BLOOD METABOLITE AND HORMONE CONCENTRATIONS OF DAIRY CALVES DIFFERING IN GENETIC POTENTIAL FOR MILK FAT PRODUCTION

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ABSTRACT

The present study was conducted at the Massey University Dairy Research Unit to investigate the effect of genetic merit for milk fat production on the physiology and metabolism of Friesian calves.

Twenty four Friesian calves divided into four groups namely High Breeding Index (HBI) heifers, HBI bulls, Low Breeding Index (LBI) heifers, and LBI bulls were challenged with four different experimental treatments, ie. fasting, feeding, intravenous arginine infusion, and subcutaneous synthetic corticosteroid injection at ten to eighteen days of age. A total of eighteen blood samples were collected from each calf through an indwelling jugular cannula and the concentrations of plasma glucose, insulin, GH and cortisol were determined.

Some statistically significant differences were found in plasma metabolite and hormone concentrations between the HBI and LBI groups.

1. The basal glucose concentration in HBI group was significantly higher than that in LBI group ($P<0.05$). The basal plasma insulin concentration was also significantly higher in HBI group than in LBI group ($P<0.01$). The basal GH concentration in HBI calves was higher in HBI calves than in LBI calves, but the difference was not quite significance at 5% level ($P=0.059$).

2. Following feeding, plasma insulin and GH concentrations in HBI group were significantly higher than those in LBI group ($P<0.01$, $P<0.05$ respectively).

3. Acute intravenous arginine infusion induced hyperinsulinemia and hypoglycemia in all calves. LBI calves had significantly higher increments of plasma insulin measured as a percentage of basal levels than HBI calves. The response of GH concentration to arginine challenge differed significantly in terms of level and pattern between HBI and LBI groups, with the HBI calves having more prolonged higher GH concentration than LBI calves ($P<0.05$).

4. Subcutaneous injection of synthetic corticosteroid resulted in significant increments in plasma glucose and insulin concentrations, and a significant decrease in endogenous cortisol production in all calves. ($P<0.01$, $P=0.05$, $P<0.01$ respectively). But no significant differences were detected between HBI and LBI groups.

Effects of sex on plasma metabolite and hormone concentrations were also found in the present study. Plasma insulin concentration was consistantly higher in bulls than in heifers and the differences were significant at the time of fasting, after feeding, and after arginine infusion ($P<0.01$). Plasma glucose concentrations following feeding were significantly higher in bulls than in heifers ($P<0.05$). GH concentration was slightly but not significantly higher in bulls than in heifers for most of the experiment.

It was concluded that differences exist in some important metabolic and endocrinological characteristics between HBI and LBI calves, and these differences could become significant under certain physiological conditions and experimental treatments such as those applied in the present study. This study also showed the promise of identifying genetically superior Friesian dairy cattle at an early age by using physiological markers. However this possibility has yet to be tested by carring out measurements on calves for which breeding index value for milk fat production will be determined by methods such as progeny test.

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TABLE OF CONTENTS

page

LIST OF FIGURES

LIST OF TABLES

Table page

Table page

CHAPTER ONE: INTRODUCTION

The Usefulness of physiological markers in dairy cattle breeding

It has long been desired ,as recently discussed in several papers (Gorski, 1979; Kiddy 1979; Linstrom, 1982; Peterson et al,1982; Land et al, 1981) to be able to identify genetically superior dairy cattle from their contemporaries at an early stage of their life by using physiological or biochemical markers which are genetically correlated with milk production.

A dairy breeding system using such markers would have advantages over the conventional breeding technique, progeny test, in several respects:1) reduced breeding cost , 2) more intensive selection of bulls, as a large population of animals can be tested for the marker, 3) shorter generation intervals, due to the early intensive use of the identified bulls in the artificial insemination scheme.

Animal breeding programmes for dairy cattle, so far, have been based on the principles of quantitive genetics. These programmes are expensive and genetic improvement is slow. In New Zealand, contracts are made each year by the Dairy Board with individual farmers to purchase about 150 newborn dairy bulls (including both Friesian and Jersey breeds) for progeny testing. All these bulls are kept in the breeding centre till they are 5 years old, waiting the assessment of their daughters production. Thereafter only a few of the proven bulls are used intensively in the AI scheme while others are culled. Nevertheless this conventional breeding method has proved reliable and rewarding in dairy herd improvement as witnessed by the great improvement in milk production of Friesian cattle both around the world (Jasiorowski et al, 1983) and within New Zealand (Bryant et al, 1981; Davey et al, 1983; Wickham et al, 1978). This provides the opportunity to compare the physiological differences between two groups of genetically diversed Friesian cattle, a study of which may lead to the identification of suitable gene markers which could , in turn, aid in further improvement of dairy cattle production.

Previous work on early identification of genetically superior dairy cattle

Though the idea might not be new, the literature contains very few reports on early identification of dairy cattle which are genetically superior for milk production. A study reported by Tilakaratne et al (1980) indicated that dairy calves with different genetic merit might be identified by measuring the concentrations of certain plasma metabolites because they found significant differences in the concentrations of total protein and urea between the high and low genetic merit calves.

Osmond et al (1981) showed however, that simple spot estimates of plasma concentrations of tri-iodothyronine (T3), thyroxine (T4) and insulin from 2 to 7 years old Friesian bulls did not supply useful predictors of their daughters milk production.

Studies with lactating Friesian cows of known genetic merit in this Department showed that high breeding index (HBI) cows differed from low breeding index (LBI) cows in certain important metabolic aspects (Flux et al, 1984). Plasma glucose,insulin and growth hormone (GH) concentrations in HBI cows were significantly higher than in LBI cows when feed intake was restricted.

The present study

The aim of the present study was to investigate the physiological attributes of young pre-ruminant HBI and LBI calves with similar genetic backgrounds to those of the cows used by Flux et al (1984).The means of the breeding indices based on ancestry were 134.8 and 104.3 for HBI and LBI calves respectively. Plasma concentrations of glucose,insulin,GH,and cortisol were chosen as the markers to be examined , not only because they are of great metabolic importance to calf development in general,but also due to the fact they are at least equally important to the metabolic economy of lactating cows (Cowie et al, 1980). Thus differences in the physiological attributes between the HBI and LBI calves may also persist in the adult animals. Therefore the review of the literature is focused on the mechanisms controlling the plasma concentration of the metabolite and hormones of particular interest to the present experiments.

CHAPTER TWO: REVIEW OF THE LITERATURE

2.1 Regulation of plasma insulin, GH, and cortisol concentrations

2.1.1 Regulation of plasma insulin concentration

2.1.1.1 Plasma metabolites and ions:

Changes in plasma glucose concentration are of recognised importance in regulating insulin secretion in ruminants (Bassett 1975,1981), as well as in other species (O'Connor et al, 1977). The exact mechanism by which glucose stimulates insulin secretion is still unknown. Two mechanisms so far have been proposed: 1) glucose may act by interacting with specific membrane receptors on the B-cell from which a secondary signal may originate , regardless of the further glucose metabolism within B-cell. 2)glucose may act as a substrate, the stimulus for insulin release being due to a metabolite or cofactor arising during its metabolism within the B-cell (Belfiore, 1980). Studies with isolated pancreatic B-cells show that prolonged stimulation with glucose induces a biphasic insulin secretory response characterised by an initial rapid phase lasting about 5 minutes during which about 2-3 percent of the pancreatic insulin content is released, followed by a slow increase in insulin secretion which results in the liberation of up to 20 percent of total pancreatic insulin content (O'Connor et al, 1977). It has been suggested that two, one small and one larger, pancreatic components of insulin are responsible for the minor and major insulin secretion peaks respectively (Belfiore, 1980). Glucose besides its stimulating effect on insulin release, also stimulate insulin synthesis probably at the transcriptional and post-transcriptional levels but most of the insulin released initially upon glucose stimuli comes from the hormone stored in the secretory granules (Randal and Hales, 1972).

Some amino acids (AA) especially arginine,lysine, leucine, and phenylalanine, are very effective in stimulating insulin secretion in several species (reviewed by Parman, 1979) including ruminants (Hertelendy et al, 1970, Davis, 1972; Stern et al, 1971; Bohn 1978). Amino acids do not appear to act as insulin secretagogues by

serving as fuels for B-cells (Hellman et al, 1971) but probably by potentiating glucose-stimulated insulin release (Basabe et al, 1971). It has been found amino acids greatly stimulate lactate formation from glucose (Pace et al, 1975). This effect may play a significant role in regulating the extent to which glucose serves as a stimulus for insulin secretion by pancreatic islets.

Free fatty acids (FFA) in man enhance the secretory response of insulin to glucose (Balasse and Ooms, 1973). Medium chain triglycerides also induced insulin secretion in dogs (Bach et al, 1974). In adult and young ruminants, volatile fatty acid (VFA), especially propionic acid and butyric acid are better stimuli for insulin release than glucose (Bassett, 1975; Manns and Boda, 1967; Horino et al, 1968; Ambo et al, 1973; Bohn, 1978).

High and normal extracellular calcium ion concentrations stimulate insulin release by increasing the amount of calcium ions entering into the B-cell (reviewed by Wollheim, 1981). The full development of the insulin response to physiological stimuli requires the presence of extracellular calcium. It is inferred that glucose increases the cytosolic calcium ion concentration of the B-cell by stimulating a rapid entry of calcium ions across the plasma membrane into the cell after an imbalance of calcium ion flux across the membrane. It is proposed that the biphasic pattern of glucose-induced insulin secretion can be accounted for by a biphasic changes in the concentration of calcium across the B-cell membrane. The amino acids (AA) also require the presence of extracellular calcium to stimulate insulin release (Wollheim, 1981).

The physiological significance of insulin responses to nutrient entry into the circulation lie in that insulin is the major anabolic hormone. Its initial short-term action is to maintain nutrient homeostasis by promoting uptake of glucose, FFA, and AA by the peripheral tissue (cells), especially muscle, adipose and connective tissues, and increasing the synthesis of lipid and protein in these tissues. It also minimizes the endogenous production of glucose, FFA and AA by antagonizing the catabolic effect of many other hormones, thus leading to a decrease in the plasma concentration of the metabolites.

2.1.1.2 Enteric and pancreatic hormones

Gut hormones: Many gastrointestinal hormones have been demonstrated to have a stimulatory effect on insulin release following feeding. These include gastrin, cholecystokinin (CCK), secretin, gut-glucagon, cerulein, vasoactive intestinal peptide (VIP), and gastric inhibitory polypeptide (GIP) (Misra, 1980; Bertaccini, 1982 Adrain and Blom, 1982). However so far only GIP has been shown to be physiologically significant. The mechanisms as to how these gut hormones affect insulin release is still poorly defined. Neurotensin has also been considered a very potent regulator of insulin and glucagon secretion in the calf (Blackburn et al, 1980).

Somatostatin (SLI): Soon after the discovery of the presence of somatostatin (SLI) in the hypothalamus, it was found that SLI is also present in many other tissues. It is now established that SLI has a widespread influence on the overall metabolism of the animal by its inhibitory effect on the secretion of many hormones, including GH, thyrotropin, prolactin, insulin, glucagon, and most of the gut hormones (Gerich, 1981). It has been proposed that the insulin release in response to a meal may be attenuated by SLI (Schusdziarra, 1980) to avoid abrupt metabolic disturbances which could be induced by hyperinsulinemia during a meal. SLI may also decrease insulin response to a meal by inhibiting the secretion of gut hormanes that control the digestion and absorption of nutrients. Thus reducing gut motility, gastric and pepsin secretion, pancreatic fluid, electrolyte and enzyme production, splanchnic blood flow as well as affecting many other vital processes (Vale et al, 1975; Gerich , 1977; Gerich, 1981; Effendic et al, 1978). At present, however, there is a lack of knowledge concerning the role of SLI in the regulation of insulin secretion in ruminants.

Glucagon: The role of glucagon as an antagonist of insulin action in blood glucose homeostasis has been discussed in several papers (Bassett, 1975, 1978, 1980; McDowell 1983; Unger et al, 1977; Lefebvre and Luyckx, 1979). It is established firmly that glucagon has a direct stimulatory effect on insulin secretion (Samols

et al, 1965; Mayhew et al, 1969). Insulin release in response to glucagon infusion has also been observed in ruminants (Bassett,1971). It is proposed that there is a insulin-glucagon negative feedback mechanism in which insulin suppresses glucagon while glucagon stimulates insulin secretion (Samols et al, 1972). A recent morphological study of the islets of Langerhans suggested that insulin release from the B-cell may be less inhibited by SLI from the D-cell than glucagon from the A-cells (Unger and Orci 1976). So far, however, it is not clear to what extent insulin secretion is stimulated by levels of circulating nutrients and by glucagon.

2.1.1.3 Other hormones and the central nervous system (CNS):

GH: Whether the naturally occuring sudden rise in GH release is capable of stimulating pancreatic insulin secretion has not been established. It has been reported that perfusion of an isolated rat pancreas preparation with bovine or ovine GH produced a transient insulin and glucagon secretion (Tai and Pek, 1976). Acute pancreatic insulin and glucagon release was also observed in dogs following intravenous injection of GH (Sirek et al, 1979). Insulin concentration was raised in cows following GH treatment in one study (Bines et al, 1980), but not in another (Peel et al, 1983). Another study showed that highly purified hGH did not affect insulin release (Larson et al, 1978). Thus there is no conclusive evidence that GH has a direct effect on the endocrine pancreas. There is the possibility that contaminants in the GH administered to the animals in the above experiments may be responsible for the observed pancreatropic effect. Somatomedins should be ruled out from these contaminants as it requires several hours to attain a measurable increase in its activity (Wyk et al, 1973). It is also possible GH can affect insulin concentration by regulating the affinity of insulin receptors (Lwasaki, 1982). Thus the mutual relationship between GH and insulin is still poorly understood as it appears that certain amino acids stimulate the secretion of both insulin and GH, but for insulin the relationship to acute energy substrate levels may be more overiding than for GH. High insulin concentrations were found in acromegalic patients whose GH level was high (Yallow and Berson, 1960). Higher insulin and GH levels were also found in

energy restricted HBI cows than in LBI cows (Flux et al 1984). In these cases insulin secretion may be stimulated to antagonize the diabetogenic and lipolytic effect of GH.

Cortisol: It is known that alteration in adrenal cortical function can dramatically perturb insulin secretion in experimental animals and in man. Decreased concentration of glucocorticoids are associated with decreased insulin secretion while either endogenous or exogenous hyperadrenocorticoidism is associated with increased insulin secretion (Kinash and Hasit, 1954; Malaisse et al, 1967a, 1967b; Rastogi and Campell, 1970; Arkerblom et al, 1973; Curry and Bennett, 1973; Bassett and Wallace, 1967) and these alterations in insulin concentration could be due to either a change in insulin secretion or a change of insulin binding to its receptor on peripheral tissue. Recent studies have demonstrated the presence of steroid receptors in islet cells (Green et al, 1978; Tesone et al, 1979). While other experiments have demonstrated the direct effects of glucocorticoids on insulin secretion (Billaudel et al, 1978; Brunstedt et al, 1979, 1981; Borelli et al, 1982). Insulin secretion may be stimulated by corticosteroids through the following ways:1) increased glucose metabolism in B-cells. 2) increased calcium uptake by B-cells. 3) increased CAMP production by B-cells. 4) hyperglycemia. Glucocorticoids may also affect insulin concentration by acting on insulin receptors on peripheral tissue.

Catecholamines: It is now firmly established that α-adrenergic activation has an inhibitory control, and β-adrenergic activation has a stimulatiory effect on insulin secretion (Mayhew et al, 1969; Rossini and Buse, 1973). Noradrenalin is an almost pure α-adrenergin agonist, while adrenalin can stimulate both α- and β-adrenergic receptors in the islets equally well. Both catecholamines are directly under sympathetic nervous control. However the physiological relevance of both adrenalin and noradrenalin released from nerve terminals in response to sympathetic nervous activity is thought to be negligible except during acute muscle exercise,or in response to stress when the dual effect of stimulating glucagon and inhibiting insulin would be expected to encourage hepatic glucose release. Because of the short half-life of catecholamines in plasma,

their effect on the pancreatic endocrine function may also be a temporary one.

Central Nervous System (CNS): Recent studies show that the functions of the islets are under the integrative control of the CNS (Pearse and Polak, 1978). The islets of Langerhans are extensively innervated by adrenergic, cholinergic and peptidergic nerve fibers. It has been suggested that the first phase of insulin secretion in response to a meal or oral glucose is due to the cooperativity between sympathetic and parasympathetic systems (Burr et al, 1976). However, the CNS involvement in regulation of pancreatic secretion is yet to be fully investigated.

2.1.1.4 Insulin receptor:

The insulin receptor is defined as the molecular structure on the cell surface which is capable of recogniszing and binding insulin in a specific manner. Insulin and the receptor then forms a complex which is involved in the initiation of the stimulus-response sequence. Insulin binding to the receptor is a necessary but not sufficient condition for insulin action. The hormone receptor complex must be linked to an effector system for a biological response to be expressed (Kahn, 1976). It is now believed that the number and affinity of insulin receptors can be affected by several factors. Insulin and analogoes can directly down regulate the number of insulin receptors (Bar et al, 1979; Olefsky and Reaven, 1977; Olefsky and Ciaraldi, 1981). The binding characteristic of remaining receptors are unaltered. Short term fasting leads to a striking increase in insulin binding to its receptors (Olefsky, 1976) due to an increase in receptor affinity. In both man and animals, a high carbohydrate intake will result in a decreased affinity of insulin receptors on adipose tissue (Olefsky and Saekow, 1978). It was also found that in the long term insulin binding to the receptor may be controlled by altering the receptor number with binding affinity returning to normal (Kolterman et al, 1980; Yasuda and Kitabchi, 1980).

It is proposed that maximal insulin stimulation of glucose uptake occurs when only a small fraction ,about ten percent, of total

adipose insulin receptors are occupied (Kono and Barharm, 1971; Gammeltoft and Gliemann, 1973). This suggests that the cellular response to insulin increases with receptor occupancy until a certain level of occupancy is reached (10% of available receptors) and that further increases in receptor occupancy will not lead to further increases in cellular response. This also suggests that increased plasma insulin level may be either due to an increase in insulin secretion or a decrease in insulin binding to the receptor. Thus some factor which can affect insulin concentration may act on the regulation of insulin receptor affinity. Decreased insulin binding has been found in animals treated with glucocorticods (Olefsky et al, 1975; Kahn et al, 1978). In acromegaly insulin binding to its receptor may be enhanced by an elevated GH level (Muggeo et al, 1977).

2.1.2 Regulation of plasma GH concentration

2.1.2.1 Hypothalamic control:

The mechanism controlling GH secretion is complex and work in this field is far from conclusive for any species, but it is now accepted that hypothalamic control is mainly responsible for the rhythmic release of GH and similar mechanisms exist in all species (reviewed by Chiodini and Liuzzi, 1979; Sonksen and West, 1979; Cockram et al, 1983).

GH secretion from the anterior pituitary is regulated by a dua of hypothalamic control. Growth hormone releasing factor (GHRF) stimulates and GH release inhibitory factor (GHRIF) or somatostatin (SLI) inhibits GH secretion. The basal GH level is thought to be regulated by SLI while the episodic spike of GH release occurs when GHRF predominates over the inhibitory action of SLI which has a short half life in the circulation. Besides supressing GH release SLI has also been found to have widespread inhibitory effects on the release of other hormones, such as thyrotrophin, (TSH), prolactin, insulin, glucagon and many gut hormones which might indirectly influence GH secretion (Gerich, 1981). GH concentration can be depressed by SLI treatment in sheep (Redekopp et al, 1980). Depressing SLI concentration with antibody against SLI has resulted in increased GH

level in lambs. (Varner et al, 1980). It was believed that SLI regulates GH secretion by a short loop negative feedback mechanism.

The neural mechanisms which control GH secretion via their influence on hypothalamic release of GHRF and SLI has also been extensively reviewed in the literature (Sonksen and West 1979; Cockram et al, 1983; Chiodin and Liuzzi, 1979). In summary, the high concentration of monoamines (noradrenalin, dopamine, and serotonine) in the hypothalamus, particularly in the median eminence region, suggested that these may be important in the regulation of hypothalamo-hypophysiotrophic hormones. In man and primates there is a stimulating effect of the central noradrenergic system operating through α-adrenergic receptors, while β-receptors appear to be inhibitory. The central noradrenergic system appears to represent the major drive for GH secretion in the primate and rat. Recent evidence in rats suggest the noradrenergic system mediates GH secretion by stimulating GHRF release rather than by inhibiting SLI release (Eden et al, 1981).

The central serotoninergic system also has a stimulatory effect on GH release and it is suggested that the serotoninergic system is involved in the physiological regulation of GH as blockade of serotonin receptors in man suppress both sleep related GH secretion (Chihara et al, 1976) and GH release in response to insulin hypoglycemia (Smythe and Lazarus, 1974).

The role of dopaminergic system is less clearly defined. L-dopa which crosses the block-brain barrier, generally evokes GH secretion although it may reduce the GH response to hypoglycemia, suggesting a dual action in GH release. The dopaminergic system possibly acts to augment basal and reduce stimulated GH release (Cockram et al, 1983).

Vasoactive intestinal polypeptide (VIP) now widely accepted as a gastrointestinal hormone has been found in the hypothalamus of many species. Evidence shows that VIP secreted from the hypothalamus possibly acts as a hypothalamic neurohormone. GH release can be stimulated by VIP both at the hypothalamic and anterior pituitary levels (reviewed by Nicosia et al, 1983). This stimulating effect of VIP may due to its inhibition of SLI release as shown in rat (Epelbaum et al, 1979). However the physiological role of VIP in regulating GH release is yet to be defined.

Other hypothalamic hormones may also influence GH release

directly or indirectly. Thyrotrophin releasing hormone is a potent stimulus for GH release. Overall it appears that GH secretion is regulated by the integrated action of may hypothalamic hormones.

2.1.2.2 Somatomedins (SM):

Considerable evidence suggests the existence of short loop negative feedback regulation of GH secretion. Though SLI may regulate GH release by such a mechanism at the hypothalamic level (Molitch and Hlivyak, 1980), many studies suggested that somatomedins (SM) may be partly responsible for the negative feedback control of GH secretion (Berelowitz et al, 1981; Abe et al, 1983; Tannenbaum et al, 1983). Low concentrations of the SM and high concentration of GH are found in Larson Dawrfism, in which GH does not stimulate SM production. Somatomedin C not only stimulates SLI release from the rat hypothalamus, but also acts directly on the anterior pituitory cells to suppress GH release (Berelowitz et al, 1981). In these studies insulin-like growth factor II was less potent. It is also suggested that SM may be generated locally in the pituitary or hypothalamus which is involved in the feedback control of GH release (Abe et al, 1983). GH also appeared to exert negative feedback effects on its own secretion. It is proposed that short term autoregulation is via GH effects on hypothalamus SLI or possibly GHRF secretion, whereas longer term effects may be via the somatomedins (Abe et al, 1983).

2.1.2.3 Glucocorticoids:

The effect of glucocorticoids on GH secretion has been reviewed by Chiodin and Liuzzi (1979). In summary, administration of glucocorticoids can impair the secretion of GH in humans as well as in other species. Chronic administration of cortisol resulted in impaired release of GH in response to insulin hypoglycemia, and smaller dosage of cortisol resulted in less consistent inhibition. Large and acute doses of cortisol can also suppress GH release induced by hypoglycemia. Sleep-related GH secretion is not affected by acute cortisol administration but is impaired by chronic treatment. There is at present no satisfactory explanation for the inhibitory activity of glucocorticoids on GH secretion. Cortisol in low concentration stimulated GH release from normal human pituitary in vitro (Bridson and Kohler 1970) but inhibited GH release from rat pituitary when given in high concentrations (Birge et al, 1967). It is not know at what stage GH concentration is affected by cortisol, synthesis, secretion or after secretion events.

2.1.2.4 Metabolites:

Energy substrate: In relation to plasma levels other than amino acids GH appears to be diametrically opposed to insulin. Whereas insulin is secreted in response to high glucose concentration, GH is often stimulated by hypoglycemia including that induced by administration of insulin. It is noted that a rapid decrease in blood glucose was always effective in inducing GH release, regardless of the absolute glucose levels involved. (Roth et al, 1964). Similar responses were observed in ruminants (Stern et al, 1971; Wallace and Bassett., 1970). It is suggested that there is a hypoglycemic threshold for GH release (West and Sonksen, 1977). Conversely, hypoglycemia has been found to inhibit basal GH secretion and arginine or exercise induced GH secretion, but does not block sleep or stress induced GH release. It is believed that the hypothalamus contains areas which act as glucoreceptors and are important in relation to GH secretion. It has been observed that some adaptation of glucoreceptors occurs in relation to chronic blood

glucose changes during recovery from hyperglycemia even though absolute blood glucose levels are high (Irie et al, 1967). Glucose perfusion of the median eminence region has blocked GH response to insulin hypoglycemia in monkeys (Blanco et al, 1966) and induction of intracellular glucopenia in the hypothalamus has stimulated GH secretion in monkeys (Himsworth et al, 1972). These data suggest that the lateral hypothalamus is sensitive to a fall in blood glucose and the ventro medial nucleus to a rise in blood glucose concentration.

Prolonged fasting in many species is characterized by rises in plasma concentration of free fatty acids (FFA) and ketone bodies. It is currently held that the increased FFA concentration in starvation results from the lipolytic effect of the raised GH concentration and less of the antilipolytic action of insulin due to reduced insulin secretion. So far, however, there is no convincing evidence that FFA concentration directly affects GH release. Some evidence shows that an inverse relationship exists between plasma concentration of FFA and GH (Quabbe et al, 1977; Hertelendy and Kipnis, 1973). It is thought that the inhibitory effect of FFA on GH release may be transferred through serotonin.

Increased GH secretion as a result of low plasma energy substrate levels has been found in fasted laborotroy animals (Gonzales et al, 1981). In ruminants limited energy intake caused higher GH levels in control animals than in animals fed ad lib (Purchas et al, 1971; Hove and Blom, 1973; Flux et al, 1984). The main chronic effect of GH on energy substrate is to decrease the transport of glucose into cells and to promote lipolysis perhaps by increasing the responsiveness of the adipose tissue to other lipolytic agents (Peel et al, 1981, 1983). Thus plasma energy substrate levels are raised as a result of GH secretion occuring when they were low. The action of GH in this respect has been found to be biphasic. It initially exerts a relatively short-term insulin like effect prior to exerting its insulin antagonistic effect (Altszuler et al, 1968; Ahren and Hjalmarsson, 1968). The other effect of GH in maintenance of plasma energy level is thought to be lipolytic. Studies showed that GH stimulated lipolysis in animals under certain conditions notably with the fact that it synergised with glucocorticoids, resulting in far more lipolysis than the additive

effect of each alone (Caldwell and Fain, 1970; Goodman et al, 1970). GH stimulates lipolysis in the absence of insulin, such as in fasting, to prevent energy substrate from decline. It is also known that the lipolytic effect of GH can be inhibited by insulin.

Amino acids: Intravenous infusion of certain amino acids, particularly arginine, which stimulate insulin secretion, also stimulate GH release, but larger doses are required for a GH response. In cases where the concentrations of both hormones were estimated (Hertelendy et al, 1970; Davis, 1972; Bohn, 1978), the insulin peak preceded the GH peak by 15 or 30 minutes. It is possible that GH release is induced by the insulin hypoglycemia. It was suggested that arginine may have a direct stimulatory effect on GH release (Bohn, 1978), but the mechanism is still unknown. The physiological significance of the GH response to amino acid challenge may lie in the fact that GH is the major anabolic hormone which promotes the transport of amino acids into cells and increases protein and nucleic acid synthesis causing somatic growth (Hart, 1980; Bauman and McCutcheon, 1984).

It is also know that cAMP and calcium ion are effective in stimulating GH release and probably act directly on the pituitary somatotrophs. However highly purified GHRF did not increase cAMP within the pituitary but increased cGMP levels which in turn promote calcium uptake into the pituitary causing GH release (Peake, 1973).

Metabolic factors are less important in the day to day regulation of GH secretion than the complex neuroendocrine mechanisms although metabolic factors may act in concert with neuroendocrine and other endocrine secretions in emergency.

2.1.2.5 Different physiological stage:

Age:It is has been observed in the study of human and other species that plasma GH concentration are high at birth and decrease rapidly during the first few weeks of life and then more slowly towards the end of weaning (Cornblath et al, 1965; Machlin et al, 1968; Tsushima et al, 1971; Tucker et al, 1974; Reyneart et al, 1976; Roy et al, 1983; Bassett and Alexander, 1971; Hertelendy et al, 1969) and young adults appear to have higher GH concentrations

than old adults. These changes are due to differences in secretion rather than metabolism of GH because of the absence of a change in the metabolic clearance rate of GH with increasing age (Taylor et al, 1969). As the young animal reaches puberty, there is evidence that the GH secretion is again enhanced (Finkelstein et al, 1972; Dudl et al, 1973).The pubertal increase in GH level may be directly related to increased levels of gonadal steroids. It has been observed that both androgens and oestrogens have an enhancing effect on GH concentration (Illig and Prader, 1970).

Sex: The literature contains some reports that females have higher plasma GH concentration than males in some species, but studies with laboratory animals indicated that plasma GH levels bear little relationship to pituitary GH concentration (Schindler et al, 1972). Thus higher plasma GH level in the females may result from the inhibitory effect of oestrogen on GH removal rather than by stimulation of secretion. But studies in human (Merimee et al, 1969) suggested that oestrogen may have a stimulatory effect on GH secretion.

Pregnancy: GH levels were found to increase progressively in maternal plasma as pregnancy advanced (rat: Saunders et al, 1976; cow: Koprowski and Tucker, 1973). The GH content of the pituitary of the fetus of various animal species undergoes a continuous increase throughout intrauterine life, reaching a maximum just before birth (Contopoulos et al, 1967). The plasma concentration of GH in fetus is also greatly in excess of the maternal plasma GH concentration at comparable stages of gestation. This may be due to a relatively poorly developed hypothalamic controlling system in the fetus.

Lactation: While prolactin is the major pituitary hormone responsible for the maintenance of established lactation in non-ruminants it is now clear that GH is more important than prolactin as a lactogenic hormone in ruminants (Cowie et al, 1980; Bines and Hart, 1982; Bauman and McCutcheon, 1984). The higher GH concentration found in early lactation (Hart, et al, 1978) and in high yielders (Flux et al, 1984; Hart et al, 1978) may lie in the

fact that the lactogenic and lipolytic effect of GH favour the milk synthesis in the mammary gland by increasing the availability of precursors for synthesis. However the precise mechanisms of how GH acts on the different target tissue (eg. adipose tissue, muscle and mammary gland) is still poorly defined.

2.1.2.6 Secretory rhythm of GH

The episodic secretion of GH has been well documented in the literature. For example, GH release occurs with a cyclicity of 3.3 hr in rats, independent of other physiological factors (Tannenbaum and Martin, 1976a, 1976b). Similar rhythmic secretion has also been found in other species, but the biological significance is still ill defined. In man, a most striking increase in GH secretion occurs during sleep and appears to be linked closely to slow wave sleep (SWS) (Pawel et al, 1972). It is not known to what extent the sleep induced GH release is facilitated by the nocturnal state of fasting, though central nerve system (CNS) certainly plays a dominant role in GH release. GH concentration also varies greatly during the day in ruminants. It may be due to the fluctuation in plasma energy status as GH level is normally minimized after the start of feeding (Blom et al, 1976; Bassett, 1975).

2.1.3 Regulation of cortisol concentration

2.1.3.1 ACTH and CRF

Corticortropin (ACTH) has long been known to be intimately involved in the regulation of plasma cortisol levels in animals. In response to increased levels of ACTH, the adrenals increases in size, adrenal blood flow increases and biosynthesis of corticosteroids and androgens in the adrenals increases. The response of the adrenal to ACTH is very rapid, occuring in a few minutes. It is believed that increased cAMP production and increased permeability of the membrane to certain ions are responsible for the rapid response (Fahmy 1983). In addition to its immediate effect, ACTH also exerts a pronounced trophic effect on the adrenal cortex. ACTH is necessary for normal growth, development and maintenance of the adrenal cortex which

atrophies very quickly after removal of the pituitary unless ACTH is administered. Thus ACTH regulates glucocorticoid concentration by increasing glucocorticoid production rather than by affecting its secretion because adrenal steroid hormones are released as soon as they are synthesized and only trace amounts can be detected in the gland (reviewed by Gower, 1979; Fahmy 1983, Hardy 1981; Yates, 1967).

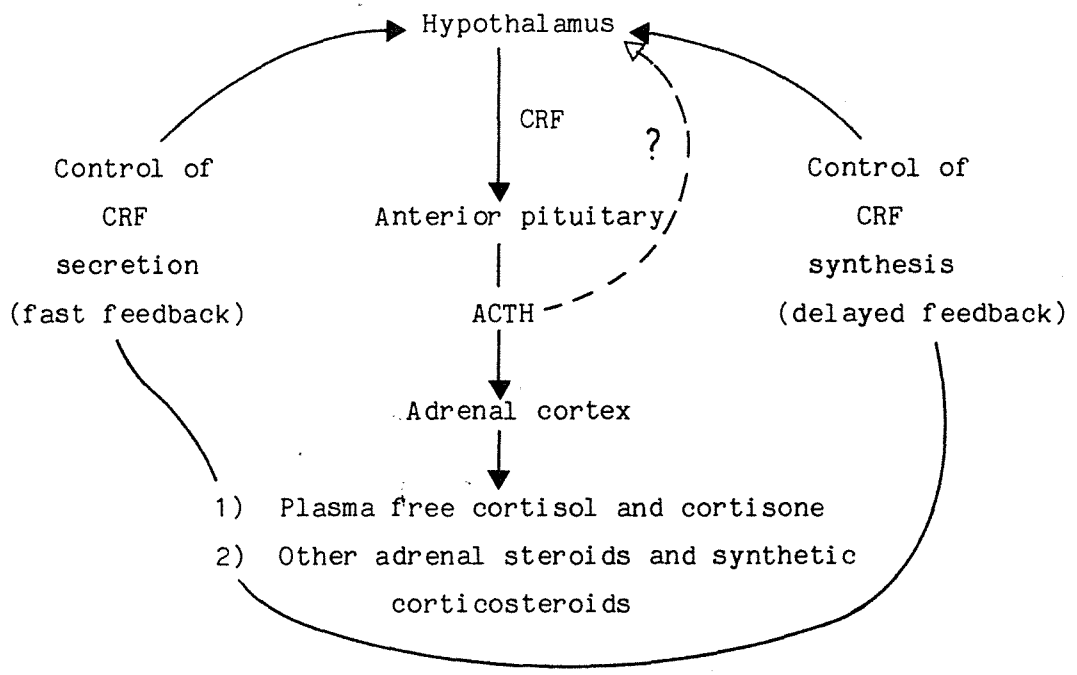
It is also established that secretion of ACTH is under hypothalamic control. Stimulation of higher neural areas by various stresses such as mental disturbance, infection, insulin hypoglycemia, cold and other enviromental challenges can cause the release of corticortropin releasing factor (CRF) from the hypothalamus which stimulates the release of ACTH. ACTH secretion is also regulated by both fast and delayed feedback systems (Jones, 1979). Increased cortisol and cortisone are fast feedback agonists whereas other adrenal steroids and many synthetic glucocorticoids have little or no effect on this system. However higher concentrations of the latter may be involved in the delayed feedback mechanism. The fast feedback reduces the amount of CRF released whereas the delayed feedback reduces the synthesis of CRF. Fig.1 shows the control of hypothalamic - pituitary - adrenal function.

2.1.3.2 Cortisol binding globulin (CBG)

Corticosteroid circulating in plasma are largely bound to plasma protein, particularly to albumin, but cortisol and cortisone are also bound by a specific cortisol - binding globulin (CBG). In human more than eighty percent of circulating of cortisol is bound to CBG, ten percent to albumin, and ten percent as free steriod (Fahmy, 1983). The binding percentage in ruminant is not clear. The biological activity of cortisol is solely accounted for by the free cortisol as it is only form which can be taken up by the target tissue. The high affinity of cortisol for CBG may be of importance in buffering the high amplitude fluctuation of the "free" steriod due to the episodic secretion of cortisol (Fahmy, 1983).

Figure 1 Control of hypothalamus-pituitary-adrenal function

Stimulation of higher neural areas by stresses, mental disturbance, insulin-induced hypoglycemia etc.



(Adapted from Gower, 1979)

2.1.3.3 Circadian factors

Glucocorticoids secretion shows a diurnal fluctuation in many species (man: Rose et al, 1972; pig: and horse: Bottoms et al, 1972; rats: Guileman, 1959; cat: Krieger et al, 1968; cattle: Macadam and Eberhart, 1972); with the maximal production occuring in the early morning and the minimal production in the late evening; Patterns are sometime difficult to discern. The diurnal fluctuation of glucocorticoids concentration involves a parallel fluctuation in ACTH and CRF release though the source of the rhythm is not clearly defined. It is possibly due to the changes in plasma energy substrate levels as it is known that glucocorticoids play an important role in the control of carbohydrate metabolism. Administration of glucocorticoids has a hyperglycemic effect in animals (Ford, 1971; Bassett and Wallace, 1967). This has been attributed to both an increased endogenous glucose production and impaired peripheral glucose metabolism (Reilly, 1971), as well as antagonism of insulin action by cortisol (Bassett and Wallace, 1967).

Glucocorticoids stimulate glucose production by two separate gluconeogenic effects. First they stimulate the breakdown of protein in skeletal muscle and the release of amino acids, leading to increased plasma precusor levels for hepatic and renal gluconeogenesis. Secondly, they induce the formation of specific proteins in the liver, such as the transaminases which catalyse the transfer of α-amino moieties to α-ketoglutarate, thereby providing a pathway for conversion to carbohydrate (Gower, 1979; Hardy 1981). Thus there is a simultaneous increase in the release of amino acid from skeletal muscle and the rate at which the amino acid are trapped by the liver and converted to carbohydrate.

The anti-insulin effect of glucocorticoids in peripheral tissue may lie in that glucocorticoids can effectively inhibit glucose uptake especially by adipose tissue (Belfiore, 1980) probably by reducing the affinity of insulin receptors for insulin (see section 2.1.1.4). Therefore the maximal glucocorticoid production in the early morning as reported in the literature can be partly explained by the fasting hypoglycemia which is sensed by the CNS, causing the release of CRF and ACTH.

2.1.3.4 Stress:

Increased cortisol secretion has been observed in animals under various stress conditions, such as fear, cold, insulin hypoglycemia etc. (Falconer,1976; Johnson and Vanjonack,1976; Dooley and Williams,1975). It is believed that CNS senses these stressful stimuli and the activity of the higher nerve area causes the release of CRF ,thus the release of ACTH.

2.1.3.5 Age:

Young animals are normally born with high cortisol levels, but these decline rapidly after birth within the first few weeks of life. Morphologically, newborn animals have a large adrenal relative to body weight which is due to the trophic stimuli from the fetal pituitary (Orti 1979; Neville and O'Hare, 1982). However , the adrenals involute rapidly after birth with a concomitant fall in plasma cortisol concentration. This pattern resembles that of GH but the evolutionary significance has not been defined.

2.2 Digestive metabolism and glucose homeostasis in young milk fed calves

2.2.1 The digestive process:

The digestive system of a young calf fed solely milk is essentially monogastric since the milk by-passes the rumen-reticulum and its gastric digestion commences in the abomasum. Once in the abomasum, the milk is quickly coagulated by renin to form a clot of casein and fat. The whey is released, assisted by the motility of the abomasom into the duodenum. The passage of the whey into the duodenum, which is the major absorption site of the nutrients, decreases over time and complete passage of whey may require about 7 to 9 hours. The breakdown products from the casein clot are released slowly during the first 6 hours after feeding but thereafter at a more rapid rate as the curd disintegrates (Roy, 1980; Thivend et al,

1979).

2.2.2 Blood glucose homeostasis:

a) After feeding: In the pre-ruminant calf, blood glucose concentration after feeding rises rapidly to a peak of about 130 mg/dl at 1-2 hr after feeding and then declines to pre-feeding values 5-6 hr after feeding (Dollar and Porter, 1957). The rapid increase in glucose concentration is due to the rapid entry of nutrients from the gut. In contrast, blood glucose concentrations are more stable in the ruminating calf, a slight depression occurs about 3 hr after feeding followed by a gradual rise to maximum values 8 - 12 hr after feeding. (Preston and Ndumber, 1961).

It is established that insulin is the most important anabolic hormone involved in the post-prandial regulation of glucose homeostasis. It inhibits gluconeogenesis and glycogenolysis, promotes glucose uptake by insulin-dependent tissues, such as muscle, adipose and connective tissue. As a result of these actions insulin lowers the circulating glucose concentration. The predominant insulin secretion together with hyperglycemia in the post-prandial period also antagonize the action of many other hormones such as glucagon, glucocorticoids, catecholamines, and GH, which promote endogenous glucose and fatty acid production (Bassett 1975, 1981; Belfiore 1980; Marks and Ross 1981; McDowell, 1983).

Blood glucose homeostasis,in the post-prandial period, no doubt is also regulated by some gut hormones and somatostatin from the D-cells present both in pancreas and gut. It is well accepted that several of the gut hormones and peptides have a more or less clearly defined physiological role in digestion and absorption as well as metabolism, while the majority of these gut hormones still await further characterization of their physiological role (Adrain and Bloom, 1982). Increased rate of digestion and absorption of the nutrients and changed motility of the gut will certainly affect the nutrient entry rate into the circulation. It is also recognized that gut hormones and SLI may indirectly regulate metabolic homeostasis by stimulating or inhibiting the secretion of glucagon and insulin. GIP now appears to be more important in regulating insulin and glucagon secretion than other gut hormones such as CCK, gastrin, secretin etc.

(Adrain and Bloom, 1982). The mechanism by which gut hormones affect insulin and glucagon is still not clear. Somatostatin (SLI) has also been proposed to be a major factor in integrating information from ongoing digestive and metabolic events and providing a fine tuning of metabolic homeostasis in the way that certain nutrients enter the organism in balance with their rate of disposal (Reviewed by Schusdziarra 1980). There is evidence to shown that SLI slows intestinal carbohydrate absorption (Wahren and Felig, 1976, Bratusch-Marrain et al, 1981), while the inhibitory effect of SLI on the secretion of pancreatic, pituitary and gut hormones is well established (in section 2.1) . It should be emphasised that most of the work concerning gut hormones and SLI so far has been conducted on laboratory animals but they are assumed to have similar roles in ruminants.

b) Fasting: In the fasting animal when the supply of glucose from the gut is below the requirement of the body, the hormonal regulation of metabolic homeostasis would favour the endogenous production of glucose. It is well known that insulin concentration is diminished in fasted animals and hormones with antagonizing effects on insulin action, such as glucocorticoids and GH, appear to peak when plasma energy substrate levels decline. A historical study (Long et al, 1940) shows that glucocorticoid hormones are essential for maintaining blood glucose levels during fasting, and this appears to be achieved by increasing the rate of protein degradation and mobilising amino acid from skeletal muscle while decreasing protein synthesis in tissues (Tischler, 1981). In fasting, a fall in insulin concentration and a rise in the plasma molar ratio of glucocorticoids to insulin may be important for expression of the glucotropic effect of the corticoids. Glucocorticoids may also have a permissive influence on the insulin antagonizing actions of other hormones such as GH and glucagon.

GH has also been considered a hormone of fasting as its plasma concentration is inversely related to energy status of the animal (Trenkle 1971, 1976; Bassett, 1974). Though there is no evidence that GH promotes gluconeogenesis in the fasting animal, it may help to increase plasma glucose concentration by 1) promoting mobilization and oxidation of FFA from the adipose tissue. 2) reducing glucose

uptake and utilization by peripheral tissue to antagonize the effect of insulin on glucose metabolism.

The principle role of glucagon is to balance the effect of insulin in maintaining glucose homeostasis. It acts primarily to increase hepatic glucose output by promoting gluconeogenesis from amino acids and other glucose precursors, such as lactate and propionate, and by promoting hepatic glycogenolysis. The importance of glucagon in regulating glucose homeostasis may also lie in the fact it is a fasting hormone, when insulin secretion is suppressed or glucagon secretion is increased, lipolysis in adipose tissue may also be faciliated (Trenkle, 1981). The molar ratio of insulin and glucagon has been considered of greater physiological significance than the absolute concentration of the two hormones in maintaining glucose homeostasis in both ruminants (Bassett, 1975) and non ruminants (Unger, 1971). A recent study in lactating sheep indicated that the decreased molar ratio of insulin to glucagon during fasting is due to a decreased insulin concentration rather than increased glucagon value (Gow et al, 1981).

The literature contains few reports concerning the regulation of blood glucose homeostasis in young milk fed calves. It may be expected that such a regulating system in the young calf would bear more resemblance to the one of monogastrics rather than of adult ruminants.

2.3 Objectives of the present study

In the present study, 24 young milk fed Friesian calves of HBI and LBI groups were studied to investigate the effect of genetic divergence for milk production on metabolic and endocrinological status of the calves, and the possibility of finding some suitable markers that can be used in early identification of superior dairy cattle.

Four different experimental treatments i.e. fasting, feeding, arginine challenge and synthetic corticosteroid injection were applied to all calves in order to find out the differences in the metabolite and hormone concentrations between HBI and LBI calves.

It is well known that in fasting, the metabolic homeostasis of

animals favours endogenous production of energy substrates and mobilization of body energy reserve such as glucose and FFA through endocrine system (Section 2.2.2)

Studies with lactating cows suggested that the differnce in milk fat production between HBI and LBI cows might be due to the difference in their ability to mobilize body energy reserve when chronic negative energy balance occurred in lactation (Davey et al, 1983). And this was most probably paralleled by difference in metabolite and hormone concentrations in the blood as significant differences were found in plasma metabolite and hormone concentrations between HBI and LBI cows (Flux et al, 1984) when energy intake was restricted. So it appeared that it was worthwhile ,in the present study, to investigate the effect of fasting which could partly mimic the status of negative energy balance incurred by dairy cows in peak lactation on the metabolite and hormone concentrations in the calves.

The effect of feeding on metabolic homeostasis has been intensively studied by nutritionists in many species, and the knowledge in the literature is abundant. However, it might be also rewarding to investigate its effect from the viewpoint of breeding, as Hart et al (1978) found that a low milk yielding breed had a more vigorous insulin response to feeding than a high yielding breed. Thus, the effect of feeding was studied in the present experiment.

Arginine challenge is a good stimulus for the release of GH and insulin, as well as other hormones in many species, including dairy cattle. It seemed appropriate to measure the response of the calves of different breeding index to arginine injection since GH and insulin secretion and their responsiveness to metabolic signals are essential factors in the control of metabolism.

The role of cortisol in lactating dairy cattle has not been clearly defined. Cortisol may be a determinant factor for the established lactation (Cowie et al, 1980). Furthermore, Johnson and Vanjonack (1976) found that blood cortisol level was higher in higher yielding cows than in low yielding cows and it was spectulated that the difference in plasma glucose concentrations between HBI and LBI cows was caused partly by a difference in plasma cortisol concentration (Flux et al, 1984). Thus the cortisol concentration and the responsivenesses of plasma glucose, insulin and GH

concentration to synthetic corticosteroid injection were investigated in the present study.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Animals

A total of 24 Friesian calves aged from 10 to 18 days old were used in this experiment. Eleven of the 24 calves were classified as high breeding index (HBI) calves the other 13 calves were low breeding index (LBI) calves. Of the 11 HBI calves six were bulls (HBIB) and 5 were heifers (HBIH). Seven of the 13 LBI calves were bulls (LBIB) and 6 were heifers (LBIH). Some general information about these calves ie., date of birth, birth weight, breeding index (BI), age and body weight at the begining of cannulation and sex are given in Table 3-1. The BI for each calf was estimated from the BIs of the calf's parents. The mean values, the standard deviations, and the standard errors of the mean of the breeding index for both HBI and LBI groups are presented in Table 3-2.

All the calves were born on the Dairy Cattle Research Unit at Massey University in Spring 1983 (from 31st July to 8th September). Only healthy calves were used and twin calves or calves with birth weights under 30kg were rejected from this experiment.

3.2 Rearing of calves

After birth all calves were left with their dams for three days after which they were transported to the calf rearing shed and fed by bucket. Normally 2 or 3 days were required before they learnt to suck milk from a bucket through a teat properly, then the calves were transported to the Animal Physiology Unit (APU) and isolated from each other by being housed in individual pens. All calves spent 3 days in these pens, and were fed regularly twice daily at 9.00 and 16.00 hr. This feeding practice was observed until the end of the experiment. Once in the APU the diet was gradually changed from tank-stored colostrum to reconstituted milk made of Ancalf milk powder (The New Zealand Co-Operative Dairy Co.Ltd., Hamilton).

A mixture of half colostrum and half reconstituted milk was fed at each meal for the first 3 days in the APU. They also received a sulphonamides tablet (Scourban Bolus, Vetco Products Ltd) at each

Table 3.1 Number,Breeding Index,sex,date of birth,birth weight, body weight and age at cannulation of calves used in the experiment.

Calf No	BI	Sex	Birth Date	Birth Wt kg	Body cannulated (kg)	AGE cannulated (days)
6	134	M	6-8-83	38.6	43.0	11
13	138	M	8-8-83	45.9	46.5	13
16	133	M	9-8-83	38.1	41.8	15
33	130	M	21-8-83	44.0	43.0	15
40	137	M	27-8-83	51.8	58.0	17
42	130	M	30-8-83	43.6	47.0	13
5	135	F	5-8-83	41.8	47.3	12
7	137	F	6-8-83	37.7	40.0	11
12	133	F	7-8-83	36.3	38.3	14
34	132	F	21-8-83	43.1	44.5	15
50	142	F	8-9-83	46.4	46.5	11
1	103	M	31-7-83	47.2	49.5	14
8	105	M	7-8-83	43.0	47.0	14
10	103	M	7-8-83	46.3	48.5	10
14	99	M	8-8-83	45.0	44.5	13
17	101	M	10-8-83	44.0	45.5	14
18	105	M	11-8-83	40.0	47.0	18
43	108	M	30-8-83	45.9	48.5	13
15	106	F	9-8-83	42.7	44.8	15
20	106	F	13-8-83	44.5	49.0	16
21	103	F	13-8-83	41.8	44.3	16
26	105	F	16-8-83	37.7	43.8	14
29	107	F	17-8-83	36.3	41.5	13
31	102	F	19-8-83	36.0	40.0	17

Table 3.2 Expected means, standard deviations, ranges and the standard errors in Breeding Index units of the HBI and LBI group.

Calf group	Number of calves	Mean of BI	Standard deviation {individuals}	Range for 95% calves	Standard error of the mean
HBI	11	134.8	7.3	120.2-149.4	2.2
LBI	13	104.3	7.3	89.7-118.9	2.0

Table 3.3 General calf rearing procedure.

Stage	Approx age of calves (days)	Rearing site	Feeding regime
1	1-3	with dam on pasture	Colostrum ad lib.
2	4-6	in calf shed.	Tank-stored colostrum fed twice daily using bucket and teat.
3	7-9	Individual craters in APU	Feeding time: 9.00 and 16.00 hr. Diet: half tank-stored colostrum plus half reconstituted milk. Intake: see Table 3.4.
4	10-12	Individual pens in APU	Feeding time: 9.00 and 16.00 hr. Diet: reconstituted milk only. Intake: as above for days 7-9.
5	13-15 approx age at cannulation	as above for days 10-12	as above for day 0-12.

evening meal for the first 3 days to prevent the occurence of calf diarrhea. By the end of the 3rd day the calves were fed completely with reconstituted milk and they were transferred to separate pens in another room where they stayed for 6 days till the end of the experiment. The general calf rearing procedure was summarised in Table 3-3.

Throughout this experiment good hygiene practices were strictly observed: each calf had its own bucket and teat which were rinsed and disinfected after each meal. Calf pens were washed down after every evening meal. The health of the calves appeared very good during the experiment.

3.3 Reconstituted milk:

The reconstituted milk was made up of Ancalf milk powder. The nutritive composition of Ancalf is listed in appendix I. Before feeding, a condensed reconstituted milk was made up by mixing 4.5kg milk powder to 10kg water and stored in a refrigerated room. The condensed milk was diluted with warm water in the ratio 1:1 for each meal.

The calf intake at each meal was calculated according to Table 3-4.

Table 3-4 Milk Intake of Calves at Each Meal

weight of calf (kg)	condensed milk (g)	warm water (g)	total reconstituted milk (g)
30	806	806	1612
35	903	903	1806
40	1000	1000	2000
45	1095	1095	2190
50	1182	1182	2364

3.4 Procedure for cannulation of the jugular vein.

The cannulae (E-ZCATH, 16 gauge,90 cm long, Desert Pharmaceutical Co.,Inc, Sandy, UTAH 84070) were prepared before cannulation with adhesive stuck to the cannulae 15-20cm from the tip. The neck and shoulder of the calves were clipped and then washed using disfectant solution. The position to be cannulated was marked with twink and injected with a local anesthetic (Xylocaine).

The jugular vein of the calf was enlarged by pressing a thumb on it and preventing blood flow to the heart. The tip of cannula was aimed at the centre of the vein and pushed down with an appropriate pressure. Once the blood was obtained from the vein, the needle within the cannula was withdrawn gradually and the cannula inserted into the vein about 15-20cm. Before the start of suturing, sampling was tested and 2ml of heparinised saline injected via the cannula into the vein, then the cannula was plugged. Adhesive tape was sutured to the skin above the cannulating site to prevent the cannula from folding and slipping-out. Another 2 or 3 suturings were also necessary on the neck and back of the calf to support the cannula. Finally a puff of antibiotic powder (Aureomgcin, Cyanamid of New Zealand Ltd., Auckland) was applied at places of trauma to prevent infection.

In some cases a 13 or 14 gauge hypodermic needle was inserted into the jugular vein when difficulty was experienced with the usual procedure. The cannula was passed into the jugular vein through the needle. Calves 29, 31, 43 and 50 were cannulated in this way. They usually lost more blood than calves cannulated with the normal procedure. However, these two different cannulation methods were not expected to have a substantial effect on the physiological status of the calves and the results of the experiment because all calves were sampled on the following day. Twenty four hours was assumed to be adequate for recovery from the surgery.

3.5 Blood sampling

Each calf had its own sampling and flushing syringes. Clean centrifuge tubes were labelled according to calf numbers, sample number, and date of sampling and filled with 5ul of heparin (100,000

iu/ml) prior to the start of sampling. The first 2 ml of mainly heparinised saline was discarded at each sampling then a 10 ml sample of blood was withdrawn. The cannula was then flushed with 2 ml heparinised saline from the flushing syringe. The blood sample was immediately transferred to the centrifuge tube containing 5ul of heparin.The tube was topped with parafilm and inverted gently, mixing the sample with the heparin. Then the sample tube was put into ice bucket or centrifuged immediately after sampling.

The supernatant plasma from the centrifuged sample was removed by using a transfer pipette and aliquoted into 3 vials appropriately labelled. The vials were than capped and stored in a deep freezer. It is imperative to have all samples processed quickly and kept cold to minimize the degradation of plasma components.

3.6 Measurment of haematocrit

Haematocrit were measured on sample 1 and 15 from each calf, using microhaematocrit tubes, centrifuge and reader (International-micro-capillary-reader,International Equipment Company,Boston 35, Massechusetts) following the manufacturers instructions.

3.7 Measurement of Immunoglobulin G1 (IgG1)

Immunoglobulin G1 (IgG1) concentration in the plasma of each calf was determined in a sample collected one day ahead of cannulation at 8.30 am before feeding, using the radial immunodiffusion method described by Fahey and McKelvey (1965). The plasma samples from most calves were diluted 1:40 before assay. Calves 20,29,31, and 43 had low IgG1 concentrations and no measurable precipitate rings were found even when their plasma samples were diluted 1:10.

3.8 Plasma glucose assay:

Plasma glucose concentrations were measured with a Yellow Springs Instrument Model 27 Industrial Analyzer (Yellow Springs Instrument Co. Inc. Ohio). The YSI model 27 was calibrated using five concentrations of standard glucose solutions (25, 50, 75, 100 and 150 mg/dl).The instrument 27 was recalibrated using the standard of 150 mg/dl after every five samples. All samples were assayed twice for glucose concentration with an interval of approximate 1 week between duplicate assays. The result, from the two assays matched each other closely and the mean values of glucose concentration were used for later statistical analysis.

3.9 Radio immunoassays (RIA) of plasma insulin, GH and cortisol.

1) Hormones used in RIA for iodination, standards and raising antibodies.

GH: BGH NIH GH B8 donated to Prof.Flux by Prof. A.E. Wilhelimi (National Institute of Health, U.S.A) was further purified on DEAE cellulose to remove prolactin (Flux, et al, 1984).

Insulin: Bovine crystal insulin, Sigma Chemical Co. no I-5500 10t 121 C-1350 (26.4 iu/mg). No further purification was made.

Cortisol: Stock 1, 2-^{3}H cortisol(New England Nuclear no NET-185).

2) Iodination

The bovine GH and insulin were iodinated by the chloramine T method (Greenwood et al, 1963). The BGH was dissolved in 2M urea at room temperature for 1 hr. before iodination. The iodinated GH was purified from the iodide on a Sephadex G50 Column and was used within two weeks of iodination. A further purification on a Sephadex G100 column was carried out for iodinated GH used more than two weeks after iodination.

3) Antisera

Professor Flux raised the 1st antibody to BGH in guinea pigs in 1974 and the second antibody in Romney sheep in 1980, and in 1982 (personal communication). Antisera was stored at -15°C.

The antisera to insulin was raised in guinea pigs by Bohn (1978) using bovine insulin polymerised with diethyl-pyrocarbonate. The second antibody was raised in sheep. Cortisol antiserum no F3-314 was purchased from Endocrine Science (18418 Oxnard St.,Tarzana, California, 91356).

The GH RIA showed no significant cross-reaction with any of the following anterior pituitary hormones, ACTH, FSH, LH, prolactin and thyrotropin. The insulin assay showed a small cross-reaction with a glucagon preparation. Glucagon has approximately 0.03 percent the effect of insulin, with a non-parallel dose-response line. This degree of cross reaction was considered unimportant (Flux et al, 1984). The cross-reaction of various steroids with cortisol antiserum F3-314 is given in Appendix II.

4) RIA procedures for GH, insulin and cortisol

Plasma GH concentration was determined by RIA using the method of Hart et al (1975). Insulin concentration was determined by a double-antibody RIA (Hales and Randle, 1963). Cortisol level was assayed by the method recommended by the suppliers of the antiserum (Endocrine Science, 18418, Oxnard Street,Tarzana, California,91356, 1982). The detailed RIA procedures for GH,insulin and cortisol are presented in Appendix III.

5) RIA Data Transformation

The original RIA results expressed as counts/min were further processed on a computer (Sord M222), using a program written by Prof. Munford based on a method developed by Burger et al (1972). The untransformed standard carve data were represented by the equation:

$$Y = \frac{A}{C + X^{E}} + e$$

Y --amount of radioactive hormone bound by 1st antibody.

X --amount of non-radioactive hormone present in the tube.

A, C & E --constants specific for each assay.

e - random error.

A,C, and E were determined by an iterative technique and the estimate of the amount of hormone present in each sample. (X) was calculated from the equation:

$$X = \left(\frac{A}{Y} - C\right)^{\frac{1}{E}}$$

X - estimate of amount of hormone present in each of the 50 or 100 ul sample.

The estimate of GH concentration from the Sord printout required two further processing steps:

1) Since each sample had two estimates one from the two 50ul subsamples, and the other from the two 100ul subsamples the estimate from the Sord subsamples was doubled to make it comparable with that from the 100 ul subsamples. But doubling the estimate from the Sord subsample also doubles the error. To cope with this a weighted estimate (W) of the unknown hormone concentration in the 50 and 100 ul samples were calculated according to:

$$W = \frac{Y50 + Y100}{1.5}$$

where Y 50 and Y 100 are the estimate of hormone concentration from the 50 and 100 ul subsamples respectively.

2) The Sord estimates were satisfactory as far as parallelism was concerned, but the standard curve based on the GH standards is displaced because the standard samples have less plasma in them than in the unknown samples. GH assay 82 and 83 both included a set of samples in which known amounts of GH were added to plasma from a somatostatin treated sheep. The correlation between the Sord estimations for the GH concentration in the "normal" plasma samples with known amounts of GH added and these estimations for the GH in the somatostatin treated sheep plasma was high, $r = 0.987$ for assay 82 and $r = 0.978$ for assay 83 when GH concentrations were expressed

in log units (pg/ml). So a simple regression was used to adjust the Sord estimate of GH to compare with the added GH in somatostatin treated sheep plasma. The adjusted Sord GH estimations were used in further statistical analysis.

Since each cortisol sample only had two equal volume subsamples the mean of them were used for further statistical analysis.

6) Parallelism in RIA

Logically the estimate from the 100 ul subsample should be double that from the 50 ul subsamples. This can be checked by plotting the levels of the unknown sample estimates adjusted to the standard curve so that they are the same distance apart on the X axis. If the slope of the line joining the 50 and 100 ul estimate is not parallel to the standard curve such a deviation from parallelism can indicate that:

a) The immunoreactivity of the standard hormone differs from that of the natural hormones in the plasma sample;

b) Or some factor in the assay buffer is depressing or stimulating the affinity of the antisera to the natural hormone to a different degree compared to the standard hormone (Bohn 1978);

c) The suppressing or stimulating effect of some assay factors on the affinity of 50 ul and 100 ul subsamples to the antisera differs;

d) The standard curve in the assay is not capable of providing equal precision over the whole range of the curve as it is often observed that the precision is much reduced at both ends of a standard curve, where the regression line curves away quickly from the major central section (Snedecor and Cochran, 1980).

If lack of parallelism occurs and its cause is detected the problem can be minimised, for example, problem a) can be solved by using a suitable standard hormone; problems b) and c) can be minimised by eliminating or balancing the disturbing factors, or if the disturbing effect is systematic , the estimate from 50 ul and 100 ul may still be valid; problem d) can be tackled by diluting the first antibody at a different ratio, a high ratio increases the sensitivity of the standard curve at low concentrations while a low ratio increases the sensitivity of the standard curve at high

concentrations.

Parallelism for GH and insulin RIA were checked in this experiment (Appendix IV). Samples used for checking were chosen randomly and as no serious parallelism problem was encounted, no further investigation was necessary.

10 Experimental procedure and Statistical Analysis

1) Procedure

Before the start it had been planned to used 24 young pre-ruminant calves in this experiment with 6 calves in each of the four groups, HBIB HBIH, LBIB and LBIH. However, 6 calves in HBIB, 5 in HBIH, 7 in LBIB and 6 in LBIH were actually used as there were only 5 healthy HBI heifers available.

The aim of the experiment was to examine the metabolic and endocrinological differences between the high breeding index (HBI) and low breeding index (LBI) groups of Friesian calves under different physiological conditions and experimental challenges. Special attention was given to the bull calves.

Four treatments were applied to each calf and 18 blood samples were collected from each calf during the experiment. The timing of the treatments (fasting, feeding, acute arginine infusion, and cortisol injection) and samplings are presented in Table 3-5. On day one, the following treatment were given to the calves and fourteen samples were collected.

Fasting: Each calf was fasted for approximately 16 hrs before two blood samples were collected in the morning at an interval of 30 minutes. The calves were fed immediately after the second sample.

Feeding: Calves were fed reconstituted milk according to their body weight (section 3.3). Six samples were collected after feeding at unequal intervals with more intense sampling immediately after feeding as indicated in Table 3-5.

Arginine infusion: Three and a half hours after the start of feeding each calf received an intravenous infusion of 0.04g arginine/kg body weight. Another 6 samples were collected from each calf at unequal intervals (see table 3-5).

Corticosteroid injection: Subcutaneous injection of the

Table 3.5 Summary of experimental procedure.

Treatment	Time of sampling	Sample number	Time intervals (minutes)
Day One			
Fasting			16 (hours)
	8:30	1	-30
	9:00	2	-1
Fed	9:00 to 9:03		0
	9:05	3	5
	9:10	4	10
	9:20	5	20
	9:40	6	40
	11:00	7	120
	12:25	8	205 (or -5)
Infuse arginine			
(0.04g/kg bwt)	12:30		0
	12:35	9	5
	12:40	10	10
	12:50	11	20
	13:10	12	40
	13:50	13	80
	15:55	14	205 (or -5)
Inject Betsalan			
(50ug/kg bwt)	16:00		0
Day Two			
	8:30	15	-30
Fed	9:00		0
	9:20	16	20
	9:40	17	40
	16:00	18	380

synthetic corticosteroid (Betsalan,Glaxo,New Zealand Ltd.) was given to each calf at 16:00hr. The dosage was 0.05g/kg body weight. Four samples were collected on the following day at the time 8:30, 9:20, 9:40 and 16:00 hr respectively.

Plasma glucose, insulin, GH and cortisol concentration were analysed using the methods described in sections 3.8 and 3.9.

2) Data analysis:

The data of plasma metabolite and hormone concentration from this experiment were analysed using a generalized linear model computing package (REG) (Gilmour, 1983). Multivariate analysis was conducted to measure the variance between the repeated observations (i.e. samplings) within each calf and the variance between the main factors, BI and sex, assuming an underlying linear model, which included BI, sex and the appropriate interaction effects.

The form of the model used to describe the observations pertaining to the first sample measured in the BI and belonging to the sex was:

$$Y_{pijk} = U_p + A_{pi} + B_{pj} + AB_{pij} + e_{pijk}$$

U = overall mean together with the effect of the pth sampling.
A = effect of the ith BI in the pth sampling.
B = effect of the jth sex in the pth sampling.
AB = effect of the interaction of the ith BI and jth sex in the pth sampling.
e = random residual effect specific to pij combination of BI and sex in the pth sampling.

In the analysis pertaining to the repeated-measurement models, null hypotheses were tested using the Chi-squared statistic, the basis for so doing being fully described in section 5 - 8 of Morrison (1976).

The following abbreviation are used to denote the significant level of analysis results.

NS = not significant or $P>0.05$

* = $P<0.05$

** = $P<0.01$

*** = $P<0.001$

CHAPTER FOUR: RESULTS

4.0 Results:

Mean values of plasma glucose, insulin, GH and cortisol concentrations for each group of different BI and sex in samples collected before and after feeding, and after arginine infusion are plotted in Figures 2-5 . Mean values of these metabolite and hormone concentrations in samples collected after synthetic corticosteroid (Betsalan) injection are plotted in Figures 6-9 . Results of the statistical analysis of the plasma metabolite and hormone concentrations are as follows.

4.1 Before feeding (fasting status)

Results of statistical analysis of the plasma metabolite and hormone concentrations in prefeeding samples (sample 1 and 2) are presented in Tables 4.1-4.4 which show that:

a) HBI calves had significantly higher basal plasma glucose concentration than LBI calves ($P < 0.05$) (103 vs 93, 102 vs 93 mg/dl for sample 1 and 2 respectively)(Fig.2).

b) HBI calves had significantly higher basal plasma insulin concentration than the LBI calves ($P < 0.01$) (22 vs 12, 21 vs 14 ng/ml, for samples 1 and 2 respectively). Basal insulin concentration in bull calves were also significantly higher than in heifers ($P < 0.01$) (20 vs 13, 20 vs 12 ng/ml, for samples 1 and 2 respectively). Of the four groups of calves, HBIB had the highest basal plasma insulin concentration followed by HBIH, LBIB and LBIH (Fig3).

c) Basal GH concentrations in the HBI calves were also higher than in LBI calves (Fig 4, Table 4.3) at a significance level of 5.1% (11.4 vs 10.5, 11.2 vs 10.3 Lnpg/ml for samples 1 and 2 respectively) though the variance within each group was large.

d) Basal cortisol concentrations in HBI calves were slightly but not significantly higher than that in LBI group ($P > 0.05$) (9.9 vs 8.9, 8.2 vs 7.7 ng/ml for sample 1 and 2 respectively) (Table 4.4).

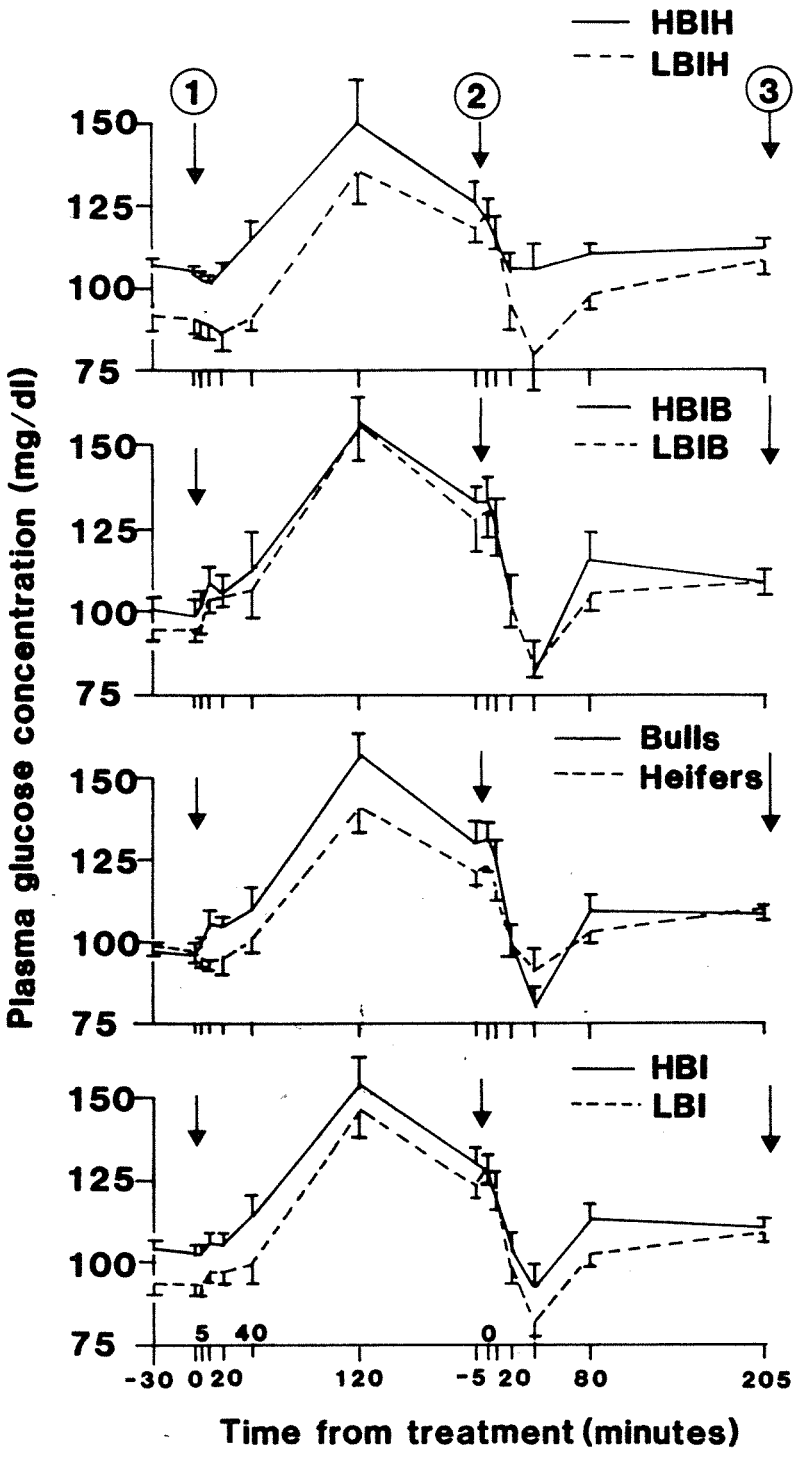


Figure 2 Effects of fasting, feeding, and arginine infusion on plasma glucose concentrations in groups of calves of different BI and sex.

(Vertical bar = standard error of the mean)

(1) = Fed (2) = arginine infusion (3) = steroid injection

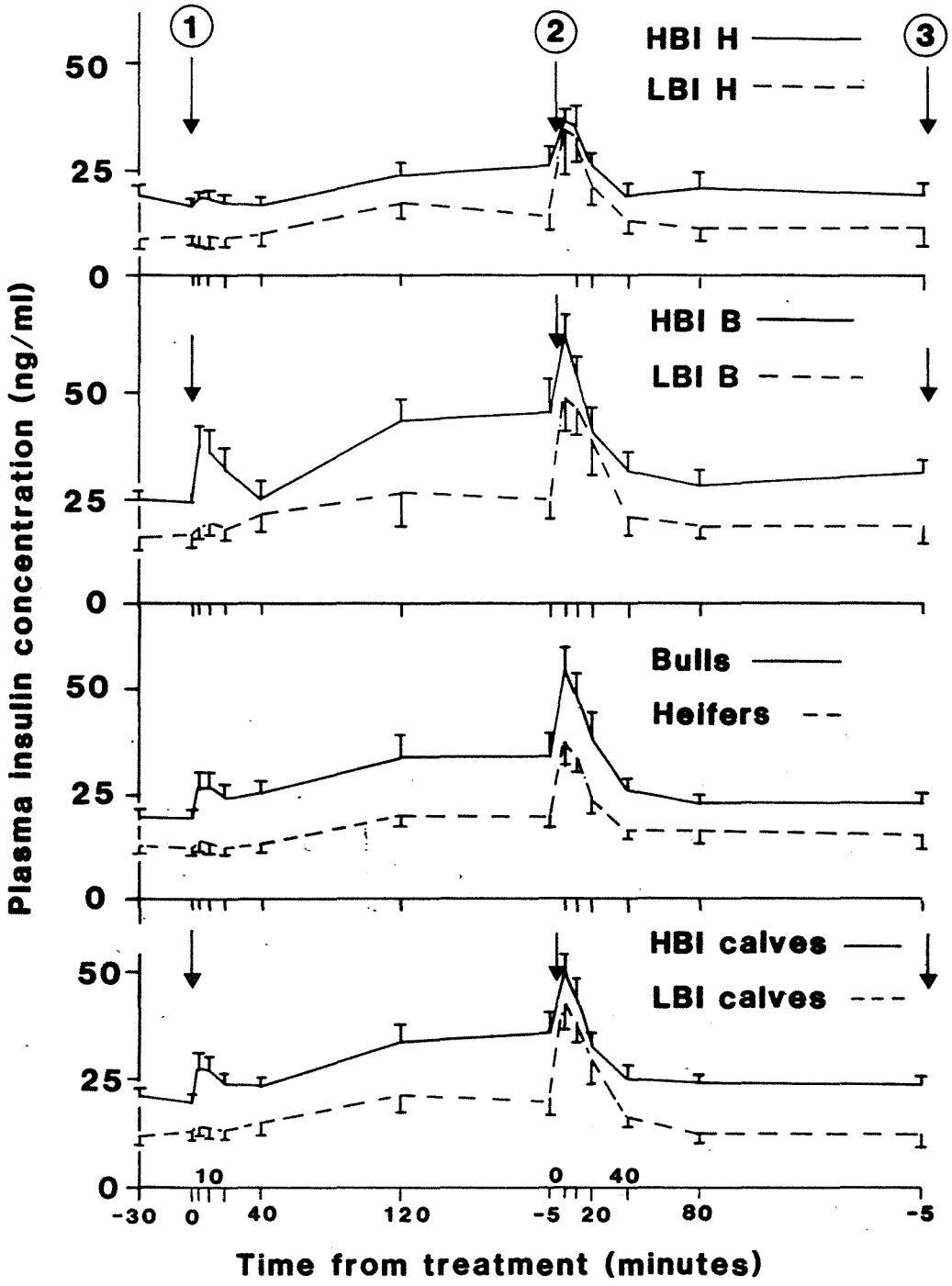


Figure 3 Effects of fasting, feeding, and arginine infusion on plasma insulin concentrations in groups of calves of different BI and sex.

(Vertical bar = standard error of the mean)

(1) = Fed (2) = arginine infusion (3) = steroid injection

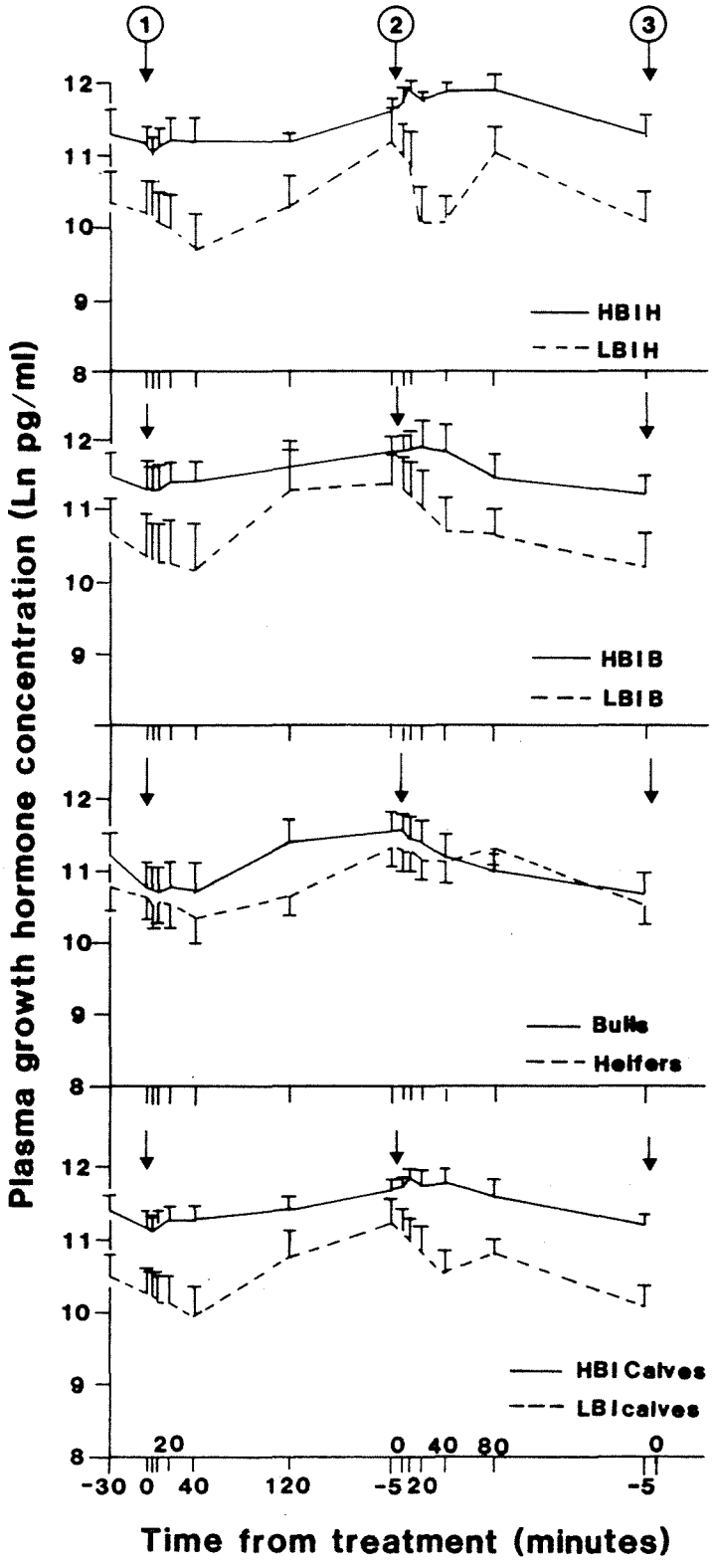


Figure 4 Effects of fasting, feeding, and arginine infusion on plasma GH concentrations in groups of calves of different BI and sex.

(Vertical bar = standard error of the mean)

(1) = Fed (2) = arginine infusion (3) = steroid injection

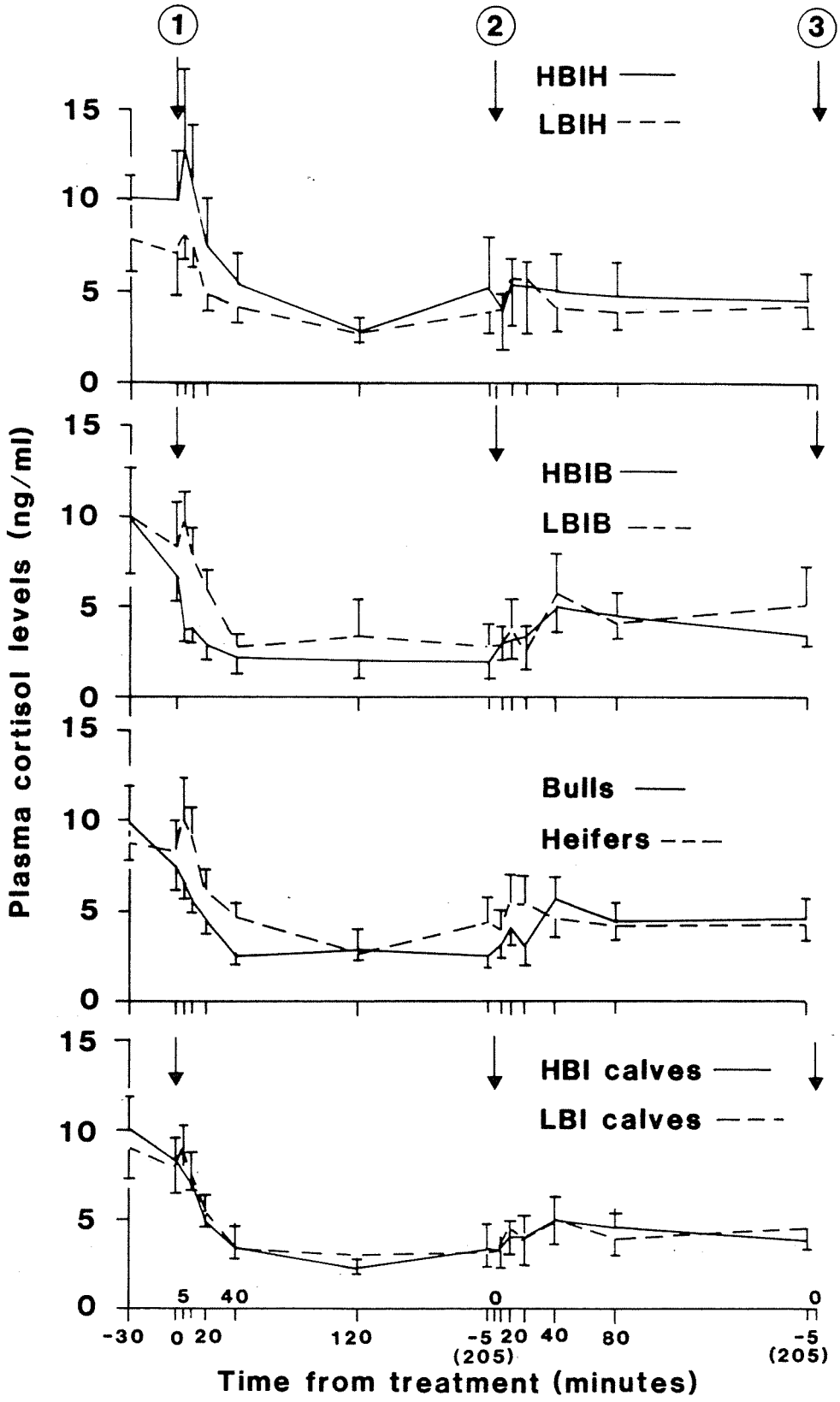


Figure 5 Effects of fasting, feeding, and arginine infusion on plasma cortisol concentrations in groups of calves of different BI and sex.

(Vertical bar = standard error of the mean)

(1) = Fed (2) = arginine infusion (3) = steroid injection

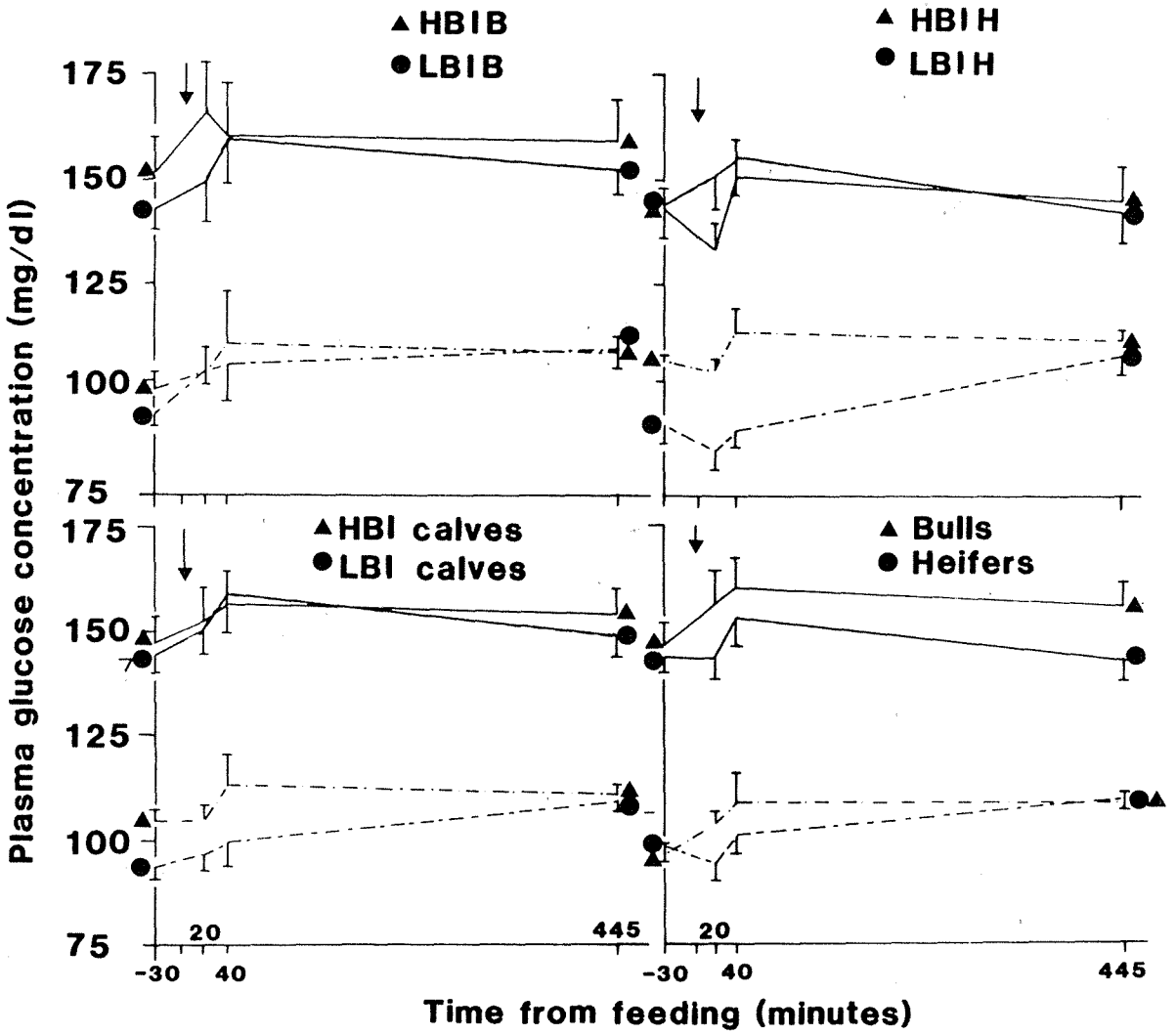


Figure 6 Effect of synthetic corticosteroid injection on plasma glucose concentrations in groups of calves of different BI and sex

Solid line (—) represents values obtained on day 2 following betsalan injection. Broken line (---) represents values obtained on day 1 before betsalian injection.
↓ = fed

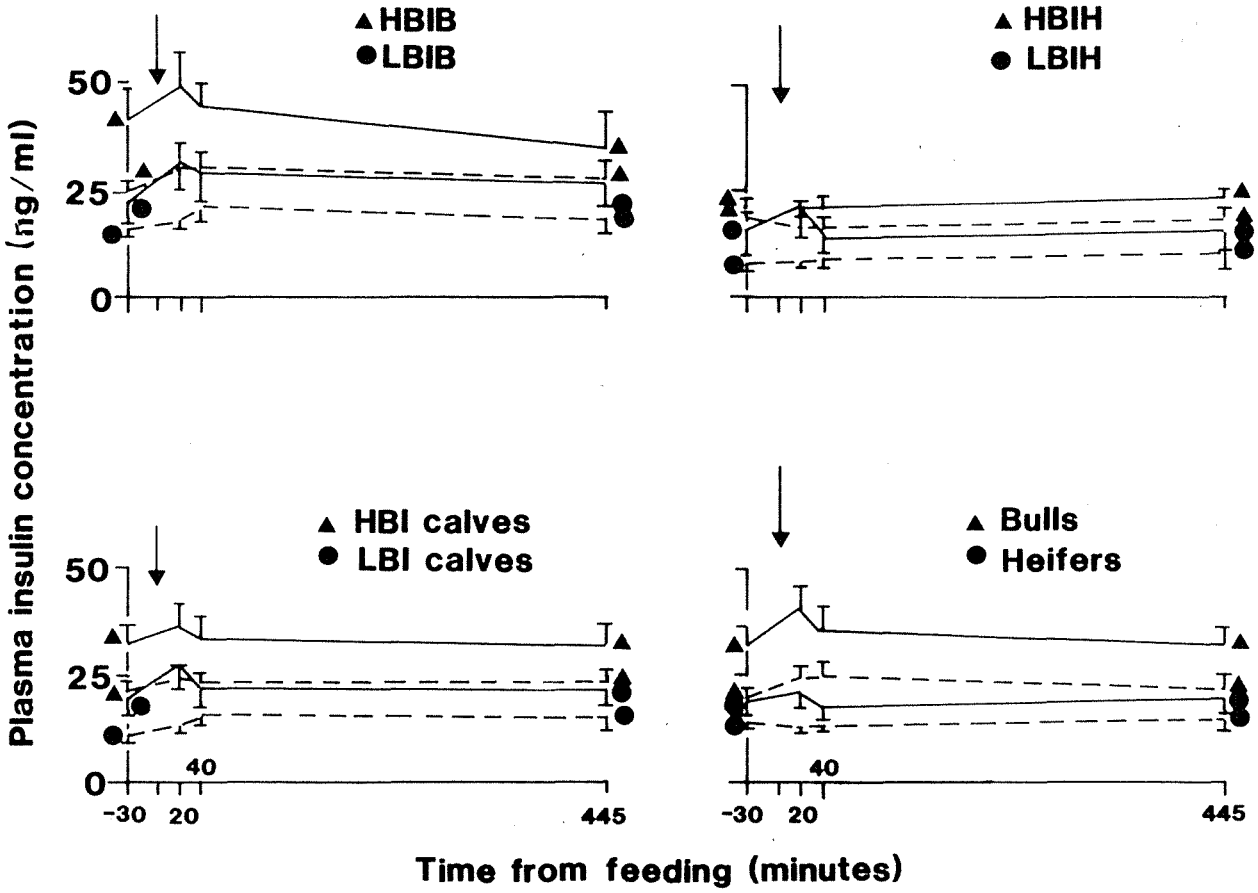


Figure 7 Effect of corticosteroid injection on plasma insulin concentrations in groups of calves of different BI and sex.

Solid line (___) represents values obtained on day 2 following betsalan injection. Broken line (---) represents values obtained on day 1 before betsalian injection.
↓ = fed

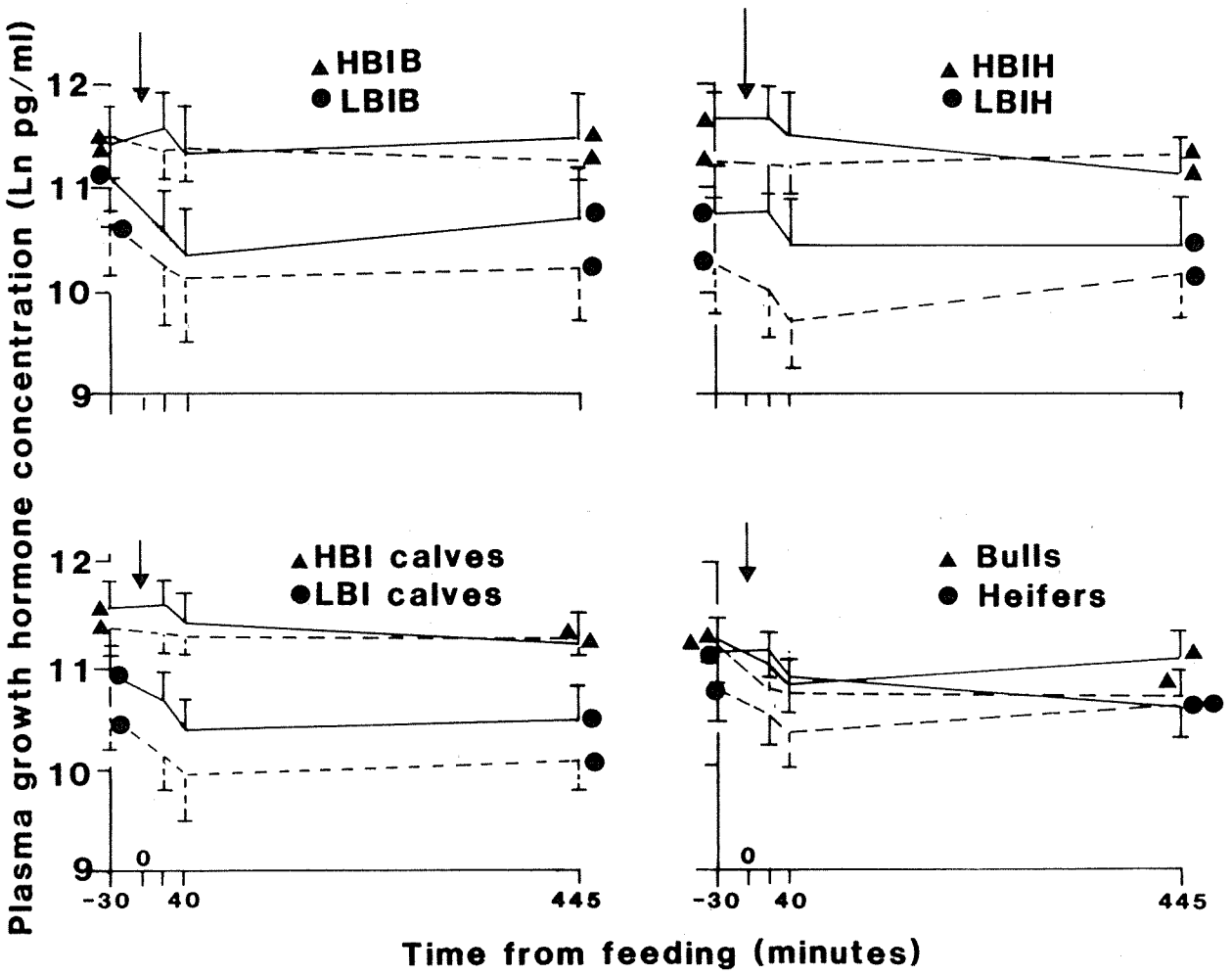


Figure 8 Effect of synthetic corticosteroid injection on plasma GH concentrations in groups of calves of different BI and sex

Solid line (___) represents values obtained on day 2 following betsalan injection. Broken line (---) represents values obtained on day 1 before betsalian injection.
↓ = fed

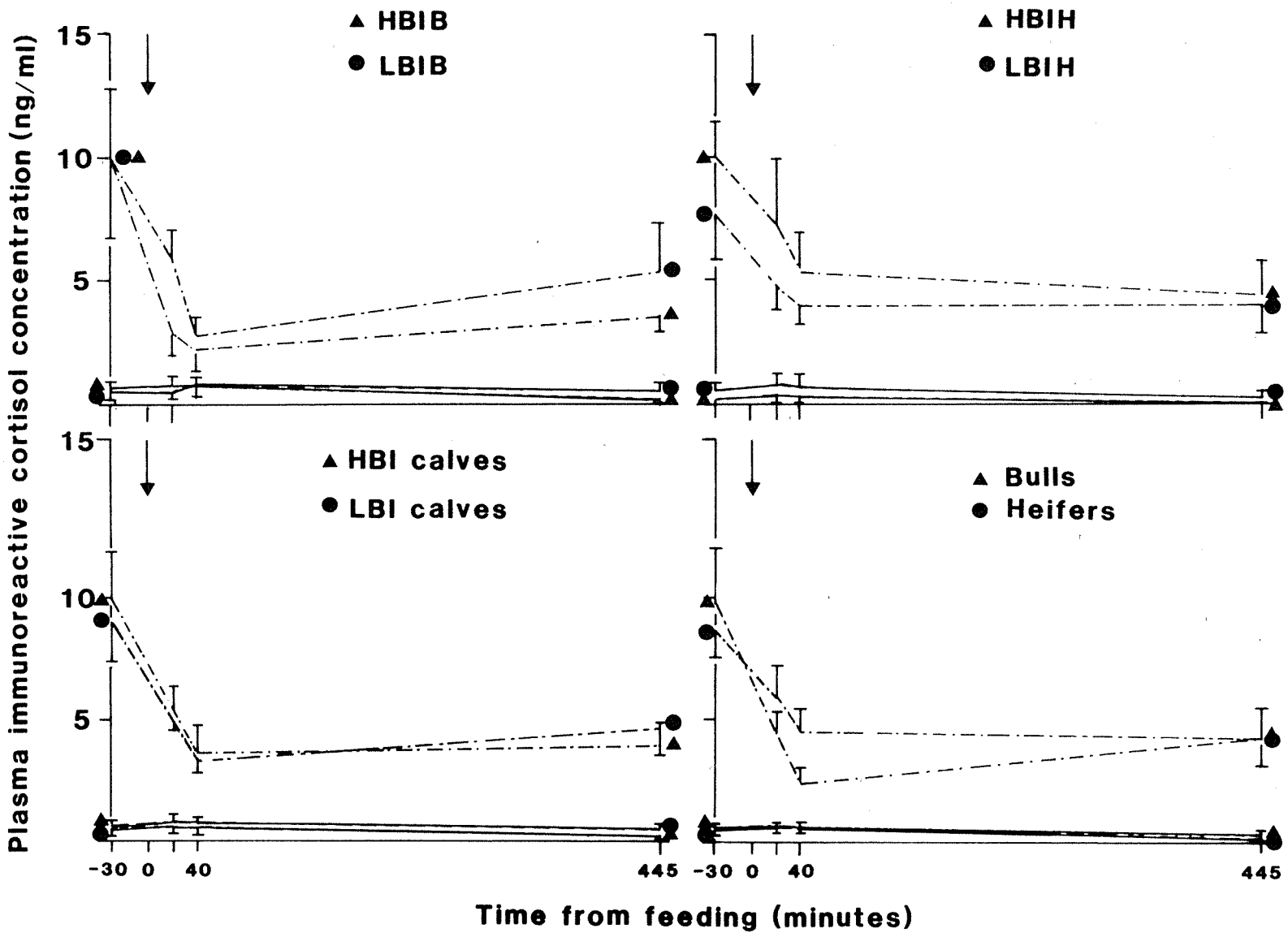


Figure 9 Effect of synthetic corticosteroid injection on plasma cortisol concentrations in groups of calves of different BI and sex

Solid line (—) represents values obtained on day 2 following betsalan injection. Broken line (---) represents values obtained on day 1 before betsalian injection.
↓ = fed

Table 4.1 Analysis of variance of repeated measurements of basal glucose concentration between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Time	1	2.57	NS
BI	1	4.16	*
Sex	1	0.09	NS
BI x Sex	1	1.26	NS
Time x BI	1	0.74	NS
Time x Sex	1	0.44	NS
Time x BI x Sex	1	0.08	NS
Number of observations at per stage	24		

Table 4.2 Analysis of variance of repeated measurements of basal insulin concentration between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Time	1	0.17	NS
BI	1	10.33	**
Sex	1	6.91	**
BI x Sex	1	0.05	NS
Time x BI	1	1.57	NS
Time x Sex	1	0.28	NS
Time x BI x Sex	1	0.01	NS
Number of observations at per stage	24		

Table 4.3 Analysis of variance of repeated measurements of basal GH concentration between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Time	1	4.15	*
BI	1	3.57	NS (P<5.9%)
Sex	1	0.19	NS
BI x Sex	1	0.00	NS
Time x BI	1	0.08	NS
Time x Sex	1	0.51	NS
Time x BI x Sex	1	0.13	NS
Number of observations at per stage	24		

Table 4.4 Analysis of variance of repeated measurements of basal cortisol concentration between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Time	1	1.96	NS
BI	1	0.10	NS
Sex	1	0.00	NS
BI x Sex	1	0.50	NS
Time x BI	1	0.07	NS
Time x Sex	1	0.84	NS
Time x BI x Sex	1	0.21	NS
Number of observations at per stage	24		

Table 4.5 Analysis of variance of repeated measurements of after-feeding glucose concentrations between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Time	5	42.00	***
BI	1	2.98	NS (P<8.5%)
Sex	1	3.80	NS (P<5.2%)
BI x Sex	1	1.57	NS
Time x BI	5	1.20	NS
Time x Sex	5	9.45	NS
Time x BI x Sex	5	1.74	NS
Number of observations at per stage	24		

Table 4.6 Analysis of variance of repeated measurements of after-feeding insulin concentrations between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Time	5	16.81	**
BI	1	8.14	**
Sex	1	8.45	**
BI x Sex	1	0.91	NS
Time x BI	5	6.83	NS
Time x Sex	5	1.56	NS
Time x BI x Sex	5	9.52	NS
Number of observations at per stage	24		

Table 4.7 Analysis of variance of repeated measurements of after-feeding GH concentrations between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Time	5	21.71	***
BI	1	4.01	*
Sex	1	0.48	NS
BI x Sex	1	0.03	NS
Time x BI	5	7.60	NS
Time x Sex	5	5.03	NS
Time x BI x Sex	5	1.92	NS
Number of observations at per stage	24		

Table 4.8 Analysis of variance of repeated measurements of after-feeding cortisol concentrations between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Time	5	24.17	***
BI	1	0.19	NS
Sex	1	2.36	NS
BI x Sex	1	3.29	NS
Time x BI	5	1.80	NS
Time x Sex	5	4.01	NS
Time x BI x Sex	5	7.69	NS
Number of observations at per stage	24		

Table 4.9 Analysis of variance of repeated measurements of glucose concentrations in response to arginine challenge between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Time	5	32.06	***
BI	1	0.72	NS
Sex	1	0.19	NS
BI x Sex	1	0.36	NS
Time x BI	5	2.70	NS
Time x Sex	5	5.29	NS
Time x BI x Sex	5	3.80	NS
Number of observations at per stage	24		

Table 4.10 Analysis of variance of repeated measurements of insulin concentrations in response to arginine challenge between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Time	5	34.69	***
BI	1	1.94	NS
Sex	1	4.72	*
BI x Sex	1	0.21	NS
Time x BI	5	1.98	NS
Time x Sex	5	7.92	NS
Time x BI x Sex	5	9.19	NS
Number of observations at per stage	24		

Table 4.11 Analysis of variance of repeated measurements of GH concentrations in response to arginine challenge between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Time	5	17.69	**
BI	1	5.46	*
Sex	1	0.04	NS
BI x Sex	1	0.09	NS
Time x BI	5	12.95	*
Time x Sex	5	4.64	NS
Time x BI x Sex	5	2.19	NS
Number of observations at per stage	24		

Table 4.12 Analysis of variance of repeated measurements of cortisol concentrations in response to arginine challenge between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Time	5	6.65	NS
BI	1	0.01	NS
Sex	1	0.27	NS
BI x Sex	1	0.05	NS
Time x BI	5	1.22	NS
Time x Sex	5	3.50	NS
Time x BI x Sex	5	0.97	NS
Number of observations at per stage	24		

Table 4.13 Analysis of variance of repeated measurements of glucose concentrations in response to synthetic corticosteroid injection between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Time	3	6.55	NS
BI	1	0.06	NS
Sex	1	1.38	NS
BI x Sex	1	0.73	NS
Time x BI	3	0.46	NS
Time x Sex	3	5.87	NS
Time x BI x Sex	3	6.02	NS
Number of observations at per stage	24		

Table 4.14 Analysis of variance of repeated measurements of insulin concentrations in response to synthetic corticosteroid injection between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Time	3	12.55	**
BI	1	2.88	NS
Sex	1	5.67	*
BI x Sex	1	0.85	NS
Time x BI	3	1.69	NS
Time x Sex	3	1.79	NS
Time x BI x Sex	3	3.25	NS
Number of observations at per stage	24		

Table 4.15 Analysis of variance of repeated measurements of GH concentrations in response to synthetic corticosteroid injection between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Time	3	10.62	*
BI	1	3.51	NS (P<6.2%)
Sex	1	0.03	NS
BI x Sex	1	0.01	NS
Time x BI	3	3.83	NS
Time x Sex	3	7.67	NS (P<5.4%)
Time x BI x Sex	3	2.66	NS
Number of observations at per stage	24		

Table 4.16 Analysis of variance of repeated measurements of cortisol concentrations in response to synthetic corticosteroid injection between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Time	3	2.38	NS
BI	1	0.41	NS
Sex	1	0.29	NS
BI x Sex	1	0.30	NS
Time x BI	3	1.76	NS
Time x Sex	3	0.92	NS
Time x BI x Sex	3	0.16	NS
Number of observations at per stage	24		

4.2 After feeding:

Results of the statistical analysis of the plasma metabolite and hormone concentrations in the after feeding samples (samples 3 - 8) as presented in Tables 4.5-4.8 show that:

a) HBI calves still maintained higher glucose concentrations than LBI calves during the time after-feeding (117.9 vs 109.6 mg/dl for means only) but the difference was significant only at the 8% level (Table 4.5). The mean value of plasma glucose concentration in bulls was 117.8 mg/dl which is significantly higher than that of the heifers of 108.3 mg/dl ($P<0.05$). In response to feeding there were notable fluctuations of blood glucose homeostasis in most of the calves with both elevated and depressed glucose concentrations recorded in the first 20 minutes, but after 20 minutes hyperglycemia developed in all calves with a peak occuring 2-3 hours after feeding.

b) The post-prandial insulin secretion pattern was bi-phasic with a first minor peak occuring within half an hour from the start of feeding, and a second large peak occuring 2-3 hours after feeding. For most of the calves, their second insulin peaks matched the post-prandial glucose apogee very well.

Although the pattern of insulin concentration following feeding was similar between groups (Fig 3), the mean concentration of insulin remained significantly higher in HBI and bull calves (29.3 and 28.4 ng/ml respectively) than those in LBI and heifer calves (16.6 and 15.4 ng/ml respectively) ($P< 0.01$, Table 4.6) following feeding.

c) It was observed that GH concentration was stablized or reduced in the time immediately after feeding in most of the calves. It appeared that GH concentration pattern resembled those of glucose and insulin in response to feeding in that GH concentration in most calves was at its highest about 3 hours after feeding.

GH concentration after feeding was significantly higher in HBI calves (11.3 Ln pg/ml) than in LBI calves (10.4 Ln pg/ml) ($P < 0.05$,). However, no such difference was detected between bulls and heifers (11 vs 10.7 Ln pg/ml) ($P > 0.05$).

d) There was a rapid response of plasma cortisol concentration to feeding in most of the calves. The mean plasma cortisol concentrations in HBIH, LBIB and LBIH were increased, but decreased in the HBIB group (Fig.5). Then cortisol concentration was depressed in

Table 4.17 Analysis of covariance of repeated measuements of glucose concentrations in response to feeding between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Basal	1	9.76	
Time	5	41.95	***
BI	1	0.57	NS
Sex	1	5.38	*
BI x Sex	1	0.52	NS
Time x BI	5	3.96	NS
Time x Sex	5	9.76	NS
Time x BI x Sex	5	2.98	NS
Number of observations at per stage	24		

Table 4.18 Analysis of covariance of repeated measurements of insulin concentrations in response to feeding between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Basal	1	26.98	
Time	5	18.15	***
BI	1	0.18	NS
Sex	1	1.56	NS
BI x Sex	1	2.37	NS
Time x BI	5	5.71	NS
Time x Sex	5	2.30	NS
Time x BI x Sex	5	8.94	NS
Number of observations at per stage	24		

Table 4.19 Analysis of covariance of repeated measurements of GH concentrations in response to feeding between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Basal	1	43.60	
Time	5	26.45	***
BI	1	0.67	NS
Sex	1	1.52	NS
BI x Sex	1	0.25	NS
Time x BI	5	4.62	NS
Time x Sex	5	4.93	NS
Time x BI x Sex	5	1.83	NS
Number of observations at per stage	24		

Table 4.20 Analysis of covariance of repeated measurements of cortisol concentrations in response to feeding between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Basal	1	20.85	
Time	5	27.04	***
BI	1	0.62	NS
Sex	1	3.83	*
BI x Sex	1	3.19	NS
Time x BI	5	2.13	NS
Time x Sex	5	3.89	NS
Time x BI x Sex	5	6.69	NS
Number of observations at per stage	24		

all groups reaching a nadir when the plasma glucose and insulin peaked i.e. about 2-3 hours after feeding. LBI and heifer calves had slightly higher cortisol concentrations (5.3 and 6.2 ng/ml respectively) than HBI and bull calves (4.9 and 4.2 ng/ml respectively). However neither of the differences were significant ($P > 0.05$).

e) The post-prandial plasma glucose, insulin, GH and cortisol concentrations were further analysed by covariance analysis, using the value of sample 2 as a covariate. The adjusted results by covariance analysis are presented in Tables 4.17-4.20 . Comparison of this result with the unadjusted results in Tables 4.5-4.8 shows that:

i) After adjustment, glucose concentration remained significantly higher in bulls than in heifers ($P < 0.05$), i.e. feeding *per se* produced a relative hyperglycemia in bulls when both bulls and heifers were fed at the same level.

ii) The apparently significantly higher insulin concentrations after feeding in HBI calves and bulls were due to their higher basal insulin concentrations. Feeding *per se* did not produce an additional significant difference in insulin concentration between different BI groups and between different sex groups as there was no significant difference in the adjusted results.

iii) Feeding *per se* did not produce additional differences in plasma GH concentrations between calves of different BI and sex. The significant difference between HBI and LBI calves which existed in the unadjusted result for the post-feeding period resulted from the differences existed before feeding.

iv) After adjustment by covariance analysis, the difference in plasma cortisol concentrations between bulls and heifers become statistically significant ($P = 0.053$) with heifers having higher cortisol concentrations than bulls in response to feeding.

4.3 After arginine infusion

The patterns of the mean plasma metabolite and hormone concentrations in response to arginine infusion in different calf groups are presented in Figures 2 and 5, and the responses of individual calves within each group are plotted in Figures 11-14 . Results of statistical analysis of the mean values are presented in

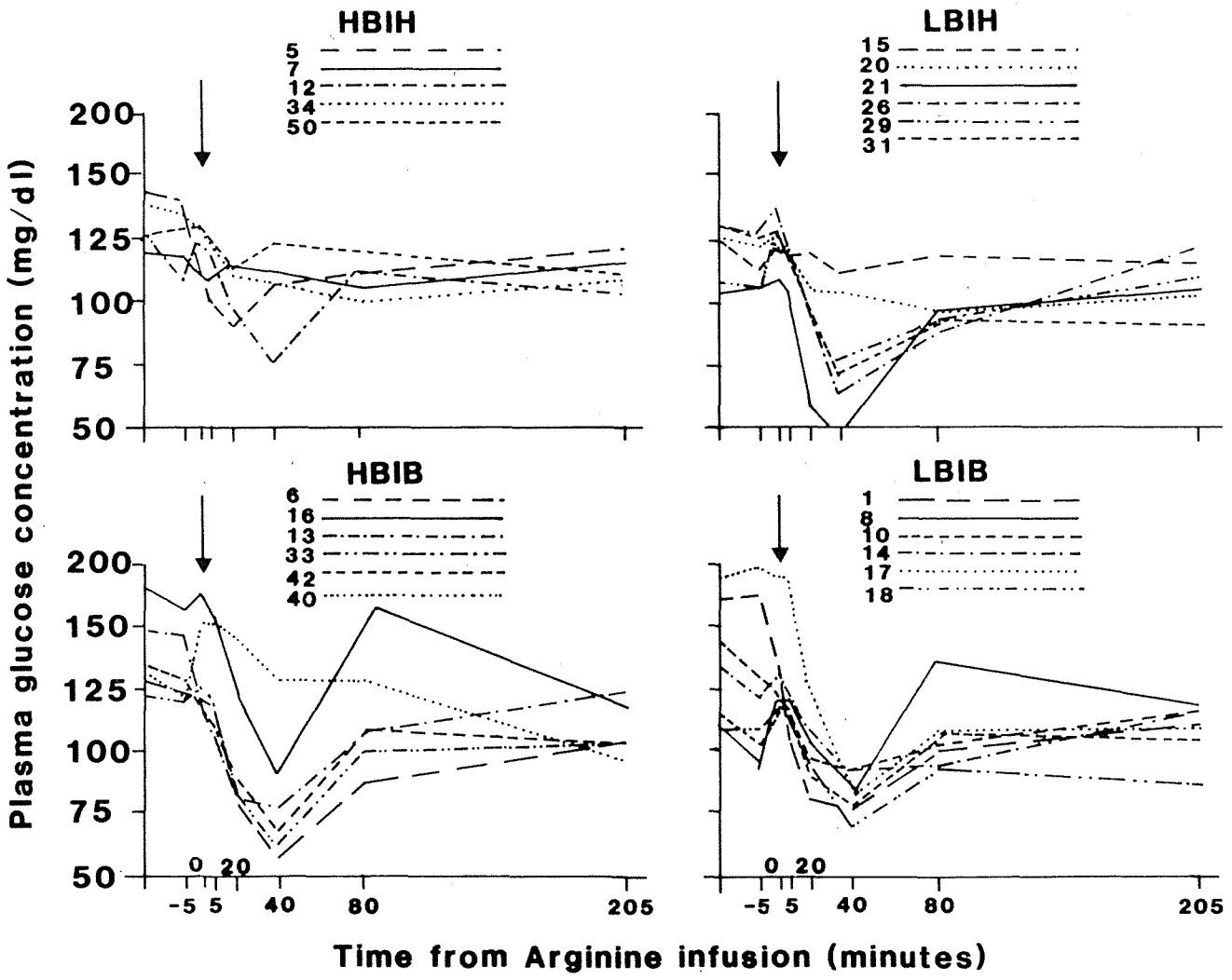


Figure 11 Effect of arginine infusion on plasma glucose concentrations in individual calves of different BI and sex group

↓ = arginine infusion

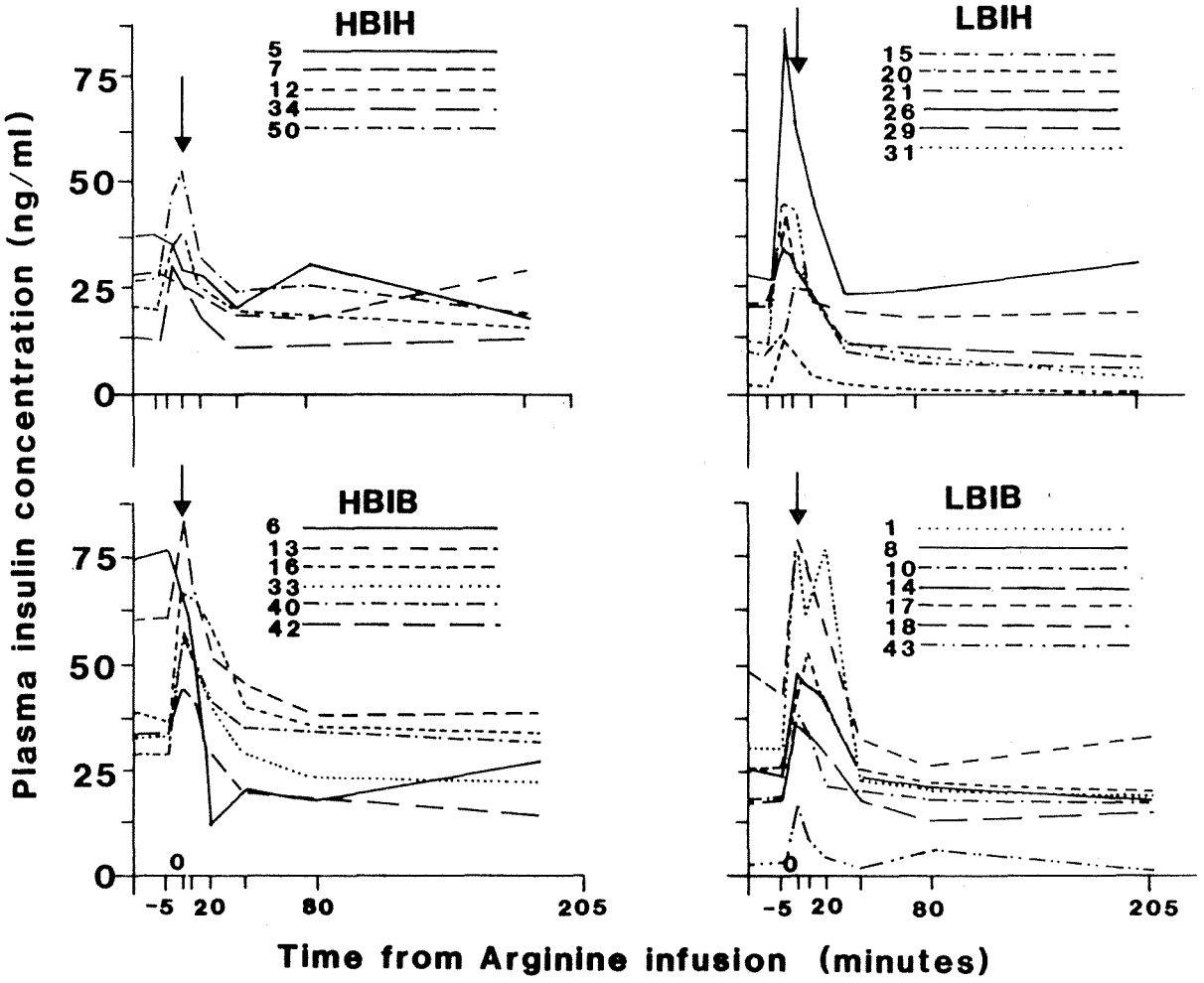


Figure 12 Effect of arginine infusion on plasma insulin concentrations in individual calves of different BI and sex group

↓ = arginine infusion

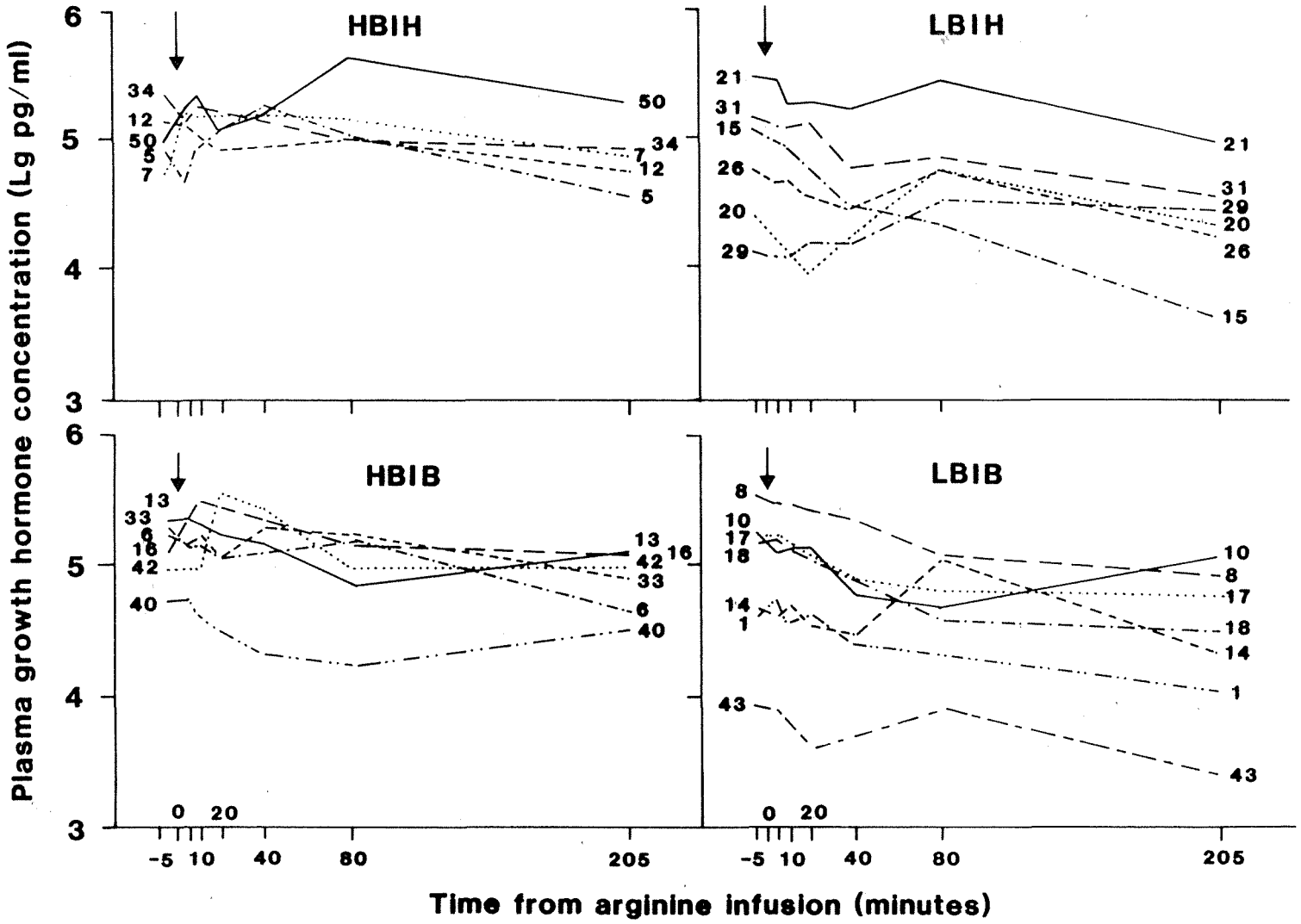


Figure 13 Effect of arginine infusion on plasma GH concentrations in individual calves of different BI and sex group

↓ = arginine infusion

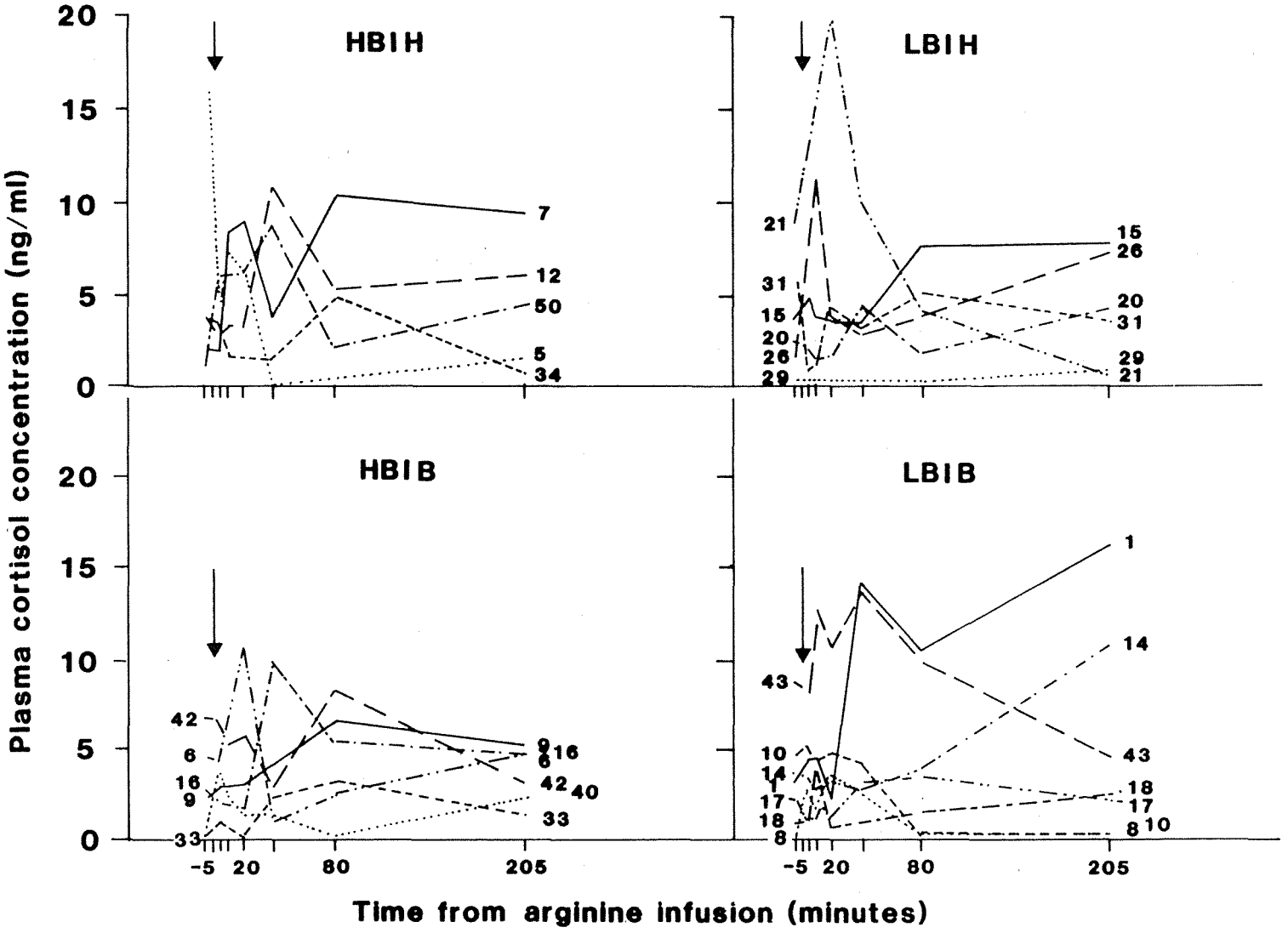


Figure 14 Effect of arginine infusion on plasma cortisol concentrations in individual calves of different BI and sex group

↓ = arginine infusion

Tables 4.9-4.12 which shows that:

a) Significant hypoglycemia developed in all calves following arginine challenge, reaching a nadir about 40 minutes after infusion. It was noted that most LBI calves established a short-lived increment in glucose concentration before the occurence of hypoglycemia while HBI calves did not show a similar response. Nevertheless no significant differences were found between calves of different BI and different sex in the plasma glucose concentration and its pattern ($P > 0.05$) in response to arginine challenge.

b) Following arginine infusion, there was a rapid insulin release in all calves. Peaks were detected within 5 to 10 minutes of arginine infusion. When compared with the pretreatment concentration, peak concentration of insulin in HBI group increased by 40%, in LBI group by 120%, in bulls by 60% and heifers by 100%, and fell to the pre-infusion concentration about 40 minutes after infusion when the glucose concentration was also at its nadir. Though there was no significant differences in plasma insulin concentration and insulin pattern between different BI and between different sex groups, the percentage increment of plasma insulin concentration in the LBI group was significantly higher (120%) than in the HBI group (40%) ($P < 0.05$).

c) The response of GH concentration to arginine infusion was significantly different between HBI and LBI calves in terms of pattern and absolute concentration. LBI calves showed rapid decreases in GH concentration following arginine infusion, while GH concentrations in HBI calves were slightly increased and remained stable for about 30 minutes before falling (Figure 13).

d) Cortisol concentration fluctuated after arginine infusion (Figure 14) in all calves but no significant differences were found between HBI and LBI calves or between bulls and heifers.

e) Covariance analysis was also conducted on the plasma metabolite and hormone concentrations following arginine treatment, using the value of sample 8 (pre-treatment sample) as a covariate. The adjusted results are presented in Tables 4.21-4.24 which show that:

i) Arginine challenge produced a significant difference in GH concentration between HBI and LBI calves ($P < 0.01$) with HBI calves having the higher concentration. Arginine stimulation resulted in a

Table 4.21 Analysis of covariance of repeated measurements of glucose concentrations in response to arginine challenge between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Basal	1	2.87	
Time	5	35.81	***
BI	1	0.34	NS
Sex	1	0.01	NS
BI x Sex	1	0.29	NS
Time x BI	5	4.75	NS
Time x Sex	5	5.72	NS
Time x BI x Sex	5	4.11	NS
Number of observations at per stage	24		

Table 4.22 Analysis of covariance of repeated measurements of insulin concentrations in response to arginine challenge between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Basal	1	11.65	
Time	5	30.87	***
BI	1	0.15	NS
Sex	1	0.95	NS
BI x Sex	1	0.00	NS
Time x BI	5	5.29	NS
Time x Sex	5	5.80	NS
Time x BI x Sex	5	8.41	NS
Number of observations at per stage	24		

Table 4.23 Analysis of covariance of repeated measurements of GH concentrations in response to arginine challenge ,between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Basal	1	32.12	
Time	5	21.03	***
BI	1	8.10	**
Sex	1	0.30	NS
BI x Sex	1	2.20	NS
Time x BI	5	13.59	*
Time x Sex	5	4.42	NS
Time x BI x Sex	5	6.64	NS
Number of observations at per stage	24		

Table 4.24 Analysis of covariance of repeated measurements of cortisol concentrations in response to arginine challenge between groups of calves of different BI and sex.

Source of Variation	DF	Chi square	Significance
Basal	1	2.72	
Time	5	6.29	NS
BI	1	0.01	NS
Sex	1	0.01	NS
BI x Sex	1	0.00	NS
Time x BI	5	1.16	NS
Time x Sex	5	2.54	NS
Time x BI x Sex	5	1.36	NS
Number of observations at per stage	24		

different GH response pattern between HBI and LBI groups ($P < 0.05$).

ii) Arginine infusion per se did not result in a significant difference in plasma glucose, insulin, and cortisol concentrations between HBI and LBI or between bull and heifer calves.

4.4 After synthetic corticosteroid (Betsalan) injection

Mean values of plasma glucose, insulin, GH and cortisol concentrations of different calf groups before and after Betsalan injection are plotted in Figs 6-9.

4.4.1) Comparison of pre-injection value with the after-injection value:

Results of the statistical analysis of the corresponding means of metabolite and hormone concentration on day one (before injection) and day two (after injection) are in Table 4.25 which show:

a) The mean plasma glucose concentration were significantly elevated following Betsalan injection for all groups of calves ($P < 0.01$).

b) Plasma insulin concentration was also significantly raised by Betsalan injection ($P=0.054$) for all calves but the increment in individual groups was not significant even though glucose concentration was increased by 41% in the HBI group; 63% in the LBI group; 52% in bulls and 44% in heifers.

c) GH concentrations were also enhanced in all group of calves following Betsalan treatment. However none of the increments were significant ($P > 0.05$).

d) Endogenous cortisol production was significantly suppressed after the injection of synthetic corticosteroid (Betsalan) in calves of different BI and sex group. ($P < 0.01$).

4.4.2 Comparison between calves of different BI and sex

Results of statistical analysis of the plasma metabolite and hormone concentration in the samples collected after Betsalan injection are presented in Tables 4.13-4.16 which shows that:

a) HBI calves still had a higher GH concentration than LBI calves

Table 4.25 Mean plasma glucose, insulin, GH and cortisol concentrations of calves on day one before, and on day two after synthetic corticosteroid injection, together with the statistical significance of the difference.

Hormones	Group	before Injectio	After Injection	Significance
Insulin	Overall	18.5	27.8	*
(ng/ml)	HBI	23.7	33.6	NS
	LBI	14.1	23.0	NS (P<6.5%)
	Bulls	22.9	34.9	NS
	Heifers	13.4	19.4	NS
Glucose	Overall	103.4	151.0	***
(mg/dl)	HBI	107.8	152.0	***
	LBI	99.6	150.1	***
	Bulls	105.0	155.0	***
	Heifers	101.4	146.1	***
GH	Overall	10.7	11.0	NS
(ln pg/ml)	HBI	11.3	11.4	NS
	LBI	10.2	10.6	NS
	Bulls	10.8	11.0	NS
	Heifers	10.6	10.9	NS
Cortisol	Overall	5.6	0.5	***
(ng/ml)	HBI	5.6	0.4	***
	LBI	5.6	0.6	***
	Bulls	5.3	0.6	***
	Heifers	5.9	0.5	***

(11.4 vs 10.6 Ln pg/ml; P = 0.06) following Betsalan injection and the interaction of sampling and calf sex was significant at the 5.3% level.

b) HBI calves still maintained higher, but not at the 5% significance level, plasma insulin concentrations than LBI calves (33.6 vs 23 ng/ml P= 0.09) and the bulls had significantly higher insulin concentrations than the heifers (34.9 vs 19.4 ng/ml P< 0.05).

c) Plasma glucose concentrations were slightly higher in HBI and bull calves (152 and 155 mg/dl respectively) than those in LBI and heifer calves (150 and 146 mg/dl respectively), but the difference were not significant (P > 0.05).

d) Cortisol concentration was lower in all groups of calves and no significant difference was found between HBI and LBI calves or between bulls and heifers.

4.5 The relationship between calf BI and plasma metabolite and hormone concentrations:

The correlation coefficients of calf BI estimated from ancestry and the plasma metabolite and hormone concentrations estimated at four different stages of the experiment for different BI and sex groups are presented in Table 4.26 and the simple regression lines for the BI and basal (pre-feeding) metabolite and hormone concentrations are plotted in Figure 10 . It is noted that:

a) The correlation coefficients of BI and basal insulin concentration were significant for all calves (r=0.54, P< 0.01); and for the HBIB group (r=0.82, P < 0.05). The value of the correlation coefficient between BI and basal insulin were not significant for LBIB (r=0.74, P= 0.07), for HBIH (r = 0.59, P>0.05) and for LBIH (r=0.37, P>0.05).

The correlation coefficients of the estimated BI and basal glucose, GH and cortisol concentrations were not significant for all group of calves with the exception of the relationship between BI and basal glucose in LBIB and basal GH in LBIH. The correlation coefficient of BI and basal glucose value was significant for LBIB (r = -0.78, P = 0.05). The correlation coefficient of the estimated BI and basal GH was significant for LBIH (r=-0.81,P<0.05).

b) The correlation coefficients of the estimated BI and plasma

Table 4.26 Correlation coefficients between BI and concentrations of glucose, insulin, GH and cortisol in calf plasma sampled at four different stages of the experiment.

Experimental stages	Calf group	Glucose (mg/dl)	Insulin (ng/ml)	Cortisol (ng/ml)	GH (ln pg/ml)
Pre-feeding	Overall	0.33	0.54**	0.11	0.31
	HBIB	-0.04	0.82*	0.31	-0.56
	HBIH	0.17	0.59	-0.12	0.46
	LBIB	-0.78*	0.74*	0.21	0.47
	LBIH	-0.25	0.37	-0.17	-0.81*
After-feeding	Overall	0.13	0.38*	0.03	0.27
	HBIB	0.25	0.38	0.03	0.27
	HBIH	-0.10	0.46	-0.15	0.39
	LBIB	-0.25	0.49	0.20	0.42
	LBIH	-0.06	-0.40	-0.24	-0.71
After arginine infusion	Overall	0.11	0.11	0.02	0.34
	HBIB	0.23	0.38	-0.16	-0.36
	HBIH	0.33	0.43	0.34	0.45
	LBIB	-0.29	0.27	0.27	0.35
	LBIH	0.21	-0.03	-0.38	-0.68
After Betsalan injection	Overall	0.01	0.21	-0.11	0.29
	HBIB	0.83*	0.41	0.33	-0.62
	HBIH	-0.11	0.36	-0.34	0.38
	LBIB	-0.29	0.42	-0.17	-0.09
	LBIH	0.05	-0.13	-0.38	-0.64

Note: the concentrations of insulin from calf 20 and GH from calf 43 (both were LBI calves) were not used in the calculation of correlation coefficients as their concentrations were anomolously lower than the corresponding means of the LBI group.

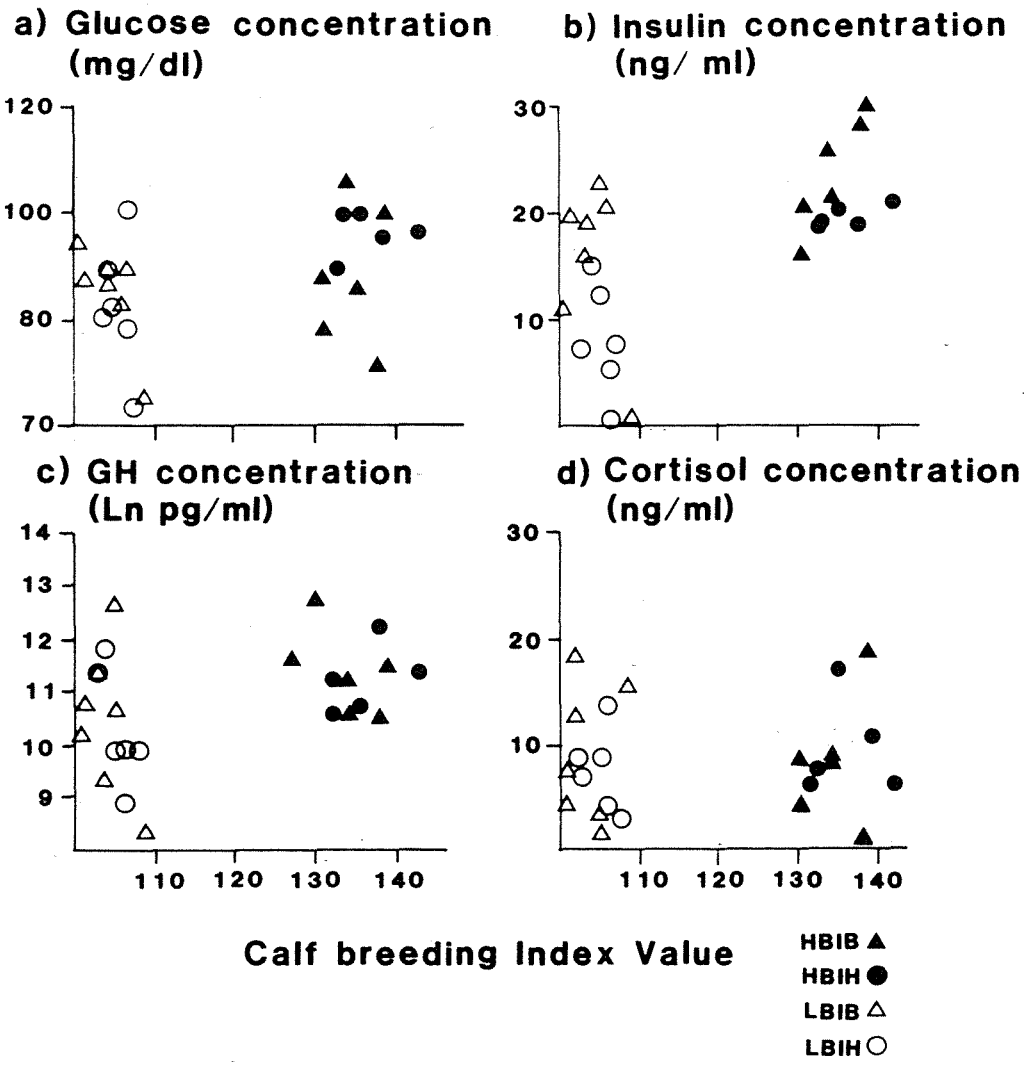


Figure 10 Relationship between breeding index of calves and the basal concentration of (a) glucose, (b) insulin, (c)GH, and (d) cortisol in the plasma within BI and sex group

glucose, insulin, GH and cortisol concentration after feeding were not significant in any group of calves.

c) The correlation coefficients of the estimated BI and plasma glucose, insulin, GH and cortisol concentration after arginine infusion and after Betsalan injection were not significant for all groups with the exception of a significant relationship between BI and glucose concentration following Betsalan injection in the HBIB group ($r=0.83$, $P<0.05$).

4.6 Other physiological traits

Mean values of birth weight, blood immunoglobulin (Ig) G1 concentration and haematocrit for different calf groups are presented in Table 4.27. Statistical analysis of these results shows that the birth weight of bulls (44 kg) was significantly higher than that of heifers (40 kg) ($P<0.05$). IgG1 concentration in bulls was significantly higher than in heifers (22.5 vs 15.3 mg/ml, $P<0.05$). Four LBI calves 20, 29, 31, 43 had such low IgG1 concentrations in their blood that it was below the sensitivity of the assay. When these low IgG1 values were included in the statistical analysis IgG1 concentration in HBI calves was significantly higher than in LBI calves (19.5 vs 14.5 mg/ml, $P<0.05$) but no such difference was present after the rejection of these values from LBI group (19.5 vs 20 mg/ml for HBI and LBI respectively). Mean value of haematocrit in HBI group was slightly higher than that in LBI group (38.9 vs 37.8), and these value agreed well with other studies (Bradley *et al*, 1982).

Table 4.27 Mean values of birth weight, IgG1 concentration and haematocrit in calf groups of different BI and sex.

Variable	Mean	BI of calves			Sex of calves		
		HBI	LBI	significance (HBI-LBI)	M	F	Significance (M -F)
Number	24	11	13				
Birth Weight (kg)	42.4	42.5	42.5	NS	44.1	40.4	*
IgG1 (mg/ml)	19.7	19.5	20	NS	22.5	17.3	*
Haematocrit (%)	38.3	38.9	37.8	NS	37.5	39.3	NS

CHAPTER FIVE: DISCUSSION

5.1 General discussion

All calves appeared to have acquired adequate Immunogloblin G1 (IgG1) from their dam's colostrum after birth except 4 calves in the LBI group whose IgG1 concentration was less than 2 mg/ml. It would appear that there was a failure in the transfer of immunoglobulins from dam to circulation in these four calves. Further studies would be needed to ascertain if this was affected by breeding index of the cow and the calf. Even so no problems of calf health was incurred during the experiment possibly due to the strict hygiene practice applied. It is hard to explain the higher IgG1 concentration observed in the bulls. It could be caused by a greater consumption of colostrum by the bulls or higher IgG1 concentration in the colostrum of bull's dam at the time when the calf was born. Differences in the closing time of the intestinal barrier for IgG1 absorption and in the turnover rate of IgG1 may be responsible for the different IgG1 concentrations between the bulls and heifers. It is not known if the endogenous IgG1 production differs in quantity between bulls or heifers, but it is normally accepted that endogenous IgG production will not become dominant until many weeks after birth (Roy, 1980; Butler, 1969).

The greater birth weight of the bulls observed in this study agreed with the report of others (Appleman et al, 1974).

The concentration of the various hormones and metabolite in the plasma samples collected in the present experiment were within the range of values reported in the literature. Thus comparable values have been measured for glucose (Bradley et al, 1982), insulin and GH (Bohn, 1978; Roy et al, 1983; Flux et al, 1984) and cortisol (Venkalaseshu and Estergreen, 1970).

5.2 Effects of fasting, feeding, intravenous arginine infusion, and subcutaneous synthetic corticosteroid injection on plasma glucose and hormone concentrations

5.2.1 Effect of fasting

After 16 to 17 hours fasting, the basal plasma glucose and insulin concentrations were significantly lower than those observed in subsequent samples collected after feeding (Figures 2-5). This is in agreement with the established knowledge that plasma energy substrate concentrations, especially glucose concentration, are the main stimuli for insulin secretion. In fasting animals decreased absorption of nutrient from the gut will lead to hypoglycemia, thus hypoinsulinemia.

The prefeeding plasma cortisol concentrations in the calves were significantly higher than those after feeding (Figure 5). Maximal cortisol production has also been reported to occur in the early morning in many species (see section 2.1.3) but the pattern is less discernable in adult ruminants. Glucocorticoids are known to have a major role in glucose homeostasis during fasting (Trenkle, 1981; McDowell, 1983, Tischler, 1981). They promote endogeneous glucose production by facilitating gluconeogenesis and antagonize the anabolic action of insulin probably by reducing the affinity of insulin receptors (Kahn et al, 1978), thus leading to increased blood glucose concentration.

Increased GH concentration as a result of low plasma energy concentrations has been found in fasted animals of many species (Roth et al, 1964; Machlin et al, 1968; McIntyre and Odell, 1974; Purchas et al, 1971; Hove and Blom, 1973). However in the present study, no such increment has been found in basal GH concentrations when compared to the value in the subsequent samples. This may due to the infrequent samplings over the pre-feeding time. Great variation were found in the basal GH concentrations in all calves which is consistent with an episodic release of GH.

5.2.2 Effect of feeding

Changes in both the glucose and insulin concentrations in response to feeding in the present study were bi-phasic (Figures 2-3). This was very apparent in bull calves. These patterns are almost identical to those of non-ruminants (Mayhew et al, 1969) and similar responses were observed in young ruminants (Stern et al, 1971; Bohn, 1978). Though increased plasma glucose concentration is primarily due to the absorbed digestive products from the gut, the initial glucose response to feeding may also be endogenous caused by feeding-induced glucagon and catecholamines release.

Insulin response in some calves preceded the changes in glucose concentration. Similar observations were also made in steers (Chase et al, 1977). It was suggested that a direct neural stimulation of the pancreatic B-cells or the release of some gut hormones such as GIP, or CCK on the ingestion of food were responsible for the rapid insulin release (Bassett, 1981; Parman, 1979). Another explanation for the post-prandical insulin secretion pattern was proposed by Schusdziarra (1980) who suggested that feeding-induced insulin release was modulated by somatostatin (SLI) secreted from the gastrointestinal and pancreatic area. In spite of many other factors, the post-prandial insulin concentration is primarily controlled by the increased glucose concentration as observed in the present study in which the major insulin peak coincided with the maximal plasma glucose concentration.

The observed fall of GH concentration after feeding (Figure 4) is consistent with other reports (Bassett, 1974, 1975; Blom et al, 1976; Hove and Blom, 1973). The diminished episodic GH secretion after feeding does not correlate with the energy concentration, a case which might occur in animals sustaining chronic energy deficit. In fact GH concentration peaked in most calves about 3 hr after feeding when plasma glucose concentration was still significantly elevated in response to feeding. So far the literature contains very few satisfactory explanations for the stablized GH concentration immediately after feeding. Bassett (1975) proposed that the secretion of hypothalamic GHRF and SLI may be influenced by feeding. However, there is no direct evidence to support that statement. Recent evidence shows that plasma SLI concentration was elevated in dogs

following feeding (Shusidziarr et al, 1978a, 1978b) due to the secretion of SLI from gastrointestinal and pancreatic area and it is established that increased blood SLI concentration has a powerfull inhibitory effect on GH secretion (Fernandez-Durango et al, 1978; Siler et al, 1973; Mortimer et al, 1974). So it is postulated here that the suppressed GH concentration after feeding may be due to the inhibitory effect of SLI released from gastrointestinal and pancreatic area rather than from the hypothalamus. However as reviewed in chapter two, the regulation of GH secretion is complex, it would not be suprising if many other factors are involved in the regulation of post-prandial GH release.

Cortisol concentration was depressed in all calves following feeding with a nadir developed 2 - 3 hr later (Figure 5) when plasma glucose and insulin reached their maximal values. The reduced cortisol production may be due to the inhibition of hypothalamus-pituitary release of CRF and ACTH which is sensitive to various stresses and hypoglycemia. The stimulatory effect of hypoglycemia on CRF-ACTH release, thus cortisol production, may be antagonized by the hyperglycemia developed following feeding as observed in the present study. Rapid short-lived cortisol release was also recorded in some calves during the time immediately after feeding. This was probably caused by the frequent handling of the calf during sampling or in response to drinking.

5.2.3 After arginine infusion

Arginine infusion triggered a burst of insulin release in all calves (Figure 12). This was consistent with other studies (Bohn, 1978, Mayhew et al, 1969; Hertelendy et al, 1970; Davis, 1972). The mechanisms by which the pancreatic B-cell is stimulated by arginine and other amino acids is still unknown, possibly by potentiating the effect of glucose on insulin release. In the present studies, arginine was infused into the calf at a time when the glucose concentration was still high following feeding. It is known that the combination of glucose and certain amino acids is a better stimulus for insulin release then either of them alone (Pagliara et al, 1974).

Following arginine infusion none of the calves showed significant increases of blood glucose concentration (Figure 11). In fact,

glucose concentration declined steadily in most calves. This result was in contrast to the reports of others who observed increased glucose concentration following arginine infusion (Bohn 1978, Gerich et al, 1974; Pagliara et al, 1974) and attributed it to the effect of concurrent release of glucagon. The discrepancy in the results between this study and others may be due to the lower dosage of arginine used in the present experiment (0.04g/kg bwt vs 0.5g/kg bwt).

A significant hypoglycemia development in all calves soon after arginine challenge (Figure 11). This probably was a secondary effect of the hyperinsulinemia as the insulin response preceded the occurence of hypoglycemia. A similar observation was also made by Bohn (1978).

Arginine infusion has been shown to stimulate GH release in animals in many studies (Hertelendy et al, 1970; Stern et al, 1971; Davis, 1972; Bohn,1978). However, GH response to arginine challenge was not consistent in the calves of present study (Figure 13). This may due to the lower dosage of arginine given. The apparent differential effect of arginine on GH release between calves of different BI will be discussed later (in secton 5.3).

No significant cortisol responses to arginine challenge were found in the present study (Figure 14). The small fluctuation of cortisol concentration may be due to the restraint of the calves during the period of intensive sampling or due to the insulin hypoglycemia as increased plasma corticosteroid concentration was found in sheep with insulin hypoglycemia (Dooley and Williams, 1975).

5.2.4 After injection of synthetic corticosteroids (Betsalan)

Plasma glucose concentration in all calves were significantly elevated after Betsalan injection (Figure 6). This effect of synthetic corticosteroids on glucose concentration may be achieved by two ways: a) an increase in the rate of endogenous glucose production; b) a decrease of peripheral utilization of glucose. Studies by Ford (1971) indicated that the increased rate of gluconeogenesis in the liver and kidney of sheep are responsible for a betamethason injection induced hyperglycemia and the peripheral utilization of glucose was not reduced at the time 24 hr after injection. A study by Bassett (1963) suggested that a progressive impairment of glucose utilization relative to the blood glucose pool,

but not a reduction in total glucose utilization occurred in sheep after cortisol injection. There are other data which suggested that glucose utilization by muscle (Morgan et al, 1959) and adipose tissue (Feldman, 1977) were inhibited by administration of corticosteroids.

The increased insulin concentration following Betsalan injection in the present study was consistent with the observation by Bassett and Wallace (1967). The elevated insulin concentrations may be caused by: a) cortisol induced hyperglycemia as high glucose concentration is well known as a good stimulus for insulin release and synthesis; b) direct effect of cortisol on B-cell. Recent studies showed increased insulin response to oral glucose and intravenous tolbutamide infusion in man (Parley et al, 1968) and to intravenous glucose administration in sheep (Bassett and wallace, 1967) after cortisol treatment. More recent investigations have demonstrated the presence of steroid receptors on the islet cells (Green et al, 1978; Tesone et al, 1979) and the direct influence of insulin secretion by circulating glucocorticoids (Borelli et al, 1982); c) increased half-life of plasma insulin due to the reduced binding to receptor in the peripheral tissue. Decreased insulin binding has been observed in animals following glucocorticoid treatment (Olefskey et al; 1975, Kahn, 1976).

Endogenous immunoreactive cortisol concentration fell significantly in all calves following Betsalan injection (Figure 9). This fall was most probably accompanied by corresponding decreases in CRF and ACTH release as it is well known that cortisol production in adrenal cortex is under the direct control of ACTH whose secretion in turn is under the regulation of CRF (section 2.1.3). It is suggested that the synthetic corticosteroids (in the present study Betsalan) regulate ACTH secretion probably through a delayed feedback system, i.e. by reducing the rate of CRF synthesis, while endogenous cortisol and cortisone regulate ACTH release through a fast feedback system i.e. by regulating CRF release (Jones, 1979).

GH concentration was not significantly affected following Betsalan injection. This result is not conclusive. In view of the episodic nature of GH release the number of samples taken after the injection of Betsalan were insufficient for an accurate assessment of GH concentration. It is currently believed that administration of glucocorticoids impair GH release (Chiodin and Liuzzi, 1979).

5.3 Effect of genetic merit for milk fat production on plasma metabolite and hormone concentrations

The impact of genetic divergence for milk production on the plasma metabolite and hormone concentrations of the calf has been clearly demonstrated by the present study. Calves of high genetic merit (HBI) had higher plasma glucose, insulin, and GH concentrations than LBI calves for most of the time of the experiment (Figures 2-9). Similar difference were also found between HBI and LBI cows with similar genetic background to the calves when feed intake of the cows was restricted (Flux et al, 1984). The present experiment for the first time showed that such differences also exist between bull calves of different genetic merit.

1) Glucose: Glucose concentrations were significantly different between HBI and LBI calves after 16 hr fasting ($p < 0.05$), but the difference was minimized after feeding, arginine infusion and synthetic corticosteriod injection ($P > 0.05$). One recent study in England (Tilakaratne et al, 1980) also showed that glucose concentration concentration was constantly higher in calves of high genetic merit than in calves of low genetic merit for milk production. The prolonged fasting (30 to 40 hours) in that experiment also resulted in a significant difference in plasma glucose concentrations between the two groups of calves. Studies with the lactating cows showed that LBI cows had significantly lower plasma glucose value than HBI cows when the animals were underfed, while the underfed HBI cows maintained glucose concentration comparable to that of better fed cows (Flux et al, 1984). This evidence suggests that dairy cattle of high genetic merit have the ability to maintain higher glucose concentrations than that of low genetic merit especially under conditions of energy deficit, regardless of the age of animals. It is known that glucose is a limiting factor in determining milk yield of lactating ruminant, as glucose is the precusor of lactose in milk .

The higher basal glucose concentration in HBI calves may be due to either an increased endogenous glucose production or decreased peripheral utilization of glucose. As gluconeogenesis is stimulated by glucagon and glucocorticoids, both hormones might be involved in

the present study. Administration of cortisol to sheep (Bassett and Wallace, 1967) and Betsalan to calves (present experiment) resulted in hyperglycemia and hyperinsulinemia. The increased glucose concentration is caused by increased gluconeogenesis from amino acids in liver and decreased glucose turnover (Reilly and Black, 1973). In the present study, however, basal cortisol concentration were only slightly and not significantly higher in HBI calves than in LBI calves. Thus it is unlikely that the higher basal glucose concentration in HBI calves can be attributed to an increase of glucose production stimulated by cortisol, but the role of glucagon requires further investigation. Administration of glucagon to sheep also resulted in a significant increase in blood glucose concentration (Bassett, 1971).

A decreased glucose utilization in HBI calves in comparison with LBI calves could be caused by:

a) Increased GH concentration: Chronic administration of exogenous GH increased blood glucose concentration in non-lactating animals (Wagner and Veenhuizen, 1978, Wagner et al, 1970, Wallace and Bassett 1966; Bassett, 1978) and insulin is less active in GH treated sheep (Wagner et al, 1970). In the fasting state the effect of GH is though to be lipolytic. It promotes the mobilization and oxidation of FFA, thus reducing the peripheral utilization of glucose. In the present study basal GH concentrations were significantly higher in HBI calves though great variation existed within both BI groups. It may be the major factor accounting for the higher glucose concentration found in the HBI group. High GH and glucose concentrations were also found in HBI cows (Flux et al, 1984) when feed intake was restricted.The lactogenic effect of GH in ruminant has been excellently reviewed (Cowie et al, 1980; Hart, 1981; Bauman and McCutchen, 1984).

b) Increased molar ratio of glucagon to insulin due to a large decrease in the insulin concentration in the fasting state. Glucagon action on adipose tissue is also lipolytic when insulin concentration is kept low (Brockman, 1976). Both of these would facilitate gluconeogenesis and reduce peripheral utilization of glucose.

c) Increased energy substrate concentrations other than glucose. Increased FFA concentrations were found in calves of high genetic merit in prolonged fasting (Tilakaratne et al, 1980). In lactating

cows high FFA concentrations were also found to be positively associated with milk yield (Hart et al, 1978). It may be inferred from these studies that basal FFA concentrations were also higher in HBI calves than in LBI calves in this experiment. A higher FFA concentration together with high GH concentration in the HBI group would reduce the requirement for glucose by the peripheral tissue.

d) Decreased anabolic action of insulin: This may be due to the decreased binding of insulin to its receptor on the peripheral tissues. High insulin concentration does not necessarily mean an increased insulin action, as the affinity of the insulin receptor is down regulated by the amount of circulating insulin (Olefsky and Reaven, 1977; Olefsky, 1981).

e) Increased glucocorticoid production: Administration of cortisol was found to cause hyperglycemia in animals (Bassett and Wallace, 1967) by impairing glucose utilization. This is probably achieved by altering the affinity and number of insulin receptors on the target tissue (Kahn et al, 1978). However, mean cortisol concentration was only slightly higher in the prefeeding samples in HBI calves than in LBI calves (Figure 5).

2) Insulin: Plasma insulin concentration was consistently higher in HBI calves than in LBI calves (Figures 3 and 7) and this difference was significant during fasting, after feeding and after synthetic corticosteroid (Betsalan) injection. Higher insulin concentrations were also found in HBI cows which were under fed (Flux et al, 1984). This is hard to explain. The higher insulin concentration may be caused by higher glucose concentration observed in HBI calves and HBI cows as it is well accepted that glucose is the most efficient stimulus for B-cell function. Higher GH concentration found in HBI calves might also contribute to the difference in insulin concentration between HBI and LBI groups. Acute pancreatic insulin release has been observed in dogs following intravenous GH injection (Sirek et al, 1979). GH treatment of cows caused elevation of insulin secretion in one study (Bines et al, 1980) but was not recorded in another (Peel et al, 1983). So far however, there is no conclusive evidence to show that GH can stimulate insulin release from the B-cells as purified hGH did not affect insulin secretion (Larson, 1978). It is still possible that GH may affect plasma insulin

concentration by changing the affinity of insulin receptors, probably by promoting the production of somatomedins, and insulin like growth factors. Recent studies implied that some of the peptide hormones may compete with insulin for insulin receptors in the peripheral tissues (Czech et al, 1983). Investigation in this field is still going on. Injection of cortisol has been found to increase the concentration of insulin as well as glucose in ruminants (sheep: Bassett and Wallace, 1967; calf: the present experiment). However,it is unlikely that the consistently higher insulin concentrations found in HBI calves were caused by the cortisol , as there was no significant difference in plasma cortisol concentration between the HBI and LBI groups. The higher insulin concentration found in HBI calves was most probably accompanied by a higher glucagon concentration as parallel changes were found in plasma insulin and glucagon concentrations of sheep following feeding and injection of energy substrates (Bassett, 1972). Glucagon infusion also resulted in a rapid increase in insulin concentration in sheep (Bassett, 1971). Further investigation of glucagon concentration in HBI and LBI calves would be desirable.

3) Growth Hormone: Higher plasma GH concentration in HBI calves in the present study were consistent with the observation in HBI cows (Flux et al, 1984). Higher GH concentrations were also found in a high milk yielding breed than in a low yielding breed (Hart et al, 1978). It is postulated here that the hypothalamic GHRF and SLI secretion may differ between HBI and LBI calves. In the present study the higher GH concentration in HBI group was apparently not caused by insulin hypoglycemia as the concentration of insulin and glucose were consistently higher in HBI calves. Glucagon, again, may play a role in determining GH concentrations in calves as glucagon administration to sheep increased plasma GH, insulin and glucose concentrations (Bassett, 1971).

In response to arginine challenge the differences in plasma GH concentrations between HBI and LBI groups become significantly different . HBI calves not only had higher GH concentrations but maintained such concentrations for a longer time than LBI calves (Figures 4 and 13). The biological relevance of this might be very important in that HBI calves could release more GH than LBI calves when the same amounts of amino acid are available to the animals. GH

is known to be a major hormone for the preservation of body protein, particularly during periods of energy deficit by stimulating the incorporation of amino acids into protein while directing glucose away from tissue deposition and by possibly stimulating lipolysis (Raben, 1973). The different responses of GH concentration to arginine challenge of a relatively low dosage under the condition of the present study might result from a difference in hypothalamic sensitivity to arginine challenge between HBI and LBI calves. However, a possibility that the episodic nature of GH release caused the different GH response still exists. An accurate estimate of GH may not have been obtained with the infrequent sampling regime used in the present study, and further experiments are required to reach a reliable conclusion.

5.4 Sex effect:

The present study showed that bull calves had consistently higher insulin concentrations than heifers (Figures 3 and 7), and the differences were significant throughout the experiment. Glucose concentration was also significantly higher in bulls than in heifers during the post-prandial period (Figure 2). Cortisol concentration, however, was significantly lower in bulls than in heifers following feeding (adjusted result Table 4.20, Figure 5).

The effect of sex on plasma metabolite and hormone concentrations has been less investigated in calves. Tilakaratne et al (1980) reported that sex had little effect on plasma concentrations of BHBA, glucose, FFA, urea, total protein and albumin, only globulin concentration was significantly higher in bulls ($P<0.05$). In the present study, higher glucose concentration developed in bulls which coincided with the lower cortisol concentration occuring in bulls at the same time. This suggests that glucose entry rate from the gut might be faster in bulls than in heifers. It is not clear if the lower cortisol concentration in bulls after feeding was caused by the higher glucose concentration in bulls or by mechanisms other than hyperglycemia.

The observed higher insulin concentrations in bulls is also hard to explain. This is probaly caused by the sex steroids which might act on the pancreatic B-cells and peripheral insulin receptor (Tesone

et al, 1979). This is yet to be tested. It may be inferred from this experiment that pancreatic B-cells were more mature in bulls than in heifers at the time of the study. This is indirectly supported by the fact that the body weight of bulls was significantly higher than that of heifers.

GH concentration was slightly but not significantly higher in bulls than in heifers as observed in the study (Figures 4 and 8). This suggested insulin instead of GH is the major anabolic hormone for the early development of the young calf.

5.5 Estimation of calf genetic merit by using metabolite and hormone concentrations as gene markers

1) The primary aim of this experiment was to investigate the metabolic and endocrinological response to various treatments in HBI and LBI calves with particular emphasis on the early identification of genetically superior bulls by using the metabolite and hormones as markers to predict the BI of the calf. As the BI of each calf in the present study was estimated from their ancestry BI (instead of true BI from a progeny test), some discrepencies between the estimated and true BIs for each calves are expected. This is because:

a) for a calf born to a sire which had been used very widely in AI and to a dam itself born to genetically superior parents and with a good lactation performance the upper limit for accuracy of BI estimated from ancestry would be about 0.4. The standard deviation of the BI for each calf would be about 7 BI units (Table 3.2).

b) For a large number of calves all with estimated BIs of 130, 95% of their actual BIs would be expected to lie in the range of 116 to 144. Thus a high correlation between a measurement of hormone or metabolite concentration and the estimated BI could not be expected even if there was actually a high correlation between the hormone or metabolite measurement and the true BI.

c) The number of calves used in this experiment was not very large and the estimated BIs are not evenly distributed within each BI groups.

2) Nevertheless results of the present study suggest that basal glucose and insulin concentration may be used as markers to discriminate between HBI and LBI calves in general. GH concentration and pattern after arginine challenge may also be useful criteria for selecting HBI calves. All of these will be discussed in the following section.

i) Basal glucose: Though fasting glucose concentration was significantly higher in the HBI group than in the LBI group (102 vs 93 mg/dl, $P< 0.05$), the correlation coefficients of the estimated BI and basal glucose concentration for all calves was not significant ($r = 0.33, P> 0.05$, Table 4.26, and Figure 10). Calves with the highest estimated BI did not have the highest fasting glucose concentration within each group. The correlation coefficients of the BI and basal glucose within each group is either small or negative. Thus it appears that glucose would not serve as a good marker to predict the estimated BI of individual calves, though it appears that basal glucose concentration may be used to discriminate HBI and LBI calves as a group.

ii) Basal insulin: Basal insulin in the preliminary results showed promise as a gene marker to estimate the genetic merit of the young Friesian calf as the correlation coefficents of BI and basal insulin were significant for all calves ($r=0.54$, $P<0.01$); for HBIB ($r=0.84$, $P<0.05$); for LBIB ($r=0.78$, $p=0.07$) (Figure 10, Table 4.26). However, the value of this result in the early identification of superior dairy bull has yet to be tested using bulls whose BI has been estimated in the progeny testing programme.

iii) GH response to arginine challenge: In response to arginine challenge GH concentration differed significantly in terms of absolute level and pattern between HBI and LBI calves (Figure 4, Tables 4.11 and 4.23). It appears that HBI calves can be discriminated from LBI calves by their differing GH response to arginine challenge. In general HBI calves had higher GH concentration and remained elevated after arginine infusion while GH concentration fell rapidly in LBI calves after arginine infusion.

CHAPTER SIX: SUMMARY AND CONCLUSION

Some important metabolic and endocrinological differences were found between HBI and LBI calves in the present study.

HBI calves were characterized as having higher plasma glucose, insulin and GH concentrations than LBI calves. Similar observations were also made in mature cows of similar genetic background (Flux et al, 1984). These differences may be associated with the differences in genotype for milk fat production.

1. After 16 hours fasting basal plasma glucose and insulin concentrations were significantly higher in HBI calves than in LBI calves ($P < 0.05$, $P < 0.01$, $P = 0.59$ respectively). GH concentrations were also higher in HBI calves but not at 5% significance level ($P<0.06$).

2. The changes in concentrations of glucose , GH and insulin with time in response to feeding was very similar for each group calves of different BI and sex. However,insulin concentration was significantly higher in HBI calves than LBI calves ($P<0.01$). GH concentration in HBI group was also significantly higher than in LBI group ($P<0.05$). But the differences in GH and insulin concentrations between HBI and LBI group resulted from differences which existed before feeding rather than caused by feeding per se because no significant differences were found when the results were adjusted by the prefeeding values. Cortisol concentration declined rapidly following feeding in all calves but this could have been related to the circadin nature of cortisol secretion rather than be a response to feeding. No significant difference in cortisol concentration was found between HBI and LBI calves.

3. Acute intravenous arginine infusion induced significant hypoglycemia and hyperinsulinemia in all calves and the former was apparently due to the effect of the later. Following arginine challenge calves in the LBI group showed a greater absolute increment of insulin concentration and a significantly higher percentage increase of insulin release than HBI calves ($P<0.05$). But insulin

concentration was not significantly different between HBI and LBI groups. Neither was glucose concentration.

The response of GH concentration to arginine challenge was significantly different between HBI and LBI calves. In the HBI group GH concentration was slightly increased and remained elevated whereas in the LBI group GH fell rapidly after arginine challenge . The biological relevance of the different response is not clear.

Plasma cortisol concentration fluctuated to a small extent within each group of calves following arginine infusion, but no significant difference was detected between HBI and LBI groups.

4. Subcutaneous injection of synthetic corticosteroid (Betsalan) caused significant elevations of plasma glucose and insulin concentrations and a significant decrease of endogenous immunoreactive cortisol concentration in all calves, but none of the metabolite and hormone concentrations were significantly different between HBI and LBI calves. GH concentration was slightly elevated in most calves but the change in concentration was not significant. Following synthetic corticosteroid treatment, GH concentration was higher in the HBI group than in the LBI group, but the difference was not quite significant at the five percent level (P=0.061). This difference was similar to that observed before Betsalan injection, which suggests that the difference in GH concentration is not due to the effect of Betsalan treatment.

5. The significant positive correlations between BI and fasting plasma insulin concentration for all calves, for HBIB and LBIB groups indicate that basal insulin concentration could be used as a gene marker for the early identification of genetically superior Friesian dairy cattle. Basal GH concentration was also positively though not significantly correlated with BI for all calves. As the spot estimation of basal GH concentration may not represent the real status of GH concentration in a calf the possibility that the mean concentration in a intensive series of samples may serve as a useful marker in identifying superior dairy cattle could not be ruled out. Basal glucose concentrations were poorly correlated with the BI of each group, possibly because the blood glucose levels were maintained by homeostasic mechanisms within a relatively narrow range. Very

small differences in mean basal plasma cortisol concentrations were found between HBI and LBI calves in the present study and no significant correlation existed for basal cortisol level and calf BI.

6. Effect of sex on plasma metabolite and hormone concentrations were found in the present study. Plasma insulin concentration was consistently higher in bulls than in heifers and the differences were significant at the time of fasting, after feeding, and after arginine infusion. Following feeding, glucose concentration was significantly higher and cortisol concentration was lower but not at a significantly level in bulls than those in heifers respectively. GH concentration was slightly not significantly higher in bulls than in heifers during most of the experiment.

It was concluded that HBI and LBI calves differ significantly from each other in certain metabolic and endocrinological aspects and there is a promise of identifying superior Friesien dairy cattle at an early age by measuring the concentrations of some metabolites and hormones as gene markers.

Appendix I Analysis of Ancalf milk powder

component	quantity g/100g	component	quantity (amount/kg)
Lipid	18	Vitamin A	22,000IU
Protein	27	Vitamin D	6,600IU
Lactose	43	Vitamin C	66mg
NaCl	1.5	Vitamin E	22IU
Fibre	0		

note: calcium, iron, phosphorous, iodine, manganese, cobalt, and zinc were also blended in ancalf by the manufacturer.

Appendix II Percent cross-reaction of various steroids with antiserum F3-314

Compound	percent cross-reactivity
Corticosterone	0.015
Cortisone	3.3
Deoxycorticoserone	< 0.01
Estradiol	< 0.01
Estriol	< 0.01
17-hydroxyprogesterone	0.23
Prednisone	3.1
Pregesterone	< 0.01
Testosterone	< 0.01
Tetrahydrocortisone	0.3

From Endocrin Science, 18418 Oxnard Street, Tarzana, California 91356 (1982).

Appendix III

RIA procedures for GH, insulin and cortisol

i) GH RIA: Assay buffer: 0.01M pH 7.5, phosphate buffered saline (PBS) made 0.01M with respect to NaEDTA and 2% with respect to inert protein (plasma from a somatostatin (SLI) treated sheep and filtered using a millipore filter before use) (MPBS).

Plastic tubes (Luckhams LP3) were used in both GH and insulin assays. Thirty tubes were required for the standards of GH (0, 0.5, 1, 2, 4, 8, 16, 32, 64, and 128 ng/ml) and 4 tubes for each unknown sample (2 tubes for 100 µl plasma samples, 2 tubes for 50 µl plasma samples).

The antisera to bGH (1st antibody) was diluted at 1:16,000 in PBS solution. Guinea-pig-gamma globulin (gpg-r-globulin) was also made to 1 ug/ml in PBS. This is the final make-up of PBS (FPBS).

The FPBS reagents were added to LP3 tubes in the following order:

1) 100 µl of FPBS containing first antibody and gpg-r-globulin to each tube;

2) 100 µl of standard GH solution or 100 µl of a sample, or 50 µl of a sample plus 50 µl of MPBS.

Then the reagents were mixed on a vortex mixer and incubated at laboratory temperature for 24 hours. Then 50 µl per tube of 125-I labelled GH (about 6,000 counts/min) was added, mixed and followed by a further 24 hours incubation at room temperature.

On the third day, 50 ul/tube of the second antibody (to gpg-r-globulin raised from a sheep and diluted at 1:40 before use) was added and mixed on a vortex mixer, then the tubes were transferred to a refrigerator and incubated at 4°C for 72 hours.

After that the tubes were centrifuged at 2,000 rpm for 30 min at 4°C. The supernatant was removed with a pipet attached to a vaccumm, then the radioactivity of the precipatate on the bottom of the tube was counted in a NE 1600 gamma counter.

ii) Insulin RIA: The assay buffer was the same as that used in the GH RIA: 0.01M pH 7.5 PBS with respect to 0.01 NaEDTA (NaSalt) with 2% added plasma from a somatostatin treated sheep.

To each tube were added :

1) 100 µl of assay buffer containing 1st antibody;

2) 100 µl standard insulin solution or 100 µl sample or 50 µl of a sample plus 50 µl assay buffer. The tubes were incubated at room temperature for 24 hours.

Then 50 µl 125-I labelled insulin (6,000 counts/min) were added to each tube and incubated for a further 24 hours at room temperature. On the third day 50 µl of ovine antiserum to gpg-r-glubolin diluted at 1:40 in assay buffer was added to each tube, and mixed and incubated for 72 hours at 4°C.

The remaining steps of centrifuging and counting were identical to those for the GH RIA.

iii) Cortisol RIA: Three days were required for each batch of cortisol assays.

Day One: Extraction of plasma cortisol: To each tube were added:

1) 100 µl sample;

2) 2 ml Dichloromethane (DCM).

All tubes were mixed on a vortex mixer for 30 seconds and frozen overnight.

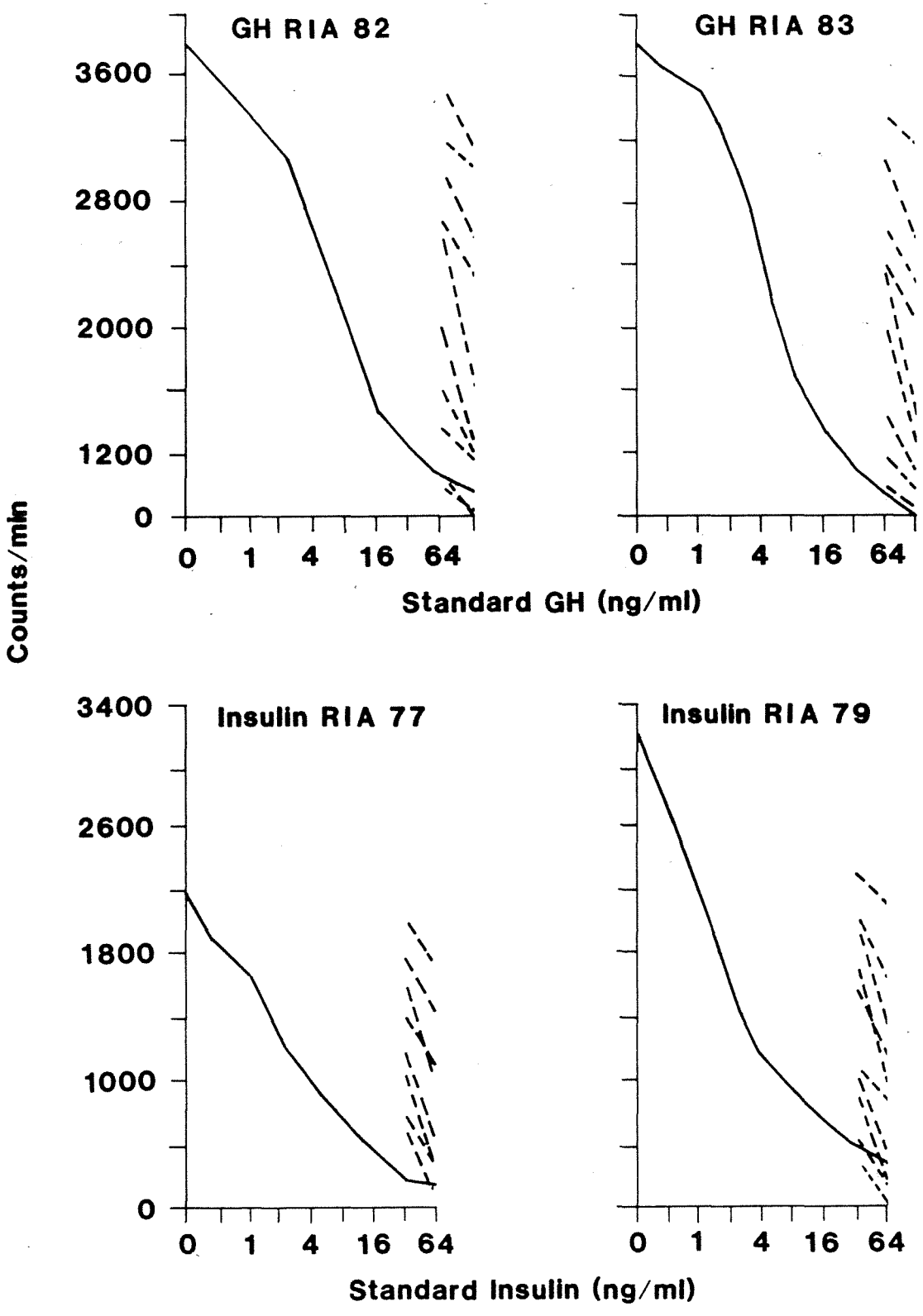
Day Two: The DCM containing the extracted cortisol was poured into glass tubes and dried down under air flow. The extract was redissolved in 1 ml ethanol and mixed for 5 seconds on a vortex mixer (to minimize evaporation, only 10 tubes were processed at a time). A set of standards containing five concentrations of cortisol (0.3125, 0.625, 1.25, 2.5 and 5 ng/ml) were also suspended in 1 ml ethanol. 2 x 100 µl eliquots of each ethanol sample or standard were dispensed into assay tubes and then dried under a flow of air.

Antibody was diluted as recommended by the suppliers of the antiserum (Endocrine Science, 18418 Oxnard street, Tarzana, California 91356,1982). 200 µl reagent mixture containing borate buffer, stock tritiated cortisol (6,000-7,000 counts/min), bovine gamma globulin (BGG), bovine serum albumin (BSA), and antiserum were added to each tube and incubated at room temperature.

Day Three: Separation of bound radioactivity: From this point every step was done at 4°C. Tubes and reagents were kept on ice or in a referigerator.

A suspension of charcoal/dextran mixture (7.5ug/ml) was stirred and 500 µl of the suspension was added to each of the tubes. All tubes were centrifuged at 2,500 rpm for 20 minutes at 4°C. Immediately after centrifuging, 500 µl of supernatant was transferred into scintillation vials. 5 ml of scintillation fluid was added to each vial. Vials were placed in the counter for several minutes before counting. Each vial was counted for 2 minutes in the counter.

Appendix IV Standard curves of GH and Insulin RIAs and check of parallelism



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