PLASMA METABOLITE AND HORMONE CONCENTRATIONS IN FRIESIAN CALVES OF LOW OR HIGH GENETIC MERIT :EFFECTS OF SEX AND AGE

A thesis presented in partial fulfilment of
the requirements for the degree of Master of
Agricultural Science in Animal Science
at Massey University

SEOK-HONG MIN

1990

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LIST OF ABBREVIATIONS

AI	artificial insemination
ANOVA	analysis of variance
BI	breeding index
BW	body weight
CS	creatinine space (%)
DM	dry matter
F	female
g	gram
GFR	glomerular filtration rate
IU	international unit
h	hour
HBI	high breeding index
IGF-1	insulin-like growth factor-1
K	fractional decay constant
KC	fractional decay constant for creatinine
kg	kilogram
KU	fractional decay constant for urea
lwt	liveweight
M	male
MANOVA	multivariate analysis of variance
ME	metabolisable energy
mg	milligram
MJ	megajoules
NEFA	non-esterified fatty acids
l	liter
ml	milliliter
mm	millimetre
mmol	millimole
ng	nanogram
P	probability
PRL	prolactin
r	correlation

r^2	coefficient of determination
RIA	radioimmunoassay
se	standard error
T_3	triiodothyronine
T_4	thyroxine
ueq	micro equivalents
US	urea space (%)

Levels of Statistical Significance

NS	P>0.10 (not significant)
+	0.10>P>0.05
*	0.05>P>0.01
**	0.01>P>0.001
***	0.001>P

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CHAPTER I: INTRODUCTION

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INTRODUCTION

Over the last 30 years the ability of dairy cows to produce milk has been improved considerably. Although this partly reflects improvements in nutrition, health and management programmes, genetic improvement through selection programmes is no doubt one of the major contibutors to this improved milk yield. In New Zealand, selection programmes based on artificial insemination started in 1955 and emphasised milk fat production. Since then (1955 - 1987), a 24% improvement in the average genetic merit of cows has been achieved (Anonymous, 1986/1987). This genetic progress appears to have contributed about 80% of the total increase in milk yield over the same period (Holmes, 1988).

Animal breeding programmes for dairy cattle worldwide have been based on the principles of quantitative genetics. Although these programmes are the most reliable and accurate methods available at present, they are expensive and genetic improvement is very slow. In New Zealand, contracts are made each year by the Dairy Board with individual farmers to purchase about 150 newborn bulls (mainly consisting of Holstein - Friesian and Jersey) for testing. All these bulls are kept in the breeding centre until they are 5 years old, waiting the assessment of their daughters' performance. Thereafter, only a few of the proven bulls are used extensively in the AI scheme while the others are culled. The long generation interval involved in this progeny testing is a major limitation to high rates of genetic gain, despite the improved accuracy of selection possible (as opposed to selection on ancestry information alone).

In an attempt to overcome these problems of the conventional breeding system, there is growing interest in developing new techniques which will lead to faster genetic responses. One of these techniques is to attempt prediction of genetic merit using physiological characteristics. The basic concept of this approach is that milk production reflects the net effect of numerous biochemical pathways which are under genetic control. Thus variation in genetic merit might be reflected in different plasma levels of metabolites or regulatory hormones involved in these metabolic pathways. If

it were possible to identify "physiological markers" which were strongly associated with genetic merit but independent of age and sex, then the efficiency of genetic improvement of dairy cattle could be enhanced considerably by increasing the accuracy of selection and/or reducing the generation interval. Additionally, an understanding of the physiological basis of dairy merit might lead to the development of exogenous promoting agents for milk production and, in the long term, to the identification and manipulation of specific genes controlling lactational performance.

Active research programmes into the identification of possible genetic markers in dairying are currently being undertaken in several countries including New Zealand. In most cases, scientists are attempting to identify possible markers by studying calves from divergent selection lines of high and low genetic merit. The use of animals from divergent selection lines in identifying potential genetic markers has the great advantage that there is generally a substantial difference in average genetic merit between the selection lines even though the genetic merit of individual calves within the lines is not known. The major problem in studies of this type is the fact that lines are generally established within a breed, starting with a limited initial base population. As a result, genetic variation between a particular pair of lines may not necessarily apply to other similar experiments. Furthermore, the number of animals per line is usually small for economic reasons. Selection lines are thus subject to effects of random genetic drift which cannot be accurately estimated (i.e differences between the lines in metabolic characteristics may reflect the effect of genetic drift rather than being correlated responses to the selection process).

An alternative approach to use of selection lines is to employ progeny testing schemes in identifying genetic markers. In this system, metabolic characteristics are identified in young bulls which are then progeny tested to allow accurate estimation of their genetic merit. The ability of metabolic characteristics to predict genetic merit can then be evaluated directly. The great disadvantage of this system is the long interval between when markers can be measured and when genetic merit can be evaluated via the progeny test.

The possibility that genetic merit may be recognizible in terms of physiological characteristics has been examined under a wide range of experimental conditions. This range of conditions may reflect the desire to develop physiological markers which are

easily measured and repeatable under a variety of conditions. In general, these can be divided into three major types; basal measurements, metabolic challenges and fasting/refeeding.

1 Basal measurements

Measurement of basal concentrations of plasma hormones and/or metabolites is a common first step in the study of physiological differences between high and low genetic merit dairy cattle. Measurement of these concentrations of hormones and metabolites is done under normal feeding conditions and is thus relatively simple, requiring only periodic blood samples via venous cannulae or venipuncture. The rationale behind the use of basal concentrations of plasma hormones as potential genetic markers is that they may relate indirectly to the expression of dairy merit because they control the partitioning of nutrients between body tissues and the mammary gland. Similarly, basal metabolite concentrations may indicate the availability of intermediary metabolites for the relative function of these tissues or levels of byproducts from this function. The plasma concentrations generally are a function of:

a) the rate of turnover in the plasma pool,

b) the size of the plasma pool, and

c) the set point at which secretory and/or clearance processes are invoked thus modifying the concentration.

1.1 Hormones

1.1.1 Somatotropin

Many studies have investigated somatotropin as a potential genetic marker. This is not surprising since the effect of exogenous somatotropin on regulating milk production of dairy cattle is well established (Bauman *et al.*, 1986; Gluckman *et al.*, 1987; Peel *et al.*, 1987). Furthermore, injecting somatotropin into cows causes them to increase milk production and use nutrients in a manner similar to that observed in cows of high genetic merit (McCutcheon *et al.*, 1985).

Hart *et al.* (1975) were one of the first groups to study the possibility that genetic merit for milk potential may be expressed in terms of somatotropin level. In this study, which compared basal concentrations of somatotropin in lactating dairy and beef cattle, the concentrations of somatotropin were greater in the dairy cattle. Three years later, differences in basal somatotropin among cows of unequal genetic merit were again confirmed by the same group with high and low producing dairy cattle (Hart *et al.*, 1978). They suggested that somatotropin may be a physiological mediator of selection pressure for milk yield. Although this suggestion has been supported by several later studies with young calves (Barnes *et al.*, 1985), heifers (Barnes *et al.*, 1985; Xing, 1989), bred heifers (Barnes *et al.*, 1985) and lactating cows (Barnes *et al.*, 1985; Kazmer *et al.*, 1986; Bonczek *et al.*, 1988; Lukes *et al.*, 1988; Sartin *et al.*, 1988), it is now also clear that some of these results, including the earlier studies, were due to differences in energy balance rather than to differences in genetic merit (Hart *et al.*, 1975, 1978; Bonczek *et al.*, 1988; Lukes *et al.*, 1988; Sartin *et al.*, 1988). As a result, differences in basal somatotropin observed at the earlier study (Hart *et al.*, 1978) were no longer apparent when animals were fed to maintain equal energy status (Hart, 1983). Furthermore, the secretion of somatotropin is pulsatile in nature (Gluckman *et al.*, 1987; Millard, 1989). For this reason, even in those studies showing a genetic effect, results are of limited value because assessment of somatotropin levels was based either on infrequent sampling or on sampling over very short periods. Therefore, there is no conclusive evidence for the use of basal somatotropin as a possible genetic marker.

1.1.2 Insulin-like growth factor-1 (IGF-1)

Ahlborn-Breier *et al.* (1987) have shown that the basal concentrations of IGF-1 in the blood of mature bulls are associated with their genetic merit as determined by progeny testing. While this result suggests the use of IGF-1 as a potential genetic marker, subsequent studies with young bulls (Mackenzie *et al.*, 1988) and heifers (Davis *et al.*, 1989) failed to find consistent differences between lines of cattle divergently selected on the basis of milkfat yield. Nevertheless, there is growing interest in the use of IGF-1 as a genetic marker for dairying since the action of somatotropin on milk production may be partly mediated by IGF-1 (Gluckman *et al.*, 1987). Furthermore, the IGF-1 concentrations in the peripheral circulation of mammals, including dairy cattle, are relatively stable with no obvious diurnal rhythm (Gluckman *et al.*, 1987; Elsasser *et al.*, 1989; Ronge *et al.*, 1988) although this

hormone is affected by nutrition (Ronge *et al.*, 1989; Steele *et al.*, 1989). Therefore, a single blood sample during the course of the day seems to be sufficient to characterize the IGF-1 status of individual animals (Roberts *et al.*, 1990).

1.1.3 Prolactin (PRL)

Barnes *et al.* (1985) found that plasma concentrations of PRL were lower in selection group than in control Holstein calves, yearlings, bred heifers and primiparous cows after feeding and insulin administration. Similarly, Klindt *et al.* (1988) estimated the relationship between PRL secretory parameters in Holstein bulls and performance of their daughters and reported that frequency of PRL peaks was negatively correlated with daughters' predicted difference(PD) for milk, fat and protein. While these data strongly indicate the use of plasma PRL concentrations as a possible genetic marker, there is other conflicting evidence in the literature. Several studies with pregnant heifers (Bitman *et al.*, 1984) and lactating cows (Kazmer *et al.*, 1986; Lukes *et al.*, 1988; Bonczek *et al.* 1988) have reported that selection for milk yield does not affect the basal plasma concentrations of PRL. The lack of any effect of genetic selection for milk yield on blood PRL is consistent with other studies showing little correlation between basal PRL and milk production (Koprowski *et al.*, 1973; Hart *et al.*, 1978, 1979). Plaut *et al.* (1987) also reported that administration of exogenous PRL failed to increase milk production in dairy cattle. Therefore, these studies generally support the established concept that, in ruminants, PRL is essential for full lactogenesis but not for maintenance of lactation (Cowie *et al.*, 1980).

1.1.4 Thyroid hormones

The role of thyroid hormones (T_3, T_4) in milk production is well demonstrated in dairy cattle (Cowie *et al.*, 1980; Davis *et al.*, 1988a,b). Furthermore, there is some evidence that thyroid hormones are under genetic control (Edfors-Lilja *et al.*, 1980; Land, 1981; Almlid *et al.*, 1982). Therefore, a number of studies have attempted to detect differences between high and low genetic merit cattle in the level of thyroid activity by measuring either serum thyroid hormones or thyroid hormone degradation rates.

The first evidence for the use of thyroid activity as a potential genetic marker was provided by Joakimsen *et al.* (1971). In this study, involving 82 mature Finnish bulls, the thyroxine degradation rate in bulls was positively correlated with fat-corrected milk production of their daughters (r=0.28 to 0.57). Sorensen *et al.* (1981) also studied thyroxine degradation rate in young bulls (1.5 - 11 months of age) of Danish dual-purpose breeds in relation to performance of their daughters and reported that thyroxine degradation rate had a relatively large and positive genetic correlation with breeding value for butter-fat production (r=0.42). Since thyroid activity reflects the basal metabolic rate of animals, this positive correlation between thyroid degradation rate and breeding value for milk yield may indicate that bulls with high breeding value have a greater metabolic activity. This suggestion is partly supported by the positive correlation between thyroxine degradation rate and growth rate in both of the above studies. It is further substantiated by a recent study in Denmark (Jensen *et al.*, 1988). This study involved 650 young bulls, the progeny of 31 sires of Holstein or Brown Swiss descent. Genetic parameters for 51 traits, including estimated energy requirements, growth, total feed intake, and carcass composition were estimated by a multitrait model in relation to milk yield of female sibs. Results showed that breeding value for milk production was significantly correlated with maintenance requirement before 200 kg but not after 200 kg liveweight. Therefore, European bulls with high breeding value for milk yield, particularly young ones, appear to be characterized by a greater metabolic rate.

While measurement of thyroxine degradation rate was successful in establishing positive correlations between thyroid activity and breeding value for milk yield, simultaneous measurement of serum thyroid hormones failed to confirm this relationship in the same studies. These observations strongly indicate that serum thyroid hormones are, unlike thyroid degradation rate, not useful predictors of genetic merit. This suggestion is generally supported by other studies examined the relationship between breeding value and serum thyroid hormones concentrations. All of these studies except one (Graf *et al.*, 1979) have been unable to demonstrate a significant correlation between the basal concentrations of plasma thyroid hormones and genetic merit of calves (Land *et al.*, 1983; Sejrsen *et al.*, 1984), lactating cows (Bodoh *et al.*, 1972; Hart *et al.*, 1978; Bonczek *et al.*, 1988) or bulls (Osmond *et al.*, 1981). Even in the study of Graf *et al.* (1979), a positive correlation between serum thyroxine concentrations in bulls and performance of their daughters observed at 8 months of age disappeared at 11 and 14 months of age. Failure to detect genetic merit

for milk yield in terms of serum thyroid hormones is not surprising since the effect of thyroid hormones on metabolism is influenced by several factors such as the level of feed intake (Christopherson *et al.*, 1979; Macari *et al.*, 1983), energy balance (Tveit *et al.*, 1980; Blum *et al.*, 1980; Blum *et al.*, 1981a,b; Blum *et al.*, 1985; Kunz *et al.*, 1985; Ellenberger *et al.*, 1989), environmental temperature (Yousef *et al.* 1985), season (Aceves et al., 1985) and age (Blum *et al.*, 1983). These factors were poorly controlled in the majority of the above studies. Therefore, efforts in future research should be devoted to minimizing these effects with a view to more accurately assessing the relationship between genetic merit and serum thyroid hormones. If these effects cannot be overcome, then it may be better to use thyroid hormone degradation rate, rather than serum thyroid hormone levels, as genetic markers because the former seems to be more stable than the latter.

1.1.5 Insulin

In all overseas studies except three (Hart *et al.*, 1978, 1979; Bonczek *et al.*, 1988), selection for milk yield failed to alter the basal plasma concentrations of insulin in young calves, yearling heifers, bred heifers, lactating cows or mature bulls (Osmond *et al.*, 1981; Land *et al.*, 1983; Sejrsen *et al.*, 1984; Sinnett-Smith *et al.*, 1987). Even in the studies of Hart *et al.* (1978, 1979) and Bonczek *et al.* (1988), differences in basal concentrations of insulin between the two groups were, as was the case for somatotropin, due to differences in energy balance rather than to differences in genetic merit. As a consequence, there is no evidence for differences in plasma insulin between high and low genetic merit groups in overseas research.

In contrast to overseas studies, research with the Massey University selection lines has shown that basal concentrations of insulin are greater in the high breeding index (HBI) group than in the low breeding index (LBI) group. These results have been obtained with young calves (Xing *et al.*, 1988), young bulls (Mackenzie *et al.*, 1988), heifers (Xing, 1989) and lactating cows (Flux *et al.*, 1984) although in the last case the differences were only apparent at 70% of ad libitum feeding. Thus, high plasma insulin appears to be a consistent characteristic of the Massey University HBI animals. This greater plasma concentration of insulin in the selection group at Massey University is difficult to explain since insulin is anabolic in nature (McDowell, 1983) and the commencement of lactation is usually associated with a decline in plasma concentration of insulin in dairy cows (Ronge *et al.*, 1988). However, Flux *et al.*

(1984) also noted a greater plasma concentration of glucose in HBI cows and suggested that the higher levels of insulin in the HBI cows may be attributed to a decreased sensitivity of peripheral tissues to insulin. This relative insulin insensitivity is possibly a result of decreased numbers of insulin receptors (Xing *et al.*, 1988).

1.1.6 Glucagon

Measurement of this hormone in blood is complicated by problems of specificity (i.e ability of antibodies to distinguish between pancreatic and gut glucagon) and available commercial kits are expensive. As a result, few experiments have been conducted to study the effect of selection for milk yield on plasma concentrations of glucagon.

The first study examining the relationship between genetic potential for milk yield and plasma glucagon was conducted by Barnes *et al.* (1985). In this study, employing 48 dairy cattle of 6, 12, 18 and 24 months of age, there was no effect of genetic merit on basal plasma concentrations of glucagon at any age except 6 months. At 6 months of age, the basal concentrations of glucagon were greater in the control than in the selection group. Similarly, Xing (1989) and Sartin *et al.* (1988) measured plasma glucagon in yearling heifers and lactating cows, respectively, and reported that selection for milk yield was not accompanied by corresponding changes in plasma glucagon. The lack of any response of plasma glucagon to selection for milk yield is not surprising since others have also been unable to obtain a close correlation between blood glucagon and milk production (Herebein *et al.*, 1985; Sartin *et al.*, 1985a,b). However, failure to detect a genetic effect on the circulating level of glucagon does not necessarily indicate a similar glucose metabolism between high and low genetic merit groups. Glucose availability is regulated not only by glucagon but also by other hormones such as somatotropin and insulin. The ratio of glucagon to insulin is particularly important in regulation of blood glucose concentrations since the glycogenolytic and gluconeogenic actions of glucagon are counter-balanced by the glycogenic and antigluconeogenic actions of insulin (Bassett, 1978; Annison *et al.*, 1982). Therefore, possible differences in glucose metabolism between the groups of high and low genetic merit should be examined in terms of the ratio of glucagon to insulin rather than circulating levels of glucagon alone.

1.2 Metabolites

1.2.1 Glucose

The availability of glucose to the mammary gland plays a vital role in regulating milk secretion through lactose synthesis. Thus the use of plasma glucose as a potential genetic marker for dairying has been the subject of many studies over the last 10 years.

These efforts have generally not been successful when animals are studied under basal conditions. Although several studies with milk-fed calves (Xing *et al.*, 1988), young heifers (Barnes *et al.*, 1985), yearling heifers (Xing, 1989) and pregnant heifers (Gibson *et al.*, 1987) have shown that the basal plasma concentrations of glucose are greater in the selection group than in the controls, differences reached a significant ($P<0.05$) level in only one of these studies (Xing, 1989). Even in the study of Xing (1989),the results may have a limited value since a only small number of animals per line (n=8) were employed in this study. In addition, the greater plasma concentrations of glucose in the high genetic group were observed with lactating cows (Flux *et al.*, 1984), but the differences were again only apparent at 70% of ad libitum feeding. Conversely, a large number of studies based on either a small number of animals (Tilakaratne *et al.*, 1980; Sejrsen *et al.*, 1984; Barnes *et al.*, 1985) or a population (Stark *et al.*, 1978; Rowlands *et al.*, 1986; Gibson *et al.*, 1986) have reported that selection for milk yield dose not change the basal level of plasma glucose in well-fed calves, heifers, lactating cows and bulls. Thus, plasma glucose was poorly correlated with breeding value for milk yield when the genetic correlations for blood glucose and milk yield were estimated in bulls or heifers of different ages (Stark *et al.*, 1978; Sejrsen *et al.*, 1984; Rowlands *et al.*, 1986; Gibson *et al.*, 1986). Lack of glucose response to selection pressure for milk, regardless of its vital role in milk production, may reflect the fact that glucose is an important metabolite for many key functions (eg as an energy source for the central nervous system). Thus its plasma levels are under tight homeostatic control with the result that little genetic variation may be expressed in these levels.

1.2.2 Non-esterified fatty acids (NEFA)

Sejrsen *et al.* (1984) measured the basal plasma concentrations of NEFA in Red Danish bull calves with different genetic potential for butterfat production and found that these concentrations were positively correlated with breeding value at 3.5 months of age (r=0.52). Consistent with this result, Barnes *et al.* (1985) reported that the basal level of NEFA was greater in high genetic merit heifers at 6 and 24 months of age, but not at 12 and 18 months of age. However, such differences have not been found in most other studies with calves (Tilakaratne *et al.*, 1980; Gibson *et al.*, 1986; Sinnett-Smith *et al.*, 1987), heifers (Gibson *et al.*, 1986; Xing, 1989) and lactating cows (Flux *et al.*, 1984). Instead, these studies have reported that the concentrations of plasma NEFA do not show any selection line effect. Furthermore, the pattern of plasma NEFA in response to somatotropin injection has not been found to vary between Fresian calves sired by bulls of high or low genetic merit (Land *et al.*, 1983). Therefore, the measurement of plasma NEFA under full feeding conditions is unlikely to provide a useful predictor of genetic merit for milk yield.

1.2.3 Urea

As with other potential genetic markers, attempts to detect the genetic potential for milk yield in terms of plasma level of urea during basal conditions have met with only limited success. While some studies have identified greater basal levels of plasma urea in calves, heifers, cows or bulls of high genetic merit (Stark *et al.*, 1978; Freeman *et al.*, 1978; Barnes *et al.*, 1985; Xing, 1989), others have not (Tilakaratne *et al.*, 1980; Blum *et al.*, 1983; Sejrsen *et al.*, 1984; Rowlands *et al.*, 1986; Sinnett-Smith *et al.*, 1987). These inconsistent results among studies are not surprising since plasma urea, like other metabolites, is subject to the influence of non-genetic factors including nutrition, hormonal state and age (Rowlands, 1980). As a result, the repeatability of plasma urea has been found to be low in lactating cows (Peterson *et al.*, 1982) and bulls (Rowlands *et al.*, 1986) although, in the former case, a positive genetic correlation between plasma urea and production of milk, fat and protein was observed. Nevertheless, the possibility of using plasma urea as a potential genetic marker is still being pursued partly because of a moderate genetic correlation between blood urea levels and milk production (Kitchenham *et al.*, 1975, 1976; Peterson *et al.*, 1982). Furthermore, compared with most other potential physiological markers, plasma urea concentrations have the advantage of being easily measured and relatively resistant to variation due to handling stress.

1.2.4 Creatinine

Peterson *et al.* (1982) estimated the genetic correlations for serum and production traits of 545 Holstein cows in 35 herds and found that the basal concentrations of plasma creatinine had large genetic correlations with production traits such as yields of milk (r=-0.60), milk fat (r=-0.60) and milk protein (r=-0.68). They also reported from the same study that plasma creatinine, unlike urea, is highly heritable (h=0.44). Since the use of physiological markers for dairying relies on the strength of both heritability and genetic correlation, these results strongly suggest that the genetic potential for milk yield could be predicted by measuring the level of plasma creatinine. This suggestion has been confirmed by recent studies with yearling heifers (Xing, 1989) that found lower plasma concentrations of creatinine in yearling heifers of the HBI group. The use of plasma creatinine as a possible genetic marker for dairying is, therefore, worth pursuing further.

2 Metabolic challenges

It is clear from the previous section that measurement of hormones or/and metabolites under basal conditions may not be an ideal method of screening potential genetic markers for dairying. This is not surprising since basal concentrations of hormones and metabolites are easily affected by a variety of non-genetic factors. For this reason, even in those studies showing a selection line effect, the results are sometimes difficult to interpret (for example, somatotropin). Furthermore, concentrations of some metabolites under basal conditions are being constantly controlled by homeostatic regulators with the result that their levels remain relatively constant. Therefore, differences in metabolic characteristics between high and low genetic merit dairy cattle may not be expressed under these conditions.

An alternative approach to detecting potential genetic markers for dairying is to use simple "metabolic challenges" in which the animal is injected intravenously with a hormone or metabolite. The main aim of this technique is to detect differences between the genetic groups in sensitivity to homeostatic signals by disturbing their homeostatic state. Interest in this approach has been generated by the proposal that dairy cattle achieve dynamic control of nutrient partitioning by altering the sensitivity of specific

target tissues to homeostatic regulators (Bauman *et al.*, 1980). In general, studies of this type involve the measurement of basal levels of hormones or/and metabolites prior to the administration of the challenge and subsequent monitoring of responses in a further series of samples until basal concentrations are again reached or approached.

2.1 Insulin challenge

As noted previously, the higher basal glucose concentrations and simultaneously greater basal concentrations of insulin found in the HBI dairy cattle at Massey University (Flux *et al.*, 1984; Xing *et al.*, 1988; Mackenzie *et al.*, 1988; Xing, 1989) may reflect a reduced sensitivity of peripheral tissues to insulin. If this were the case, the clearance of blood glucose in response to an insulin challenge would be expected to be less rapid in the HBI dairy cattle. When young heifers from these lines were challenged with insulin, no such differences were evident (Xing, 1989). Consistent with this result, overseas studies with calves (Land *et al.*, 1983) and heifers (Barnes *et al.*, 1985) reported that glucose response to the injection of insulin was not affected by the genetic potential for milk yield. However, Mackenzie *et al.* (1988) found with young bulls (8 months old) that, following an insulin challenge, glucose clearance from plasma was faster in the HBI group than the LBI group. In the same study, a similar pattern of plasma glucose was observed between the lines after a glucose challenge. Thus there appear to be no consistent differences between high and low genetic merit dairy cattle in peripheral sensitivity to circulating insulin.

2.2 Glucagon challenge

An alternative explanation for the higher basal plasma concentrations of glucose in the HBI dairy cattle (Flux *et al.*, 1984; Xing *et al.*, 1988; Mackenzie *et al.*, 1988; Xing, 1989) is that the glycogenolytic or gluconeogenic response to circulating glucagon is greater in the HBI dairy cattle than the LBI dairy cattle. However, such an hypothesis has not been confirmed by the study of Xing (1989) which showed with young heifers that there was no differences between the two lines in glycogenolytic or gluconeogenic response to a glucagon challenge at two feeding levels (75% and 125% maintenance energy requirement). Mackenzie *et al.* (1988) reported that HBI bulls had a reduced glucose response to the glucagon challenge, suggesting that they are less sensitive to the stimulation of glycogenolysis or gluconeogenesis by this hormone.

Given these results, and those from the insulin challenge discussed above, it is difficult to explain how the HBI group maintain their relatively higher blood glucose levels while simultaneously exhibiting elevated circulating concentrations of insulin.

2.3 Glucose challenge

The higher basal insulin concentrations of the HBI dairy cattle from the Massey University (Flux *et al.*, 1984; Xing *et al.*, 1988; Mackenzie *et al.*, 1988; Xing, 1989) could also be interpreted as suggesting that the pancreatic B-cells of the HBI dairy cattle are more sensitive to circulating glucose than those of the LBI dairy cattle. Insulin response to a glucose challenge has been used to measure this effect. While some studies with young bulls (Mackenzie *et al.*, 1988), yearling heifers (Xing, 1989) and lactating cows (Michel *et al.*, 1990) have shown a greater pancreatic sensitivity in the HBI group, others with young heifers (Xing, 1989) have not. However, it is important to note that in the study of Xing (1989), the basal plasma concentrations of insulin tended to be lower in the HBI calves than in the LBI calves (i.e. the reverse of the situation most commonly observed). Furthermore, another study with milk-fed calves (Xing *et al.* 1988) showed that insulin response to an arginine challenge was greater in the HBI calves, indicating differences in pancreatic sensitivity between the two lines. Therefore, it can be suggested from the above discussion that the higher basal plasma concentrations of insulin in the HBI dairy cattle from the Massey University selection lines can, at least in part, be explained by their greater pancreatic sensitivity to circulating glucose.

2.4 Adrenalin challenge

Xing (1989) reported that, in young heifers (6-8 months old), the plasma NEFA release in response to an adrenalin challenge was greater in HBI animals than in LBI animals when they were subjected to chronic underfeeding conditions (75% maintenance energy requirement). Since one of the major actions of adrenalin is to promote lipolysis, this result suggests that the responsiveness of adipose tissue to this hormone is greater in the high genetic merit dairy cattle during an energy deficit (i.e. that they are better equipped to break down fat and use it as an energy source in times of energy deficit). However, such differences were not apparent when the animals were well fed (125% maintenance energy requirement). Similarly, other studies with heifers (Bridges *et al.*, 1987; Xing, 1989) and bulls (Mackenzie *et al.*, 1988) failed to

identify differences between the lines in lipolytic response to an adrenalin challenge under either well-fed conditions or fasting. However,in one of these studies (Bridges *et al.*, 1987), the glucose response to adrenalin was greater in the HBI heifers.

2.5 Propionate challenge

Some studies have attempted to detect possible differences in carbohydrate metabolism between high and low genetic merit dairy cattle by infusion of propionate, the major precursor for gluconeogenesis in ruminants. These attempts were generally not successful in separating the genetic groups when animals were challenged after feeding (Sinnett-Smith *et al.*, 1987) or during fasting conditions (Tilakaratne *et al.*, 1980; Land *et al.*, 1983).

3 Fasting/refeeding

While "metabolic challenges" offer a potentially useful means of detecting differences between the genetic groups in sensitivity to homeostatic signals, such regimes are often associated with some difficulties. One major problem is that the challenges require carefully controlled experimental conditions and intensive blood sampling. Furthermore, use of exogenous hormones required in studies of this type may be commercially expensive or limited by supply/purity/disease control requirements. Hence, it is questionable whether they could be used to screen large number of animals.

The disturbance of homeostasis achieved by metabolic challenges can be also be achieved by fasting/refeeding. This has the advantage of requiring relatively simple blood sampling regimes and of being a "challenge" which induces changes in many body tissues. As a result, fasting/refeeding, unlike metabolic challenges, can be used to screen large number of animals. Furthermore, costs involved in these studies are much less than those involved in metabolic challenges. Therefore, a large number of studies have adopted these techniques to screen potential genetic markers for dairying.

3.1 Hormones

3.1.1 Somatotropin

Mackenzie *et al.* (1988) reported that the plasma concentrations of somatotropin were greater in the HBI bulls than in the LBI bulls during fasting conditions. Other studies with milk-fed calves (Xing *et al.*, 1988), heifers (Xing, 1989) and lactating cows (Flux *et al.*, 1984) have also shown that HBI dairy cattle tend to have higher somatotropin concentrations during energy deficits such as those occurring during fasting or underfeeding. Given that somatotropin is an important catabolic hormone, these results generally support the established concept that dairy cattle of high genetic merit have a greater ability to mobilize body reserves (largely fat) to meet the demands of the mammary gland during periods of negative energy balance such as in early lactation (Bryant *et al.*, 1981; Bauman *et al.*, 1983). However, such differences were not always consistent between and even within studies (Land *et al.*, 1983; Xing *et al.*, 1988), probably because of episodic secretion of somatotropin. This again emphasizes the difficulty of using somatotropin as a potential genetic marker for dairying.

3.1.2 Thyroid hormones

Sejrsen *et al.* (1984) measured the blood thyroxine levels of Red Danish bull calves (3.5 and 7 months old) during a fast and reported that the plasma concentrations of thyroxine (at both ages) were not different between high and low genetic merit animals throughout five days of fasting. However, when the measurement of thyroxine was extended into refeeding conditions, thyroxine levels were negatively correlated with the breeding value of the bulls for butter-fat yield at both ages. Since thyroid activity is an indicator of metabolic status of animals, these results may imply a lower metabolic rate and thus greater energetic efficiency in high line animals in this state. This hypothesis is further supported by the recent study of Sinnett-Smith *et al.* (1987) which reported with calves that the heart rate following fasting remainded lower in calves of high genetic merit for milk yield. However, such differences were not apparent in another study with calves (Land *et al.*, 1983) in which the plasma thyroxine concentrations during fasting and refeeding tended to be greater in the high line animals.

3.1.3 Insulin

Differences in circulating insulin concentrations between high and low producing dairy cattle, particularly in early lactation (i.e. the periods of negative energy balance) strongly suggest that expression of genetic merit for dairying in terms of plasma level of insulin may be enhanced when animals are subjected to a nutritional stress (Hart *et al.*, 1975, 1978, 1979; Bonczek *et al.*, 1988). Therefore, several studies have attempted to detect the genetic potential for milk yield in the plasma level of insulin at times of nutritional challenges such as during fasting (or underfeeding) and refeeding.

In overseas studies, efforts to enhance the expression of genetic merit for dairying by imposing a stressful environment on animals were not successful with respect to plasma insulin. Sejrsen *et al.* (1984) showed that, in male calves, there was a significant correlation between the plasma concentrations of insulin on the first day of fasting and breeding value of calves for butter-fat production at both 3.5 and 7 months old. However, such trends were not evident in other studies with calves of similar ages (Land *et al.*, 1983; Sinnett-Smith *et al.*, 1987). Even in the study of Sejrsen *et al.* (1984), the results are difficult to interpret since the correlation was positive or negative, depending on age of the animals. Moreover, only one sire was represented in each of the lines in that study.

While nutritional stimuli have generally failed to separate the genetic groups in overseas studies, such techniques have successfully separated the two Massey University lines in the plasma level of insulin. This occurred in milk-fed calves (Xing *et al.*, 1988), lactating cows (Flux *et al.*, 1984) and young bulls (Mackenzie *et al.*, 1988). In each case, the fasting plasma concentrations of insulin were greater in the high breeding index (HBI) group. These results support the previous notion that high plasma insulin levels are a characteristic of the Massey University HBI animals, although recent studies with young heifers (Xing, 1989) have failed to confirm such trends under similar conditions.

3.1.4 Glucagon

Previous reports (Herebein *et al.*, 1985; Sartin *et al.* 1985a,b; Sartin *et al.*, 1988) that plasma concentrations of glucagon are stable irrespective of stage of lactation may indicate that nutritional stimuli such as fasting or refeeding are unlikely to separate the genetic groups in the plasma level of glucagon. As expected, a recent study with yearling heifers (Xing, 1989) failed to find differences between the high and low breeding index groups in the levels of blood glucagon when animals were subjected to a 3-day fast and refeeding. Thus, plasma glucagon alone is, as noted previously, not a useful marker of genetic potential for milk yield.

3.2 Metabolites

3.2.1 Glucose

In the previous section (1.2.1 Glucose), failure to detect differences between dairy cattle of high and low genetic merit in the plasma level of glucose under well-fed conditions was considered likely to be a consequence of tight homeostatic control. Thus, nutritional stimuli such as fasting (or underfeeding) and refeeding have been used to enhance expression of genetic merit for milk yield in terms of the plasma level of glucose.

The effectiveness of these regimens has been verified by studies at Massey University. The first evidence for differences in glucose metabolism between the high breeding index (HBI) and low breeding index (LBI) dairy cattle was provided by Flux *et al.* (1984). In that study, involving 32 lactating cows, they reported that the plasma concentrations of glucose were greater in the HBI than LBI cows at a restricted feeding level (70% of ad libitum). Since then, the study of glucose metabolism between the HBI and LBI animals under different nutritional conditions has been extended into milk-fed calves (Xing *et al.*, 1988), young bulls (Mackenzie *et al.*, 1988) and yearling heifers (Xing, 1989). In each case, the plasma concentrations of glucose were greater in the HBI animals. This relatively higher level of blood glucose in the HBI dairy cattle could be a result of either increased glucose production or decreased peripheral glucose utilization. Whatever the reasons, differences in glucose metabolism between the HBI and LBI animals under fasting/refeeding conditions promise the possibility of using plasma glucose as a potential genetic marker for dairying.

Such a possibility is not, however, apparent in results from overseas studies. Although Sejrsen *et al.* (1984) observed a positive correlation between the increase in plasma glucose during refeeding and breeding value of bull calves at both 3.5 and 7 months old, other studies with calves (Tilakaratne *et al.*, 1980; Sinnett-Smith *et al.*, 1987) have failed to confirm these observations.

3.2.2 Non-esterified fatty acids (NEFA)

Tilakaratne *et al.* (1980) reported that, during a two-day fast, the plasma concentrations of NEFA were greater in high genetic merit calves than in low genetic merit calves. Consistent with this result, Sejrsen *et al.* (1984) found that the circulating blood NEFA levels of calves at 3.5 and 7 months old were positively correlated with breeding value although this relationship was sustained only in the early stage of fasting (until the second day). Since increased plasma NEFA is indicative of mobilization of fat from adipose tissue, these results suggest that animals with high genetic merit for milk yield have a greater capacity to mobilize body reserves during an energy deficit. Nevertheless, the use of fasting plasma NEFA as a genetic marker seems to be unreliable since subsequent studies with calves (Sinnett-Smith *et al.* 1987), young bulls (Mackenzie *et al.*, 1988), heifers (Xing, 1989) and lactating cows (Flux *et al.*, 1984) have failed to confirm these observations under similar conditions. Failure to detect consistent differences in circulating NEFA between the genetic groups in different studies may partially reflect the sensitivity of NEFA concentrations to environmental stress, thus underlining the difficulties of using such concentrations as a genetic marker.

3.2.3 Urea

The study by Tilakaratne *et al.* (1980) reported that the fasting plasma urea concentrations were lower in high genetic merit calves than in low genetic merit calves. Similarly, in the study with Red Danish bull calves, Sejrsen *et al.* (1984) found a negative correlation between fasting plasma urea levels and breeding value at 3.5 and 7 months of age. In addition, several other studies have shown low levels of blood urea during a fast in dairy calves of high genetic merit (Sinnett-Smith *et al.*, 1987; Woolliams *et al.*, 1988; Xing, 1989). Thus, differences in fasting plasma urea levels between the high and low genetic merit dairy cattle appear to be quite consistent.

Considering that each experiment involves different breeds, genetic backgrounds of cattle and in some cases different fasting regimes, these consistent results suggest that fasting urea concentrations is a repeatable genetic marker and not a peculiarity of one breed, line or sire. Therefore, blood urea concentration during a fast is one of the most promising genetic markers for dairying available at the present time.

3.2.4 Creatinine

There has been only one report regarding the effect of genetic merit for dairying on plasma creatinine levels during fasting and refeeding. In that study, involving 16 yearling heifers (Xing, 1989), the plasma concentrations of creatinine were monitored during a three-day fast and a refeeding period. Results showed that the plasma creatinine levels were lower in the high breeding index (HBI) heifers at both periods although differences were significant only at 41 and 56 hours of fasting. This result suggests that dairy cattle with high breeding values have lower rates of protein catabolism, since plasma creatinine arises from breakdown of body protein.

4 Line differences in plasma urea and their possible mechanisms

Of the numerous potential genetic markers studied, only a few seem to be promising as predictors of dairy merit. These include glucose, insulin, creatinine and urea. Among these potential genetic markers for dairying, plasma urea is of particular interest since differences in this metabolite are consistent among studies despite differences in breed, genetic background of dairy cattle and fasting regimes (Tilakaratne *et al.*, 1980; Sejrsen *et al.* 1984; Sinnett-Smith *et al.*, 1987; Woolliams *et al.*, 1988; Xing, 1989). Furthermore, similar differences in plasma urea have also been reported in pig lines selected for low vs high fatness (Mersmann *et al.*, 1984); in Romney sheep selected for high fleeceweight vs controls (McCutcheon *et al.*, 1987; Thomson *et al.*, 1989; Clark *et al.*, 1989); and in Southdown sheep selected for low vs high levels of subcutaneous fatness (Bremmers *et al.*, 1988; Carter *et al.*, 1989; Van Maanen *et al.*, 1989). In each case, lines of high genetic merit have low plasma urea concentrations. Therefore, plasma urea has potential as a predictor of genetic merit for productivity across a range of species and productive functions.

In general, plasma concentration of a metabolite such as urea is a function of the entry rate into the plasma pool, the size of the plasma pool and clearance rate from the plasma pool. The observed differences between the lines in plasma urea could therefore be explained by variation in one or more of these factors. Of these, pool size has received little attention with respect to selection line differences.

4.1 Entry rate

The principal source of urea is from liver conversion of ammonia produced by the rumen degradation of dietary protein and the deamination of absorbed amino acids. Therefore, the observed differences between the lines in plasma urea concentration could be partly due to differences in the relative importance of these components. However, there is little evidence that variation in entry rate of urea from rumen ammonia contributes to the line differences since the response of plasma urea to feeding was not different between the lines in any of the above studies. Conversely, some evidence indicates that the genetic differences in plasma urea are likely to reflect variation in the rate of amino acid deamination and thus utilisation of amino acids for productive functions. First, in studies with dairy cattle (Tilakaratne *et al.*, 1980; Sejrsen *et al.*, 1984; Sinnett-Smith *et al.*, 1987; Woolliams *et al.*, 1988; Xing, 1989), the plasma concentrations of urea during a fast were greater in the low genetic merit than high genetic merit animals. Since plasma urea during times of energy deficit arises primarily from catabolism of amino acids derived from labile protein, Tilakaratne *et al.* (1980) suggested that dairy cattle of high genetic merit preferentially derive energy from fat stores and thus spare amino acids. Second, in studies with pigs (Mersmann *et al.*, 1984) and sheep (Bremmers *et al.*, 1988; Carter *et al.*, 1989; Van Maanen *et al.*, 1989), plasma urea was greater in animals selected for high fatness than in those selected for low fatness at different feeding levels. These results have been interpreted as indicating that lean animals utilize amino acids more efficiently for protein synthesis, with consequent reduced requirement to deaminate amino acids (Mersmann *et al.*, 1984). Third, in studies with the control and high fleeceweight sheep (McCutcheon *et al.*, 1987; Thomson *et al.*, 1989; Clark *et al.*, 1989), plasma urea concentrations were greater in the control than in the fleeceweight-selected groups. The higher plasma urea levels in the control group were again suggested to be a consequence of inefficient utilization of amino acids for wool production. This hypothesis was substantiated by the study of Clark *et al.* (1989) which showed that infusion of methionine (a limiting amino acid for wool growth) reduced the plasma urea concentration in the control group, while having no effect in the selection group.

4.2 Clearance rate

Differences between the lines in plasma urea may also reflect variation in kidney function. It has been found that, in the studies with Romney fleeceweight-selected and control lines (McCutcheon *et al.*, 1987; Clark *et al.*, 1989), the plasma creatinine levels were also lower in the fleeceweight line. These results indicate that the control group had a lower glomerular filtration rate (GFR) since this is often a characteristic of animals with high levels of substances such as urea and creatinine which are not actively secreted or reabsorbed in the kidney nephron. However, differences in circulating creatinine were not apparent in the studies with Southdown sheep selected for high or low fatness when animals were exposed to similar experimental conditions (Bremmers *et al.*, 1988; Van Maanen *et al.*, 1989). This occurred in spite of an apparently greater clearance rate of urea in the high genetic merit(lean) group. Therefore, the differences between the lines in plasma urea can not be explained entirely by differences in GFR and further studies are required to adequately define differences in kidney function between the lines.

5 Purpose and Scope of the investigation

The identification of markers, such as blood metabolite and hormone concentrations, which accurately predicted genetic merit of dairy cattle at an early age could greatly benefit the dairy industry by increasing the rate of genetic improvement. Such markers should, at a minimum, meet the three major criteria. First, a marker must be easily measured, its assay being both inexpensive and manageable with respect to ease and time involved. Second, a marker must be correlated with genetic merit for dairying and repeatable under a varity of conditions. Finally, use of the marker must not have negative effects on other economic traits.

The desire to identify markers satisfying the above criteria has led scientists to screen numerous potential genetic markers under a variety of environmental conditions. Of the markers examined, plasma urea concentration has emerged as one of the most promising markers since consistent differences in plasma urea have been reported not only in dairy cattle but also in other selection lines (i.e lean meat and wool). Nevertheless, as discussed previously, mechanisms responsible for these

differences are still not fully understood. Understanding of these mechanisms is of considerable importance since this could assist in the identification of experimental conditions which minimize effects of environmental factors, thus maximizing the expression of genetic merit in terms of the level of plasma urea. One objective of this study was therefore to elucidate mechanisms responsible for differences in circulating urea between dairy selection lines in terms of pool size and clearance rate by using intravenous urea/creatinine loads. Relationships between plasma urea concentrations and age were also examined in dairy calves from the Massey University selection lines since Sejrsen *et al.* (1984) have shown that, in Red Danish bull calves, the expression of genetic differences in plasma urea concentration varies with age of the animals.

In addition to having low plasma urea concentrations, high genetic merit dairy cattle have been found to maintain high concentrations of insulin, glucose and NEFA. Such differences were particularly apparent when energy homeostasis of animals was altered by nutritional stimuli such as fasting or refeeding. The opportunity was therefore taken in this study to further investigate circulating metabolite and hormone concentrations of dairy calves in the fasted and fed states.

CHAPTER II: EXPERIMENTAL

CHAPTER II

EXPERIMENTAL

PLASMA METABOLITE AND HORMONE CONCENTRATIONS IN FRIESIAN CALVES OF LOW OR HIGH GENETIC MERIT : EFFECTS OF SEX AND AGE

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ABSTRACT

The aim of the present study was to investigate the potential use of blood metabolite and hormone concentrations as genetic markers for milk fat production and their possible interactions with sex and age. Two groups of calves, one from the Massey University High Breeding Index (HBI) line of dairy cattle (7 males and 8 females) and the other from the Low Breeding Index (LBI) line (4 males and 11 females), were studied at 3.5 months and 7 months of age. The average breeding indices (BI) of the calves based on ancestry BI in the two groups were 138 and 111 respectively.

Serial blood sampling regimens were conducted in relation to feeding (1.3 times maintenance energy requirement, Period I), during an intravenous urea (120mg/kg liveweight) and creatinine (1.81mg/kg liveweight) challenge (Period II) and during fasting (63 hours) and refeeding (Period III). Urea and creatinine determinations were made on blood samples from all three periods, glucose and insulin on samples from periods I and III, and NEFA on samples from period III.

Plasma concentrations of urea, creatinine and NEFA were not significantly different between the lines in any of periods examined and at either age. In contrast, plasma concentrations of glucose and insulin were greater in the HBI calves than in the LBI calves although these differences were mainly restricted to the period immediately

after feeding. Urea space at 7 months of age and creatinine space at both ages were also greater in the HBI animals than in the LBI animals. Spaces were calculated by back-extrapolation of baseline-adjusted post-challenge metabolite concentrations.

Plasma concentrations of all hormones and metabolites except insulin were significantly influenced by sex and/or age. Variation between animals in plasma glucose was less than that in plasma concentrations of other hormones and metabolites, reflecting the fact that circulating glucose is under tight homeostatic control.

In conclusion, this study does not confirm previous findings that genetic merit for dairying is expressed in plasma levels of urea and NEFA, particularly those during a fast. However, the results of the present study are consistent with previous Massey University observations of differences in glucose and insulin metabolism between the two genetic merit lines. Such traits may therefore have potential as genetic markers for milk fat production.

INTRODUCTION

Several studies have reported differences between calves of low or high genetic merit in circulating concentrations of metabolites and hormones, particularly during fasting and refeeding (Tilakaratne, Alliston, Carr, Land and Osmond, 1980; Sejrsen, Larsen and Andersen, 1984; Sinnett-Smith, Slee and Woolliams, 1987; Woolliams and Smith, 1988; Mackenzie, Wilson, McCutcheon and Peterson, 1988). The most consistent difference observed to date has been in concentration of urea.

Tilakaratne *et al.* (1980) found that calves of high genetic merit had low plasma levels of urea but increased circulating non-esterified fatty acid (NEFA) concentrations during a fast, compared with calves of low genetic merit. They suggested that high genetic merit animals preferentially used NEFA as an energy source and thus spared amino acids. Subsequent studies (Sejrsen *et al.*, 1984; Sinnett-Smith *et al.*, 1987; Woolliams *et al.*, 1988) have also shown that plasma urea concentrations of high genetic merit calves are lower than those of low genetic merit calves. The observations of Sejrsen *et al.* (1984) suggest that the effect of genetic merit on plasma urea concentrations is more apparent at 3.5 months than at 7 months of age. Similar differences have been observed in other species, lower plasma urea concentrations

being associated with selection for increased fleeceweight in Romney sheep (McCutcheon, Mackenzie and Blair, 1987; Clark, Mackenzie, McCutcheon and Blair, 1989; Thomson, Dellow and Barry, 1989), and for reduced fatness in Southdown ram hoggets (Bremmers, Morgan, McCutcheon and Purchas, 1988; Carter, McCutcheon and Purchas, 1989; Van Maanen, McCutcheon and Purchas, 1989) and in pigs (Mersmann, Pond and Yen, 1984). Plasma urea concentration may therefore be a useful predictor of genetic merit for milk yield (Woolliams *et al.*, 1988) and other traits.

Studies of the Massey University Friesian selection lines have failed to show the same relationship between circulating urea concentration and dairy merit. Mackenzie *et al.* (1988) showed that there were no differences between low breeding index (LBI) and high breeding index (HBI) calves in plasma urea during fasting but that urea increased more rapidly in HBI calves during refeeding. More recent observations in these lines (Xing, 1989) suggest that this may be due to differences in urea space.

The objective of this study was therefore to examine differences in plasma concentration of urea and other metabolites/hormones between the Massey University low and high BI calves at two ages and under a variety of nutritional regimens. The physiological basis of differences in circulating urea was examined using an intravenous urea load to determine pool size and clearance rate.

MATERIAL AND METHODS

1 Animals

Thirty Friesian calves from the Massey University high breeding index (HBI) and low breeding index (LBI) selection lines were used. Two groups, one HBI and the other LBI, were selected from 62 calves born in spring 1987. Calves were selected to ensure that, as far as possible, sex and sire groups were balanced. The average estimated breeding index of HBI calves was 137.9 (range from 132 to 144 with s.e. 4.4) while the corresponding value of the LBI group was 110.6 (range from 105 to 113 with s.e. 2.3). Further information on these animals, including BI, sex, weight, age, and dam and sire BI are presented in Appendix 1.

The calves were divided at random into two blocks (A and B) to facilitate housing and administration of metabolic challenges (see Appendix 1). The first experiment commenced at a nominal age of 3.5 months (November/December) with blocks A and B being studied 3 weeks apart. Studies were then repeated at a nominal age of 7 months (March). During the interval between the first and second experiments animals were divided on the basis of sex and grazed separately on mixed ryegrass/white clover pastures.

2 Housing and Feeding

Twelve days before the start of each experiment the animals were transferred from grazing to the Animal Physiology Unit, weighed and penned individually. For the first experiment (3.5 months) animals were housed in a calf-shed with sawdust bedding while for the second experiment (7 months) they were housed in a free stall barn with scoria bedding. Bedding was cleaned and, where necessary, replaced each morning. Fresh water was available ad libitum. Animals were allowed a 12-day adjustment period before the start of each experiment. During this period they were fed chaffed lucerne hay (at 130% of maintenance requirement) once daily at 1200h. Maintenance requirement was calculated as 0.5 MJ ME/kg$^{0.75}$ and feed was assumed to contain 9.5 MJ ME/kg DM (Holmes and Wilson, 1984). The calves were supplemented with a mineral mix (59% sodium chloride, 37% sodium sulphate, 4% sodium molybdate) to avoid possible copper toxicity. This was mixed with the feed at a rate of 1g/day for 3.5 month old animals and 2g/day for 7 month old animals. Animals were reweighed at cannulation (day 11) and feed allowances recalculated.

3 Cannulation procedure

On day 10 of the adjustment period, the neck and shoulder of the animal was clipped and on the following day the clipped area of the neck was washed using 70% ethanol.A local anaesthetic (10% Xylocaine, Astra Pharmaceuticals Ltd.,N.S.W, Australia) was then sprayed on the skin over the jugular vein. To facilitate cannulation the jugular vein was occluded by pressing below the cannulation site with the thumb and an Angiocath intravenous placement unit (12G needle and sleeve; Sherwood Medical, St.Louis, Mo., U.S.A) was inserted through the skin into the vein. Once blood was obtained from the vein, the needle within the sleeve was withdrawn slowly and a sterile cannula (Internal diameter 1.0mm, external diameter 1.5mm, Dural Plastics and

Engineering, N.S.W, Australia) was passed through the sleeve and 100mm into the vein. After testing blood flow, the sleeve was withdrawn and the cannula fixed in place by wrapping at the wound site with plastic tape (Sleek Tape, Smith and Nephew Med.Ltd., Hull, England), embedding suture material in the tape and passing a single suture through the skin. After suturing, Aureomycin powder was applied to the wound to prevent infection. Finally the cannula was passed to a point behind behind the neck and held in place with 75mm elastic adhesive bandage. Before the animal was returned to its pen, 5ml of antibiotic (Streptopen, Lot 312620, Glaxo,N.Z) was injected to prevent infection. After cannulation rectal temperature was monitored daily.No signs of elevated rectal temperature were observed during the experiment.Patency of cannulae was maintained with 0.9% saline containing 100 IU/ml sodium heparin (Batch 128B, Wendal Pharmaceuticals Ltd, London) and 0.4ml/l oxytetracycline (Terramycin Q-100, Pfizer laboratories, Auckland, N.Z).

4 General experimental procedure

At each age,the experiment was conducted in three periods, commencing on day 13 of the study.

Period I (day 13). Calves were given their daily ration at 1200h. Blood samples were withdrawn at -60, -30 and -10 minutes relative to the time of feeding then at hourly intervals for 12h after feeding.

Period II (day 14). On the second day of the experiment calves were subjected to intravenous urea (120mg/Kg liveweight) and creatinine (1.81mg/kg liveweight) challenges administered as a single bolus in approximately 4ml of saline. The challenge was administered at 1000h and blood samples collected at -60, -40, -20, 15, 30, 60, 120, 180, 240, 300, 360 and 420 minutes relative to the time of injection. The daily feed allowance was then offered at 1700h.

Period III (days 15-18). Calves were fasted for 63h at the end of period II. During this period blood samples were collected at 12h intervals, commencing 15h after the last feed. At the end of the fast calves were offered half their daily ration at 0900h. Blood samples were collected 30, 60, 120 and 240 min after refeeding.

5 Collection and Processing of blood

Blood samples (10ml) were withdrawn via the indwelling jugular cannulae and transferred to centrifuge tubes containing 0.1 ml of 35% (w/v) sodium citrate as the anticoagulant. The centrifuge tubes were kept on ice and within one hour were centrifuged at 2500g and 4°C for 20 minutes. Plasma was then pipetted off into duplicate vials and stored at -20°C until analysed.

6 Chemical analyses

Urea and creatinine concentrations in plasma were determined by the autoanalyser methods of Marsh, Fingerhut and Miller (1965) and Chasson, Grady and Stanley (1961), respectively. Plasma glucose was determined by a Peridochrom Auto Analyser (Boehringer Mannheim Gmbh, Diagnostica) based on the enzymatic colourimetric method of Trinder (1969) and Hoffmeister and Junge (1970). Plasma was also analysed for non-esterified fatty acids, using the modified colourimetric method described by McCutcheon and Bauman (1986). Intra- and inter-assay coefficients of variation for urea, creatinine, glucose and NEFA were 3.5 and 1.3, 0.4 and 1.9, 1.2 and 4.9, 2.6 and 3.8%, respectively. Insulin was assayed using the double antibody radioimmunoassay (RIA) method described by Flux *et al.* (1984). Bovine insulin (Sigma Chemical Co. Catalogue No I-5500, Lot No: 55F-0536, 23.4 IU/mg) was used for iodination and as the reference standard in insulin RIA. The intra- and inter-assay coefficients of variation for insulin RIA were 8.2 and 12.4%.

7 Statistical analysis

Liveweight and intake were subjected to multivarate analysis of variance (MANOVA) to test for the significance of main effects (line, sex and block), nominal age and their interactions. Effects of line, sex and block on actual age were tested by univariate analysis of variance. Block effects were found to be non-significant ($P>0.05$) and have therefore not been presented.

Plasma hormone and metabolite concentrations arising from sequential blood sampling during feeding (Period I) and fasting/refeeding (Period III) were subjected to MANOVA to test the main effects (line, sex and block) and the repeated factor (age),

together with their interactions, within each sampling time. Corresponding analyses were initially used to examine the challenge (Period II) data either uncorrected or corrected for prechallenge concentrations (by subtraction). Note that these analyses must be interpreted conservatively because while they take account of the repeated measures nature of the age effect, they do not account for the repeated nature of the sampling time effect. In all analyses block effects were fitted first in the model to permit comparisons of other main effects after adjustment for block. The block effects have not reported since they were an experimental convenience not central to the hypotheses being tested.

Post-challenge curves (corrected for baseline) of urea and creatinine were further analysed to determine the distribution space and fractional decay constant (K) by a single-compartment model (i.e $Y_{(t)}=Y_{(0)}e^{-kt}$ where $Y_{(t)}$ is the concentration of urea or creatinine at time t, $Y_{(0)}$ is the concentration at t=0 by back extrapolation and e is the base of natural logarithms). This model was fitted to the urea response data in the period from 30 to 300 minutes following the urea injection since plasma urea concentrations in most calves were approaching basal concentrations by 300 minutes following the injection. For creatinine, the corresponding intervals for this model were from 30 to 180 minutes post-challenge. Over 90% of the within-calf variation in plasma urea and creatinine concentrations during the chosen intervals was accounted for by this model. The distribution space of both urea and creatinine were calculated as the ratio of injected dose to $Y_{(0)}$. It was later found by analysis of the injected solution that the amount of creatinine injected was 50% greater than intended at 7 months of age. Thus, the calculation of creatinine space of the older animals was based on this greater value. The effect of line, sex, age and their interactions on distribution space and K value were then tested by MANOVA as described previously. All statistical analayses were performed using the generalised linear model computer packages 'REG' (Gilmour, 1985) and SAS (SAS, 1986).

RESULTS

1 Weight, feed intake and age

Effects of line, sex and nominal age on liveweight, feed intake and actual age are presented in Table 1. There were no significant differences between the lines in liveweight but HBI calves had greater intakes ($P<0.05$) than LBI calves. Thus HBI calves consumed a greater proportion of the feed offered since the feed allowance was proportional to live weight.

Male calves were heavier than females($P<0.01$), the between-sex difference in liveweight at 3.5 months being less than that at 7 months of age. As a result, the sex x age interaction was significant ($P<0.001$). Intakes were also greater in males at both ages, this effect being largely accounted for by their greater liveweights.

There were no differences between the lines or sexes in actual age at the time of the experiment. Effects of line and sex on weight and intake were additive at both nominal ages.

TABLE 1

Effects of selection line and sex on weight, feed intake and age of calves (Mean±se)

	Nominal	Selection line		Sex	
	Age (months)	LBI (15)	HBI (15)	Female (19)	Male (11)
Weight (kg)	3.5	94.4±2.9	97.5±2.5	92.9±2.1	101.1±3.4
	7.0	146.8±6.4	156.6±6.3	140.0±3.3	171.9±7.8
Intake (kg DM/day)	3.5	2.4±0.1	2.5±0.1	2.4±0.1	2.6±0.1
	7.0	3.4±0.1	3.8±0.1	3.4±0.1	3.9±0.1
Age (days)	3.5	104.4±2.8	108.4±2.1	105.0±2.5	108.7±2.2
	7.0	216.4±2.8	220.4±2.1	217.0±2.5	220.7±2.2

	Significance level						
	Line(L)	Sex(S)	LxS	Age(A)	LxA	SxA	LxSxA
Weight (kg)	NS	**	NS	***	NS	***	NS
Intake (kg DM/day)	*	*	NS	***	NS	NS	NS
Age (days)	NS	NS	NS	N/A	N/A	N/A	N/A

NS P>0.10 * P<0.05 ** P<0.01 *** P<0.001 N/A not applicable

2 Feeding responses (Period I)

The pattern of plasma urea levels in response to feeding is illustrated in Figure 1. At both ages urea was stable before feeding, increased after feeding (reaching maximum values within 4 hours) and declined thereafter. The concentration of urea during this period was not influenced by line or sex although there was a significant sex x age interaction at most of the sampling times (Table 2). At 3.5 months of age, males generally had greater plasma urea concentrations than females, whereas at 7 months of age the reverse was true. There was also a significant difference between ages in urea level. Compared with 7 month-old animals, 3.5 month-old animals maintained greater concentrations of urea ($P<0.001$) at all sampling times.

TABLE 2

Significance of effects of line, sex, age and their interactions on plasma urea concentration in response to feeding

Effect	Significance of effect at sampling time[a]													
	-1	-0.5	-0.2[b]	1	2	3	4	5	6	7	8	9	10	11	12
Line (L)	----------						NS						----------		
Sex (S)	----------						NS						----------		
LxS	----------						NS						----------		
Age (A)	----------						***						----------		
LxA	----------						NS						----------		
SxA	**	**	**	**	------		***	----		**	**	*	NS	NS	NS
LxSxA	----------						NS						----------		

NS $P>0.10$ * $P<0.05$ ** $P<0.01$ *** $P<0.001$

[a] Sampling time (hours) relative to feeding at time zero (1200 h)

[b] Actual sampling time is -10 minutes relative to feeding

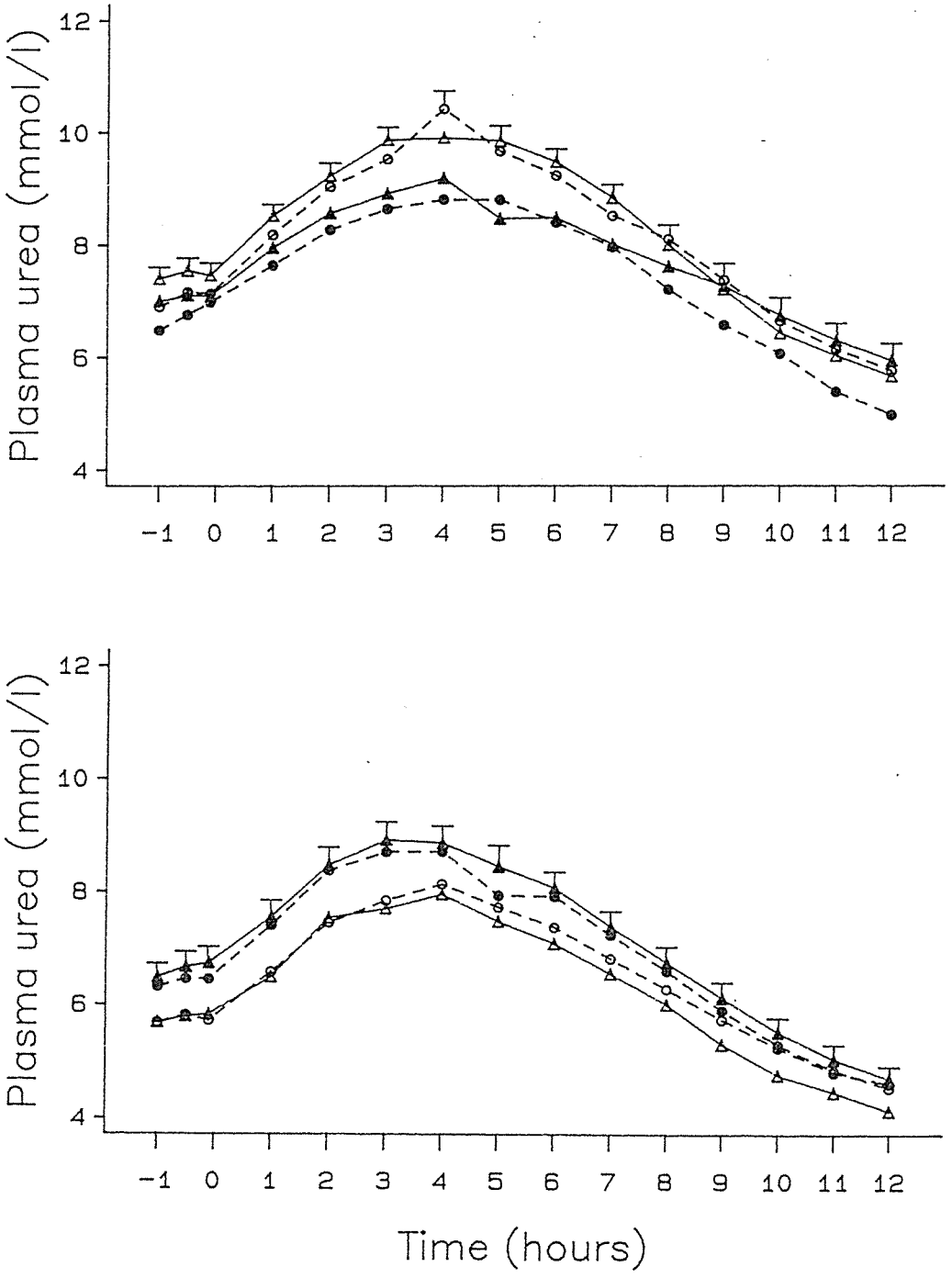

Fig. 1. Plasma concentrations of urea at 3.5 (upper panel) and 7.0 (lower panel) months of age in LBI male (open triangle) and female (closed triangle) or HBI male (open circle) and female (closed circle) calves in response to feeding. Vertical bars denote the pooled standard error at each sampling time.

Plasma creatinine concentrations, which were greater in 7-month old animals than in 3.5-month old animals (P<0.001), were similar between the lines and did not increase after feeding (Figure 2, Table 3). The effect of sex on creatinine level was apparent only in the later stages of sampling. During this period, male calves maintained greater creatinine concentrations than did female calves at both 3.5 months and 7 months of age.

TABLE 3

Significance of effects of line, sex, age and their interactions on plasma creatinine concentration in response to feeding

Effect	Significance of effect at sampling time[a]													
	-1	-0.5	-0.2[b]	1	2	3	4	5	6	7	8	9	10	11	12
Line (L)	----------						NS								----------
Sex (S)	----------		NS				*	NS	*	*	*	*	*	*	+
LxS	----------						NS						+	+	NS
Age (A)	----------						***								----------
LxA	----------						NS								----------
SxA	----------						NS								----------
LxSxA	----------						NS								----------

NS P>0.10 + P<0.10 * P<0.05 ** P<0.01 *** P<0.001

[a] Sampling time (hours) relative to feeding at time zero (1200 h)

[b] Actual sampling time is -10 minutes relative to feeding

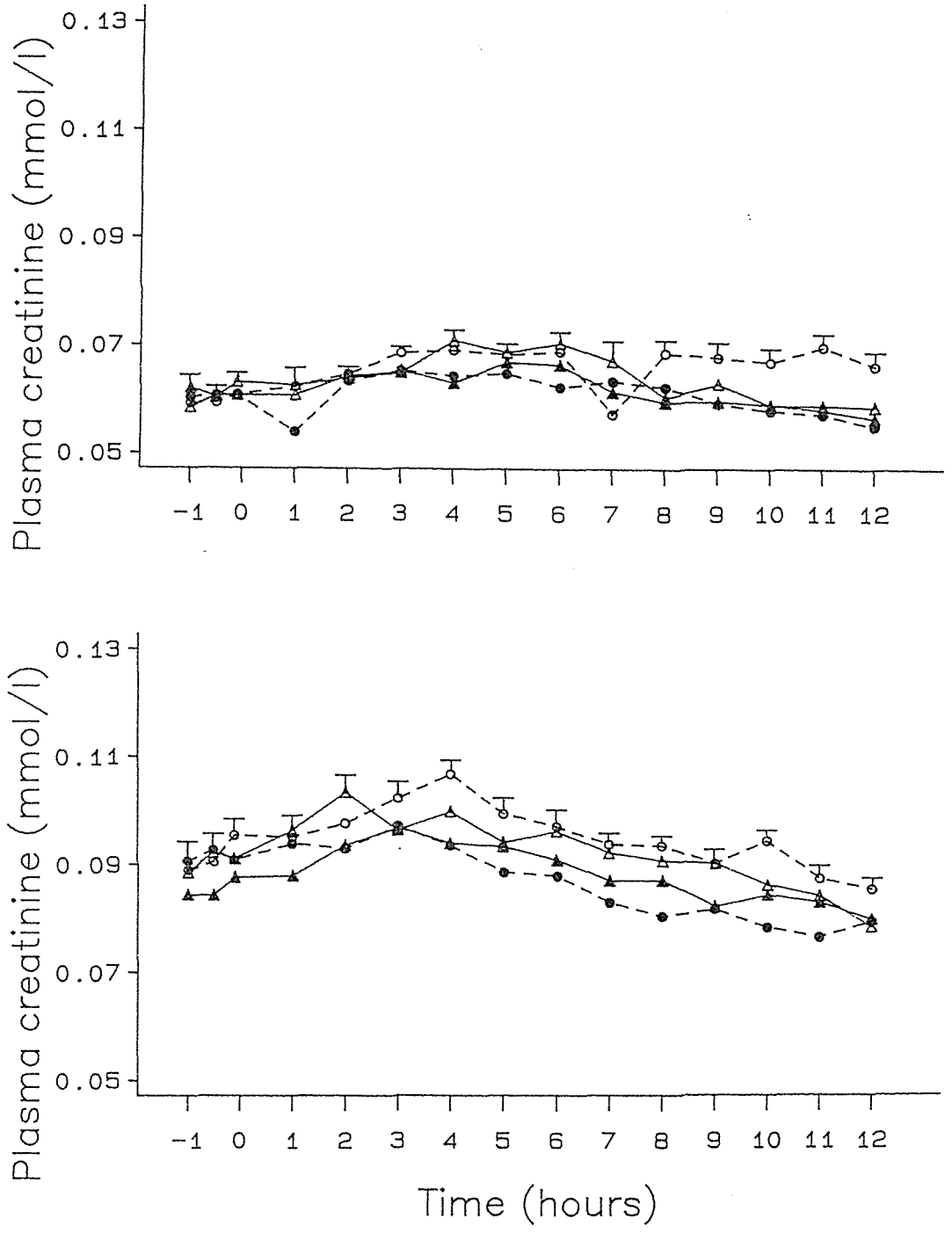

Fig. 2. Plasma concentrations of creatinine at 3.5 (upper panel) and 7.0 (lower panel) months of age in LBI male (open triangle) and female (closed triangle) or HBI male (open circle) and female (closed circle) calves in response to feeding. Vertical bars denote the pooled standard error at each sampling time.

Plasma glucose concentration declined until 1h after feeding and increased gradually thereafter at both ages (Figure 3). Plasma glucose concentrations across ages tended to be greater in the HBI than LBI group although such differences were apparent ($P<0.05$) only during the first few hours after feeding (Table 4). Similarly, during the interval 2 to 4h after feeding, male calves showed higher plasma glucose concentrations than female calves ($P<0.06$) although this situation was observed only at 7 months of age. There was also a significant ($P<0.05$) sex x age interaction at most of the sampling times. At 3.5 months of age, females generally showed greater plasma glucose concentrations than males, whereas at 7 months of age the reverse was true. However, this trend was apparent mainly in LBI calves. As a result, there was also a significant ($P<0.01$) line x sex x age interaction ($P<0.01$) prior to feeding and at 4 to 7 hours post-feeding. The difference in plasma glucose between ages was significant at most sampling times, 3.5 month-old animals having slightly higher plasma glucose levels than 7 month-old animals.

TABLE 4

Significance of effects of line, sex, age and their interactions on plasma glucose concentration in response to feeding

Effect	Significance of effect at sampling time[a]														
	-1	-0.5	-0.2[b]	1	2	3	4	5	6	7	8	9	10	11	12
Line (L)	NS	NS	+	**	*	*	NS	NS	NS	+	------		NS	--------	
Sex (S)	NS	*	NS	NS	**	*	+	----------				NS	----------		
LxS	----------						NS	----------							
Age (A)	*	*	**	**	**	*	*	*	+	*	*	*	**	**	**
LxA	NS	NS	NS	+	------		NS	----------							
SxA	**	*	*	*	**	+	*	+	NS	NS	+	*	*	*	NS
LxSxA	*	*	**	NS	NS	NS	*	**	*	**	---		NS	----------	

NS $P>0.10$ * $P<0.05$ ** $P<0.01$ *** $P<0.001$

[a] Sampling time (hours) relative to feeding at time zero (1200 h)

[b] Actual sampling time is -10 minutes relative to feeding

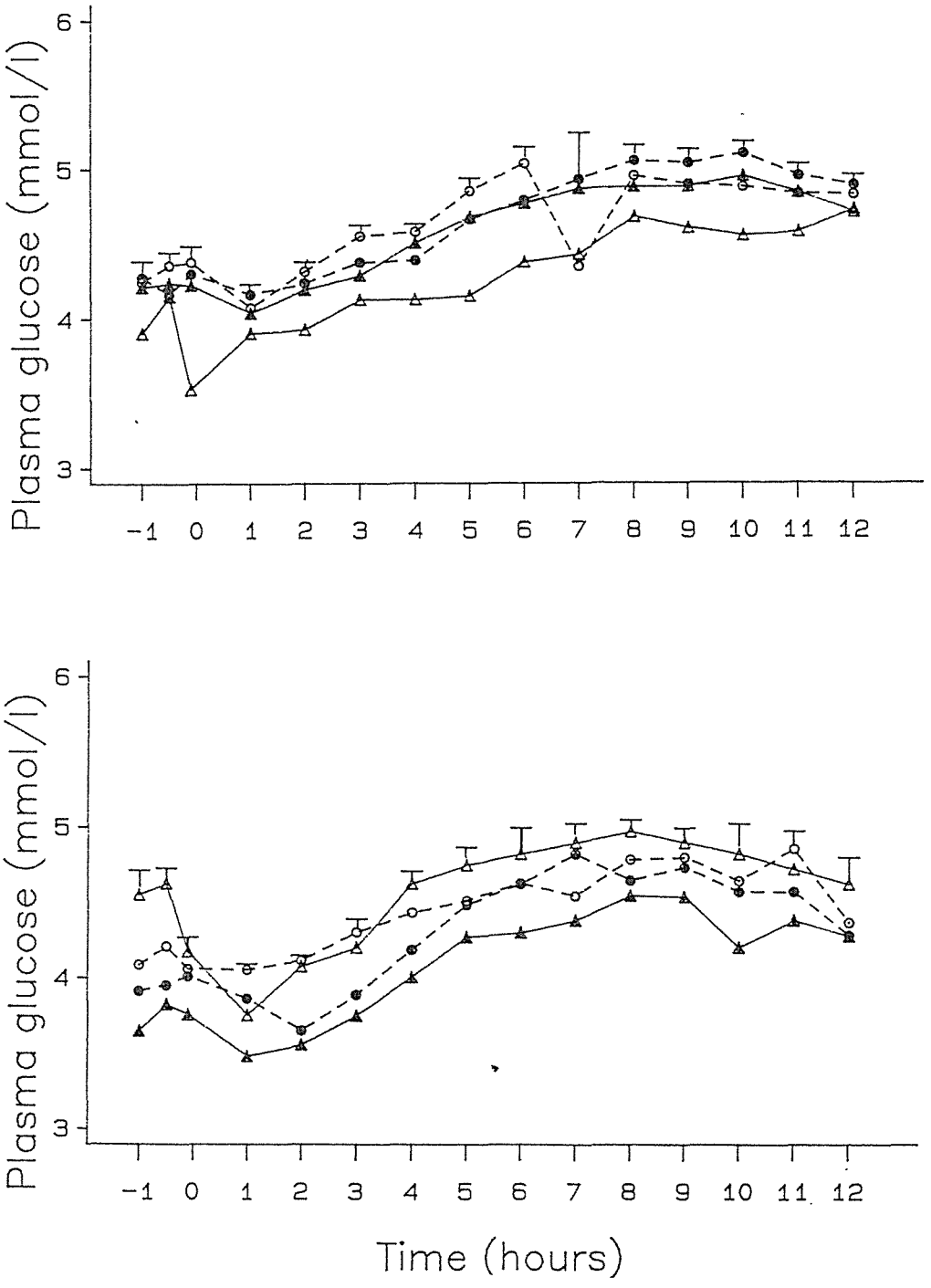

Fig. 3. Plasma concentrations of glucose at 3.5 (upper panel) and 7.0 (lower panel) months of age in LBI male (open triangle) and female (closed triangle) or HBI male (open circle) and female (closed circle) calves in response to feeding. Vertical bars denote the pooled standard error at each sampling time.

The average plasma concentrations of insulin were greater in the HBI animals than in the LBI animals at most of the sampling times (Figure 4). However, because of large variation within lines, significant differences between lines in plasma insulin were observed only at 1 hour before feeding and 1, 2, 7, 9, and 10 hours postfeeding (Table 5). Similarly, the plasma insulin concentrations were greater in female than in male calves at both 3.5 and 7 months of age although differences were again apparent only at 1, 7, 9 and 10 h after feeding. The effects of age and its interactions with line or sex on circulating blood insulin were not significant at most of the sampling times.

TABLE 5

Significance of effects of line, sex, age and their interactions on plasma insulin concentration in response to feeding

Effect	Significance of effect at sampling time[a]													
	-1	-0.5	-0.2[b]	1	2	3	4	5	6	7	8	9	10	11	12
Line (L)	**	NS	NS	**	*	NS	+	NS	NS	*	NS	**	**	+	NS
Sex (S)	NS	NS	+	*	------	NS	------			*	+	**	*	+	NS
LxS	NS	NS	*	---------	NS	----------									
Age (A)	NS	NS	NS	+	------	NS	----------								
LxA	*	----------	NS	------	+	NS	+	NS	NS	NS					
SxA	NS	NS	+	---------	NS	----------								*	NS
LxSxA	*	NS	*	---------	NS	----------								+	NS

NS P>0.10 + P<0.10 * P<0.05 ** P<0.01

[a] Sampling time (hours) relative to feeding at time zero (1200 h)

[b] Actual sampling time is -10 minutes relative to feeding

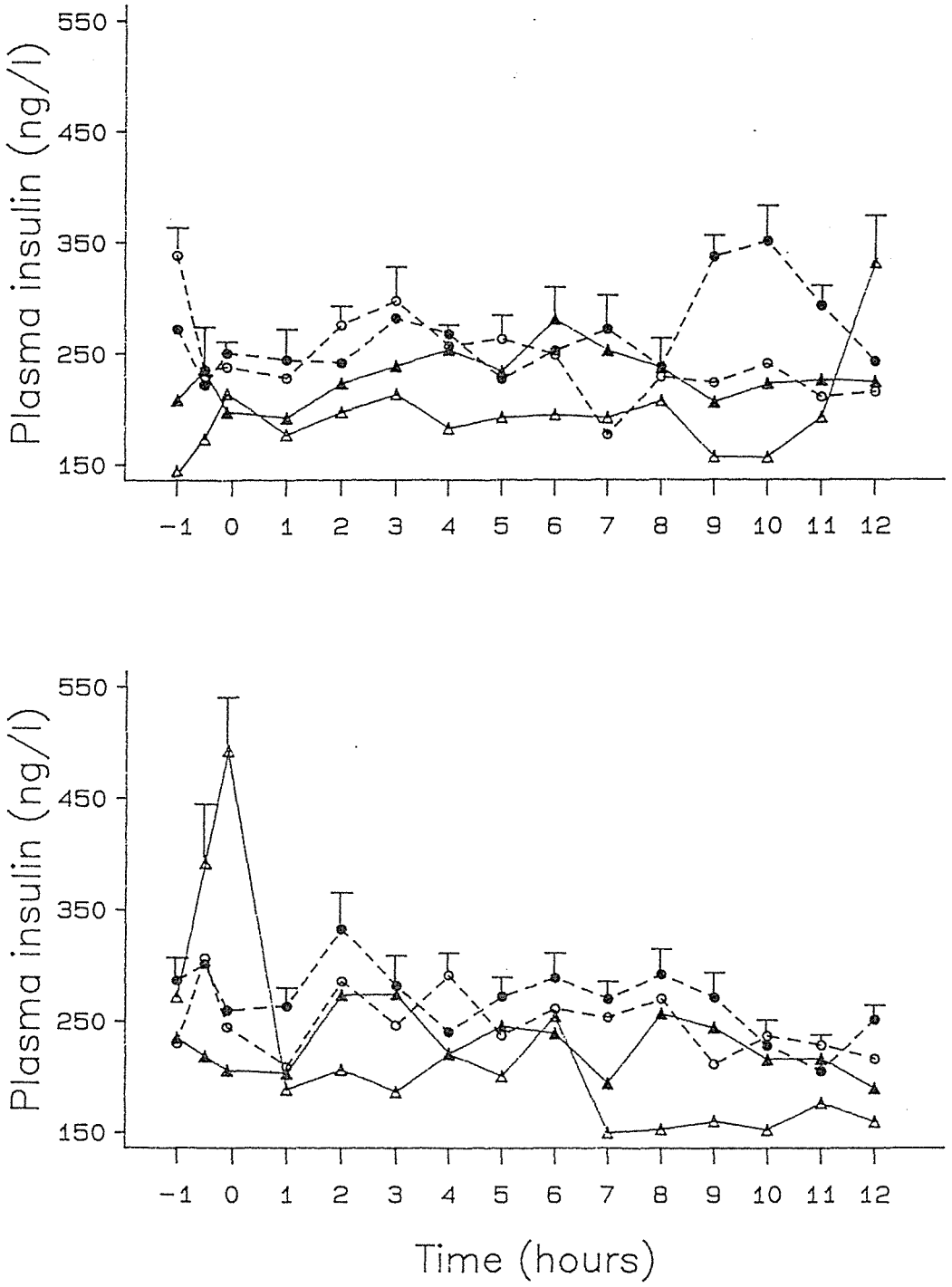

Fig. 4. Plasma concentrations of insulin at 3.5 (upper panel) and 7.0 (lower panel) months of age in LBI male (open triangle) and female (closed triangle) or HBI male (open circle) and female (closed circle) calves in response to feeding. Vertical bars denote the pooled standard error at each sampling time.

3 Fasting/Refeeding (Period III)

Plasma urea concentrations during fasting and refeeding are shown in Figure 5. At both ages plasma urea concentrations rose during the early stages of fasting and remained stable or declined slightly until refeeding when they again increased. There were no significant differences between lines in plasma urea during fasting and refeeding,but the plasma urea concentrations of young animals (3.5 months of age) were significantly (P<0.001) higher than those of old animals (7 months of age) at all sampling times (Table 6). Plasma urea concentrations were higher in male than in female calves at 3.5 months of age but not at 7 months of age. As a result, there were significant (P<0.05) sex x age interactions at most of the sampling times.

TABLE 6

Significance of effects of line, sex, age and their interactions on plasma urea concentration during fasting and refeeding

Effect	Fasting (h)					Refeeding (h)			
	15	27	39	51	63	0.5	1	2	4
Line (L)	----------	----------	----------	----------	NS	----------	----------	----------	----------
Sex (S)	----------	----------	----------	----------	NS	----------	----------	----------	----------
LxS	----------	----------	----------	----------	NS	----------	----------	----------	----------
Age	----------	----------	----------	----------	***	----------	----------	----------	----------
LxA	*	----------	----------	----------	NS	----------	----------	----------	----------
SxA	**	***	**	**	NS	*	*	*	**
LxSxA	----------	----------	----------	----------	NS	----------	----------	----------	----------

NS P>0.10 * P<0.05 ** P<0.01 *** P<0.001

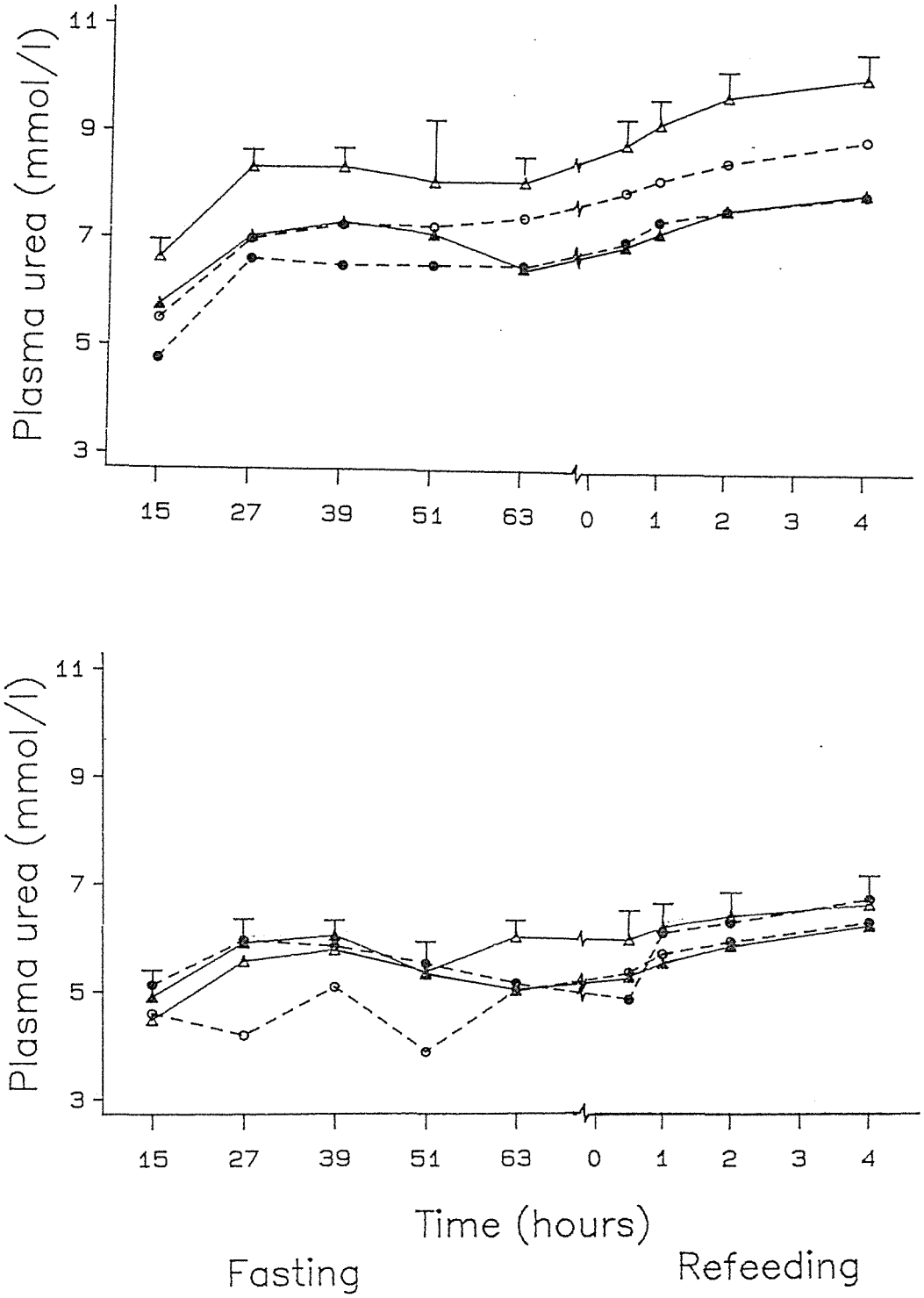

Fig. 5. Plasma concentrations of urea at 3.5 (upper panel) and 7.0 (lower panel) months of age in LBI male (open triangle) and female (closed triangle) or HBI male (open circle) and female (closed circle) calves during fasting and refeeding. Vertical bars denote the pooled standard error at each sampling time.

Plasma creatinine levels showed only a gradual increase during the fasting/refeeding period (Figure 6). Neither line nor sex had any significant effect on plasma creatinine at most of the sampling times (Table 7). Creatinine level was again affected by age (P<0.001), being greater in 7 month-old than in 3.5 month-old calves.

TABLE 7

Significance of effects of line, sex, age and their interactions on plasma creatinine concentration during fasting and refeeding

Effect	Fasting (h)					Refeeding (h)			
	15	27	39	51	63	0.5	1	2	4
Line (L)	----------	----------	----------	----------	NS	----------	----------	----------	----------
Sex (S)	NS	NS	NS	+	*	NS	NS	NS	NS
LxS	----------	----------	----------	----------	NS	----------	----------	----------	----------
Age (A)	----------	----------	----------	----------	***	----------	----------	----------	----------
LxA	----------	----------	----------	----------	NS	----------	----------	----------	*
SxA	----------	----------	----------	----------	NS	----------	----------	----------	----------
LxSxA	----------	----------	----------	----------	NS	----------	----------	----------	----------

NS P>0.10 * P<0.05 ** P<0.01 *** P<0.001

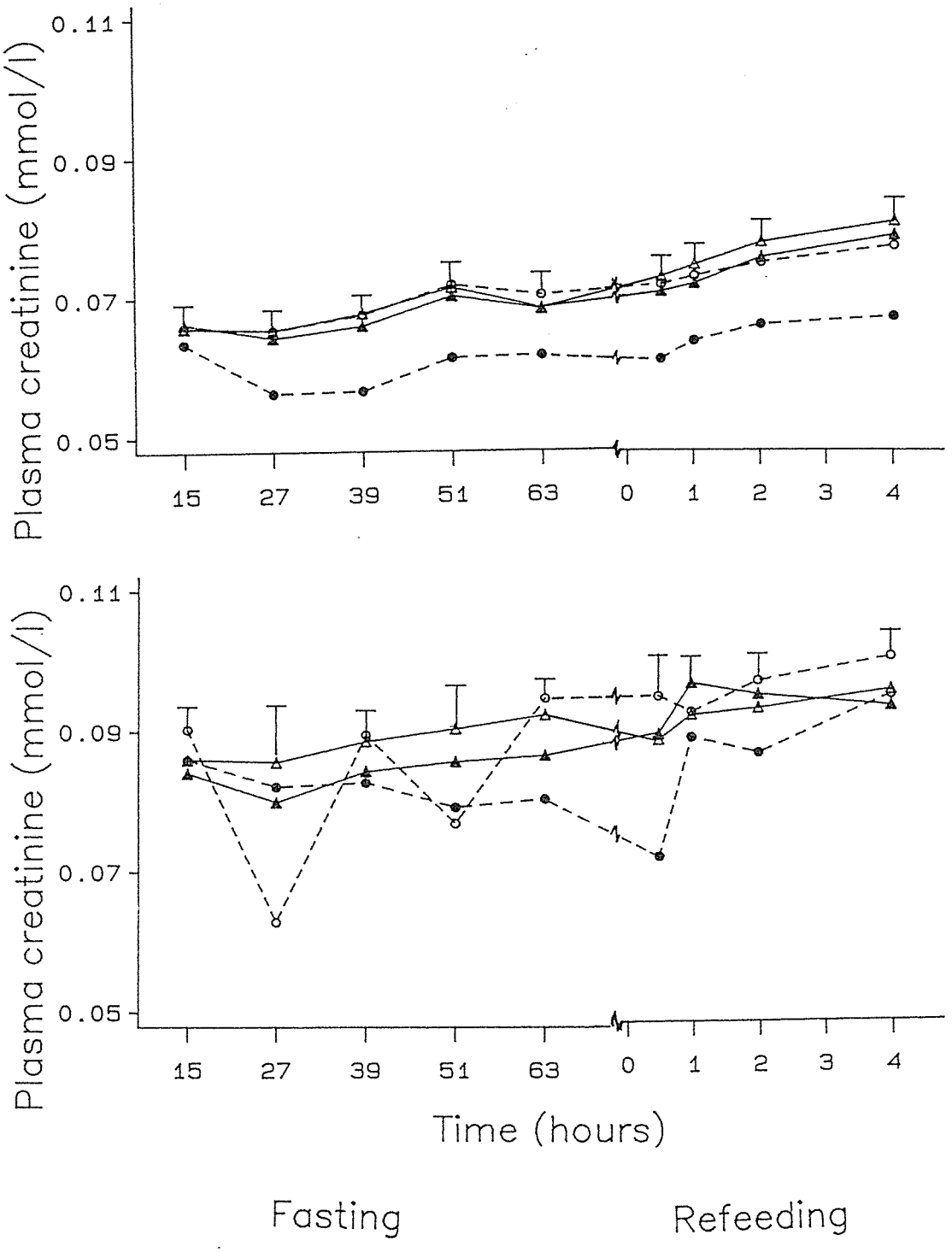

Fig. 6. Plasma concentrations of creatinine at 3.5 (upper panel) and 7.0 (lower panel) months of age in LBI male (open triangle) and female (closed triangle) or HBI male (open circle) and female (closed circle) calves during fasting and refeeding. Vertical bars denote the pooled standard error.

Plasma concentrations of glucose declined steadily during fasting and showed a small increase upon refeeding at both ages (Figure 7). There were no significant differences between the lines in plasma glucose during fasting and refeeding (Table 8), although the mean concentration tended to be higher in the HBI animals during the later stages of fasting (63h after fasting) and during refeeding. Again, there were significant ($P<0.001$) effects of age on plasma glucose at most of the sampling times, a higher level of plasma glucose being observed in animals at 7 months of age than in those at 3.5 months of age. Male calves had higher plasma concentrations of glucose at 7 months but not at 3.5 months, resulting in a significant ($P<0.01$) sex x age interaction at most sampling times.

TABLE 8

Significance of effects of line, sex, age and their interactions on plasma glucose concentration during fasting and refeeding

Effect	Fasting (h)					Refeeding (h)			
	15	27	39	51	63	0.5	1	2	4
Line (L)	----------NS----------								
Sex (S)	*	NS	*	*	+	NS	NS	+	+
LxS	----------NS----------								
Age (A)	NS	**	**	**	**	+	*	*	*
LxA	----------NS----------								
SxA	NS	**	**	*	***	**	*	**	*
LxSxA	NS	*	----------NS----------						

NS $P>0.10$ + $P<0.01$ * $P<0.05$ ** $P<0.01$ *** $P<0.001$

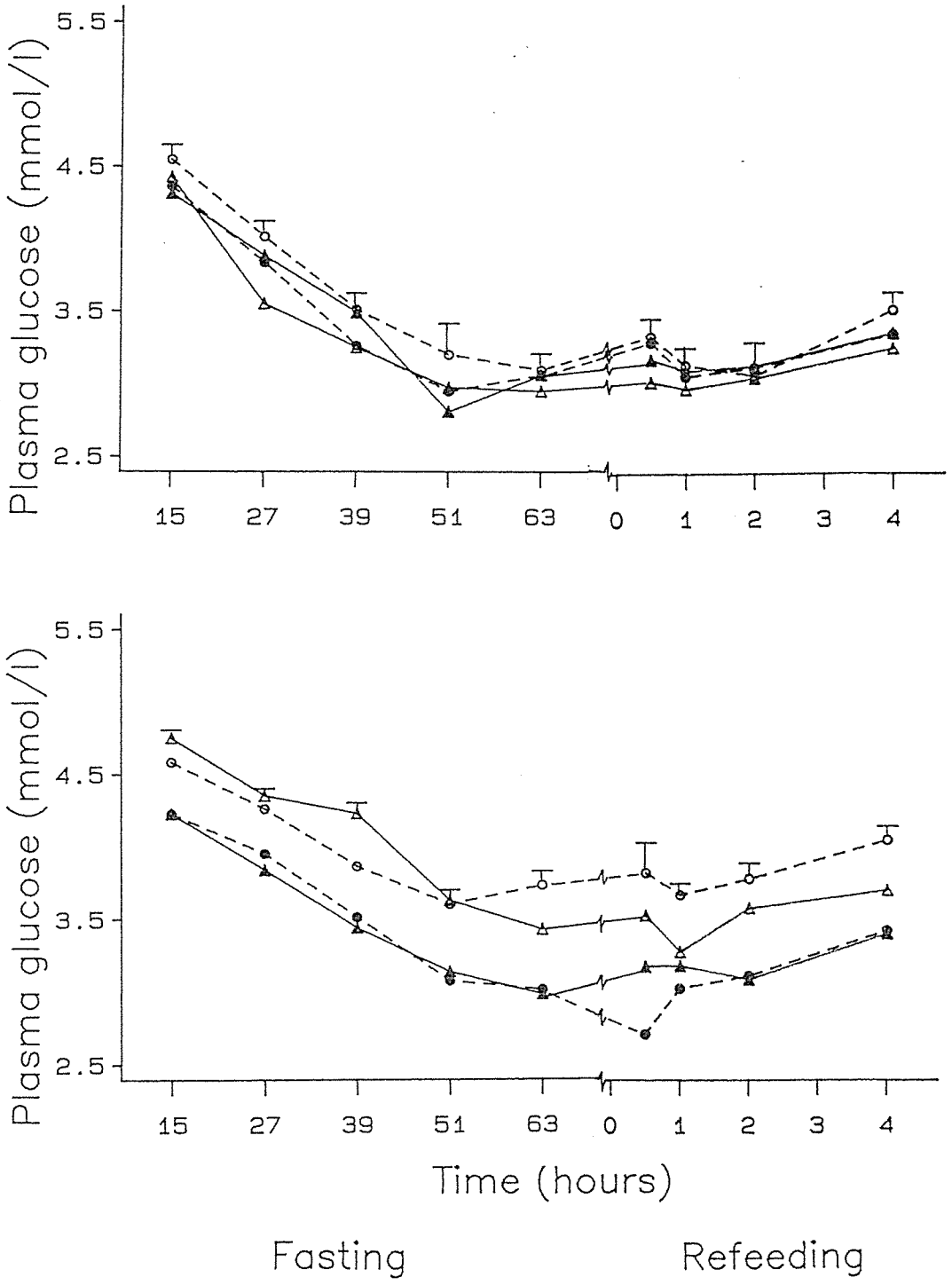

Fig. 7. Plasma concentrations of glucose at 3.5 (upper panel) and 7.0 (lower panel) months of age in LBI male (open triangle) and female (closed triangle) or HBI male (open circle) and female (closed circle) calves during fasting and refeeding. Vertical bars denote the pooled standard error at each sampling time.

Circulating concentrations of NEFA increased steadily during the fasting period and the highest values were recorded in the period just before refeeding (Figure 8). Refeeding initially resulted in a decline in plasma NEFA concentration but a plateau or small rise was observed 2 hours after feeding. In general, plasma NEFA did not show any line or sex effect during either period (Table 9), but male calves had higher NEFA levels than female calves during the early stages of fasting, particularly at 7 months of age. A significant effect of age on the plasma concentration of NEFA was observed at 39, 51, 63 hours of fasting and 30 minutes after refeeding, young animals showing slightly greater levels of NEFA than older animals.

TABLE 9

Significance of effects of line, sex, age and their interactions on plasma NEFA concentration during fasting and refeeding

Effect	Fasting (h)					Refeeding(h)			
	15	27	39	51	63	0.5	1	2	4
Line (L)	----------				NS	----------			
Sex (S)	**	*	----------		NS	----------			
LxS	----------				NS	----------			
Age (A)	NS	NS	*	***	*	**	NS	NS	*
LxA	----------				NS	----------			
SxA	*	*	NS	*	NS	+	NS	NS	NS
LxSxA	NS	NS	*	-----	NS	----------			

NS P>0.10 + P<0.10 * P<0.05 ** P<0.01 *** P<0.001

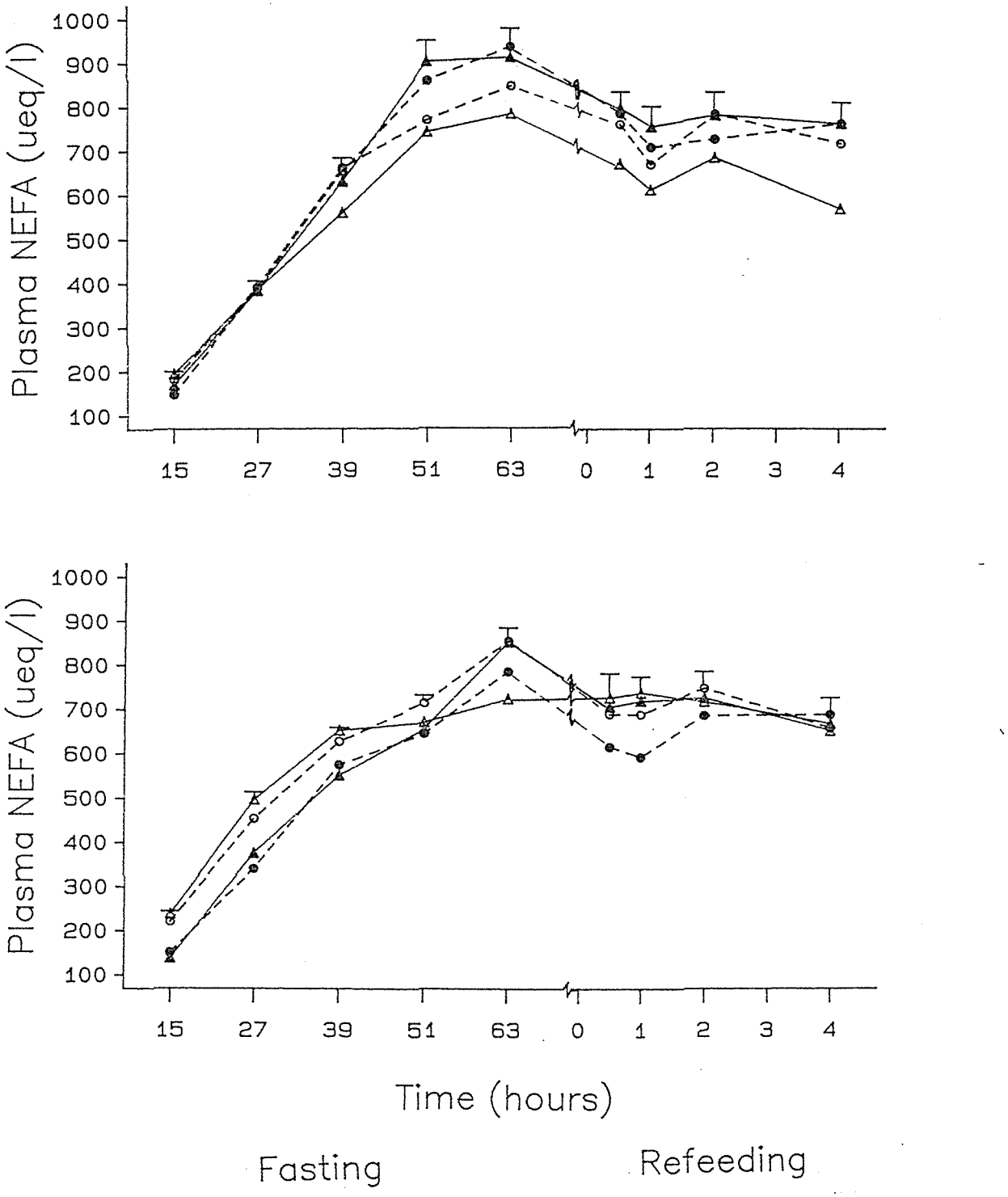

Fig. 8. Plasma concentrations of NEFA at 3.5 (upper panel) and 7.0 (lower panel) months in LBI male (open triangle) and female (closed triangle) or HBI male (open circle) and female (closed circle) calves during fasting and refeeding. Vertical bars denote the pooled standard error at each sampling time.

The plasma concentration of insulin did not show any marked changes during fasting or refeeding (Figure 9). Neither line nor sex influenced the insulin concentration (Table 10) although the HBI group tended to have greater levels of insulin during the early stages of fasting. No effect of age on the plasma concentration of insulin was observed.

TABLE 10

Significance of effects of line, sex, age and their interactions on plasma insulin concentration during fasting and refeeding

Effect	Fasting (h)					Refeeding (h)			
	15	27	39	51	63	0.5	1	2	4
Line (L)	----------				NS	----------			
Sex (S)	----------				NS	----------			
LxS	----------				NS	----------			
Age (A)	*	----------			NS	----------			
LxA	----------				NS	----------			
SxA	----------				NS	----------			
LxSxA	----------				NS	----------			

NS P>0.10 * P<0.05

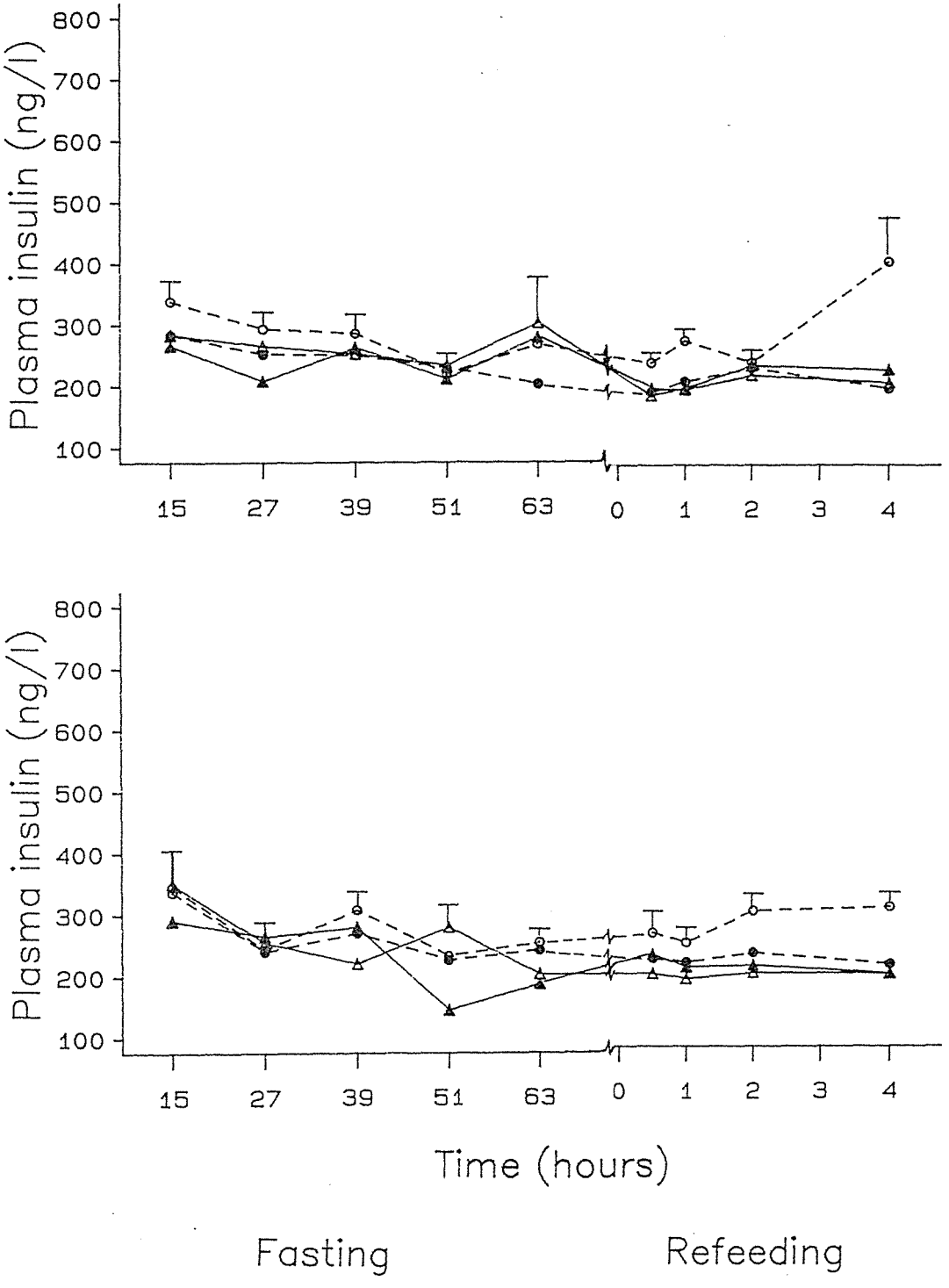

Fig. 9. Plasma concentrations of insulin at 3.5 (upper panel) and 7.0 (lower panel) months of age in LBI male (open triangle) and female (closed triangle) or HBI male (open circle) and female (closed circle) calves during fasting and refeeding. Vertical bars denote the pooled standard error at each sampling time.

4 Urea/Creatinine challenge (Period II)

Plasma concentrations of urea during the challenge are shown in Figure 10. Concentrations at both ages were stable prior to challenge. During this period plasma urea concentrations were not influenced by line or sex but were significantly (P<0.001) higher in animals at 3.5 months of age than in those at 7 months of age (Table 11). Intravenous administration of urea (120 mg/kg liveweight) resulted in a rapid increase in plasma urea concentration and a progressive decline thereafter. The absolute response to challenge (peak urea concentration minus mean of pre-challenge urea concentration) and subsequent changes in plasma urea were similar in both lines and sexes. As a result, the relative concentrations of urea in the pre-challenge period were maintained throughout the post-challenge period with line and sex effects being non-significant.

TABLE 11

Significance of effects of line, sex, age and their interactions on plasma urea concentration during the urea challenge (120 mg/kg lwt).

Effect	Significance of effect at sampling time[a]											
	-60	-40	-20	15	30	60	120	180	240	300	360	420
Line (L)	----------						NS					----------
Sex (S)	----------						NS					----------
LxS	----------						NS					----------
Age (A)	----------						***					----------
LxA	----------						NS			+	NS	NS
LxA	***	NS	**	NS	-------		NS					----------
LxSxA	----------						NS					----------

NS P>0.10 + P<0.10 ** P<0.01 *** P<0.001

[a] Sampling time (minutes) relative to challenge at time zero

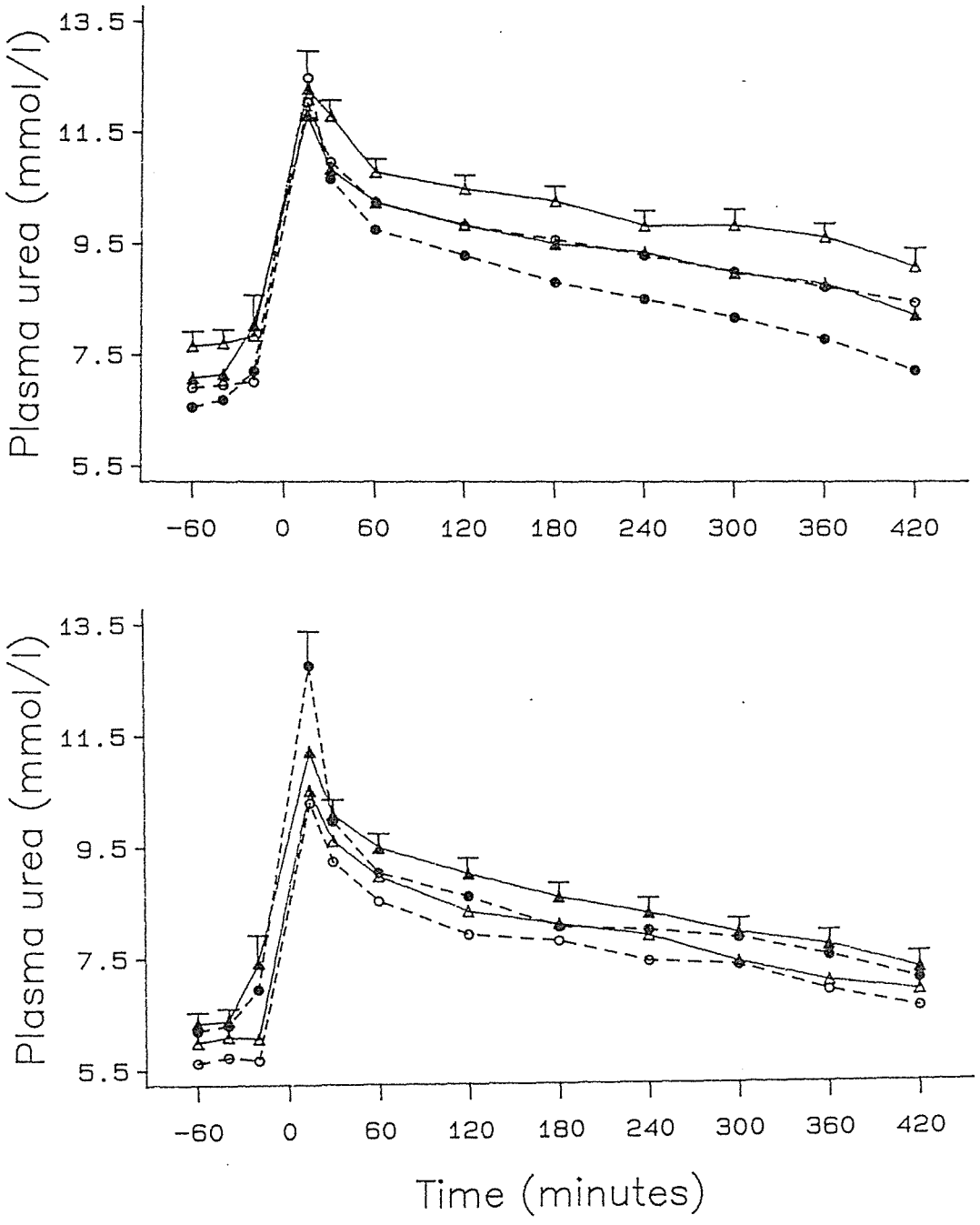

Fig. 10. Plasma concentrations of urea at 3.5 (upper panel) and 7.0 (lower panel) months of age in LBI male (open triangle) and female (closed triangle) or HBI male (open circle) and female (closed circle) calves during the urea challenge (120 mg/kg lwt). Vertical bars denote the pooled standard error at each sampling time.

Responses to the urea challenge were further analysed to determine the urea space and the fractional decay constant (Table 12). Urea space (corrected for baseline) was influenced by line, depending on age. While urea spaces at 3.5 months of age were not different between the lines, those at 7 months of age were greater in the HBI than in the LBI animals ($P<0.06$). As a result, there was a significant ($P<0.01$) line x age interaction on zero-time urea space. The older animals had higher urea space than young animals ($P<0.05$). The fractional decay constant (K) of urea was not influenced by line, sex or age.

TABLE 12

Effects of line, sex, age and their interactions on urea and creatinine spaces and fractional decay constants (Mean±se)

				Age (months)	
Metabolite	Parameter	Line	Sex	3.5	7.0
Urea	Space[a]	LBI	M	50.7±2.5	53.1±2.5
			F	52.3±1.5	51.5±1.5
		HBI	M	50.8±1.9	57.9±1.9
			F	47.4±1.7	54.4±1.8
	K value[b]	LBI	M	0.0026±0.0005	0.0036±0.0005
			F	0.0024±0.0003	0.0033±0.0003
		HBI	M	0.0024±0.0004	0.0028±0.0004
			F	0.0034±0.0004	0.0032±0.0004
Creatinine	Space[a]	LBI	M	26.7±2.5	23.6±2.2
			F	24.8±1.5	22.7±1.4
		HBI	M	32.0±1.9	25.5±1.7
			F	27.8±1.7	23.6±1.6
	K value[b]	LBI	M	0.0111±0.0016	0.0117±0.0013
			F	0.0111±0.0010	0.0102±0.0008
		HBI	M	0.0079±0.0012	0.0096±0.0010
			F	0.0098±0.0011	0.0108±0.0009

(Continued overleaf)

Table 12, continued

	Significance level						
	Line (L)	Sex (S)	LxS	Age (A)	LxA	SxA	LxSxA
US	NS	NS	NS	*	**	NS	NS
KU	NS	NS	NS	*	+	NS	NS
CS	*	+	NS	**	NS	NS	NS
KC	----------NS----------						

NS P>0.10 + P<0.10 * P<0.05 ** P<0.01

[a] Spaces expressed as % liveweight

[b] K Fractional decay constants (min^{-1})

US=Urea space

KU=Fractional decay constant (K) for urea

CS=Creatinine space

KC=Fractional decay constant (K) for creatinine

Creatinine concentrations during challenge did not show any effect of line, sex or line x sex interactions (Figure 11, Table 13). The only factor to influence plasma creatinine concentrations was age, older animals having greater plasma concentrations of creatinine than young animals (P<0.001).

TABLE 13

Significance of effects of line, sex, age and their interactions on plasma creatinine concentration during creatinine challenge(1.81 mg/kg lwt for 3.5-month old and 2.75 mg/kg lwt for 7-month old)

Effect	Significance of effect at sampling time[a]											
	-60	-40	-20	15	30	60	120	180	240	300	360	420
Line (L)	----------					NS						----------
Sex (S)	----------					NS						----------
LxS	----------					NS						----------
Age (A)	----------					***						----------
LxA	---------	NS	----------		+	NS	*	+	NS	NS	NS	
SxA	----------					NS						----------
LxSxA	----------					NS						----------

NS P>0.10 + P<0.10 * P<0.05 *** P<0.001

[a] Sampling time (minutes) relative to creatinine challenge

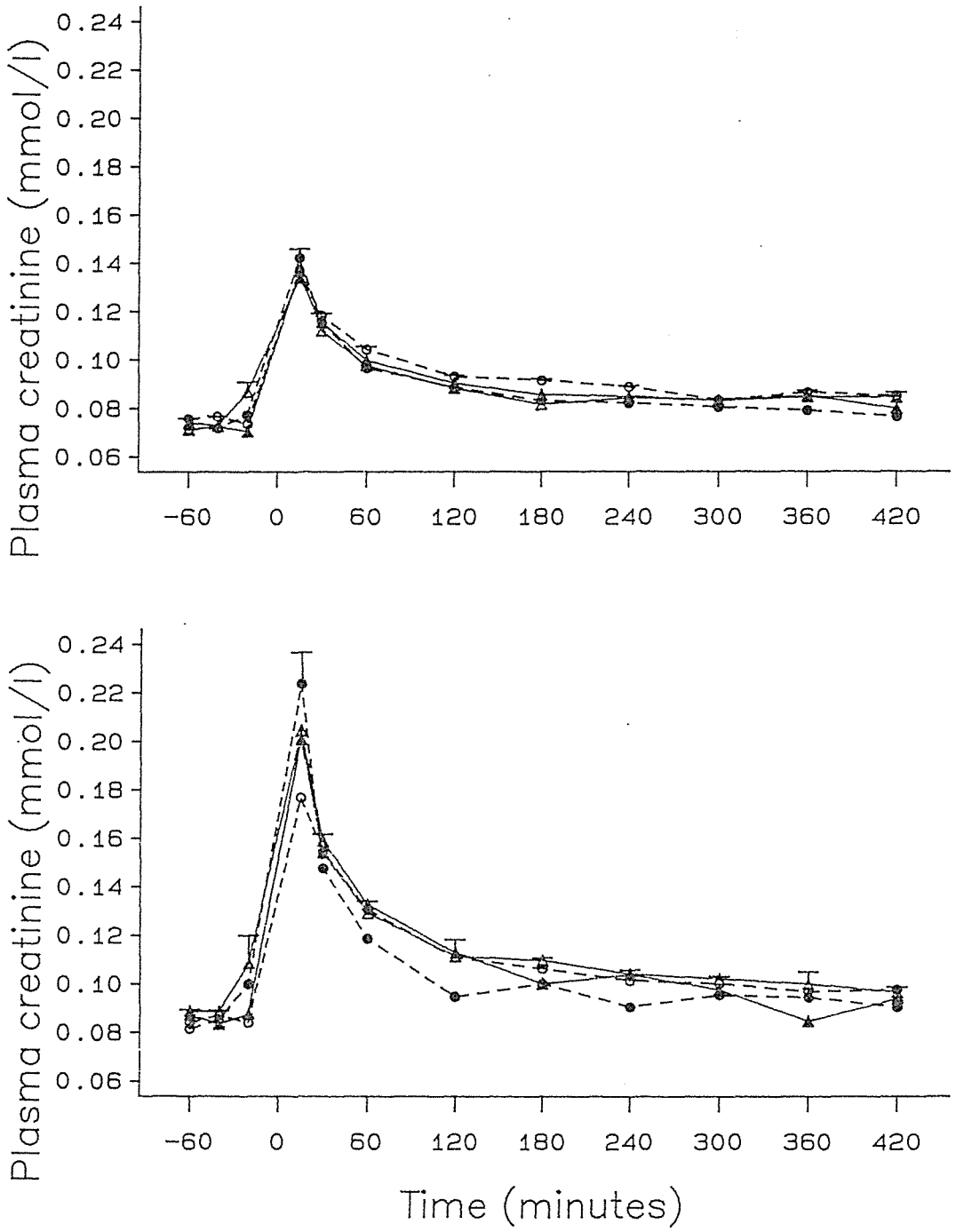

Fig. 11. Plasma concentrations of creatinine at 3.5 (upper panel) and 7.0 (lower panel) months of age in LBI male (open triangle) and female (closed triangle) or HBI male (open circle) and female (closed circle) calves during creatinine challenge (1.81 mg/kg lwt for 3.5-month old and 2.75 mg/kg lwt for 7-month old). Vertical bars denote the pooled standard error.

In order to estimate the creatinine space and fractional decay constant, data from the creatinine challenge were subjected to the same model used in analysing urea data. Creatinine spaces were significantly ($P<0.05$) greater in the HBI than the LBI calves at both ages. Creatinine space also showed an age effect, younger animals having a greater creatinine space than old animals ($P<0.01$) even after correction for the different doses of creatinine. The effect of sex on creatinine space was not significant although male calves tended to have a larger creatinine space ($P<0.10$). Similarly, the fractional decay constant (K) for creatinine was not influenced by line, sex or age although the K values tended to be lower in the HBI than in the LBI animals at both ages.

DISCUSSION

The aim of present study was to investigate the potential use of blood metabolite and hormone concentrations as genetic markers with particular reference to possible differences in both energy and protein metabolism between animals of different genetic merit for milk fat production. Previously Sejrsen *et al.* (1984) showed marked differences in the magnitude of selection line effects at 3.5 and 7 months of age, particularly in plasma urea concentrations. Therefore in the present experiments calves at two different ages (3.5 and 7 months of ages) were exposed to several nutritional and metabolic challenges which in other studies were the most effective means of identifying animals of high genetic merit (Tilakaratne *et al.*, 1980; Flux *et al.*, 1984; Sejrsen *et al.*, 1984; Xing *et al.*, 1988; Mackenzie *et al.* 1988). The present study met with limited success in that the feeding regime (Period I) resulted in a separation of the genetic groups in terms of plasma levels of glucose and insulin. In addition, differences between the lines in plasma concentrations of both glucose and insulin were apparent over short periods within the 12h sampling interval but these were influenced by factors such as sex and age.

Tilakaratne *et al.* (1980) reported that, during a two-day fast, plasma urea concentrations were lower in high genetic merit calves than in low genetic merit calves. Similarly, in a study with Red Danish bull calves, Sejrsen *et al.* (1984) found a negative correlation between fasting plasma urea levels and breeding value at 3.5 and, to a lesser extent, 7 months of age. In addition, several other studies have shown low levels of blood urea during a fast in dairy cattle of high genetic merit (Sinnett-Smith *et al.*, 1987; Woolliams *et al.*, 1988; Xing, 1989). In the present study, however, there was no evidence of differences between the high genetic merit and low genetic merit calves in urea metabolism, responses in plasma urea to several nutritional stimuli such as feeding and fasting/refeeding being similar between the two groups at both ages. The differences between present findings and previous studies are difficult to explain, but may partially reflect the sensitivity of plasma urea levels to environmental factors such as nutrition (Rowlands, 1980). For example, animals in the majority of the previous studies (Tilakaratne *et al.*, 1980; Sejrsen *et al.*, 1984; Sinnett-Smith *et al.*, 1987; Woolliams *et al.*, 1988) were fed limited amounts of concentrates plus ad

libitum hay, whereas those in the present study were fed lucerne chaff at 130% of maintenance energy requirement. The influence of such dietary factors on plasma urea concentration should theoretically be eliminated during a 63h fast. However, levels of plasma urea prior to fasting in the present study (6-7 mmol/l) were greater than those observed in previous studies (3-4 mmol/l) (Tilakaratne *et al.*, 1980; Sejrsen *et al.*, 1984; Sinnett-Smith *et al.*, 1987). This difference, which was probably due to different levels of protein/energy intake, may have modified the subsequent patterns of plasma urea and thus genetic expression in terms of urea levels during a fast. Furthermore, concentrations of plasma urea in the present study, unlike those in previous studies (Tilakaratne *et al.*, 1980; Sinnett-Smith *et al.*, 1987), also showed significant effects of sex and age throughout the experiment. Such differences, in addition to the effect of different feeding regimens, could be another cause of the discrepancy between results of this and other experiments.

In the present study urea spaces were between 47% and 58% of liveweight, i.e. similar to those reported by Bartle, Males and Preston (1983), Bartle, Kock, Preston, Wheeler and Davis (1987) and Bartle, Turgeon, Preston and Brink (1988). Urea space increased with age ($P<0.05$), but the magnitude of the increase was greater in the HBI calves than in the LBI calves. As a result, the HBI animals had significantly ($P<0.01$) greater urea spaces than their LBI counterparts at 7 months of age. Urea space has been used to predict body composition of ruminants, including cattle, in a large number of studies (Preston and Kock, 1973; Meissner, 1976; Kock and Preston, 1979; Meissner, van Staden and Elma Pretorius, 1980a,b; Bartle *et al.*, 1983; Bartle and Preston, 1986; Rule, Arnold, Hentges and Beitz, 1986; Bartle *et al.*, 1987; Bartle *et al.*, 1988; Abdullah, Purchas, McCutcheon and Van Dinther, 1990). Although there are some reservations concerning the reliability and usefulness of this method for estimating body composition of animals, particularly young or light-weight ones (Preston *et al.*, 1973; Kock *et al.*, 1979; Abdullah *et al.*, 1990), urea space has provided satisfactory predictions of body components such as water and protein in most reports (Preston *et al.*, 1973; Meissner, 1976; Kock *et al.*, 1979; Bartle *et al.*, 1983; Bartle *et al.*, 1986; Bartle *et al.*, 1987). Urea space in those studies was positively correlated with body protein and body water contents but negatively correlated with body fat content. Hence the difference between the HBI and LBI calves in urea space at 7 months of age suggests differences in protein and fat accumulation between the two BI groups. This finding provides the first evidence of differences in body composition of dairy cattle of high or low genetic merit. The data suggest that young dairy animals of superior

genetic merit preferentially partition nutrients into body protein rather than into fat. The greater proportion of protein deposited by high line animals may, as noted by Tilakaratne *et al.* (1980), be sustained even in periods of energy deficit by their preferentially mobilizing and oxidising fat although simultaneous measurements of plasma urea and NEFA concentrations in the present study failed to reflect such trends.

Plasma creatinine concentrations, which were greater in 7-month old animals than in 3.5-month old animals ($P<0.01$), were similar between the BI groups throughout this experiment. In contrast, previous studies with the same selection lines (Xing, 1989) have reported lower creatinine concentrations in HBI heifers than in their LBI peers. However, results obtained by Xing (1989) should be interpreted with caution since only a small number of animals (n=8) were employed in those studies. Furthermore, in one of those studies (Xing, 1989) differences between the two lines were only marginal ($P<0.10$).

Creatinine space at both ages was greater ($P<0.05$) in the HBI calves than in the LBI group but there were no differences between the two lines in the fractional decay constant for creatinine. Since creatinine space reflects extracellular volume, the results of this study suggest a greater extracellular space in the HBI animals than in the LBI animals. However, in a study with the same selection lines, Xing (1989) failed to observe similar trends, possibly because of a smaller sample size involved in his experiment. In the latter study thiocyanate was used to evaluate the extracellular space whereas creatinine distribution was used in the present study. Therefore, further experiments involving the more accurate measurement of creatinine space by the use of ^{14}C creatinine need to be undertaken and compared with the distribution of thiocyanate.

Plasma glucose concentrations during the postprandial period were significantly ($P<0.05$) greater in the high genetic merit calves than in the low genetic merit calves although these differences were restricted to the period immediately (1 to 3h) after feeding. This elevated blood glucose of HBI animals is consistent with previous Massey University studies (Flux *et al.*, 1984; Xing *et al.*, 1988; Xing, 1989) in which greater basal plasma glucose concentrations were observed in the HBI line than in their comparable LBI groups. Differences between the HBI and LBI calves were not apparent prior to feeding and may therefore reflect a more rapid entry of gluconeogenic products from the alimentary tract in the HBI groups. Alternatively, the

greater basal glucose levels in the HBI calves of this study may have arisen from a decreased glucose utilization by peripheral tissues. This could be achieved through a reduced sensitivity of peripheral tissues to circulating insulin since the elevated blood glucose concentration of HBI animals is, in most cases (Flux *et al.*, 1984; Xing *et al.*, 1988; Xing, 1989 and this experiment), accompanied by simultaneous hyperinsulinaemia in that group. Whatever the reasons, such differences in postprandial plasma glucose between the BI groups could be a useful marker for predicting genetic capacity for milk production even if they are apparent only over limited periods immediately after feeding.

The results of this study also suggest that some consideration should be given to other factors such as sex and age when using basal concentrations of plasma glucose as a potential genetic marker for dairying. Both factors had significant ($P<0.05$) effects on plasma glucose at most of the sampling times (Table 4). Such factors are of particular interest with regard to male calves of the LBI group. Whereas basal plasma glucose concentrations, in line with previous studies (Young, Otchere, Trenkle and Jacobson, 1970; Stark, Rowlands, Manston and McClintock, 1978; Rowlands, Manston, Bunch and Brookes, 1983; Wijayasinghe, Smith, and Baldwin, 1984; Rowlands, Bunch, Brookes and Manston, 1986), decreased with age for the HBI calves and LBI female calves, the reverse was observed for male calves of the LBI group. As a result, basal plasma glucose concentrations of LBI male calves, which were the lowest of all the line x sex groups at 3.5 months of age, were the greatest at 7 months of age. Therefore, the use of basal plasma glucose as a genetic marker in male calves would be strongly influenced by the age at which animals were examined. The physiological basis of this trend is not clear since simultaneous measurement of basal plasma insulin, one of the main regulators of glucose concentration, failed to show a similar pattern.

During Period I, plasma insulin concentrations were, like plasma concentrations of glucose, greater in the HBI than in the LBI calves. Although such differences were not always significant, primarily because of large variation within lines, this greater basal insulin concentration of high genetic merit calves is in good agreement with the results of previous studies (Flux *et al.*, 1984; Xing *et al.*, 1988; Mackenzie *et al.*, 1988; Xing, 1989). It is, however, important to note that such differences with respect to insulin metabolism have been obtained only with the Massey University dairy cattle selection lines, but not with other dairy cattle selection lines (Land *et al.*, 1983; Serjsen *et al.*, 1984; Barnes *et al.*, 1985; Sinnett-Smith *et al.*, 1987). This suggests that high

plasma insulin may be a peculiarity of the Massey University HBI animals. Overall, basal plasma insulin shows promise as a potential genetic marker in so far as the Massey University selection lines are representative of the New Zealand dairy cow. Furthermore, the results of the present study, together with those of other studies (Young *et al.*, 1970; Land *et al.*, 1983), suggest that insulin is, unlike other potential genetic markers described previously, influenced neither by sex nor by age. This would simplify its use as a genetic marker.

Although basal plasma insulin concentration shows potential as a genetic marker, it is also important to define the possible mechanisms for the observed differences. This could assist in the identification of experimental conditions which minimize the effects of environmenatal factors, thus maximizing the expression of genetic merit in terms of plasma levels of insulin. The relatively high plasma insulin levels in the HBI, as compared to the LBI, animals is surprising given that plasma insulin is negatively correlated with milk yield (Koprowski and Tucker, 1973; Hart, Bines, Balch and Cowie, 1975; Hart, Bines, Morant and Ridley, 1978; Hart, Bines and Morant, 1979) and that the administration of exogenous insulin reduces milk yield (Trenkle, 1978; Cowie, Forsyth and Hart, 1980). Flux *et al.* (1984) noted a greater plasma glucose concentration in the Massey University HBI cows and suggested that their higher levels of insulin may be attributed to a decreased sensitivity of peripheral tissues to insulin in this group. Xing *et al.* (1988) drew the same conclusion from milk-fed calves of the same selection lines and further suggested that this relative insensitivity may be a result of "down regulation" of insulin receptors caused by hyperinsulinaemia. Although this insulin resistance associated with hyperinsulinaemia has been demonstrated in obese heifers and sheep (McCann and Reimers 1985, 1986; McCann, Reimers and Bergman, 1987), direct measurement of the glucose response to exogenous insulin in high and low genetic merit dairy cattle failed to confirm such trends (Land, Carr, Hart, Osmond, Thompson and Tilakaratne, 1983; Barnes, Kazmer, Akers and Pearson, 1985; Mackenzie *et al.*, 1988; Xing, 1989). Conversely, in two of these studies (Mackenzie *et al.*, 1988; Xing, 1989), glucose clearance from plasma following either insulin or glucose injection was greater in the HBI group than in the LBI group, indicating an increased sensitivity of peripheral tissues to circulating insulin in the HBI animals. There is, therefore, no good evidence that the increased basal insulin levels of the Massey University HBI dairy cattle are due to the decreased sensitivity of peripheral tissues to insulin in that group. Alternatively, the greater levels of plasma insulin in the HBI calves of this study may reflect an increased sensitivity of

the pancreatic-B cells to releasing stimuli such as circulating glucose in this group. Such a possibility was not examined in the present experiment. However, it has been demonstrated from previous studies with these lines (Mackenzie *et al.*, 1988; Xing *et al.*, 1988; Xing, 1989; Michel, McCutcheon, Mackenzie, Trait and Wickham, 1990) that insulin response to glucose or arginine challenge was greater in the HBI dairy cattle than in the LBI dairy cattle. Since acute release of insulin following such a stimulus is a valid measure of the pancreatic insulin secretion (McCann *et al.*, 1987), the greater insulin concentrations observed during basal feeding in the HBI group can be explained by the increased sensitivity of the pancreas in this group.

In contrast to previous studies (Tilakaratne *et al.*, 1980; Sejrsen *et al.*, 1984; Barnes *et al.*, 1985), plasma concentrations of NEFA were similar in the two BI groups following either fasting or refeeding. This result is surprising given that selection of the two lines has been based on milk fat yield rather than milk yield. However, no differences in plasma NEFA between dairy cattle selection lines have been reported in several other studies (Sinnett-Smith *et al.*, 1987; Mackenzie *et al.*, 1988; Xing, 1989).

In conclusion, this study does not confirm previous findings that genetic merit for dairying is expressed in terms of levels of plasma urea and NEFA, particularly during a fast. Therefore, such plasma concentrations are unlikely to be useful markers for dairying, at least for the Massey University selection lines and by inference for the New Zealand dairy cow population. Results of the present study are, however, consistent with previous Massey University observations of differences between the two BI groups in terms of glucose and insulin metabolism. Although the physiological mechanisms responsible for these differences remain unclear, such characteristics, particularly with respect to insulin, provide potential as genetic markers for dairying in New Zealand Friesian cattle.

CHAPTER III: GENERAL DISCUSSION AND CONCLUSIONS

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Although evidence for genetic variation in physiological traits in dairy cattle was presented in the early 1970's (Joakimsen *et al.*, 1971; Rowlands *et al.*, 1974), no attempts to develop such traits as genetic markers for dairying were made until 1980. In that year, British scientists (Tilakaratne *et al.*, 1980) first attempted to identify genetic markers for dairying by studying variation in several metabolic traits in Friesian calves with different genetic merit for milk yield. Results of that study showed substantial differences in some of these traits between the two genetic groups although expression of such potential genetic markers was apparent only under certain physiological stresses such as fasting. Despite being based on a relatively small sample size, these findings were sufficient to draw the attention of scientists throughout the world to the possible use of physiological parameters as genetic markers in dairying. Thus considerable research has since been done in this area (Osmond *et al.*, 1981; Land *et al.*, 1983; Bitman *et al.*, 1984; Flux *et al.*, 1984; Sejrsen *et al.*, 1984; Barnes *et al.*, 1985; Gibson *et al.*, 1986; Kazmer *et al.*, 1986; Rowlands *et al.*, 1986; Ahlborn-Breier *et al.*, 1987; Gibson *et al.*, 1987; Sinnett-Smith *et al.*, 1987; Bonczek *et al.*, 1988; Klindt *et al.*, 1988; Lukes *et al.*, 1988; Mackenzie *et al.*, 1988; Sartin *et al.*, 1988; Woolliams *et al.*, 1988; Xing *et al.*, 1988; Davis *et al.*, 1989; Xing, 1989). This research into the identification of genetic markers in dairying over the last 10 years is not surprising in view of the enormous potential benefits from developing such markers (Chapter I). However, as noted in Chapter I, the use of genetic markers depends on several factors such as the correlations between the physiological trait studied and dairy production, the repeatibility of the physiological trait and the simplicity with which it may be measured. Of the many physiological traits which have been investigated, only a few seem to be promising as genetic markers. These include plasma levels of urea, creatinine, NEFA, glucose and insulin. Nevertheless, the reliability of such traits as genetic markers is still being questioned since inconsistent or sometimes conflicting results exist with regard to differences between selection lines in these traits. The present study was thus designed to further evaluate the reliability of these traits as genetic markers and, if possible, to gain some insight into the physiological basis for genetic differences in some of these traits.

The study of urea metabolism was central to this experiment. In overseas studies (Tilakaratne *et al.*, 1980; Sejrsen *et al.*, 1984; Sinnett-Smith *et al.*, 1987), this trait was considered one of the most promising genetic markers for dairying since low plasma levels of urea have been consistently found in dairy calves of high genetic merit. Conversely, in the Massey University studies, the role of plasma urea as a potential genetic marker for dairying is less clear. While some studies (Xing, 1989) have identified low levels of plasma urea in the HBI dairy cattle, others (Mackenzie *et al.*, 1988) have observed the reverse situation. This problem is further illustrated by the results of the present study which showed similar levels of plasma urea between the two BI groups throughout the experiment. It is, however, necessary to note that the blood sampling in the present study was conducted over the 12h after feeding, which is much shorter than that (over the 24h after feeding) in previous studies (Xing, 1989). Considering that differences in blood urea concentrations between the HBI and LBI dairy cattle in those studies were most apparent in the latter stages of the post-feeding period (from 11 to 23h after feeding), the inability of the present experiment to detect genetic effects on blood urea may be partly due to the relatively short periods of blood sampling. This requires further study at the ages involved here.

Results of overseas studies (Tilakaratne *et al.*, 1980; Sejrsen *et al.*, 1984; Sinnett-Simth *et al.*, 1987) also suggest that physiological stress such as fasting is one of the most effective means of enhancing expression of genetic merit in terms of plasma urea. Such a suggestion is, however, not supported by data from the Massey University studies (Mackenzie *et al.*, 1988; Xing, 1989 and this experiment) since fasting levels of plasma urea were not significantly related to breeding value in any of those studies. Therefore, fasting cattle from the Massey University studies is not effective in causing divergence between the lines in plasma urea concentration, unlike the situation observed in overseas studies (Tilakatne *et al.*, 1980; Sejrsen *et al.*, 1984; Sinnett-Smith *et al.*, 1987). It remains unclear whether the disparity between the Massey University studies and overseas studies reflects variation in genetic background of the animals or other factors such as nutrition, sex and age. However, Rowlands (1980) reviewed possible sources of variation in plasma urea concentrations of beef and dairy cattle and concluded that environmental factors, particularly nutrition, are a major source of variation in this trait. Thus nutrition is most likely to be the major factor responsible for the discrepancy between results of the Massey University and overseas experiments. The nutritional history of the animals used in the

overseas studies differs from that of animals used at Massey University. In overseas experiments the calves were fed limited amounts of concentrate (about 2 kg/head/day) with hay ad libitum, while at Massey University lucerne hay/chaff has been fed at 130% of maintenance energy requirement. In addition the crude protein content of the overseas diet was lower than that of the N.Z diet (12-13% vs 19-21%). Both of these differences reflect the different geographical approaches to feeding cows (ie concentrate-based diets in the Northern Hemisphere vs mainly roughage feeding in the Southern Hemisphere). These differences in both energy intake and the composition of the diet between the overseas studies and Massey University studies could lead to difference in animal metabolism (particularly with respect to glucose metabolism) and thus in body composition. Fermentation of the concentrate ration would be expected to produce a higher proportion of propionic acid than that produced on the lucerne hay/chaff diet. This, coupled with a higher energy intake on the concentrate plus ad libitum hay diet would provide greater quantities of gluconeogenic precursors in the form of carbohydrate (mainly propionate), whereas the animals fed lucerne would be more reliant upon gluconeogenesis from amino acids absorbed from the small intestine. This difference in source and amount of gluconeogenic precursors might be expected to lead to differences in concentrations of circulating hormones and metabolites. In particular, the animals fed concentrate diets would have higher concentrations of insulin and glucose, with lower concentrations of NEFA, urea and growth hormone than those on lucerne diets. Although such dietary factors should theoretically be eliminated by the fasting regimen, comparison between the Massey University studies and overseas studies in concentrations of these metabolites and hormones (Table 14) indicates that the influence of the prior nutritional differences may be sustained over short periods of fasting. Thus conditions for the expression of genetically based differences related to milk production may be better in a feeding regimen based on high levels of concentrate feeding. Future experiments with the Massey lines should, therefore, be designed to study such interactions between genetic merit and the level/type of feed offered.

TABLE 14

Comparisons of blood concentrations of metabolites and hormones between overseas studies and the Massey University studies during basal feeding and fasting.

		Urea (mmol/l)	Glucose (mmol/l)	NEFA (ueq/l)	Insulin (ng/l)
12±2 h post-feeding:					
European studies					
Tilakaratne *et al.* (1980)		2.2-2.8	4.8-5.0	190-200	
Sejrsen *et al.* (1984)	A	3.4-3.6	5.5-5.8	40-60	
	B	2.4-2.5	5.5-5.6	40-44	
Sinnett-Smith et al. (1987)		2.5-2.6		150-200	380-640
Massey University studies					
Mackenzie *et al.* (1988)		3.8-4.3	4.6-4.9	290-380	450-690
Xing (1989)		4.0-5.0	4.4-4.5	100-120	
The present study	A	5.4-5.8	4.7-4.8	164-182	274-298
	B	4.4-4.6	4.3-4.4	185-188	320-364
41±2 h fasting:					
European studies					
Tilakaratne *et al.* (1980)		5.5-6.7	3.4-3.7	790-900	
Sejrsen *et al.* (1984)	A	3.8-5.2	3.6-3.8	400-450	135-152
	B	3.2-3.4	4.4-4.5	300-350	114-228
Sinnett-Smith *et al.* (1987)		3.1-3.7	3.3-3.6	550-700	170-190
Massey University studies					
Mackenzie *et al.* (1988)		5.5-5.8	3.9-4.0	530-600	320-500
Xing (1989)		5.0-5.6	3.4-3.7	650-700	160-170
The present study	A	6.9-7.9	3.0-3.1	594-656	258-267
	B	5.4-5.9	3.3-3.8	602-603	260-289

A=3.5 months of age, B=7 months of age

Low plasma levels of creatinine have been previously reported in the Massey University HBI dairy cattle (Xing, 1989). In the present study plasma creatinine levels were, however, similar between the two BI groups. The disparity between this and the previous studies is difficult to explain although some of these differences may be due to differences in sample size,age and sex. Whatever the reason, the results of the present study cast doubt on the possibility of using creatinine concentrations as a genetic marker for dairying.

Urea spaces, based on back-extrapolation of urea concentrations following a urea challenge, were greater in the HBI calves than in the LBI calves. Assuming that urea space is negatively correlated to body fat content but positively correlated to body protein content, the results of the present study support the general concept proposed first by Tilakaratne *et al.* (1980) that animals of high genetic merit may utilize amino acids more efficiently than do animals of low genetic merit. These observations must, however, be considered with caution for the two reasons. First, urea distribution was calculated in this study using a single-compartment model. This model is usually subject to some errors, since the single compartment approach does not perfectly model urea decay curves, particularly where urea levels return to a baseline value different from that which existed prior to the challenge. However, in this study, model r^2 values for individual calves were generally high (90-95%). Second, animals used in this study were young dairy calves. The accuracy of estimation of body composition by the urea dilution method is relatively low in young animals because only limited variation exists in the body composition at these ages. Thus, further studies involving more reliable techniques such as tritium oxide dilution, and the study of older animals, are required to verify the present results.

Consistent with previous Massey University studies (Flux *et al.*, 1984; Mackenzie *et al.*, 1988; Xing *et al.*, 1988; Xing, 1989), the present study demonstrated differences in glucose metabolism between the two lines. In each case, plasma glucose concentrations were greater in the HBI animals than in the LBI animals. Despite these consistent differences in glucose metabolism between the lines, the suitability of plasma glucose as a genetic marker is questionable, mainly because of variation with regard to conditions in which genetic merit is expressed. For example, some studies (Xing, 1989 and this experiment) found differences in glucose metabolism between the lines during feeding, whereas others (Flux *et al.*, 1984; Mackenzie *et al.*, 1988; Xing *et*

al., 1988) observed such differences only during fasting (underfeeding) or refeeding. Even when experimental conditions were successful in causing divergence between genetic groups in terms of plasma glucose, differences were often apparent only over limited periods (Xing, 1989 and this experiment). This inconsistent and often short-lived expression of genetic merit in terms of plasma glucose is not surprising since plasma glucose is, as demonstrated in this and other studies (Rowlands 1980), greatly affected by a variety of non-genetic factors such as nutrition, sex, age and physiological state. Furthermore, glucose, which is required not only for the synthesis of milk lactose and as a precursor for fat synthesis, but also as an energy source for many key tissues (eg the central nervous system), is under tight homeostatic control so that little variation is expressed. This is demonstrated in Tables 15 and 16 which show that the coefficient of variation in plasma glucose concentation is generally lower than that for other metabolites and hormones (results from this study). Although nutritional stimuli such as fasting (or underfeeding) have been suggested as a method to perturb homeostasis and so enhance expression of genetic merit in terms of plasma glucose (Flux *et al.*, 1984; Mackenzie *et al.*, 1988; Xing *et al.*, 1988), such efforts are, as demonstrated in this and other studies(Xing 1989), not always successful. Thus, plasma glucose seems to have limited value as genetic marker for dairying and concentrations following various perturbations require further study.

Greater basal plasma insulin concentrations, and greater insulin levels after challenge of glucose and arginine, have been previously reported in the Massey University HBI dairy cattle (Mackenzie *et al.*, 1988; Xing *et al.*, 1988; Xing, 1989; Michel *et al.*, 1990). Consistent with those observations, plasma concentrations of insulin in the present study were greater in the HBI animals than in the LBI animals. Such differences in insulin levels between the two lines are most likely to be a function of differences in pancreatic-B cell sensitivity since genetic divergence is most apparent in response to endogenous and exogenous nutritional stimuli such as feeding and glucose injection (Xing *et al.*, 1988; Mackenzie *et al.*, 1988; Xing, 1989 and this experiment). Genetic control of insulin secretion has also been demonstrated elsewhere (Verde *et al.*, 1982; Bossart *et al.*, 1985). Although the physiological importance of these differences in insulin metabolism between the lines remains to be determined, such differences in insulin secretion may be useful as selection criteria for predicting genetic merit for dairying. Furthermore, plasma insulin concentration has a relatively high repeatability (Almlid *et al.*, 1982) and is independent of sex and age (Young *et*

al., 1970; Land *et al.*, 1983 and this experiment). Therefore, plasma insulin concentration, and particularly its response to feeding or exogenous glucose challenge, is the most promising genetic marker for dairying, at least for the N.Z. Friesian breed.

TABLE 15

Coefficient of variation (%) in blood concentrations of metabolites and hormones at different times relative to the onset of feeding in 3.5-and 7-month old dairy calves.

	Times relative to feeding (h)							
	-1	01	02	04	06	08	10	12
3.5-month old								
Urea	14.0	12.1	12.0	13.7	11.8	14.5	20.4	22.8
Creatinine	18.2	11.4	11.9	13.9	14.7	17.4	18.0	19.4
Glucose	11.1	8.3	7.8	6.7	9.8	9.1	7.7	7.2
Insulin	38.5	36.2	29.6	30.6	42.3	41.2	46.4	56.2
7.0-month old								
Urea	18.0	17.7	16.4	15.0	15.7	18.8	21.5	22.4
Creatinine	16.2	12.7	13.6	11.7	13.6	10.6	11.5	12.2
Glucose	15.5	6.9	6.4	9.1	14.2	7.8	16.1	16.2
Insulin	30.7	29.2	41.8	32.1	32.3	35.2	29.0	26.5

TABLE 16

Coefficient of variation (%)in blood concentrations of metabolites and hormones at different times relative to the onset of fasting in 3.5- and 7-month old dairy calves.

	Times relative to fasting (h)					
	08	15	27	39	51	63
3.5-month old						
Urea	14.5	23.6	18.5	19.3	22.0	24.6
Creatinine	17.4	16.8	19.3	17.8	18.9	17.9
Glucose	9.1	10.0	11.9	10.9	16.9	15.6
NEFA	N/M	34.9	21.7	17.3	22.4	18.9
Insulin	41.2	46.0	45.0	47.6	40.8	95.7
7-month old						
Urea	18.8	24.6	21.7	21.0	25.0	23.8
Creatinine	10.6	14.5	17.6	15.2	14.3	12.3
Glucose	7.8	7.0	7.0	8.1	10.6	13.1
NEFA	N/M	32.9	22.5	10.1	13.9	16.2
Insulin	35.2	55.6	40.9	41.5	58.9	43.1

N/M = not measured at this time

Plasma concentrations of NEFA were measured only during fasting and refeeding since expression of genetic merit in terms of plasma NEFA is apparent only under such circumstances (Tilakaratne *et al.*, 1980; Sejrsen *et al.*, 1984). It has been reported from those studies that fasting plasma NEFA concentrations were greater in the high genetic merit calves than in the low genetic merit calves. Since increased NEFA is indicative of mobilization of fat from adipose tissue, these results suggested

that the superior calves exhibited the same response to an energy deficit as might be expected from their dams. Nevertheless, the use of fasting plasma NEFA as a genetic marker for dairying seems to be unreliable since subsequent studies with calves (Xing *et al.*, 1988), young bulls (Mackenzie *et al.*, 1988), heifers (Xing, 1989) and lactating cows (Flux *et al.*, 1984) have failed to confirm such trends under similar conditions. This conclusion is further confirmed by the present study which showed similar levels of plasma NEFA between the two genetic groups although the range of fasting plasma NEFA observed in this study (from 100-200 to 800-900 ueq/l) was similar to that reported by Tilakaratne *et al.* (1980). Therefore, measurement of fasting plasma NEFA is unlikely to provide useful information on genetic capacity for dairying.

In conclusion, there were numerous differences in metabolic and endocrinological characteristics between the Massey University studies and overseas studies. As a result, overseas observations of differences between the high and low genetic merit lines in terms of urea and NEFA metabolism were not confirmed by the results of this study. However, the present study is consistent with previous Massey University observations of differences in insulin and glucose metabolism between the two lines, thus promising the possibility of using such traits as genetic markers for dairying. The disparity between the Massey University studies and overseas studies may reflect differences in non-genetic factors, particularly nutrition, although some of these differences could also be due to genetic effects. Thus, future study should be designed to investigate these possible interactions between genetic merit and nutrition.

APPENDIX

Appendix Table 1

Block	Calf No.	BI	Sex	Date of Birth	Birth Weight (kg)	Body Weight (kg) at time of cannulation		Age (days) at time of cannulation		Dam BI	Sire BI
						3.5 month	7.0 month	3.5 month	7.0 month		
A	6	132	M	28-7-1987	36	116.0	210.0	113	225	124	139
	20	134	M	8-8-1987	32	94.5	172.0	102	214	128	139
	46	140	M	14-8-1987	32	93.5	172.5	96	208	129	151
	19	132	F	8-8-1987	33	91.5	129.5	102	214	125	139
	23	138	F	9-8-1987	32	98.5	148.5	101	213	137	139
	27	142	F	10-8-1987	35	89.5	133.0	100	212	133	151
	36	144	F	11-8-1987	33	88.5	138.5	99	211	137	151
	13	113	M	4-8-1987	44	113.5	198.0	106	218	107	118
	26	112	M	10-8-1987	45	94.5	173.5	100	212	106	118
	15	112	F	5-8-1987	36	107.0	149.0	107	219	106	118
	24	110	F	10-8-1987	50	99.5	142.5	100	212	102	118
	18	111	F	8-8-1987	40	78.5	121.5	102	214	107	115
	96	111	F	3-9-1987	36	75.5	107.0	76	188	107	115
	52	110	F	17-8-1987	30	87.0	131.0	93	205	106	114
B	31	133	M	10-8-1987	50	111.0	193.5	121	233	126	139
	51	137	M	16-8-1987	39	114.0	180.0	115	227	135	139
	64	133	M	21-8-1987	35	102.0	154.5	110	222	126	139

53	136	M	17-8-1987	36	94.0	149.0	114	226	126	151
25	136	F	10-8-1987	35	105.0	160.0	121	233	133	139
48	137	F	13-8-1987	41	86.5	137.0	118	230	124	151
74	136	F	24-8-1987	32	89.0	131.0	107	219	121	151
76	143	F	24-8-1987	30	89.0	140.0	107	219	135	151
73	113	M	23-8-1987	35	81.0	115.0	108	220	108	118
61	108	M	20-8-1987	35	98.5	173.0	111	223	102	115
37	111	F	12-8-1987	41	106.0	156.0	119	231	104	118
41	111	F	12-8-1987	32	103.0	160.5	119	231	105	118
47	111	F	14-8-1987	27	102.0	147.0	117	229	105	118
85	105	F	28-8-1987	32	94.5	151.0	103	215	103	115
67	111	F	23-8-1987	36	91.5	154.5	108	220	107	115
94	110	F	3-9-1987	34	83.5	122.0	97	209	107	114

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