general the sooner an animal reaches its near maximal potential for muscle growth the sooner it would begin to commercially express intramuscular fat. A very long feeding period allows the cattle to obtain a high level of intramuscular fat since there is time for muscle maturity to be reached followed by time for the muscle to 'fill up' with fat. Shorter feeding periods will have a higher risk of failure particularly if there is a relatively short period of fattening after muscle maturity is reached. For the shorter feeding scenario a heavier live weight entry rate would help to secure the required muscle growth.

Importantly the conclusions of Pethick et al. (2000) were based on an estimate of body composition only at 2 carcass weights. The work by Aoki *et al*. (1999) has shown that carcass fat accumulation does not necessarily increase forever and probably declines when the animals begin to reach 'maturity' defined as the point on the growth curve when carcass composition remains close to constant.

On the basis of these observations it seems that fat deposition is determined by fat tissue type, and that within a cattle breed this is relatively constant. There is substantial variation between some breeds at the extremes of the normal range (Wegner *et al.,* 1998 for Belgian Blue, Angus and dual purpose breeds; Zembayashi *et al.*, 1995 for Japanese Black, Holsteins and JB/H crossbreds). Also, there is evidence of developmental variation imposed by environmental factors (perhaps nutritional factors), which may alter the distribution of fat between tissue pools (Trenkle *et al.,* 1978) and this may be affected by breed type in that Japanese Black are possibly less sensitive to nutritional change as are other breeds (Zembayashi *et al.*, 1995).

The developmental changes in fatness observed in Figs. 4 and 6 are directly associated with changes in cellularity of fat deposits (Cianzio *et al*., 1985), and as a consequence, with metabolic changes in individual adipocytes. Trenkle *et al.* (1978) showed that moderate growth to the same carcass weight leads to increased intramuscular lipid accumulation relative to rapid growth. However, this took approximately 300 days to develop because it was not apparent in the comparison at 150 d of feeding. Subsequent studies (Renk *et a*l., 1986) have not been able to repeat this observation.

10. WHAT IS MARBLING AT THE CELLULAR LEVEL? WHAT CAUSES MARBLING TO DEVELOP?

Marbling results from the development of adipocytes between the fasciculae of skeletal muscle (Moody and Cassens, 1968). Based on histological features, these cells are likely to be of the white rather than brown adipose tissue type (Moody and Cassens, 1968). The cellular differentiation events that precede development of marbling fat are represented in Fig. 7.

Cells of mesenchymal origin, such as stromo-vascular cells (connective tissue stem cells) exist within the connective tissues of many organs (Young *et al.*, 1995). Under appropriate conditions, these cells differentiate firstly into preadipocytes (also called adipoblasts or non-terminally differentiated adipocytes). These progenitor cells have the capacity to differentiate into myoblasts, osteoblasts or chondrocytes under the influence of particular genetic or environmental stimuli (Grimaldi *et al.*, 1997). Such factors as polyunsaturated fatty acids, thiazolidinedione (TZD), fetal calf serum, growth hormone, insulin-like growth factor 1, triiodothyronine, cyclic AMP, glucocorticoids, prostaglandins, arachidonic acid, retinoic acid (low concentrations) can induce the cell to proceed toward adipogenesis (Klaus, 1997; Grimaldi *et al.*, 1997), while bone morphogenetic protein-2 tends to steer the cells toward development of osteoblasts (Katagiri *et al.*, 1990). In normal tissues in steady state, the inappropriate development of stromo-vascular cells into preadipocytes is inhibited by factors such as Id3 (Moldes *et al.*, 1997), tumour necrosis factor α (Klaus, 1997), phorbol esters (Klaus, 1997) and high concentrations of retinoic acid (Ohyama *et al.*, 1998). There may

also be factors within the extracellular matrix that influence the development of the adipocyte either positively or negatively (Smas and Sul, 1993). Hence one can imagine the connective tissue of muscle as always having the potential to develop into marbling fat, but lack of appropriate stimulii, and the presence of inhibitory factors controls its development.

Given that undifferentiated preadipocytes within the endomysial and perimysial connective tissue sheets are morphologically indistinguishable from fibroblasts or myoblasts , it is not currently possible to determine the number of cells present in the young animal that will subsequently become adipocytes. The morphological homogeneity of cells within the connective tissue sheaths has led to confusion around which cells have the capacity to differentiate into preadipocytes (adipoblasts). It seems clear however that muscle tissue cannot be replaced by fat, unless there is loss of the muscle cells and replacement by new fat tissue (see section on myocyte pathology).

Microvasculature is an essential component for the development of adipose tissues (see Crandall *et al.,* 1997) and this is also true for the differentiation of fat cells located within skeletal muscles. The capillary density of lean muscle is significantly less than that found in adipose tissue (Gersh and Still, 1945), but as marbling increases, capillary density within intramuscular adipocyte clusters would be expected to increase (Fig. 1; note the capillary in the 300X image). Therefore development of marbling is likely to be closely tied to the regulation of capillary development.

Development of adipose tissue appears to depend on its location in the body. Studies with foetal adipose tissues from the pig have shown that arteriolar differentiation precedes adipocyte development in perirenal sites but this is reversed in subcutaneous sites where capillary density is less (Hausman and Thomas, 1985; Hausman, 1987). There are also differences in the development of capillaries in adipose tissue layers of subcutaneous fat in pigs (Hausman and Thomas, 1984) and it has been observed that the size and maturity of adipocyte clusters is inversely related to the extent of connective tissue deposition. Where the connective tissue is less dense, adipocytes appear to be more developed. The site-specific nature of adipose development has lead to the suggestion that interactions between capillary cells and preadipocytes may be mediated by locally produced factors (Lau *et al.,* 1996).

Where adipose tissue capillaries of foetuses from lean and genetically obese pigs were investigated for specific histochemical, traits large differences in enzyme activities were found between the two with the obese tissue showing highly activities of oxidative enzymes whereas the lean samples were unreactive. The differences were apparent prior to any changes in adipocyte hypertrophy (Hausman and Thomas, 1985).

11. CELL CULTURE SYSTEMS

Much of what is now known about adipocyte differentiation has come from tissue culture studies, and we will review this information first, before returning to what is known about fat deposition within the whole animal. The 3T3 cell, a mesenchymal stem cell derived from mouse, has been used extensively to study adipocyte differentiation (Wang *et al.*, 1994). Differentiation of 3T3 occurs reproducibly in culture after a series of specific treatments. These cells have the capacity to differentiate into either adipoblasts, which can divide or adipocytes that have lost that capacity. Both cell types are morphologically indistinguishable, though adipoblasts retain the capacity to dedifferentiate and reenter proliferative phase (Wang *et al.*, 1994). Differentiation of 3T3 cells is thought to be closely analogous to the differentiation and growth of adipocytes in adult mammalian adipose tissue (Green and Kehinde, 1979). Once terminally differentiated, adipocytes are immotile and continue to grow through the life of the animal if nutrition is not limiting.

In circumstances of hyperplasia, adipocytes develop along a defined developmental pathway (Fig. 7). Growth arrest is an absolute prerequisite for hyperplastic adipogenesis (Wang *et al.*, 1994). This can be achieved in culture through the use of deficient growth media, but it is unclear what form of arrest is required in intact tissues in whole animals. As reviewed by Mandrup and Lane (1997), members of two families of transcriptional factors are expressed early in the differentiation program and are believed to orchestrate most of the adipocyte-specific expression of genes. The peroxisome proliferator-activated receptor family (the PPARs) and the CAAT / enhancer binding protein family (the C/EBPs) interact to instigate and regulate the phenotypic changes that are recognised as adipogenesis: lipid droplet accumulation; increased glycerol -3-phosphate dehydrogenase activity; lipase activity. Insulin, glucocorticoids, fatty acids, prostaglandins and growth hormone all act through these transcriptional factors in order to modify the expression of adipocyte-specific proteins (Mandrup and Lane, 1997; and others) (Fig. 8).

Retinoic acid, or more correctly the retinoids, act against the development of adipocytes and this antagonism is mediated at the transcriptional level. Retinoids express their effects through the retinoic acid receptors (RARs) or the retinoid X receptors (RXRs). Inhibition of adipogenesis by retinoic acid is mediated through these receptors. Retinoic acid, acting as a ligand for the RXR or RAR appears to interfere with the activation of transcription by C/EBPβ and C/EBPα. Given that transcription factors act early in adipocyte differentiation, it follows that negative regulation exerted by the retinoids may only occur at particular times (Oka, 1998a and b). After these times, differentiation becomes committed and insensitive to retinoids. Other factors are also involved in the inhibition of adipogenesis and these include the Ids (Id1, 2 and 3) (Moldes *et al.*, 1997). Another pathway of note is that associated with retinoids. Evidence from some feedlots implicate low levels of plasma retinol in promoting high levels of marbling (Torii *et al.,* 1996). Some breeds may be more susceptible to this treatment, as it has not been replicated in some countries, suggesting that there is genetic variation in the pathways controlled by retinoids that influence marbling scores.

Lastly, the thyroid hormones are implicated in the fattening of carcasses since goiterogenic compounds are long known to affect the average daily gain of cattle and the dressing percentage of carcasses (eg. Burroughs *et al.,* 1958; Raun *et al.,* 1960). The administration of thyroid hormones leads to deposition of fat cells in muscle while the thyroid hormones have an impact on fat cell differentiation *in vitro*. Thyroid hormones also influence the metabolic rate and hence would affect the amount of surplus energy available for storage as fat.

These different pathways have a well known intersection, the HRE (hormone responsive elements) of the steroid, thyroid and retinoid hormone receptors (Evans, 1988; Beato, 1989; Mangelsdorf *et al.,* 1991; Smas and Sul, 1995). These response elements are composed of palindromic sequences of DNA located in the 5' untranslated regions of genes and these are important in the regulation of gene transcription. There are only minor differences between the palindromes of the different HRE and there is clearly some cross-talk between the various hormone receptors. These hormones act as important switches controlling differentiation and thus they have pervasive effects on the development of an animal.

Another pathway is that associated with growth. Genes that increase growth of lean tissue obviously have a negative effect on the percentage of fat in the carcass. The peptide hormones such as GH (growth hormone) and its intermediary, IGF1 (insulin growth factor 1), have potent effects on lean tissue growth and fat cell differentiation (Ailhaud *et al.,* 1992; Smas and Sul, 1995). Moreover, double muscling mutations in the GDF8 (myostatin--TGFB [transforming growth factor beta] family) gene have a negative impact on both the marbling score and the amount of fat in the carcass. Factors that affect the balance between lean and fat, or total fat in the carcass will clearly affect marbling scores since there is a relationship between marbling score and total fat percentage (Gregory *et al.,* 1994, 1995), though its mechanistic basis is yet to be defined.

At least in culture, other cell types also demonstrate the capacity to transdifferentiate into adipocytes, via the stem cell. For example cells that have been designated myoblasts based on expression of genes, can be induced to transdifferentiate into adipocytes under the influence of fatty acids and thiazolidinediones (Grimaldi *et al.*, 1997). It is unclear to what extent if any, transdifferentiation of this magnitude is a factor in the development of intramuscular fat.

Once terminally differentiated, the growth of adipocytes in culture is regulated by factors that reflect their regulation in the animal. Hormones such as the glucocorticoids, insulin and the retinoids, as well as nutrients including fatty acids, glucose and acetate are involved in the stimulation of fat deposition (Vernon, 1986). Fat is deposited within fat globules in the cytoplasm of the cell, and the structure of these globules is regulated to some extent by the cell itself.

In all mammals, adipocytes develop in various depots of the body. Each depot has a characteristic developmental program in terms of the number and size of the adipocytes. To some extent this development program is also reflected in the expression of key regulatory enzymes such as acetyl CoA carboxylase and lipoprotein lipase (Vernon, 1999). While there is some evidence that cell volume and level of maturity of adipocytes might be correlated, this concept is far from established.

There is a great deal less information in the literature concerning intramuscular fat than information on deposition in other depots. This has resulted to some extent on the relatively unusual (when all mammals are considered) propensity of cattle to marble, and the strong interest of the international research community in obesity, diabetes and muscular dystrophy. As mentioned above, many factors have been shown to influence the development of marbling. These are discussed in more detail below.

Finally, it is important to consider lipid deposition at sites within the muscle other than in adipocytes. Specifically this involves lipid accumulation with the cytoplasm of myocytes. While this occurs to some extent in normal and healthy myocytes, the prevalence of lipid droplets increases markedly in particular disease states, which will be dealt with in more detail below. At the cellular level, lipid droplets have been shown to consist of protein as well as lipid (Souza *et al.*, 1998). The protein is structured into a lattice which surrounds the lipid and undergoes rearrangement when the lipid droplet is being metabolised.

12. DEVELOPMENTAL TRIGGERS FOR ADIPOSE TISSUE DEVELOPMENT

12.1 Normal ageing

Unlike muscle and bone, mammalian fat continues to develop late into an animal's life. This can be shown at the biochemical level, in that lipogenic enzyme activities continue to increase in muscle as a function of age, whereas they decrease in liver and extramuscular adipose tissue (Gondret *et al.*, 1997). Acetyl-CoA-carboxylase, malic enzyme and glucose-6-phosphatase dehydrogenase each follow the same developmental program.

The continued development of adipose tissue in the adult animal, has also been explained on the basis of continued development of the microvasculature (Crandall *et al.*, 1997). Preadipocytes and stromo-vascular cells retain the capacity to develop into adipocytes throughout the animal's life. When the supply of nutrients into a particular compartment of the body becomes enhanced through development of new blood vessels, cellular differentiation proceeds. An alternative view would be that relatively anoxic conditions in a tissue induce the cells to release molecules that are stimulators of angiogenesis. Apart from

responding to the new blood vessel growth and consequent improvement in nutrient flow, the adipocytes may be responding directly to the released angiogenic stimulators.

Amongst fat depots, the intramuscular fat is stated to be the last to develop (Hood and Allen 1973). The reason for this statement was that IM adipocytes were smaller than adipocytes from other depots. When expressed as total fat content, IM fat develops at the same rate as other depots (see earlier). At the cellular level, adult fat tissue retains a proportion of its cells in the stromo-vascular and preadipocyte phases, and fully differentiated adipocytes continue to increase in size with age and unlimited nutrition, i.e. fat continues to grow by both cellular proliferation and increases in cell size throughout life.

Muscles vary in terms of 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. Growth of the adipose tissues in an adult animal occurs through a mixture of hyperplasia and hypertrophy (Leibel *et al.*, 1989), while loss of adipose tissue mass occurs primarily through reductions in adipocyte size rather than adipocyte number.

Hood and Allen suggested that adipocyte hypertrophy was the major mechanism of development of fat in ruminants of market weight and age, though they noted the possibility that preadipose cells might proliferate postnatally. They suggested that only in early postnatal development, is growth of adipose tissue due to both cellular hyperplasia and hypertrophy, however they admitted that the technical problem of counting small adipocytes was partly responsible for conflicting conclusions about how intramuscular adipocytes develop. Even though it is possible to modify the number of adipocytes in mammalian muscle, those pharmacological or immunological treatments developed to date have little practical application for meat production (Flint *et al.*, 1994).

Although fat cell size has been regarded as a reflection of past substrate availability and hence cellular activity, the link between cell size and biochemical maturation has not been established unequivocally. Not surprisingly adipocytes of different sizes and locations express different genes and biochemical constituents (Lee *et al.*, 1997). In other words, it is difficult to judge adipocyte maturity by size alone. Thus it remains unclear if intramuscular adipocytes are less “mature” than adipocytes in other fat depots of the body.

While at first glance it appears that age is a major determinant of the development of marbling, closer examination suggests that muscle is reflecting physiological age more than chronological age (Swatland, 1999). That is, periods of weight stasis or loss may suspend physiological ageing while chronological age marches on. Reduction in oxidative capacity is just one of the consequences of normal ageing of mammalian muscle, and the mitochondrial hypothesis of ageing has been extensively reviewed elsewhere (Shoffner and Wallace, 1995). Periods of weight loss or weight stasis may also block or modify the development of marbling in cattle. There is not much information available on the effects of growth history as opposed to age on the cellular basis of development of marbling. What there is will be dealt with below (see Nutrition and Growth History).

12.2 In response to muscle atrophy

While intramuscular fat deposition that results from this cause has little practical importance to the production of meat, it may be useful as an experimental system to increase our understanding of the determinants of marbling.

‘Muscle disuse’ refers to circumstances where a mammal’s muscles no longer experience the repeated stretching associated with locomotion or exercise. Examples of disuse include: extended bed-rest; microgravity (space flight); and accidental denervation. To some extent, the loss of muscle from elite athletes following cessation of exercise can be considered analogous to disuse. Studies into disuse show that the slow muscle fibres (Type 1) are

particularly susceptible to disuse atrophy (Appell, 1990). Within the muscle fibre itself, atrophy is associated with fibril disintegration, streaming Z-lines and mitochondrial damage which may involve some vacuolar degradation and lipid droplet accumulation. In the extracellular space, atrophy can be characterised by increased amounts of collagen (at least relative to the myofibrillar area), as well as increased infiltration of adipocytes, fibroblasts and phagocytes (Cooper, 1972; Tomanek and Lund, 1974; Appell, 1986). Studies performed at the ultrastructural level suggest that the myocyte is lost during atrophy but the sarcolemmal 'tube' remains. This 'tube' is then invaded by the above mentioned cell types (Appell, 1990), and may appear at times to be replaced by fat deposits. The comparative inactivity of animals in a feedlot, is unlikely to induce marbling via this mechanism.

12.3 In response to myocyte pathology

"Skeletal muscle and adipose tissue development often has a reciprocal relationship *in vivo*, particularly in myodystrophic states." (Tontonoz *et al.*, 1994). It is interesting and may be informative to consider the pathology of human diseases or inborn errors of metabolism in which depositon of fat within muscle has been documented. The justification for this approach is that it takes advantage of the enormous efforts expended in medical research to shed light on an animal phenotype that we might not otherwise be able to understand.

There are two types of relevant pathological states: where lipid accumulation occurs within muscle cells; and where fat cells infiltrate into muscle tissue. This designation may be artificial as the later may be a result of the former in some disease states. Nonetheless for the purposes of this review, this classification should be sufficient. An example of the first state is the oxidative phosphorylation diseases. The literature on these diseases is extensive and the reader is directed to an excellent review on the subject by Shoffner and Wallace (1995). The basic defect in these inborn diseases is in deficient mitochondria. Substrates that are normally metabolised through mitochondria, to liberate energy, accumulate within the cytosol. Lipids are normally degraded by β-oxidation in the mitochondria, and because the mitochondria are dysfunctional, unoxidised lipid tends to accumulate as droplets within the cytosol (Shoffner and Wallace, 1995). The cells respond to this accumulation of lipid by synthesising proteins to surround the lipid droplets and regulate their turnover (Souza *et al.*, 1998).

Lipid droplets are also seen in mature muscle fibres as a secondary consequence of metabolic disease, leading to a deficiency in the cells ability to degrade fatty acids. An example of this is in the human genetic disease, cystinosis and other diseases which exhibit renal Fanconi's syndrome. This syndrome results from dysfunction of the renal tubules, in that there is an inability to reabsorb low molecular weight species. In the case of cystinotic patients, the loss of calcium and other microions can be treated directly (Gahl, 1997). It was found that these patients have muscle weakness and lipid droplet accumulation in muscle, all as a result of loss of carnitine in the urine. When carnitine is supplemented therapeutically, the patients muscular strength returns and the lipid droplets are reduced (Gahl, 1997). It seems likely that a cellular deficiency in carnitine leads to an inability to transport fatty acids into the mitochondria. Without mitochondrial degradation, fatty acids accumulate within the cytoplasm of the cells (Shoffner and Wallace, 1995) and are organised into droplets either spontaneously or actively.

The second group of pathological indications is characterised by the accumulation of recognisable adipose tissue between fasciculae of muscle. Examples of this pathology include:

1. Duchenne muscular dystrophy which results from mutations in the dystrophin gene and spinal muscular atrophy which is a related disease but of unknown etiology. Each condition has abnormal deposition of fat, and this may be recognised using medical

resonance imaging (Leroywillig *et al.*, 1997). The range of allelic variants of this disease express different degrees of fat infiltration into muscle.

2. McArdles disease which results from defects in glycogen degradation. This disease manifests itself clinically by progressive replacement of muscle with fat. In older patients up to 25% of the muscle volume is replaced by fat (Online Mendelian Inheritance in Man, OMIM; Dekerviler *et al.,* 1996).
3. Fibrolipomatous hamartoma is a condition that results in fusiform nerve enlargement caused by fat cell proliferation and thickening of nerve bundles (Demaeseneer *et al.*, 1997). Some patients accumulate intramuscular fat, though there is great clinical variability.
4. Intramuscular lipomas are benign, unencapsulated tumorous growths that infiltrate muscle (Schellong *et al.*, 1997). They are relatively rare and apparently sporadic in occurrence. Histologically, they manifest as monovascular fat tissue between the muscle fibres. In extreme cases the entire muscle can be replaced by fat. These are unlikely to relate to marbling since they are monovascular and localised
5. Hereditary steatosis is in some cases a result of metabolic deficiencies in the enzymes of lipid metabolism in the mitochondrion (Horiuchi *et al.*, 1998). OMIM defines circumstances in which the systems supplying carnitine to the muscle are defective and hence lipid accumulates as well as cytosolic droplets. Lipid accumulation in this case is not confined to muscle.

The preceding discussion indicates that fat can accumulate in muscle for diverse reasons in relatively extreme pathological conditions. The authors believe however that marbling in cattle generally does not result from development of a disorder or disease process. This assertion may not apply to extreme cases of marbling, such as seen in the Japanese Black breed of cattle. The phenotype and development of marbling in this breed will be discussed in a latter section. In the breeds of cattle used in Australia, and under the dietary conditions normally employed, marbling is more likely to reflect the manifestations of normal adipocyte development and metabolism.

12.4 Vitamin A status

Of the relatively few studies into effects of vitamin A on the expression of the marbling phenotype, variability of results has been the only consistent result. The cause of this between experiment variation has not been established, but genetic variation within the experimental herds is likely to be a contributor.

Vitamin A content of feed seems to play a role in the determination of the final beef marbling score. Oka *et al.* (1998 a and b) and others (Naruse *et al.*, 1994) have shown using plasma vitamin A as a marker for total body vitamin A status, that reduced vitamin A levels tend to be associated with increased marbling scores at slaughter. Subsequent studies have corroborated this by showing that vitamin A supplementation at different times prior to finishing influences the subsequent marbling score. On the other hand, it has been disputed that diet is the only cause of Vitamin A deficiency. At least one study has suggested that other metabolic causes such as sickness are involved (Adachi *et al.*, 1998). As stated above, the significance of these findings may be greater in relation to the Japanese Black breed of cattle, as oppose to other breeds.

As described in a number of cell culture systems, vitamin A and the retinoids in general, have regulatory roles in expression of various genes. This may suggest a mechanism by which different concentrations of vitamin A exert contradictory effects. The growth hormone gene is under the control of a retinoic acid responsive element (Gregoire *et al.*, 1998), and hence the IGF axis may be influenced by vitamin A levels. Furthermore, the growth regulatory role of

the thyroid hormones may be synergistically regulated by retinoic acid levels (Gregoire *et al.*, 1998). Oka *et al.* (1998b) investigated T3, T4, insulin and IGF-1 levels in Japanese Black cattle and concluded that T3, IGF-1 (free) and insulin levels were significantly different between animals on high vitamin A relative to low vitamin A diets. The age of the animal at treatment or measurement seemed to be an important component of the plasma levels of each of these hormones. In pulsed infusion experiments, the group found that at 21 months of age, the low vitamin A group had significantly different dynamics of insulin and glucose, though the animals did have the capacity to respond to insulin challenge.

12.5 Nutrient and growth history

Growth history appears to be important to the development of fat deposits within muscle. A survey of studies in ruminants (Table 1) demonstrates that many workers have found that time on high energy feed increases the amount of extractable lipid in muscle. At the cellular level, this appears to correlate to increases in the size of the adipocytes rather than development of new adipocytes, though it may also reflect changes in concentrations of lipid in other tissue compartments.

A period of energy or protein restriction seems to reduce the size of the fat depots, and this occurs through reduction in the size of the constituent adipocytes. Realimentation at least initially, induces a rapid compensatory growth of the adipocytes, as they return to a size similar to animals that had not undergone a restriction. The possibility that a restricted dietary phase might induce hyperplasia of adipocytes is not well supported in the ruminant literature, although it is well established in the avian literature (Table 2). One determinant seems to be the stage of development at which the nutritional challenge is imposed.

Protein restriction during prenatal life and/or preweaning breast feeding appears to have a significant effect on the lipid metabolism of mammals (Tables 1 and 2). This has been shown clearly in rats and mice, and there is good epidemiological data to support its occurrence in humans. Early life protein restriction affects adipocyte development and hepatic function which impacts on sensitivity of the body to energy supply and hence has long term effects on fatness. There are almost certainly effects on the expression of particular genes in the adipocyte developmental pathway (Fig. 7).

Growth pattern may induce changes in the distribution of fat between intramuscular and subcutaneous depots. Recent studies conducted in the Cattle and Beef Industries Cooperative Research Centre (Beef CRC) have identified that intramuscular fat content in *Bos taurus* cattle at Japanese market specification (carcass weight > 300 kg) was reduced relative to fat thickness and carcass weight in steers that came from background growth patterns that which resulted in lighter liveweights on entry to finishing (Table 7).

Contrasts between cattle exposed to more extreme environments exhibit the same pattern. Fig. 9 shows data from *Bos indicus*, a *Bos indicus*/*Bos taurus* composite and tropically-adapted *Bos taurus* steers finished in feedlots to carcass weights of > 300kg in two different environments (temperate northern NSW and the dry tropics of Central Queensland). The steers were grown out before feedlot entry in these two environments. The reason for the different patterns of fat distribution (reported here as fat thickness, fat trim as % carcass weight and intramuscular fat) is unclear. It may include growth pattern before feedlot entry, possible differences in feedlot diets (although evidence for an effect of feedlot diets on fat distribution is sparce) and thermal environment during finishing.

Separate analysis of growth pattern of Bos taurus steers indicates that reduced growth rate during backgrounding tends to be associated with reduction in intramuscular fat content of steers, despite the steers exhibiting increased (compensatory) growth during finishing (M.J. McPhee unpublished observations). However, the effect of growth rate during backgrounding is small compared to the effect of finishing system. In all the Beef CRC

studies, intramuscular fat content is higher at the same carcass weight in steers, finished in a feedlot than at pasture.

These results, from cattle which have grown under various patterns of growth,suggest there is a time dependent change in deposition of fat in different body pools. Perhaps the only model which is consistent with these observations is a temporal difference in proliferation and hypertrophy in adipocytes.

Growth arrest is an important prerequisite to adipogenesis in cell culture. It is unclear what the analogous situation to growth arrest would be in the whole animal but growth stasis or weight loss may be related environmental stressors. Whilst some studies suggest that adiposity is increased by cycles of weight-loss and weight gain (Rodin *et al.*, 1990), this is not consistently observed (Farrell and Williams, 1989).

Vitamin A status and stage of development may be confounded with as shown in studies by Oka *et al.* (1998a). These authors showed that retinoic acid supplementation only had an effect on marbling scores in Japanese Black cattle before the age of 23 months. After that time, the animals were not influenced by retinoid supplementation.

One possible mechanism by which growth path/nutrition could influence marbling is through systemic factors such as plasma fatty acids. These may help stimulate the development of marbling through intermediaries such as the prostaglandins (Reginato, 1998), or free fatty acids which directly influence the PPAR system. In cell culture, high concentrations of free fatty acids stimulate PPAR$_2$ (Gregoire *et al*., 1998). Fasting, stress and disease, elevate plasma free fatty acid concentrations in cattle to levels which in culture can initiate commitment of preadipocytes to adipogenesis. PPAR$_2$ exerts its effects through transcriptional activation and this is potentiated by a variety of lipids and lipid-like compounds, including naturally occurring polyunsaturated fatty acids. The physiological role of PPAR$_2$ is to regulate adipogenesis in response to lipid activators in the blood (Tontonoz *et al.*, 1994).

Cell culture studies have shown that regulation of differentiation preadipocytes into mature adipocytes requires growth (cell cycle) arrest (Fig. 7). Unlike cell line studies, adipose tissue in living animals are populations of cells at many stages of development. Thus an external trigger (such as reduction in dietary energy intake which affects, for example free fatty acid and insulin concentration) would be expected to have different effects on different cells in the population. During fasting an increase in lipolysis from mature adipocytes is expected. This in turn stimulates preadipocytes to undergo growth cycle arrest and become commited to development into mature adipocytes. Such a schema suggests that factors which impinge on commitment of preadipocytes lead indirectly to the proliferation of mature adipocytes and are dependent on a) timing of nutritionally induced “trigger” relative to growth state of all cells, and b) the proportion of susceptible cells in the total population of cells. It would be expected that the effect of nutrition is therefore likely to be proportionately greatest when imposed at a developmental stage when the proportion of susceptible cells in the population is large. This will be greatest when the animal is at an early stage of development. It has been shown in chickens (Meluzzi *et al.,* 1998) that nutritional deprivation in the first 4 weeks of life leads to proliferation in adipocytes -in the abdominal fat pad, within 2 weeks of refeeding. There is a subsequent increase in the carcass fat content 4 weeks after nutrient restriction has been lifted.

Weight loss is a frequent occurrence in diseased animals. In terms of marbling, it is unclear whether disease stress can influence the level or distribution of intramuscular fat. While it seems likely that severe weight loss is likely to reduce the size of the fat depots, it is interesting to consider the long term effects of short term weight loss induced by disease.

Corah *et al.* (1995) found that treatment of one of a pair of genetically identical (cloned) steers with dexamethasone did not enhance intramuscular fat deposition, though previous

reports had suggested that administration of exogenous glucocorticoids enhanced deposition of intramuscular fat in cattle.

Harvey *et al.* (1993) used Simmental and Charolais steers which were actively immunized against growth hormone-releasing factor (GRFi) to evaluate the effect of the growth hormone axis on intramuscular fat deposition. Animals immunized against human serum albumin were used as controls (HSAi). Based on an effect on weight gain, the immunisation was judged to have had the desired effect on the growth hormone axis. Marbling scores were lower for the GRFi steers that for the matched HSAi controls. Rib sections of GRFi steers contained more fat (31.2 vs 25.0%) and less lean (63.3 vs 68.4%) than those of control steers. These data suggest that elevated growth hormone concentration as observed in control animals has a negative effect on marbling scores. An interaction between breed and treatment was also identified in that study.

Gerken *et al.* (1995) used six sets of four genetically identical Brangus steers to study the effects of estradiol and trenbolone acetate on beef quality characteristics, including 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.

12.6 Environmental temperature, humidity and oxygen tension

There is little information available on the effects of environmental temperature on intramuscular fat development in ruminants, so we will need to rely on the information available for other species and in other body depots. Pigs held at 14°C, ate 20% more energy and deposited fat more rapidly than pigs held at 32°C (Campbell and Taverner 1988). Differences between the rates of development between animals held at the different temperatures increased as the energy intake of the animals was restricted.

Ain Baziz *et al*. (1996) studied the effect of chronic heat exposure on carcass quality of broiler chickens. At 7 wks of age, heat-exposed chickens (32°C) had a lower average daily weight gain than comparable control birds. Abdominal, subcutaneous, and intermuscular fat deposits were enhanced in hot conditions, but lipid contents of muscles were not affected by heat exposure.

12.7 Anecdotal in origin

There has also been a range of claims made by producers and breeders about other methods with which to encourage marbling: beer feeding; massage during finishing. While a small amount of data suggests interactions between liver vitamin A levels and alcohol consumption (Itabashi, pers. comms.), it seems clear that these practices only serve to stimulate the appetite of animals, which are flagging late in the production period, and are not solely responsible for the high marbling. It is not impossible however that physical manipulation does stimulate circulation in muscles and hence increase the probability that marbling will develop around well-developed capillary beds. Whether it is possible is however outside the context of this review, since such a procedure would not be applied in the Australian production system.

Another commonly held view is that marbling can be rapidly lost from a carcass, if the living animal is stressed prior to slaughter. Shorthose (1977) addressed this issue by fasting sheep for 72 hours prior to slaughter and then measuring the % fat in the *longissimus dorsi* muscle. Animals that had been deprived of feed and water for 72 hrs had total fat % or 23.4

compared with 18.1 in the control, replete animals. So stress alone is not likely to induce a reduction in intramuscular lipid contents.

Finally, there has been the view that intramuscular fat is late developing, and we have address this issue in previous sections.

13. ROLE OF MARBLING IN OVERALL MEAT QUALITY

As a result of negative health implications regarding saturated fat there has been a tendency over the years to move towards the production and consumption of meat having lower total fat content. This is particularly evident in the pig industry where carcass fat and intramuscular fat have been reduced to such an extent that juiciness, flavour and tenderness may have been compromised (Schwörer *et al.,* 1994). Recently the negative perceptions regarding eating quality of excessively lean meat have been addressed and there has been a move towards acceptance that a certain amount of intramuscular fat is required for optimal palatability.

Savell and Cross (1988) have described a *Window of Acceptability* for beef based on a relationship between intramuscular fat content and overall palatability. They have proposed that more than 3% fat is necessary to be acceptable. However, in keeping with health concerns they have set an upper limit for fat content of 7.3%.

It is generally accepted that intramuscular fat contributes positively to juiciness, flavour and overall palatability of meat and may also result in more tender meat through direct and indirect effects (see Miller, 1994). Importantly, for meat quality, the distribution of fat in the muscle tissue is just as important as the fat content and may be a factor in explaining some different relationships between fat content and tenderness (Monin and Ouali, 1991; Christensen *et al.,* 1991).

There is a popular misconception, resulting from a number of non-scientific reports, that meat containing marbling or intramuscular fat is more nutritious because it contains higher levels of mono- unsaturated and polyunsaturated fatty acids whereas in fact, the opposite is true. Lean meat contains very low amounts of fat with much of the lipid being present as phospholipids (0.5-0.7%) in the structural components of muscle cell membranes. These phospholipids are highly unsaturated, containing polyunsaturated fatty acids such as C18:2n-6, C18:3n-3, C20:4n-6 and C22:5n-3 up to 20% of the total fatty acids. When the fat content of meat increases as with increased marbling, the content of phospholipids does not change significantly as most of the additional lipid is triacylglycerol. These neutral lipids contain more saturated fatty acids and so as marbling increases the proportion of polyunsaturated fatty acids in meat diminishes (Sinclair and O'Dea, 1987).

Some of the other relationships between and among carcass characteristics of Australian cattle have been reviewed by Baud *et al.* (1998a).

14. METABOLISM OF FAT

Fat accretion in any depot is the summation of triacylglycerol (TAG) synthesis and degradation. Synthesis of TAG requires both nonesterified fatty acids (NEFA) and glycerol. Glycerol is derived from glucose but the NEFA can be obtained from a variety of sources including (i) de novo synthesis from either glucose, lactate or acetate and (ii) they can be acquired as pre-formed fatty acids in the diet and delivered to the fat depot as lipoprotein TAG. Degradation of TAG within fat depots involves lipolysis and release of NEFA and glycerol into the circulation.

14.1 Fat Accretion

The rate of fat accretion in finishing steers is determined primarily by (i) the basal energy expenditure of the animal (ii) the intake of metabolisable energy and (iii) the age of the animal (Owens *et al*. 1995). Pathways for fat biosynthesis are either accretion of preformed fat in the diet or by synthesis *de novo*. The intake of fat by ruminants is generally limited since diets containing more than about 4-5% added fat tend to depress intake. Ruminants (Bauman and Davis, 1975) synthesise fat *de novo* in adipose tissue rather than the liver and so regulation of lipogenesis within adipose tissue is a key factor when considering fat accretion in the growing animal.

The substrates for lipogenesis in ruminants are acetate and glucose/lactate. Diets which are extensively fermented in the rumen (i.e. most diets) promote acetate as the major source of carbon and reducing power for lipogenesis with a smaller contribution from glucose for some of the reducing power and all of the glycerol (Vernon,1981). An alternative pathway for lipogenesis with glucose as the primary substrate is typically seen in monogastric animals when glucose is a major end product of digestion.

Two key enzymes of the glucose lipogenic pathway are ATP citrate lyase and NADP malate dehydrogenase. In ruminants both of these enzymes are induced by intravenous glucose infusion, by an increased intake of metabolisable energy and by diets which promote direct glucose absorption from the small intestine (Ballard *et al.* 1972; Lindsay 1970, Pethick *et al*. 1995, Smith et al. 1992)

Regardless of the substrates used, the overall rate of lipogenesis in controlled by the activity of acetylCoA carboxylase which is under complex substrate, allosteric and hormonal control. Accordingly the activity of acetylCoA carboxylase is an indicator of the total capacity for lipogenesis and its activity is strongly correlated with changes in body composition Harris *et al.* (1994). Future work should focus on the regulation of this enzyme in intramuscular fat.

14.2 Glucose availability and lipogenesis

The availability of glucose has long been thought a limiting factor for fat accretion in ruminants. Thus Preston and Leng (1987) speculated that diets high in roughage promote an excess of acetate with respect to glucose and so induce a reduced rate of lipogenesis for a given intake of metabolisable energy. Few studies have critically tested this hypothesis. Two groups (Ballard *et al.* 1972; Prior & Jacobson 1979) have found an increased rate of lipogenesis *in vitro* when glucose was infused into sheep or cattle - the results are equivocal however since glucose was infused in addition to the basal diet and so the experimental design was not isojoulic.

Different adipose tissue sites have been found to have different rates of lipogenesis from acetate versus glucose or lactate. Thus marbling adipocytes show a preference for glucose/lactate carbon while subcutaneous adipose tissue uses mainly acetate as a source of acetyl units for lipogenesis (Smith & Crouse 1984; Whitehurst *et al.* 1981). This difference in substrate preference is clearly shown in Fig. 10.

The results suggest that the relative availability of glucose might influence the marbling response. Glucose availability in ruminants is largely driven by the intake of metabolisable energy with higher ME intake promoting greater rates of gluconeogenesis (Lindsay 1970). One possible site of manipulation might be to use diets which promote a greater rate of starch digestion in the small intestine with resultant direct absorption of glucose. This concept parallels the observation in humans that diets with a high glycaemic index (i.e. diets that allow rapid glucose absorption and concomitant high insulin levels) promote obesity (Ludwig 2000).

There is some evidence to support a link between the development of intramuscular fat and glucose supply. Thus diets based on maize grain promote greater fattening and in particular earlier development of intramuscular fat (Pethick et al. 1997, Mitsumoto et al. 1993, Reddy *et al.* 1975). In the work of Pethick et al. (1997) the maize feeding was associated with a greater activity of ATP citrate lyase in subcutaneous adipose tissue indicating greater glucose supply due a combination of gluconeogenesis and direct absorption of glucose. However these studies do note conclusively show that the glucose/insulin axis can be used to manipulate rates of intramuscular fat deposition. Firstly it is difficult to divorce glucose supply and starch digestion in the small intestine from changes in net energy. Thus maize based diets could well increase the net energy available for lipogenesis. Secondly Gilbert et al. (2000) showed that intramuscular fat deposition was not increased when 'protected starch and/or lipid' was fed to steers consuming a corn based diet. This was despite observed changes in other depots including greater rates of glucose metabolism in subcutaneous adipocytes.

14.3 Preformed dietary fat

It is possible that the marbling response seen for maize based diets is due to maize containing more lipid than other grains such as wheat or barley. However the lipid axis seems unlikely to be important since in the work of Gilbert et al. (2000) lipid intake was varied over a wide range with no changes in the rate of intramuscular fat deposition. In addition the work of Pethick et al. (1997) suggests that the composition of intramuscular fat is not readily affected by diet. Thus maize feeding increased the degree of unsaturated fatty acids in subcutaneous fat when compared to barley but the intramuscular fat was not changed and also was substantially more saturated. It is tempting to suggest that the basis for this is a low activity of lipoprotein lipase within intramuscular adipocytes.

14.4 Lipolysis within adipose tissue

The rate of lipolysis, when measured in a controlled animal house situation, are low when measured in a trained, resting ruminant fed at or above maintenance and housed in thermo neutral conditions. Indeed Pethick and Dunshea (1993) calculated that in such situations lipolysis within adipose tissue would be minimal and instead NEFA would be derived from TAG breakdown due to lipoprotein lipase. In the 'real world' animals are not resting, they are not at thermo neutrality and they are not free of other stress scenarios suggesting that hormone sensitive lipase might well be more active than the laboratory data might suggest. Therefore it is very likely that the turnover of TAG might well be an area that needs further investigation. Indeed the estimate of NEFAS turnover (=NEFA concentration) has never been measured on a systematic basis in feedlot cattle and given the newer more successful 'drac pac' systems it should now be possible to examine this control axis more closely.

15. GENETIC ASPECTS

Numerous experiments have shown there is genetic variation in intramuscular fat content and marbling in beef cattle and that (with some exceptions) marbling is positively related to various aspects of profitability. Estimates of genetic parameters for marbling has been widely reported over the last thirty years, with numerous studies examining the genetic parameters and its association with other carcass characteristics. The reader is referred to Marshall (1994), Koots *et al.,* (1994a) for reviews and Gregory *et al.,* (1995) for a comprehensive report on analysis of the USDA Meat Animal Research Center data set. Published estimates of heritability for marbling lie within the range 0.23 to 0.79 (Koot *et al*. 1994a).

Estimates of genetic parameters of marbling are dependent upon the method used to measure the trait, the method of finishing cattle, and age and weight at the time of measurement. In a data set where different measures of marbling were made on the same carcasses, the estimate of heritability of marbling assessed by the Australian AusMeat system was 0.15, compared to 0.32 for the Meat Standards Australia and USDA system and 0.43 for measure of intramuscular fat by chemical extraction from the visually assessed site (Johnston *et al.,* 1999). Although the genetic correlations between marbling estimated by different methods was >0.95, such differences should be kept in mind when comparing genetic parameter estimates from different studies. Recently, it has become possible to estimate genetic parameters of marbling in the live animal from analysis of real time ultrasound images (Wilson *et al.,* 1998). Estimates of heritability of live-animal scanned marbling as yearlings (0.33 & 0.20) are less than direct assessment on the carcass using intramuscular fat percent (0.43 & 0.36) (Reverter *et al.,* 2000). The genetic correlations between carcass assessment and live animal scanning as yearlings were low to moderate for bulls and moderate to high for heifers, suggesting that selection using ultrasound measures of intramuscular fat should result in predictable genetic improvement of marbling in the carcass (Reverter *et al.,* 2000). Ultrasound methodologies vary with instrument and operator. A recent evaluation of four commercially available systems applied to cross-bred steers (Herring *et al.,* 1998) indicated that overall correlations between IMF and marbling and ultrasound prediction were in the range from 0.2 to 0.75.

Method of finish and market end point affects estimates of genetic parameters for intramuscular fat (Johnston *et al.,* 2000). Analysis of 3428 beef carcasses from four British breeds (Angus, Hereford, Shorthorn, Murray Grey) of which approximately half were finished on pasture and half on grain indicate that heritability was 0.30 for pasture finish and 0.46 for grain finish. The genetic correlation between intramuscular fat content of the LD measured on both grass and grain finished animals was 1.0. The additive variance was 2.4 times larger for grain compared to pasture finished cattle at the same market end point (Johnston *et al.,* 2000). Most estimates of heritability of marbling or intramuscular fat are reported on a weight or age constant basis. Although this is suitable for a single market end point, the Australian beef industry produces to a range of market end points that have markedly different carcass weight end points. Estimates of heritability of intramuscular fat differ between market end points independent of method of finish. Those market end points which have heavier carcass weights are associated with higher estimates of heritability of intramuscular fat (0.37 for Australian domestic weight cattle – carcass weight 220 kg; 0.43 for Australian export weight cattle – carcass weight 280 – 350 kg).

In summary, estimates of heritability of marbling depend on method of measurement, and the extent to which the animals have been able to express the trait. Where trait expression is greatest i.e. animals finished on a grain based feedlot diet, or at heavier carcass weights, estimates of heritability are greater. These points should be borne in mind when comparing estimates across reports.

There are significant differences between breeds and their crosses in their marbling ability (Olson *et al.*, 1985). In *Bos taurus*, dairy breeds (eg Jersey, Friesian) have higher marbling scores at the same degree of finish than do British breeds (eg Angus, Shorthorn, Hereford) which in turn have higher marbling scores than the European breeds (eg Limousin, Simmental, Charolais) (Table 6). *Bos taurus* breeds tend to have higher marbling levels than do *Bos indicus* breeds. Estimates of heritability within breeds tend to be similar, although Koots *et al.* (1994a) reported that heritability of marbling in Angus, Hereford and Limousin breeds tended to be lower than in Shorthorn. However, given the dependence of heritability estimates on degree of trait expression and accuracy of measurement (discussed above) it is uncertain if such tabulated results are practically useful.

The CRC experiment contained progeny of known sires from dams in specific environments (Table 7). Steer progeny were purchased at weaning, and received either in autumn (May) or

summer (January). Steers were grown out from weaning until the group mean liveweight for the cohort reached an average of 400 kg. They were then sent for grass or grain finishing to Korean (560 kg or 100d on grain diet) or Japanese (>600 kg or 150d on grain diet) market endpoints. Animals were trucked to and slaughtered in commercial works and samples of *L. dorsi* taken for amongst other things, intramuscular fat content measurement. Intramuscular fat is measured using NIR calibrated against soxhlet extraction of freeze-dried samples using chloroform as the solvent. The standard error of estimate of IMF% using this method was <0.5%.

Data on 734 progeny from 11 herds, received as 7 cohorts over 4 years were analysed. In each analysis, sire was fitted as a random effect. This permitted removal of sire effects and particularly allowed standardisation of use of link sires within and across years and herds. The parsimonious fixed effects model accounted for 48% of the variance. The significant terms were: hot Carcass weight; finish (grass or grain); weight on pasture shortly after weaning; fat thickness at P8 site at slaughter; and sire. Sire accounted for about 10% of the total variance 47 – 37 or 20% of the variance accounted for. Of the remainder, carcass weight, finish (grain cf grass) and initial weight had the largest effect and p8 fat thickness the least.

16. CORRELATIONS BETWEEN MARBLING AND OTHER TRAITS

Given slaughter at similar ages and / or length of time in the feedlot, there was a low negative correlation (-0.31) between carcass weight and marbling level, with those breeds whose carcass weight is higher, having lower marbling levels (A. Reverter and D. Johnson, unpublished observations). The correlation between fat depth and marbling is positive (0.40) with breeds with higher fat depth having higher marbling levels. Figs. 11 and 12 show the relationship between breeds for marbling with carcass weight and fat depth (respectively). This study did not include breeds used in Japan, which are reported to have even higher marbling levels (Oikawa and Kyan, 1986; Mannen *et al.,* 1998).

The studies in South Australia are examining breed differences between fatty acid composition on meat quality traits. Early results from this study show that the relationship between breed means for marbling and intramuscular fat are high (0.97) (Malau-Aduli *et al.,* 1998; Ewers *et al*., 1999).

Marshall *et al.* (1987) showed that at a constant marbling level, there was no difference between Gelbvieh, Charolais, and Limousin for carcass weight, fat depth and eye muscle area when marbling was held constant.

It is difficult to obtain comparative information about the relative marbling levels of Japanese Black, Brown and Shorthorn cattle, compared with the breeds utilised in Australia. It is suggested the American Wagyu (from which the Australian Wagyu strains arise) is a result of crossbreeding involving Hereford, Angus and Japanese Black and does not express all the features of the Japanese Wagyu strains. There are few breed comparisons reported in the literature involving the American Wagyu. Lunt *et al.* (1993) reported there was a difference of 1 USDA unit of marbling score between the American Wagyu and the American Angus. However, the growth rates of the Angus were higher and the fat thicknesses were approximately the same.

Siebert *et al*. (1996) in a study of six breeds and their crosses found there were differences between breeds in fatty acid composition of intramuscular fat, but not subcutaneous fat.

Experiments have been conducted where Angus sires were selected on high and low EPD for marbling and production and growth traits of the progeny have been measured. Although difficult to make industry-wide conclusions (only 12 sires were used), the results indicate that

sires with high marbling EPD can produce high marbling levels at lower carcass weight and with reduced external fat (Vieselmeyer, *et al.,* 1996; Gwartney *et al.,* 1996).

17. INDIRECT SELECTION FOR MARBLING USING RTUS

RTUS offers scope to improve carcass merit through mass selection. Early attempts to develop systems to measure the marbling (or intramuscular fat percentage) of carcasses were cumbersome and not of use to the seedstock sector. More recently, developments in this field have shown promising results (Wilson *et al.,* 1998; Graser *et al.,* 1998). Heritability estimates for scanned intramuscular fat are moderate with levels of 0.37 for Angus steers and 0.26 for Angus bulls. Marbling score and percentage intramuscular fat are highly, but not perfectly correlated with genetic correlations of 0.94 and 0.82 for two consecutive years of data collection. Problems related to assessor-determined biases and other extrinsic factors can be minimised following an adequate training program for staff conducting the ultrasound scanning (Baud *et al.*, 1998b).

The work of Bruckmaier et al. (1998) is also important in this regard.

18. HETEROSIS ESTIMATES

Significant heterosis above the direct effects of breed has been found for marbling in several studies (Newman *et al.,* 1993; Bertrand *et al.,* 1983; Gregory *et al.,* 1994). However, the reported results are not consistent.

Bertrand *et al.* (1983) in a study of Angus, Hereford, Holstein-Friesian and Brown Swiss found there was significant heterosis between the beef and dairy breeds for marbling, but not for the crosses within the cattle types. That is, there was no heterosis for marbling within the Angus x Hereford animals.

A study in Japan (Uchida and Yamagishi, 1993) which fitted a regression of inbreeding to marbling score, found that there was a positive relationship between inbreeding coefficient and marbling level in Japanese Black steers. In this population, 13.4% of steers had inbreeding coefficients higher than 6.25%.

Maternal heterosis was noted for marbling score in a ½ red angus, ¼ charolais, ¼ tarentaise composite (Newman *et al.,* 1993).

19. "FIXED EFFECTS" DIFFERENCES AFFECTING MARBLING IN BEEF CATTLE

19.1 Gender differences

Many experiments have examined marbling levels in cattle of different gender: heifers; cows; bulls; and steers. Table 3 summarises the results from a number of experiments, which have examined the effect of gender on marbling levels. The general conclusion from this is that at a given slaughter weight, and time on feed, heifers have higher marbling levels than steers, which in turn have higher marbling levels than bulls. No difference has been observed in marbling levels between nulliparous and multiparous heifers (Bailey *et al.,* 1991). However, 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). Given that steers have higher marbling levels than bulls, some researchers have investigated the effect of time of castration on marbling level. Meaker *et al.* (1986) found that calves marked at birth had higher marbling levels than marked at six months. Worrell *et al.* (1987) found that castration at 70 days of age, compared with 230 days increased assessed marbling scores.

19.2 Maternal effects

Crewes and Kemp (1998), found changes in heritability estimate when maternal component traits were fitted to an animal model. The data used in this study were derived from 15 crossbred dam types, mated to Limousin sires. "The direct heritability decreased from 0.35 to 0.16, by the including maternal variance" in the statistical model.

Larsgard and Olesen (1998) looked specifically for maternal effects in growth and fat characteristics of lambs and found little evidence. The h^2 estimate of maternal effect was high for birth weight (0.42) and slightly lower for preweaning weight (0.14) than for weaning weight (0.17). The direct and maternal h^2 estimates for ultrasonic measurements were generally low except for the direct h^2 for muscle depth at weaning (0.32).

The discovery by Mannen *et al*. (1998) of an association between mitochondrial haplotype and marbling phenotype raised the possibility of a larger than expected maternal contribution to marbling phenotype. These findings have not subsequently been confirmed in larger populations of Japanese Black cattle (Tsuji, pers. comm.) and so the conclusion needs to be viewed with some skepticism.

20. GENES FOR MARBLING

The data from the CRC cross breeding study suggest that many genes affect fatness traits and IMF% in particular (Newman and Reverter, 2000). The reported genetic correlation between IMF% on purebred and cross animals from the same sires is not significantly different from 1 (Table 4).

These observations support the assertion that many of the genes influencing marbling are polygenic or oligogenic (cf. Morton and Lio, 1997). Nonetheless chromosomal regions (quantitative trait loci, QTL and even markers in linkage disequilibrium) which are associated with differences in the marbling phenotype have been identify (Barendse 1997).

Despite the large scale (and cost) of gene marker studies in beef cattle, many have been pursued or are currently underway. They include: (1) an Angus meat quality study of the University of Illinois, USA (Beever *et al.,* 1990), (2) the Angleton study of Texas A\&M University, USA (resource partly described in Yeh *et al.,* 1995), (3) the Beef Cattle Marbling evaluation study of the CSIRO Tropical Agriculture, Australia (Barendse, 1997), (4) the Meat Quality study, Cattle and Beef CRC for Meat Quality, Australia (eg. Hetzel *et al.,* 1998), (5) the Wagyu Meat Quality study of the Shirakawa Institute of Animal Genetics, Japan (Hirano *et al.,* 1998; Mizoguchi *et al.,* 1998), (6) the Limousin x Jersey study led by C. Bottema of the Waite Institute, Australia (Malau-Aduli *et al.,* 1998), (7) a Meat Quality study from the USDA Meat Animal Research Center, Nebraska, USA (eg. Casas *et al.,* 1998), and (8) a Meat Quality study of various breeds of Canadian Cattle (Fitzsimmons *et al.,* 1998). In all of these studies, except study number 3, marbling is only one of the characteristics being studied, and in some, marbling is being analysed even though the designs are not optimal to identify QTL for marbling.

Because marbling is a trait of such commercial importance in beef cattle, its genetic basis is keenly sought. The greater the variety of studies, the more likely it is that good estimates of the location and effects of QTL will be found. Genes that show differential effects over a wide range of environments are more valuable financially than those that show effects in only one environment. Given that the r_g for marbling over a range of environments is close to 1, the prospects for identifying useful marbling genes are good. In the genetics of meat quality project of the Beef CRC, some of the initial marbling work was of offspring of Charolais x Brahman sires fed on grass, and neither Charolais nor Brahman have high marbling scores and grass feeding of animals was not expected to yield high marbling scores. As many traits

as possible were studied in these animals. The Angleton study is of Angus x Brahman cross animals short fed for the US domestic market and so explores genes that operate early in the development of marbling. The Shirakawa study is of meat quality in Japanese Black cattle covering 16 Japanese prefectures, with marbling as a key element in meat quality performance (Sugimoto, pers.comm.). The Japanese system involves extended feeding of cattle for 3 or more years to attain extremely high levels of marbling. The Australian marbling evaluation project is targeted specifically at marbling in commercial production in Australia which involves moderate (circa 200 day) lengths in the feedlot often with hormonal growth promotant treatment of cattle.

The data available support the presence of discrete genes which are associated with marbling, a summary of what is currently known is presented in Table 5.

The only known major gene affecting marbling, GDF8, affects development of double muscle phenotype, and in so doing also affects IMF%. Several mutations have been described in GDF8, some are point mutations and some are deletions of the coding sequence (Grobet *et al.,* 1997, Kambadur *et al.,* 1997). GDF8 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 musculature as a result of these mutations in cattle (Hanset *et al.,* 1982; Shahin and Berg, 1985). With respect to development of IMF Wegner *et al.* (1998) demonstrated that GDF8 mutant double muscled animals have a) fewer islands of fat cell development in their LD muscle, b) a lower rate of growth of these islands and c) smaller adipocytes in marbling islands than conventional (i.e. non GDF8 mutant) cattle. There are also be differences in muscle connective tissue content (Arthur, 1995)

For the minor genes affecting marbling there is evidence for at least 5 QTL of moderate effect. Two have been confirmed. The first is a QTL on chromosome Bta 14. The closest markers agree that the QTL is towards the centromere of the chromosome. While few genes have been identified as residing on BTA 14, the TG (Thyroglobulin) gene has been localised to this genetic region and is located approximately 7 cM from CSSM66 (Barendse *et al.,* 1997). Polymorphisms at the TG gene have been shown to be associated with marbling capacity (Barendse, 1997). The TG gene spans 300 kb of DNA (Mercken *et al.,* 1985) and encodes a protein that is the molecular store for iodine. This protein is degraded to produce the thyroid hormones triiodothyronine and tetraiodothyronine (thyroxine). The levels of these 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. However, the means by which thyroid hormones influence marbling is unknown---studies so far have not differentiated the influence of gene control from that of metabolic rate---although the thyroid hormones are expected to influence adiposity in general, not marbling in particular. This marker on BTA 14 has now been commercialised and is in use in the Australian beef industry (MLA SBEF 018).

The second confirmed marbling QTL is towards the centromere on BTA 5. The closest marker is CSSM34 and it shows evidence of a consistent allelic association with marbling scores. CSSM34 appears to be very close genetically to the gene RARG (retinoic acid receptor gamma gene; Barendse, 1997) and may be within the non-coding sequences associated with RARG. All-trans retinoic acid binds to RARG which then binds to DNA resulting in an increase in transcription of the gene to which it has bound. The retinoic acid receptors (RAR) and the retinoid-X receptors (RXR) are important mediators in development of organs and tissues (e.g. Solomin *et al.,* 1998). In the same genomic region is the RDH5 gene which plays a role in the interconversion of all-trans retinoic acid and 9-cis retinoic acid. The latter is the ligand for the retinoid-X receptors and thus the RDH5 gene is potentially an

important controller of the abundance of the different iso-forms of retinoids, which could lead to differential gene control (e.g. Mertz *et al.,* 1997).

Leptin is associated with variation in fat metabolism and deposition in a wide range of species. The bovine leptin gene is on BTA 4 (Stone *et al.,* 1996). The leptin gene itself is highly polymorphic (Konfortov *et al.,* 1999) and it is considered a hypermutable region. Although there are reports of associations between microsatellite markers flanking the leptin gene and fatness traits in cattle (Fitzsimmons *et al.,* 1998) there are as yet no reports specifically linking variation in the leptin gene to variation in intramuscular fat.

Although these locations implicate genetic variation in the retinoid, thyroid and Leptin hormone pathways in individual differences in marbling capacity, the location of QTL near coding sequences for these pathways is not proof of their involvement.

Firstly, the genome is rich in coding sequences, with approximately 75,000 expected in cattle. There may be alternative candidates from other pathways, or as the Beef CRC 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, the complex held together by linkage disequilibrium. Such a complex is not stable since the linkage disequilibrium would decay over time through the usual processes of recombination. Selection could maintain the complex and marker assisted selection would require that a haplotype of DNA markers spanning the QTL be used to maintain effective selection. All of these argue strongly for extending the search beyond the QTL to identification of the gene(s) responsible for variation in the trait.

The identification of plausible candidates from these pathways, nevertheless, adds weight to the case for their involvement and leads to interesting strategies for identifying other genes affecting marbling as well as for the manipulation of the environment. The retinoid, steroid and thyroid receptors are a large family and they are not clustered all on one chromosome. Chromosomes that contain or are expected to contain these receptors, such as Bta 3, 9, 19 may also have an effect on marbling.

While a pathway may be implicated in marbling because it responds to an environmental treatment it need not have genetic variation that affects marbling in a particular environmental treatment. IGF-1 may be expected to have an effect on adiposity in general, firstly because it affects adipocyte differentiation and it a potent growth factor for myofibres. Selection for serum IGF-1 concentration in beef cattle for a decade has resulted in animals in which genetically lower serum IGF-1 concentrations are associated with higher marbling score (Davis and Simmen, 2000). Genes encoding IGF-1 are located on BTA 5. Studies to date have failed to detect any effect of polymorphisms in the IGF-1 gene on adiposity. The QTL on BTA 5 does not encompass the IGF-1 gene (Barendse 1997).

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Table 1. Survey of studies into the effects of growth path on muscle fat characteristics and marbling in ruminants.

Reference	Species, breed, sex, age range encompassed	Treatment variables	Magnitude and direction of the effects
Campion and Crouse, (1975)	Cattle, seven breed, steers, all from Angus cows,	212, 247 or 279 days on concentrate and silage, groups slaughtered at various ages.	Marbling score and % longissimus fat strongly correlated. Days on feed correlated to % fat and to a lesser extent marbling.
Tatum *et al*. (1980)	Cattle, unspecified breed, stratified on frame size and muscling so as to get equivalent finish weights	100, 130 or 160 days on a high-concentrate feed,	Highly variable levels of marbling. Cattle fed for 160 days had significantly higher marbling. Concomitant increase in Choice grades,
Dolezal *et al*. (1982)	Cattle, various breeds, grass and grain backgrounds, calves and yearlings,	Grass and grain fed groups, high concentrate diets for between 0 and 230 days.	Small increase in marbling with increased time-on-feed in both steers and heifers. At least 90 days on feed produced improvements in palatability irrespective increases in marbling.
Lee *et al*. (1983)	Cattle, Hereford and Charolais, steers, post weaning	Ad lib., 70 or 85% ad lib, and three levels of protein	Adipocyte size was correlated with total fatness. Dietary protein did not affect cell number.
Cianzio *et al.* (1985)	Cattle, crossbred steers, over 11 months,	Comparison between fat depots with constant growth path	At 17 months, the order of mean adipocyte size was: kidney fat > mesenteric > subcutaneous > intermuscular > intramuscular > brisket. Hyperplasia in the intramuscular compartment was between 11 and 15 months.

Nicastro *et al*. (1986)	Sheep, Hampshire, male and female, lambs	Comparison between diets with 12.5, 15.7 and 18.9% protein	Dietary protein led to increasing intramuscular fat at the expense of white muscle fibres.
Wheeler *et al*. (1987)	Cattle, Chianina and Hereford Angus cross, steers and heifers, 9 to 12 months of age	Contrast of breed and sex and treatments of days on concentrate feed.	Cholesterol content not affected by time on feed. Ether extractable fat increased with time, and was higher in heifers and crossbreds than steers and Chianinas. Cholesterol content was not affected by marbling score.
Waghorn *et al*. (1987)	Sheep, Romney, wethers, 11 to 12 months old, cannulated	Low and high crude protein diets, four daily intakes	Synthesis of fatty acid in adipose tissue was more rapid in sheep on high protein diets than on low protein diets.
Thompson and Butterfield, (1988)	Sheep, Dorset Horn, rams and wethers,	Comparisons in age development of subcutaneous and omental fat	Increases in chemical fat due to both hypertrophy and hyperplasia. In the omental and kidney fat partitions, hypertrophy greater in wethers as compared to rams.
Bruce *et al*. (1991)	Cattle, Charolais X, steers, 262 kg calves	Three diets of variable energy for 124 days, followed by up to 51 days high-grain finishing;	Steaks from steers fed for 175 days had more ether extractable lipid than steaks from steers fed for 124 days. There were no differences between the diets for extractable lipid.
Yambayamba and Price (1991)	Cattle, Hereford crossbred ,heifers , approx 197 days old,	Three treatments, serial slaughter. Ad libitum to market weight, 2 month restriction then ad libitum to market, 4 month restriction then ad libitum to market.	Feed restriction for two or four months had no significant effect on the total amount of fat, its proportion of total cut weight, or its proportion relative to muscle in the cut.

Wright and Russel (1991)	Cattle, steers, 8 months	Low level feeding to 350 kg followed by either high or low feeding.	Initial phases of compensatory growth led to more protein and water in the carcass, a second phase followed where incorporation of fat increased.
May *et al.* (1992)	Cattle, Angus X Hereford, steers, approximately 16 months, Compudose implanted	High energy diet for 28 – 196 days.	Time dependent increase in marbling score up until 112 days. There were no increased in marbling after this time. Marbling score related to palatability.
Duckett *et al*. (1993)	Cattle, Angus X Hereford steers, approximately 16 months,	Eight groups with various times on concentrate feed	Marbling score generally increased with time on feed with a decreasing rate of development, no significant development after 112 days, total lipid content doubled between 84 and 112 days, but was constant between 0 and 84 and 112 and 196 days. This resulted primarily from increases in neutral lipid content.
Berge, *et al*. (1993)	Cattle, Charolais, steers, 20 – 25 months	Three energy regimens incorporating variable protein; one regimen replacing soyabean-rapeseed meal with linseed meal.	
Van Koevering *et al*. (1995)	Cattle, British and Continental crossbred, steers, yearling – 18 months	High energy diet for 105, 119, 133 or 147 days.	Time dependent increase in marbling score, with a decreasing rate. Cholesterol and total lipid also increased linearly.
Zhang *et al*. (1995)	Sheep, weaned ewe lambs,	Effects of 15 or 20% crude protein in the diet	No effect of dietary protein on the number or size of the adipocytes in the kidney-pelvic fat or mammary gland fat pad

Xie *et al*. (1996)	Cattle, Wagyu X Simmental or Gelbvieh, steers, approx. 524 kg	High energy diet for 90 or 170 days.	170 days on feed produced no increase in marbling over 90 days. The extra 80 days of feed led to a decrease in stearic acid and total saturated fatty acids and generally an increase in mono and polyunsaturated fatty acids.
Cranwell *et al.* (1996)	Cattle, British breeds, non-pregnant cows, greater than 5 yrs	28 or 56 days on high energy diets and four implant treatments.	Both treatments produced carcasses with higher marbling.
Payne and Watkins (1997)	Sheep, Coopworth X Dorset lambs, four months	Age development between 16 and 34 weeks,	Steady increase in carcass fat and subcutaneous adipocyte diameter. No significant change in the total carcass adipocyte number.
Wegner *et al.* (1998)	Cattle, four diverse breeds, bulls, 2 to 24 months	Age development, semitendinosus muscle, serial slaughter and biopsy sampling, computer image analysis	Increases in intramuscular adipocyte diameter with age. Breed differences in both number of fat areas and adipocyte number.

[1] muscle abbreviations as in the text.

[2] sensory panel testing.

Table 2. Survey of recent, relevant studies into the effects of early life growth path on muscle fat characteristics in monogastric species.

Reference	Species, breed, sex, age range encompassed	Treatment variables	Magnitude and direction of the effects
Lucas *et al*. (1996)	Rats, female	Protein restricted and normally fed dams, progreny crossed over to dams from the other group.	Effects could be measured in adults, 6 months after treatment *in utero* or during lactation. Cholesterol and triacylglycerol metabolism affected.
Ozanne *et al*. (1997)	Rats, female,	Control (20%) or low (8%) protein diets to pregnant dams and during lactation	Low protein diets induced changes in the insulin responses of adipocytes that persisted into adult life.
Okuno *et al*. (1997)	Rats, male, from weaning	Diets containing perilla oil, safflower oil, olive oil and beef tallow.	Total adipocyte volume in the epididymal fat pad was lower in the perilla oil treated rats. Late genes of adipocyte differentiation were down-regulated. Early genes not affected. Suggest late phase of adipocyte differentiation suppressed.
Shepherd *et al*. (1997)	Rats	Protein restriction in both pregnant dams and neonates.	The dams protein supply during lactation was most important for long term growth. Offspring of dams that were protein restricted during both pregnancy

			and lactation had the smallest adipocytes.
Cristofori *et al*. (1997)	Chicken, female, 7 to 49 days.	Four groups: *Ad libitum* fed; early restricted; late restricted; fed once every two days.	Early restriction reduced abdominal fat at 21 days but not after 28 days. Feeding once per two days had similar but smaller effects.
Meluzzi *et al*. (1998)	Chicken, female, 7 to 49 days	Four groups: Ad libitum fed; early restricted; late restricted; fed once every two days.	Early restriction affected adipocyte size, and density in tissue at 21 days but not later. Treatments affected the distribution of cellular populations. Realimentation induced hyperplasia within 7 days. Feeding once per two days, inhibited hyperplasia which quickly began again upon realimentation.

Table 3. Relative performance[1] of gender status on marbling levels

Cows	Heifers	Steers	Bulls	Reference
		1	2	Johnson *et al.,* 1988
		1	2	Jones *et al.,* 1986
	1	2		Slanger *et al.,* 1985
		1	2	Ockerman *et al.,* 1984
1	2			Vincent *et al.,* 1991
		1	2	Huerta-Leidenz, 1991
	1	2	3	Jones *et al.,* 1991
		1	2	Shackleford *et al.,* 1992
	1	2		Deland *et al*., 1998

[1] A value of 1 indicates that in that experiment, that class of animals had the highest marbling levels. As the value increases, the relative performance of that class of animals decreased accordingly.

Table 4. Additive genetic variances (σ2A), heritabilities (h^2) and purebred – cross-bred correlations (rpc) estimated from combined purebred and cross-bred data. (From Newman and Reverter, 2000).

Trait	**Purebred**		**Crossbred**[1]		
	σ2A	**h^2**	**σ2A**	**h^2**	**rpc**
Retail yield %	2.278	0.551±0.110	1.037	0.309±0.104	0.955±0.177
Rump fat depth	3.398	0.307± 0.045	5.201	0.345 ±0.087	0.911 ±0.204
Intramuscular fat %	0.645	0.420 ± 0.086	0.447	0.442 ±0.085	0.921 ± 0.123

[1]Crossbred calves from Angus, Hereford, Shorthorn, Santa Gertrudis and Belmont Red sires out of Brahman dams.

Table 5. Summary of the chromosomal locations and number of QTL affecting marbling from a number of published studies.

Chromosome	Closest Marker	Possible Gene(s)	Reference
Bta 2	GDF8	GDF8	Hansett *et al.,* 1982; Shahin and Berg, 1985[1,3]
Bta 5	CSSM34-ETH10	RARG,RDH5	Barendse, 1997 [1]
Bta 12	B bloodgroup		Beever *et al.,* 1990
Bta 14	CSSM66	TG	Barendse, 1997[1]
Bta 14	CSSM66		Casas *et al.,* 1998 [2,1]
Bta 27	BMS2168		Casas *et al.,* 1998
4 coded Bta's	Coded		Hirano *et al.,* 1998[2]
Bta 4	BM1500	Leptin	Fitzsimmons *et al.,* 1998
Mitochondrial genome	D-loop		Mannen *et al*., 1998

[1] confirmed QTL

[2] on poster not abstract

[3] major gene (double muscling)

Table 6. Influence of genotype, age and level of feeding on gain and chemical content of the *M. longissimus* – adapted from Trenkle *et al.,* 1978.

Sire	**Slaughter Wt (kg)**	**Age (d)**	**Feed Level**	**Gain (kg/d)**	**LD wt (kg)**	**Lipid (%)**
Charolais	114.6	145	Milk + Pasture	0.55	1.59	0.63
Angus	110.8	136	"	0.56	1.52	0.91
Charolais	223.3	303	P + Grower diet	0.62	2.88	0.73
Angus	238.8	303	"	0.67	2.85	0.98
Charolais	367.2	389	Full	1.31	4.63	1.88
Angus	361.8	408	"	1.24	4.80	2.75
Charolais	361.8	457	Limit	0.85	4.49	1.78
Angus	361.0	490	"	0.72	4.90	2.60
Charolais	502.7	520	Full	1.27	6.22	3.31
Angus	503.4	572	"	1.03	6.01	4.87
Charolais	506.1	665	Limit	0.84	5.93	7.64
Angus	509.7	714	"	0.71	5.38	12.06

Table 7. The effect of different growth pathways during backgrounding on carcass weight, rib fat thickness, intramuscular fat content of *m. longissimus dorsi* at the end of finishing of Angus, Hereford, Shorthorn and Murray Grey steers. Steers were received over 3 years (1994, 5, 6), grown in three different growth patterns (P1, P2, P3) and finished in a feedlot (F) on a high energy diet, or at pasture (P) to carcass weights acceptable for the Japanese market. Data are means (in the case of fatness traits adjusted for liveweight or carcass weight) of at least 155 steers in each of P1, P2, P3 and at least 75 steers in each of the growth pattern and finishing categories. (taken from Oddy, V.H., Robinson, D.L., Dicker, R.W. and McPhee, M.J., unpublished observations).

		P3	P2	P1
Parameters at the start of finishing				
Liveweight (kg)		429	404	385
Rib fat thickness (mm) Adjusted for liveweight		5.4	4.3	4.1
Growth rate during backgrounding (kg/d)		0.89	0.76	0.67
Parameters at the end of finishing				
Carcass weight (kg)	F	328	321	316
	P	306	297	294
Rib fat thickness (mm) Adjusted for liveweight	F	12.3	12.2	12.2
	P	11.0	11.0	10.9
IM Fat (%) Adjusted for carcass weight	F	7.6	7.1	7.1
	P	6.2	5.7	5.5
Growth rate during finishing (kg/d)	F	1.16	1.22	1.28

	P	0.65	0.68	0.72

Table 8. Carcass composition data based on ribset dissection of Angus steers slaughtered at the beginning and end of a 150 day feedlotting period (mean ± sem).

Carcass attribute	Initial slaughter	Final slaughter	Ratio of final/initial	Significance *P*
HSCW (kg)	304 ± 5.0	417 ± 2.6	1.37	<0.001
Eye muscle area (cm^2)	67.1 ± 1.6	77.3 ± 0.7	1.15	<0.001
Intramuscular fat (%)	7.01 ± 0.47	11.3± 0.28	1.61	<0.001
Total Fat:Bone ratio†	2.65 ± 0.14	4.19 ± 0.08	1.58	<0.001
LT muscle/bone ratio†	0.72 ± 0.03	0.78 ± 0.01	1.08	0.082
gm fat in LT†	107 ± 7	201 ± 6	1.88	<0.001
LT fat/total fat ratio†	0.021±0.001	0.021±0.001	1	ns
LT fat/bone ratio†	0.05±0.004	0.09±0.003	1.80	<.001

† - Measured from the ribset dissection

LT = *m.longissimus thoracis*

na = not measured

Figure 1. Histological images of marbling fat in muscle, demonstating the structure of marbling fat at magnifications of 0.5X, 1X, 4X, 70X and 300X. Note the image has been reduced by a further approximately 5-fold for presentation. Images demonstrate the distribution of adipocytes in the connective tissue seams and around capillaries.

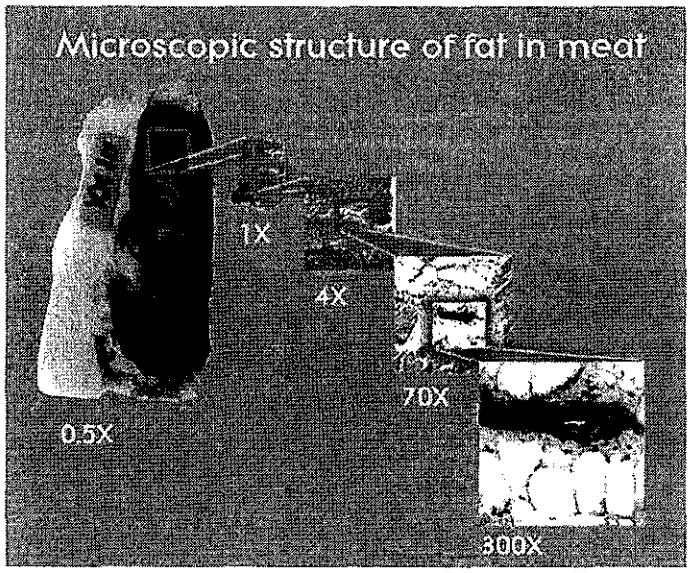
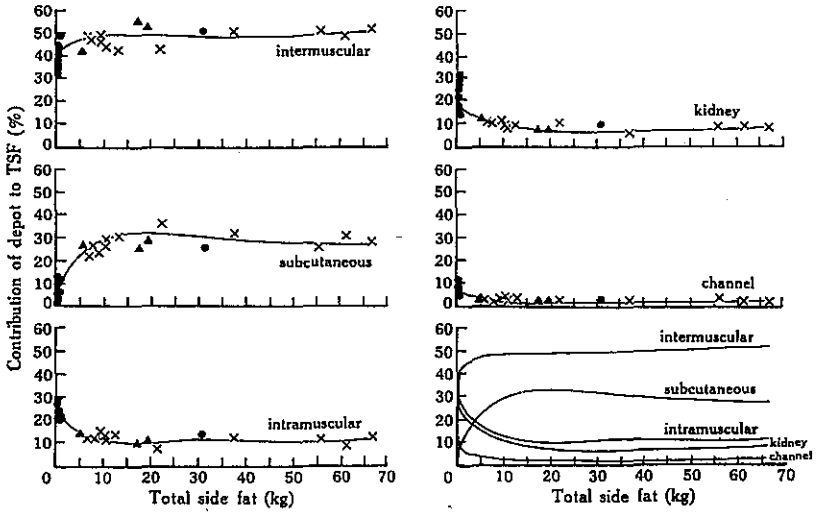


Fig. 4. Contribution of individual fat depots to the total side fat. Adapted from Johnson *et al.*, 1972.



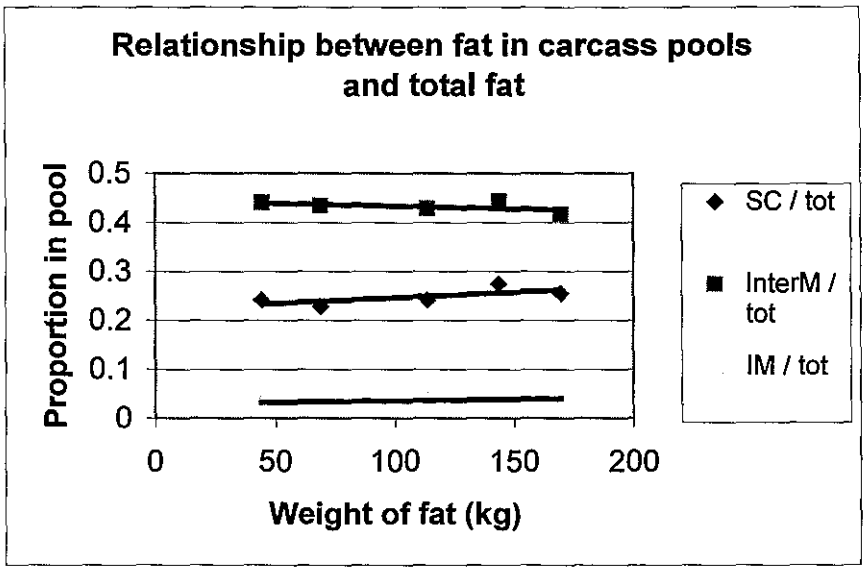


Figure 6. Distribution of fat between different carcass pools in cattle, recalculated from data presented by Cianzio *et al.* (1985).

Figure 7. Overview of stages of adipocyte differentiation showing possible pathway of transdifferentiation of myoblasts into adipocytes. Abbreviations used are pref-1 (preadipocyte factor-1), PPARγ (peroxisome proliferation-activated receptor gamma), MDF's (muscle differentiation factors), TZD's (thiazolidinediones – potent stimulators of the insulin receptor), PUFA's (polyunsaturated fatty acids), PG's (prostaglandins), RA (retanoic acid and metabolites of vitamin A). Adapted from Gregoire *et al.* (1998) and Grimaldi *et al.* (1997).

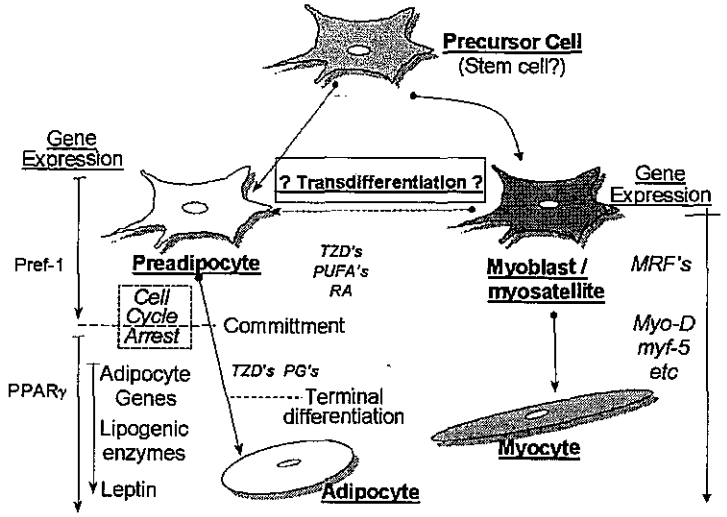


Figure 11. Relationship between marbling level and carcass weight for a number of breeds (from Marshall, 1994).

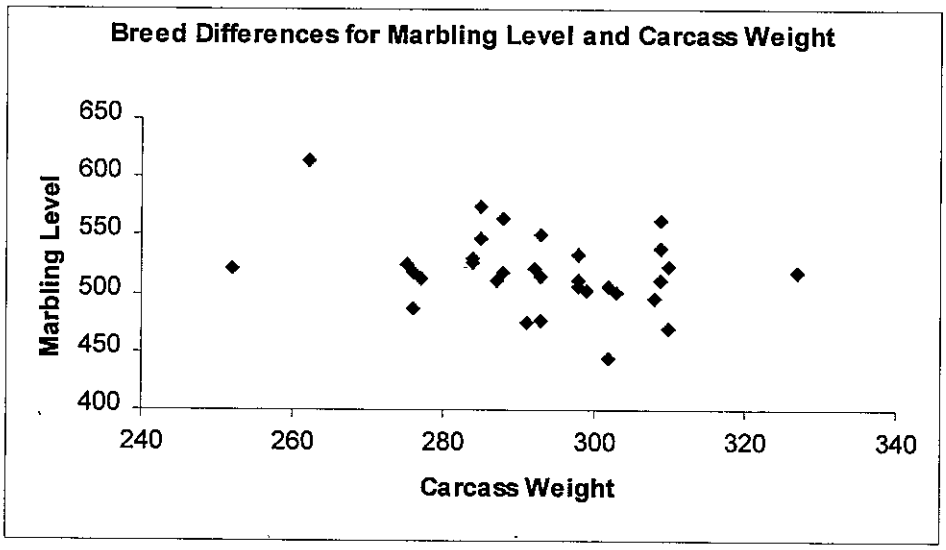


Figure 12. Relationship between marbling level and fat depth for a number of breeds of cattle (from Marshall, 1994).

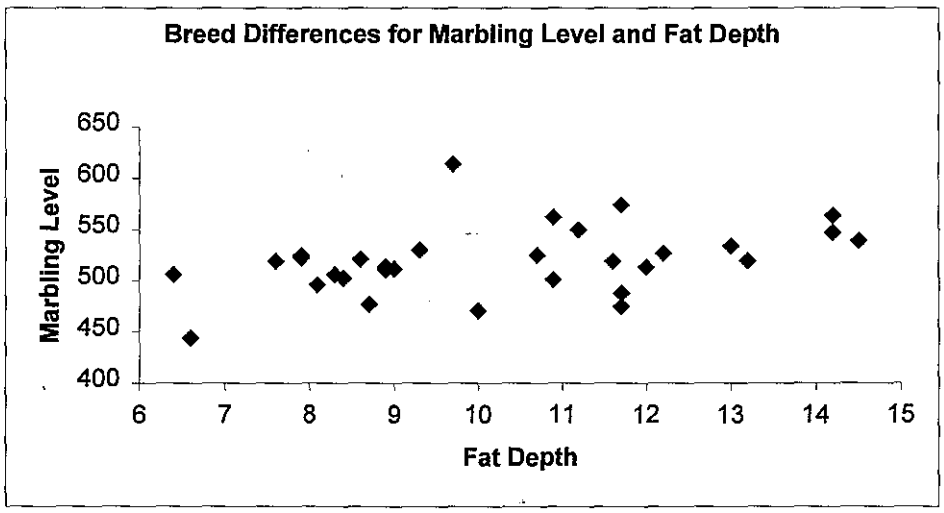


Figure 9. Fat content of the *longisimus* muscle (striploin), and carcass fat attributes in progeny of the same sires, grown out in central Queensland (Growth Check) and northern NSW (Normal Growth) and finished on a grain based feedlot diet for 150 days to Japanese specifications. Animals entered the feedlot at 400 kg and left at >600 kg. Carcass weights were the same in each group. (Unpublished data from Meat Quality CRC I.)

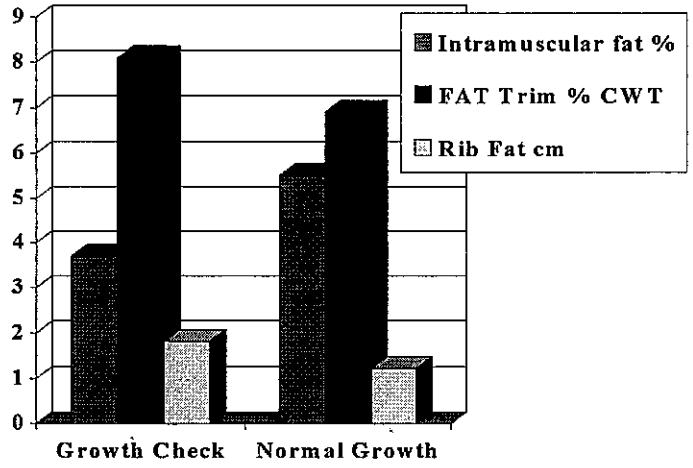


Figure 3. Comparison between chiller assessments on whole carcasses at 12°C and the same meat surfaces 24 hours later at 5°C.

Figure 10(a) Chiller assessment with whole carcass at 12°C

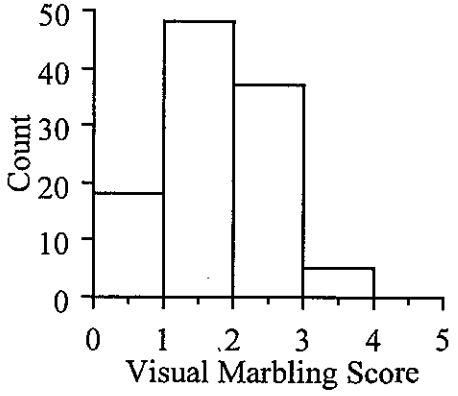


Figure 10(b) Assessment on same surface 24 hours after boning with temperature at 5°C.

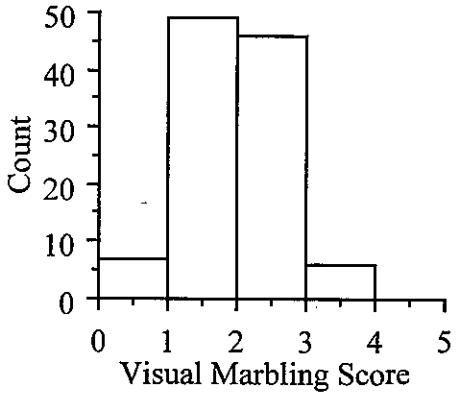


Figure 5. The relationship between carcass weight and intramuscular fat content of the m. longissimus dorsi of crossbred British (Duckett et al. 1993) and Japanese Black x Holstein cross cattle (Aoki et al. 1999).

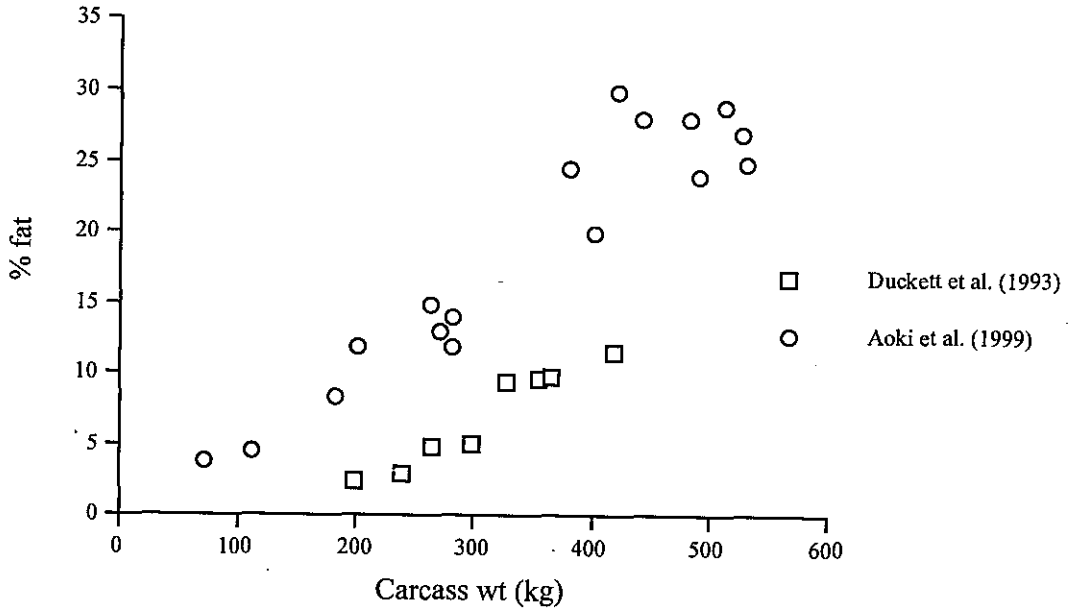


Figure 2. Comparison between the fat content of various muscles from carcasses that were either in the slight or lower marbling grades, or the slightly abundant or higher marbling grades. Data derived from Brackebusch *et al.* (1991).

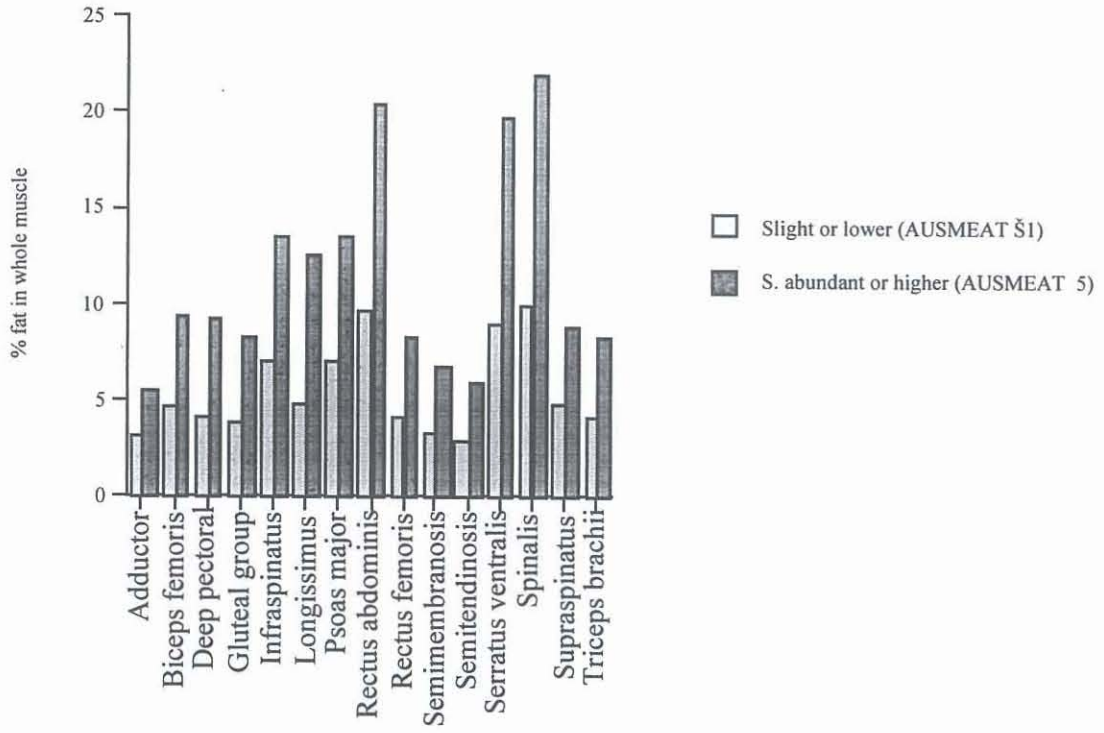


Figure 10. The relative contributions of carbon precursors to fatty acid synthesis in adipose tissue of 18 month old Angus steers (values are %±SD) (Smith and Crouse, 1984).