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METABOLIC RHYTHMS IN

ADOLESCENT DIABETES MELLITUS

by

MICHAEL DAVID HOCKING

M.B.B.S., M.R.C.P., D.C.H.

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Abstract

<u>DOCTOR OF MEDICINE</u>

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Circulating concentrations of intermediary metabolites and hormones were studied in a group of insulin dependent adolescent diabetic patients over a twenty four hour period.

Despite conventional treatment many patients were found to have high concentrations of blood glucose and other metabolites. In particular a rise in blood total ketone body concentration was identified occurring early in the night before the morning rise in glucose.

The patients were past their initial partial remission phase of diabetes but some were still secreting small amounts of endogenous insulin as indicated by circulating C-peptide. Conventional methods of assessing endogenous insulin were discussed and a new method suggested as most appropriate for these patients.

The small amounts of endogenous insulin produced by these patients was found to have only minimal effect on the metabolic profiles.

Some of the patients underwent a program of home blood glucose monitoring which lead to the use of more insulin.

Some of the metabolites improved with a lowering of total ketone bodies and glycerol concentration but blood glucose was not always significantly lower and blood lactate rose.

In patients treated similarly with either porcine or human insulin there was very little difference in overall blood glucose control but there were significant differences in other metabolites. In the formulations used the human insulin had a quicker but shorter duration of action.

In conclusion, strenuous efforts to preserve small amounts of endogenous insulin secretion in adolescent diabetic patients is unlikely to be beneficial. Modest attempts to improve control were not successful particularly at lowering the nocturnal hyperketonaemia. Insulin species had little effect on overall control.

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1.1. INTRODUCTION

Almost four hundred years ago, Richard Morton (1637-1698) wrote Phthisiologia (London, 1689, English translation 1694 and 2nd edition 1720). In it he described 'Diabetes Infantalis'. This is probably the first reference to diabetes in childhood in the English literature. He was quoted by Michael Underwood in 'A Treatise on the Diseases of Childhood' (4th edition, London, 1799): 'He speaks of it as the effect of irritation from teething, and as a family disorder, having been fatal to all the male children in one, except the last infant, to whose assistance he was called at the commencement of the thirst, and increased secretion of urine; which was sweet as in the diabetes of adults.'

Morton's treatment for the child was to 'allow only milk mixed with the Islington chalybeate water'. Symptoms recurred 'when ever a tooth was making its way so he added 'a few grains of rhubarb every morning, and a little dioscordium at bed-time'. As predicted by Morton when the first teething was complete two years later, the child enjoyed perfect health, a fact that unfortunately casts the diagnosis into doubt.

However, the paediatrician feels on home ground when he discovers that the disease he is studying has been attributed to the eruption of the dentition, for so many of childhood's maladies have been, and still are, attributed to teething. The trap of correlation being mistaken for causation is also there as a warning to the research worker.

1.2.   HISTORICAL ASPECTS OF CHILDHOOD DIABETES

1.2.1. Preinsulin

A century after Morton, John Rollo published a study on diabetes in 1797 (Papaspyros, 1964). He advocated an exclusive diet of meat which was the first effective diet. He arrived at this conclusion by careful study and it seems likely that the diet was given to patients with juvenile diabetes as he also described the odour of acetone in diabetes. Many diets and treatments were recommended in the nineteenth century not all of which included a similar reduction in carbohydrate or effectiveness.

Abraham Jacobi (1830-1919) was the first physician in the United States of America to specialize in the teaching of paediatrics. Writing towards the end of the nineteenth century (Jacobi, 1896) he recognized the disease took a more rapid course in infants and children than in adults and terminated more readily in coma and death. He recommended a similar diet to Morton: 'Milk ... forms the principal and beneficial part of their nutriment'. Persistent use of alkalis (mineral waters) was advised because of the 'facility with which cerebral symptoms are developed'. He also gave iodoform, arsenic, bromide and 'salicylate of sodium with an alkaline beverage ... has a decidedly favourable effect'. Perhaps significantly he states that the treatment was to continue for 'many weeks' rather than many years.

In 1912 Allen began developing a method of treatment based on experiments in dogs which became very popular (Allen, Stillman and Tfitz, 1919). The patient was fasted at the beginning of treatment. The subsequent diet was low in carbohydrate and high in fat but slightly under the patients calorie requirement. After the initial period fast days were recommended. The method was the basis of E P Joslin's management explained in a popular manual written for doctors and patients in the preinsulin era (Joslin, 1919).

Childhood diabetes in the immediate preinsulin era is well summarized in a case history by J L Morse in 1916 (Morse, 1916). An eleven year old boy was admitted with a one month history of polyuria and polydipsia. He had 5.9% glycosuria but no acetonuria. His prognosis was 'reckoned in months rather than years' and was 'likely to suddenly develop acid intoxication at any time and die after a few days'. Drugs were considered by this time to be of no use and treatment consisted of regulation of the diet. Carbohydrates were 'reduced until urine was free from sugar but no lower because of the risk of acid intoxication'. If the amount of acetone was small it was considered safe to neutralize the acetone bodies by giving bicarbonate of soda for a time rather than increasing the carbohydrate. 'With careful treatment he may live for a number of years'.

Thus, even before the discovery and purification of insulin, the diagnosis of diabetes in a child was not an immediate death sentence. Many children were treated with a diet and made a temporary recovery. These diets reduced the

carbohydrate load and therefore reduced the insulin requirement, but even if the child's condition improved he would eventually succumb to the disease usually after a period of gross under nutrition. The onset of ketoacidosis was an ominous sign and the emaciated dehydrated child slid into coma and died.

1.2.2. Preinsulin chemistry and physiology

Diabetes is a disease of antiquity and the knowledge of the sweet tasting urine goes back a long way. (Papaspyros, 1964).

The Greek Aretaeus named diabetes but made no reference to the sweet taste of the urine. Thomas Willis (1621-75) described it in 1674. (Fortunately, Willis's name for diabetes 'the Pissing Evil' did not stick.) It is not known if he knew of the taste from the ancient writings of Arabic medicine's Avicenna (960-1037) or Indian medicine's Sustruta (5th cent. A.D). It may have been common knowledge at the time because Moliere's comedy 'Le Medecin volant' (1650) mentions it but Willis is credited with the rediscovery. Japanese and Chinese writers of the third century A.D. knew the urine of diabetics to 'taste so sweet as to attract dogs'. No doubt it was dogs that actually made the first observation but they did not record it.

The advances in chemistry and physiology in the nineteenth century drew on these early observations. The sweetness was found to be due to glucose and then methods to measure it in urine and blood were developed. Many important

discoveries were made but it was not until Minkowski and von Mering in 1889 removed the pancreas from dogs and, unexpectedly, the dogs developed diabetes that the importance of this organ in the development of diabetes was made (Mering and Minkowski, 1890).

Once the gland was identified extracts were prepared. Some of these were effective but with toxic side effects (which may actually have been hypoglycaemia) prevented their use.

Finally, in 1922 Banting and Best succeeded in developing an extract that was free from the side effects that had hampered the previous investigators. Children were amongst the first treated and the photographs of them before treatment (Bliss, 1983) show the pitiful state they declined to before insulin was available.

1.2.3 Post-insulin

Joslin's wide experience of diabetes in Boston included children. Soon after insulin became available he wrote of the difference it made (Joslin, Root and White, 1925). More than three hundred children were seen over a period of 27yrs. Of these 164 were seen before insulin was available and all but 12 died after a mean of 2.4yrs. Of those seen after insulin was available only 10 of 130 children had died and the mean duration of treatment of the survivors was 3.1yrs. Some of the children had had a normal puberty and all were growing and putting on weight, events which were very rare before insulin.

The advent of insulin was not the end of the problems with diabetic children. It is clear from Lawrence (Lawrence, 1926) that in London in the 1920's, after the diagnosis and immediate stabilization had been made in hospital, the diabetic child was discharged back to his general practitioner. Fleming found that before setting up a special follow up clinic for diabetic children in Glasgow in 1928, nine of his first ten patients treated with insulin died but after starting the clinic only four of the next twenty died (Fleming, 1932). The first preparations of dilute soluble insulin had to be injected frequently. More concentrated solutions were made and Hagedorn developed the first longer acting insulin. The search was then for a single dose per day treatment.

1.2.4. Conclusions

From this short account of childhood diabetes and its treatment it is clear that childhood diabetes, like other childhood ailments, was treated by general physicians until relatively recently and that treatment reflected the ideas of the day. It was recognized that the disease was of a severe form in childhood but, even so, was temporarily responsive to dietary treatment. Insulin had a profound effect on the treatment of children and in the long term needed supervision by doctors with a special interest. Observations on the disease have been made by physicians since antiquity and since the development of the experimental approach in science diabetes has stimulated research.

1.3. AETIOLOGY AND PATHOGENESIS OF DIABETES MELLITUS

1.3.1.Introduction

Diabetes mellitus is a clinical syndrome and the spectrum of disease was originally thought to be one condition. The increased severity of the juvenile form has already been commented on and the first division was made on clinical grounds. Patients who developed diabetes before thirty were usually underweight on presentation and liable to develop ketosis while those over this age were likely to be overweight and unlikely to develop ketosis at least initially.

The evidence that there are indeed two major types of diabetes of different aetiologies is now overwhelming and has provided clues to the pathogenesis.

In addition to these major types, there are a large number of conditions in which glucose intolerance, sometimes leading to diabetes, may be present. As a group they form only a small number of patients but they may have different aetiologies from the main types (Rimoin and Rotter, 1982; National Diabetes Data group of the American Diabetes Association, 1979). These patients were excluded from this study.

1.3.2.Twin and family studies

Tattersall and Pyke (Tattersall and Pyke, 1972) studied identical twin pairs in whom one or both had diabetes. They and others have found that those who develop the disease in

early life are only about 50% concordant for diabetes while
those who develop the disease later approach 100%
concordance.

These data suggest that the late onset disease (Type 2)
has a much stronger genetic component than the juvenile form
(Type 1) and thus must have a different aetiology. Even
though it is less strong than type 2, there still is a
significant inherited risk for the development of diabetes
that in conjunction with other factors leads to the disease.
Similar conclusions can be made from family studies of
siblings and parents (Green, 1982).

1.3.3.HLA Types

Further evidence for a difference between the two types
of diabetes is provided by the association of type 1 with
some HLA antigens. There is no such association between any
HLA antigens and type 2 diabetes. The strongest association
in type 1 is with DR3 and DR4. Wolf et al (Wolf, Spencer and
Cudworth, 1983) showed that these antigens were present
either separately or together in 98% of patients. If present
together the risk of disease was higher than if only one was
present. The results are reported as compatible with the
existence of two susceptibility genes operating at a locus
or loci closely linked to that of HLA-DR.

1.3.4.Autoimmunity

Islet cell antibodies, particularly of the complement
fixing type, are usually present at the time of diagnosis of
type 1 diabetes but increasingly less frequent as time

passes. They are rare in type 2 diabetes. Gorsuch et al (Gorsuch, Spencer, Lister, McNally, Dean, Bottazzo and Cudworth, 1981) in the Barts-Windsor prospective study of families at risk from developing diabetes found patients positive for the antibodies before they developed diabetes. In the same study it has since been shown that the period of positivity for antibodies may be prolonged but there are some subjects who become negative again without developing the disease (Tarn, Smith, Spencer, Bottazzo and Gale, 1987; Spencer, Tarn, Dean, Lister and Bottazzo, 1984).

Insulin autoantibodies have also been found in new diabetics and genetically potential diabetics (Wilkin, Hoskins, Armitage, Rodier, Casey, Diaz, Pyke and Leslie, 1985), but they too do not seem to mark inevitable disease.

Heaton et al (Heaton, Millward, Gray, Tun, Hales, Pyke and Leslie, 1987) studied the non-diabetic identical twin discordant for diabetes for 11 years or more but who also had islet cell antibodies. They showed that there was beta cell dysfunction even though the subjects were not diabetic and could be expected to remain so.

There is further evidence for immunological processes in type 1 disease (reviews Bottazzo, 1984; Lernmark, 1985) but it is far from clear what initiates the process or even whether it is necessary for the disease to develop. If it does occur it is clear that the outcome may be clinical normality, subclinical beta cell dysfunction or diabetes mellitus

1.3.5.Virus Infection

Viruses are known to cause diabetes in susceptible animal strains. In the human it seems less common. There is a much quoted single case of Coxsackie B4 infection in a child presenting with diabetic ketoacidosis who then died. The virus was isolated from the pancreas of the patient and caused hyperglycaemia and islet inflammation in mice (Yoon, Austin, Onodera and Notkins, 1979). Banatvala et al (Banatvala, Schernthaner, Schober, De Silva, Bryant, Borkenstein, Brown, Menser and Silirk, 1985) using an IgM specific antibody found a higher incidence of recent infection in the new diabetics than the controls (30% versus 6%) in the children but in those over 16 years only 10% were positive. Most of the infections were with serotypes B4 and B5. Gamble (Gamble, 1969) in a survey of sera from new diabetics found an excess of positive titres for Coxsackie but not for mumps in those diagnosed in the previous three months. However the rate of positivity in the controls (40%) was high. It is clearly difficult to choose a suitable control group in view of the possibility of local and seasonal outbreaks of infection.

Mumps associated with the onset of diabetes was described in the last century and intermittently since. In a questionnaire survey Gamble (Gamble, 1980) found a significant excess of consultations of general practitioners for mumps in the six months before onset of diabetes, the greatest excess in the month immediately before onset. Even so this was only about 1% of patients.

Diabetes is associated with congenital rubella (Menser, Forrest, Honeyman and Burgess, 1974) but not with infection after birth. The disease may occur as late as 14 years of age (Ginsberg-Feller, 1983). Menser et al quote an incidence of 40% but Ginsberg-Feller only 3%. However, definitions of glucose intolerance may vary and congenital rubella has a broad spectrum of severity so ascertainment may not be the same.

The evidence for an acute viral cause of type 1 diabetes in the majority of cases is not strong. That is not to suggest that the added stress of a viral infection may not precipitate diabetes.

1.3.6.Histopathology

Death early in the course of diabetes is unusual so the histological material that is available is from a selected group. Foulis et al (Foulis, Liddle, Farquharson, Richmond and Weir, 1986) have recently reviewed and added to the literature. The majority (78%) of recent onset cases showed inflammatory infiltrates of insulin containing islets and this was present up to six years after onset. Insulin containing islets were more likely to be affected but the process did not affect all islets and was patchily distributed throughout the gland. There were a few cases who apparently had classical disease but did not have the classical lesions. One group of three, who were less than eighteen months at presentation, had no inflammation and

normal numbers of islets. These patients may have a different disease process from classical type 1 patients.

1.3.7.Conclusion

There is good evidence that there is an inherited risk of developing diabetes.Similarly, environmental factor must play a part but their nature and mode of action is less clear. The pathological process results in an inflammatory destruction of the insulin containing cells of the pancreas and may be caused by immune mechanisms. The process may be a long one and may continue after diagnosis but may also arrest without the development of the disease. The event or events that lead to the initiation and continuation of the destruction of the beta cell in the majority of children with diabetes is not known.

1.4. ENDOGENOUS INSULIN SECRETION

1.4.1. Introduction

The concept of endogenous insulin being present in diabetic patients was made soon after its discovery. Lawrence uses the term in his book published in 1926. Jackson and Newbergh (Boyd, 1942) recognized that the early period after starting insulin treatment could be easier than later. This could have been guessed by the survival of patients after diagnosis before insulin was discovered. The first study of the early period in childhood diabetes was published twenty years after insulin began to be used.

Lieutenant Commander J M Brush USNR described the effect of his scheme for the initial management of children with diabetes in 1944 (Brush, 1944). He showed that after stabilization a progressive reduction in the dose of insulin could be made at a fairly predictable time after starting treatment and at a fairly predictable rate. The goal was less than ten units of insulin once a day and was achieved in most patients. It is difficult to judge how good the control was but clearly these children did not all suddenly become ketoacidotic. He also recognized the temporary nature of this state "within a year or two ... most patients will come to require more than 30 units of insulin a day".

1.4.2. Measurement of endogenous insulin

The existence of endogenous insulin in the diabetic was supported by studies using the radioimmunoassay technique of Yalow and Berson (Yalow and Berson, 1960). This could measure low levels of insulin. Until that time the only methods available were biological assays such as the hypoglycaemic effect of the fluid in question on experimental diabetic rats; the effect on muscle metabolism in the rat hemi-diaphragm or the effect on fat metabolism in the rat epididymal fat pad. These methods required large amounts of material and were time consuming and expensive. They also measured insulin-like activity which was not suppressible by insulin antibodies and were also affected by insulin antagonists. Unfortunately, insulin antibodies develop quickly in diabetics and interfere with the

immunoassay methods of measuring insulin after the first few weeks.

In 1967 Steiner and Oyer (Steiner and Oyer, 1967) made the first observations on an insulin precursor and later the same group (Steiner, Clark, Nolan, Rubenstein, Margoloash, Aten, Oyer, 1969; Rubenstein, Clark, Melani, Steiner, 1969) described the biosynthesis of insulin. They found that insulin was formed from proinsulin which was converted to insulin and C-peptide and made the important observations that human insulin and C-peptide were secreted into the portal circulation in equimolar amounts (Horwitz, Starr, Mako, Blackard, Rubenstein, 1975). A little proinsulin was found to be secreted at the same time.

Insulin is metabolized by the liver, about 40% is removed in the first passage (reviewed by Field, 1973) which hampers its usefulness for estimating endogenous insulin when insulin is measured in the peripheral circulation. Stoll et al (Stoll, Touber, Menahan and Williams, 1970) showed that porcine C-peptide is only minimally affected by its passage through the rat liver but Kuhl et al (Kuhl, Faber, Hornnes, and Lindkear-Jensen, 1978) showed that as much as 12% of porcine C-peptide may be removed by the porcine liver. C-peptide seems to have no biological function after the formation of insulin. It has therefore theoretical advantages over insulin as a measure of insulin secretion (Faber, Hagen, Binder, Markussen, Naithani, Blix, Kuzuya, Horwitz, Rubenstein, Rossing, 1978) quite apart from

the impossibility of measuring insulin in the presence of endogenous insulin antibodies in the serum.

The assay and its sensitivity were improved by Heding (Heding, 1975). Not all the early assays gave the same results (Faber, Binder, Markussen, Heding, Naithani, Kuzuya, Blix, Horwitz and Rubenstein, 1978) which must be noted when reviewing the literature.

The conditions under which the blood sample is taken is not well defined in some studies. The two most usual methods are a simple single fasting level and the glucagon stimulation test (Faber and Binder, 1977). These and other suggestions are discussed in more depth later (see Chapter 4). They all have theoretical and practical problems but have enabled some conclusions to be reached.

1.4.3. Influences on endogenous insulin

Studies of normal children show that adolescents have higher C-Peptide levels (Haumont and Dorchy, 1978; Ludvigsson and Heding, 1977). It would seem that the adolescent diabetic is likely to need more insulin for a similar effect.

If there is sufficient beta cell function in a diabetic endogenous insulin may be produced in a similar way to the non-diabetic (Werther, Turner, Jenkins and Baum, 1982) although the response is usually delayed and of a lower magnitude (Madsbad, Faber, Binder, Alberti and Lloyd, 1981).

Younger patients have less endogenous insulin at diagnosis (Binder, Christiansen, Faber, Lauritsen, Madsbad and Svendsen, 1981). The decline in the ability to secrete insulin occurs more quickly in children than in adults (Madsbad, Faber, Binder, McNair, Christiansen and Transbol, 1978; Ludvigsson and Heding, 1976). Madsbad (Madsbad et al, 1978) could show a difference in residual insulin production between patients diagnosed young (10 to 20 years) and older (30 to 40 years) over the first 15 years of their disease but not later. C-peptide and therefore endogenous insulin may increase initially (Madsbad, Krarup, Faber, Binder and Regeur, 1982) as would be expected from Brush's work (Brush, 1944).

Almost all patients in the 10 to 20 years age group have been shown to have measurable C-peptide in the first two years after diagnosis (Madsbad et al, 1978). It was also present in two thirds of those of less than 5 years duration.

Madsbad et al (Madsbad, Krarup, Regeur, Faber and Binder, 1981) have shown that a short period of good control can improve the C-peptide test but that the effect is clinically short lived.

Immune mechanisms are implicated in the pathogenesis of diabetes but C-peptide status does not correlate with islet cell antibodies (Crossley, James, Elliott, Berryman and Edgar, 1981). However, insulin binding antibodies have been

implicated in beta cell destruction (Kloppel, 1976) and this may have clinical implications (Ludvigsson, 1984).

1.4.4. Complications of diabetes and endogenous insulin

After some years of disease, diabetic patients frequently develop complications namely diabetic retinopathy, nephropathy or neuropathy. Poor metabolic control is thought to be responsible for the development of these complications but proof of the association is difficult (Knowles, 1964) and much debated. One of the problems is that it is difficult to define and measure "control" over a long period of time. It would seem however, that if there were a population with some endogenous insulin present during their disease, they might be expected to have fewer complications. This idea has two main problems. Firstly, the group with the endogenous insulin are clearly different from the rest and this difference may affect the risk of complications independently from "control". Secondly, in cross-sectional analysis to speculate on the ability to secrete insulin over a number of years on the strength of a test done at an hour of one day may be hazardous, since the individual may have become negative one day or many years before.

Smith et al (Smith, Pyke, Watkins, Binder and Faber, 1979) studied 20 patients with and without complications and found one in each group with residual endogenous insulin. Bodansky et al (Bodansky, Medbak, Drury and Cudworth, 1981) studying 57 patients found, in contrast to the theory, a higher incidence in those with complications. Eff et al

(Eff, Faber and Deckert, 1978) studied a very selected group of patients who had had diabetes for more than 18 years but had a low insulin requirement (<0.5 IU/kg/day) and found significantly less severe retinopathy in those with endogenous insulin. Madsbad (Madsbad, 1983) studying over 500 patients found the degree of retinopathy was not different between those with and without endogenous insulin.

Thus, the benefit of endogenous insulin in reducing the risk of complications remains unproven.

1.4.5. Metabolic control and endogenous insulin

The liver is of major importance in intermediate metabolism and insulin is the most important hormone in its control. Insulin is produced in close proximity to the liver and it is possible that even a small amount of insulin produced in the right place may be of benefit to the patient. It is on this idea that much of the interest in endogenous insulin is based. Because this subject forms a major part of this study it is discussed in more detail in the next section after a discussion on intermediate metabolism.

1.5. INTERMEDIATE METABOLISM

1.5.1. Introduction

The early diabetic physician used his taste buds and recognized the sweet taste of diabetic urine. In the eighteenth century the sweetness of the serum was also recognized and the characteristic smell of ketoacidosis in

diabetic patients described. In the nineteenth century chemical tests were developed, Fehling's test for urinary sugar and Gerhart's test for urinary ketones.

Understandably, the study of diabetes has concentrated on glucose and its measurement. Fat and protein metabolism has not been ignored and their importance was recognized in the diet treatments of the early century.

Claude Bernard (Papaspyros, 1964) identified glycogen in the liver in the middle of the last century and it was thought that diabetes was a disturbance in the function of this organ. Since this time the metabolic pathways of the cell have been investigated in great detail and those relevant to metabolism in diabetes recently reviewed (Brownlee, 1981).

1.5.2.Intermediate Metabolism

Metabolic processes have been studied in sub-cellular systems, cellular systems, organs, whole animals and man. Whereas, in the lower order the metabolic processes may be clear, in the more complex systems it is more difficult to determine their importance and their control. In addition, the whole animal may go through periods of feeding, fasting, sleeping, exercise, emotion etc. which may have an effect on the metabolic process in question. There are also interspecies differences. Insulin has a profound effect on many of these processes.

Insulin works in a number of ways. Most tissues except the liver and brain require insulin for the entry of glucose into the cell and this increase in substrate may of itself affect the rate of intracellular processes. Insulin has a direct effect on some enzyme activities and may also increase enzyme concentration by an effect on enzyme synthesis though this is on a longer time scale. The liver, muscle and fat are the main tissues involved in fuel and energy regulation by the processes of intermediate metabolism within them.

1.5.2.a.Glycogen. Larner et al (Larner, Lawrence, Walkenbach, Roach, Hazen and Huang, 1978) have suggested that glycogen synthesis is stimulated by insulin in two ways, one directly on glycogen synthase the other by increasing glucose transport across the cell wall in those tissues such as muscle which are not freely permeable to glucose. The hepatic cell wall is permeable to glucose and does not require insulin for glucose transport. It is possible the glycogenolysis is also inhibited as has been suggested in dogs (Bishop, Steele, Altszuler, Dunn, Bjerknes, de Bodo, 1975).

1.5.2.b.Glycolysis and gluconeogenesis. The enzymes of glycolysis are present in most cells but those for gluconeogenesis are only present in the liver and kidney. The kidney does not seem to be important in gluconeogenesis except under conditions of starvation or acidosis. The substrates for gluconeogenesis are principally lactate, pyruvate, glycerol and aminoacids, especially alanine.

There are three effectively irreversible reactions in glycolysis: glucose to glucose-6-phosphate; fructose-6-phosphate to fructose-1,6-diphosphate; phosphoenolpyruvate to pyruvate. In gluconeogenesis these steps are by-passed by a different set of enzymes. It is at these points that regulation is thought to occur (Cherrington 1981). However the exact step or steps affected is not clear and the removal of the inhibition of the action of glucagon by insulin may be important. The situation is also complicated by the action of insulin in the supply of these substrates especially glycerol to the liver.

1.5.2.c.Alanine. Alanine is important in both aminoacid and carbohydrate metabolism (Sherwin,1981). It accounts for at least half the liver uptake of aminoacid and is the main aminoacid released from muscle. To a lesser extent glutamine is also released. This is largely metabolized in the gut and kidney but some is converted there to alanine.

The liver plays only a minor role in the metabolism of the branched chain aminoacids. Compared to other aminoacids there is a disproportionate rise in them after a meal. Less of the other aminoacids escape from the splanchnic bed so branched chain aminoacids account for a major part of the aminoacid uptake of muscle. The peripheral uptake of most aminoacids including the branched chain aminoacids is stimulated by insulin so there is an element of protein intolerance in insulin deficiency.

It was originally supposed that alanine was formed directly from the aminoacids in muscle because output of

alanine was greater than uptake. However, it has been shown that the carbon skeleton of alanine is from pyruvate and the alanine formed largely by transamination from the branched chain amino acids and not from protein degradation (Odessey, Khainallah and Goldberg, 1974). Pyruvate is derived from glucose and alanine is returned to the liver for gluconeogenesis. This is the glucose/alanine cycle as proposed by Exton and Felig and their co-workers (Mallette, Exton and Park, 1969; Felig, Pozefsky, Marliss and Cahill, 1970; Felig, 1973). The carbon skeletons of the branched chain aminoacids may be metabolized for energy in the muscle.

Insulin causes a rise in circulating serum alanine concentration. This seems to be mainly due to an inhibition on liver uptake for gluconeogenesis rather than an increase in muscle production (Sherwin, 1981). In insulin deficiency there is a rise in liver uptake of alanine despite a fall in serum concentration (Wahren, Felig, Cersai and Luft, 1972).

Insulin deficiency is also associated with hyperketonaemia. Infusion of ketones depresses serum alanine and glucose in diabetics but only alanine in normals (Sherwin, Hendler and Felig, 1976).

1.5.2.d.Lactate. Red cells do not have the enzymes for the citric acid cycle so the main energy source is from the metabolism of glucose by glycolysis. The lactate so produced can be taken up by the liver and recycled as glucose via gluconeogenesis. Other tissues particularly under anaerobic conditions also produce lactate. Thus, like the

alanine/glucose cycle, there is a lactate/glucose cycle (the Cori cycle).

The action of insulin on lactate production in muscle is complex because of the effect of the glucose level (Cherrington, 1981), however Jackson et al (Jackson, Peters, Advani, Perry, Rogers, Brough and Pilkington, 1973) showed enhanced lactate uptake by the forearm at the beginning of an oral glucose tolerance test in normal subjects. This suggests the rise in lactate after a meal is due to increased hepatic or splanchnic production associated with enhanced glucose utilization and is supported by Alberti et al (Alberti, Record, Williamson and Wright, 1972) who showed a correlation between serum insulin and blood lactate after intravenous glucose in normal subjects and patients with chronic active hepatitis.

Lactate is formed by the reduction of pyruvate by NADH, the reaction catalysed by lactate dehydrogenase. In this way reduced NAD+ is regenerated. The ratio between the lactate and pyruvate may therefore reflect the cell cytosol redox state.

1.5.2.e.Glycerol and ketones. Glycerol is formed from triglyceride breakdown in the adipose tissue and cannot be reconstituted to triglyceride there. It is returned to the liver and may enter the glycolytic or gluconeogenic pathways. Lipolysis also releases non-esterified fatty acids which may be re-esterified to triglyceride in situ or in the liver, or undergo beta oxidation to ketone bodies. Although the liver is the only site of ketone body production it

cannot metabolize ketones itself. Ketones can be used by other tissues, particularly muscle.

The enzyme responsible for triglyceride breakdown in adipose tissue is hormone sensitive lipase which is particularly sensitive to inhibition by insulin (Zierler and Rabinowitz, 1964). In adipose tissue in animals insulin also stimulates fatty acid synthesis from glucose by increasing cell concentration of glucose and increasing the activity of acetyl CoA carboxylase (Lee, Thrall and Kim, 1973). However in man fatty acid synthesis probably only occurs in the liver where insulin does not increase intracellular glucose.

McGarry (McGarry, 1979) has suggested the method of control of fatty acid metabolism in the liver. The fatty acid requires an enzyme system, carnitine acyl transferase to transport it across the mitochondrial membrane to the enzymes on the inside where beta oxidation to the ketones beta hydroxybutyrate and acetoacetate takes place.

The activity of the transport system is controlled by the level of an intermediate of fatty acid production malonyl CoA, high levels inhibit the system and therefore ketogenesis decreases. Malonyl CoA levels increase when a plentiful supply of glucose is being metabolized. Insulin and glucagon also affect the activity of the transport system and the enzyme that forms malonyl CoA, acetyl CoA carboxylase. It may be the ratio of the hormones that is important and the overall effect must take into consideration the effect of insulin on adipose tissue.

The effect of insulin on ketone utilization is not clear but probably it is stimulated by insulin (Sherwin, Hendler and Felig, 1976).

Acetoacetate is reduced to beta hydroxybutyrate by a dehydrogenase enzyme. Like lactate and pyruvate in the cytosol the ratio of the ketones may indicate the redox state in the mitochondria.

1.5.2.f.Summary of insulin effects. Insulin enhances the use of glucose as a fuel and its entry into most cells. Insulin causes glucose to be used for energy and the formation of fatty acids and glycogen. Hepatic glucose production by gluconeogenesis is reduced. Pyruvate dehydrogenase is activated allowing glucose oxidation and ketogenesis is inhibited. Lipolysis is inhibited and lipogenesis enhanced. Serum glycerol and ketone bodies levels fall. Glycolysis in the liver causes a rise in serum lactate and pyruvate as production is greater than utilization. Alanine also rises and this is partly due to a fall in hepatic uptake because of a reduction in gluconeogenesis. During insulin deficiency gluconeogenesis is increased and the uptake of the gluconeogenic substrates enhanced. Fat is mobilized and ketones produced. However, in the periphery the utilization of glucose is reduced, there is a reduced uptake of aminoacids and protein synthesis, fat storage is reduced and ketone utilization impaired.

The metabolic processes are influenced by different concentrations of insulin. Schade and Eaton (1977) showed that glucose production and lipolysis were suppressed at a

dose of insulin of only one-tenth that needed to stimulate glucose uptake. Using a different technique Sonksen et al (Sonksen, Brown, Grey, Hall, Jones, Saunders, Tompkins and Lowy, 1977) also showed that a low dose insulin infusion inhibited glucose production without stimulating utilization.

1.5.3. Metabolic profiles.

The complexity of intermediate metabolism, particularly during the non-steady state of normal life, makes interpretation of probable metabolic processes from single observations impossible. In order to gain an insight into the possible disordered metabolism of normal diabetic life multiple observations need to be made of the key metabolites. It is on this concept that the idea of metabolic profiles or rhythms is based. However this sort of study will in practice generate a large number of samples.

Manual methods for the measurement of intermediate metabolites have been available for some time (Bergmeyer, 1974) but Lloyd et al (Lloyd, Burrin, Smythe and Alberti, 1978) have published methods using continuous flow autoanalyser techniques which make the analysis quicker, cheaper and much easier to handle large numbers of samples.

Alberti (Alberti, 1973) has cautioned the over interpretation of this sort of data. He refers to the 'bath tub' principle, that is, one is only measuring the concentration of a metabolite - the level of the bath water - not the source or destination nor the rate of inflow or

outflow of a metabolite in the blood. The study simply samples the summation of these effects at one particular moment.

1.5.4.Application of metabolic profiles.

Metabolic profiles have been used to study many clinical situations. Stewart et al (Stewart, Johnston, Alberti, Nattrass and Wright, 1983) studied alcoholic liver disease. However, most work has been done in the two types of diabetes. Nattrass et al (Nattrass, Todd, Hinks, Lloyd and Alberti, 1977) using 12hr studies showed that biguanide therapy caused a greater derangement of the substrates of gluconeogenesis than sulphonylurea therapy. The effect was worse with phenformin than metformin. Potentially fatal lactic acidosis is a complication of this therapy (Luft, Schmulling and Eggstein, 1978) and an impairment in lactate metabolism is apparent in the metabolic profiles.

In a further paper Nattrass et al (Nattrass, Todd, Turnell and Alberti, 1978) showed that on combined treatment with sulphonylurea and phenformin significant differences in metabolites were found between groups with and without diabetic complications even though glucose control was the same. This demonstrates that patients on the same treatment with the same glucose control may have significant differences in other intermediate metabolites.

The failure of conventional insulin therapy to control glucose levels in type one diabetics has been known for some time. However, Alberti et al (Alberti, Dornhorst and Rowe,

1975b) showed with 24hr profiles that not only was glucose poorly controlled in supposedly well controlled diabetics but other metabolites were also very deranged compared to normal individuals. High lactate and pyruvate concentrations were seen during the day and elevated ketones in the night and morning. Similar results were obtained by Nattrass (Nattrass, 1982) using 12 hr studies. Alberti et al (Alberti, Dornhorst and Rowe, 1975a) showed significant differences between normal young adults and the elderly but the differences are small compared with the diabetic patients.

Griffin et al (Griffin, Spanos, Jenkins, Turner, Werther and Baum, 1980) demonstrated that adolescent children could tolerate these studies and that glucose and other metabolites were abnormal for much of the day. This study and another by the same group (Werther, Jenkins, Turner and Baum, 1980) but measuring fewer metabolites showed how different insulin regimes can affect metabolic profiles and the frequency of early morning hypoinsulinisation.

These methods have also been used to study specific situations such as insulin withdrawal (Madsbad, Alberti, Binder, Burrin, Faber, Krarup and Regeur, 1979).

Thus, as well as giving valuable information about glucose control metabolic profiles can give information about the other intermediate metabolites. Different patients and different treatments may be compared and underlying metabolic processes deduced. Children in general and

adolescents in particular have a reputation for poor control (Mann and Johnston, 1984) but have not been as widely studied as adult patients.

1.6. INTERMEDIATE METABOLITES AND ENDOGENOUS INSULIN

1.6.1. Glucose and endogenous insulin

It has been shown that endogenous insulin is more prevalent during the early years after diagnosis (Grajwer, Pildes, Horwitz and Rubenstein, 1977; Ludvigsson, Heding, Larsson and Leander, 1977; Madsbad, Faber, Binder, McNair, Christiansen and Transbol, 1978). Although endogenous insulin may improve temporarily with treatment in adult patients (Madsbad, Krarup, Regeur, Faber and Binder, 1981) the tendency is for it to decline in children (Ludvigsson and Heding, 1978).

Endogenous insulin correlates with measures of glucose control. Grajwer (Grajwer, Pildes, Horwitz and Rubenstein, 1977) included patients who were probably in partial remission. Endogenous insulin,and therefore remission, continue for a longer time in adult patients (Madsbad, Faber, Binder, McNair, Christiansen and Transbol, 1978) and some other studies also include non-insulin dependent patients (Hendriksen, Faber, Drejer and Binder, 1977; Gonen, Goldman, Baldwin, Goldberg, Ryan, Blix, Schanzlin, Fritz and Rubenstein, 1979). The application of the conclusions of these studies to the low levels of C-peptide found in post remission children should be cautious.

Ludvigsson (Ludvigsson, Heding, Larsson and Leander, 1977) only studied children past their remission period and found a correlation with control but, Madsbad (Madsbad, McNair, Faber, Binder, Christiansen and Transbol, 1980) showed that endogenous insulin may have to be considerable to have any effect on control (>0.3pmol/ml post glucagon)

Werther (Werther, Turner, Jenkins and Baum, 1982) studied 24hr profiles of C-peptide and glucose and found endogenous insulin in 6 of 15 patients. It seemed to be beneficial, particularly in four.

1.6.2. Metabolic Profiles and Endogenous Insulin.

The frequent blood sampling that is necessary to obtain a metabolic profile mean that the studies can only be relatively short. Alberti (Alberti, Dornhorst and Rowe, 1975b) has published profiles but did not measure endogenous insulin.

Madsbad (Madsbad, Faber, Binder, Alberti and Lloyd, 1981) studied mostly adult patients with less than 19 months duration of diabetes over 12hrs. He compared groups with different degrees of stimulated endogenous insulin but found very little difference between them. However, some would consider both his groups to be in remission. This study also compared the diabetic patients with non-diabetic individuals and found differences especially in glucose and ketone metabolism even with relatively large amounts of endogenous insulin. Asplin et al (Asplin, Hartog, Goldie, Alberti, Binder and Faber, 1979) studied a highly selected group of

adult patients over 14hrs and showed an improved blood glucose, particularly fasting, and some minor differences in ketones and glycerol in those with endogenous insulin.

In view of the different sensitivity of the various metabolic processes to insulin (see 1.5.2.f) it might be expected that small amounts of endogenous insulin may reduce glycerol and total ketone levels but have little effect on glucose because relatively larger concentrations of insulin are required to increase peripheral glucose uptake. On the other hand, lower levels of insulin do reduce glucose production. Metabolic profiles may help to define the action of small amounts of residual endogenous insulin.

1.7. CONCLUSION.

The metabolic rhythm is a powerful method of increasing understanding of metabolic processes in diabetes mellitus.

While the benefit of endogenous insulin on long term complications remains unproven it is reasonable to investigate its effect fully as even small amounts of insulin secreted portally may have important effects on intermediate metabolism. After the remission period endogenous insulin has little effect on blood glucose control but metabolic rhythms are a more sensitive tool giving a broader metabolic picture. The period after the partial remission phase was chosen because it has not been well studied and evidence is conflicting. If a beneficial effect was identified it would strengthen arguments for attempting to preserve even small amounts of endogenous

Chapter 2 <u>MATERIALS AND METHODS</u>

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2.1   INTRODUCTION

The recruitment of the patients, the procedure for the studies and blood sampling techniques are common to the different analyses in this thesis so to avoid repetition details are given in this chapter.

The data from some of the patients has been used in more than one study. Full details of the non-metabolic rhythm data are presented here and summaries are given in the relevant chapters.

The assay methods and analyses were the same in each of the studies so they are described once in this chapter. Any modifications are given in the methods section of the relevant study.

2.2   PATIENTS

2.2.1.Recruitment

This thesis reports studies on 28 patients of whom 16 were studied on two occasions. All but one of the patients were recruited from the diabetic clinic at the Childrens Hospital, Birmingham. The clinic has about two hundred patients. A few patients were excluded because they also had other diseases, for example cystic fibrosis.

It is clear that endogenous insulin production is important in the metabolic control of diabetes during the partial remission period and that it may change over time (Madsbad, Krarup,Regeur, Faber and Binder, 1981). A few patients with the high levels of endogenous insulin which

may be present soon after the disease is diagnosed would
have made the group less homogeneous and would present
particular difficulties as this endogenous insulin may be
increasing or declining. There is little information on the
importance of endogenous secretion after the partial
remission period and one of the aims of these studies was to
investigate this. The study therefore excluded patients in
the partial remission phase of their disease. Unfortunately,
there is no widely accepted definition of the remission
period and not many of our paediatric patients ever fulfil
the criteria suggested by the International Study Group of
Diabetes in Children and Adolescents (Akerblom, 1980).
Therefore, we somewhat arbitrarily chose to exclude patients
who had been diagnosed in the previous two years or were
being treated with less than 0.5u/kg/day insulin and had
good control (Hba1 less than 10%). In fact no patient was
excluded on the latter criteria. Finally, only patients that
were ten or more years old were approached.

A standard letter was sent to all the eligible
patients. Further discussion was offered in the clinic and
frequently taken up. Parental pressure was watched for and
doubtful patients not recruited. Only one patient withdrew
after recruitment and that was because her father became
seriously ill.

Part of the study was concerned with endogenous
insulin, particularly after improved control. For this
reason, slightly more patients were chosen from the
volunteers who had had diabetes for between two and six

years. One patient from the General Hospital adolescent clinic was recruited to complete this group.

In summary, the patients were recruited from a group of adolescent insulin dependent diabetics uncomplicated by other diseases. They were all over ten years of age and had been diagnosed for more than two years. Rather more (sixteen) were studied from those diagnosed for between two and six years and the rest (twelve) from those diagnosed for more than six years.

Ethical approval was obtained from Central Birmingham Health Authority Ethical Committee.

2.2.2.Patient details

All patients were studied at least once. Age, duration of diabetes, height, weight, prescribed carbohydrate rations and details of insulin treatment at the time of the first study are given in Table 2.1. The average age was 13.8yrs (range 10.8yrs to 17.4yrs) and duration of diabetes 6.7yrs (range 2.1yrs to 12.9yrs). They were treated with an average of 1.09u/kg (range 0.47u/kg to 1.84u/kg).

There is no widely accepted formula for an index of obesity in children that is equivalent to the adult body mass index or percent ideal body weight. Such formula are particularly difficult to use in adolescence because of the effects of puberty. The obesity index used in Table 2.1 is simply the standard deviation score for weight minus the standard deviation score for height. (The standard deviation

Table 2.1
Details of all 28 patients at time of first study.
CHO - Carbohydrate; Obesity Index - see text.

Patient Number	Age	Sex	Duration diabetes	Height	Weight	Obesity Index	Diet CHO	Insulin
	yrs		yr	cm	kg		X10 Gms	u/kg
1	12.6	M	4.8	145.0	35.7	0.51	24	1.26
2	12.0	F	5.3	141.0	43.4	1.82	19	1.71
3	13.1	F	4.0	148.0	48.6	1.55	20	1.28
4	15.8	F	5.1	159.2	64.6	1.38	20	0.65
5	11.7	F	3.8	144.7	39.9	0.83	26	0.88
6	12.7	F	6.3	167.2	54.0	-1.10	20	1.52
7	12.3	M	5.2	143.5	38.8	0.80	20	0.72
8	10.8	M	5.0	130.9	28.7	1.15	23	1.67
9	14.0	M	2.1	174.1	62.1	-0.64	27	0.68
10	15.0	F	2.4	168.0	53.5	-1.19	24	1.12
11	15.5	F	5.5	164.6	60.1	0.02	19	0.80
12	12.3	F	4.1	161.6	61.2	0.25	20	1.37
13	11.1	M	6.0	133.5	26.5	0.46	23	0.98
14	15.0	M	3.6	168.5	54.6	-0.15	25	1.37
15	12.1	M	5.3	160.1	49.3	-0.53	22	0.89
16	12.9	M	4.6	152.6	37.6	-0.43	22	0.74
17	17.4	M	11.6	186.0	77.7	-0.13	28	1.00
18	15.0	F	10.0	154.7	53.8	1.06	20	0.89
19	14.2	F	12.0	159.2	52.6	0.19	20	1.06
20	15.8	F	7.3	146.6	51.3	2.12	20	0.47
21	12.8	F	9.0	146.3	38.4	0.79	20	1.38
22	14.0	F	10.4	143.0	40.3	1.64	20	1.84
23	14.4	F	8.1	157.8	50.8	0.24	23	1.02
24	12.9	M	12.9	141.4	34.7	0.94	18	1.18
25	13.7	F	7.8	159.3	57.7	0.70	21	1.13
26	17.0	F	10.5	167.0	56.6	-0.78	18	0.71
27	14.3	F	6.4	158.3	50.0	0.08	22	0.88
28	15.0	F	7.7	158.8	65.7	1.59	21	1.29
Mean	13.8		6.7	155.0	49.6	0.47	22	1.09
Maximum	17.4		12.9	186.0	77.7	2.12	28	1.84
Minimum	10.8		2.1	130.9	26.5	-1.19	18	0.47

score for a variable is the measured variable minus the mean for the patient's age divided by the standard deviation of the variable at that age.) Thus a positive value is relatively over weight and a negative value relatively underweight. However, a normal post pubertal girl will weigh more than a prepubertal girl of the same height. Interpretation of individual values is therefore difficult but overall this column shows that the group was not particularly over weight.

The details of the insulin treatment at the time of the first profile are given in Table 2.2. Most patients had the majority of their insulin in the morning and about two fifths of the insulin was given as soluble insulin either as Actrapid or Velosulin or in a fixed mixture such as Initard. Patients 1 to 16 were studied twice. The second measurements and the changes in height, weight and insulin dose are given in Table 2.3 and the details of the changes in insulin in Table 2.4.

2.3   PROCEDURE FOR A METABOLIC RHYTHM

After the patients had been recruited they were asked to continue their usual regime and no special effort was made to try to improve their control above that normally made in the clinic.

The patients were admitted to a side ward during the evening before the study. Soon after 0730hrs the following day an intravenous teflon cannula (22G Abbocath, Abbott

Table 2.2
Details of insulin treatment in all 28 patients at time of first study.
Final columns: numbers - units insulin; IN - Initard; V - Velosulin; I - Insulotard;
A - Actrapid; M - Monotard; S - Semitard

Pt No	Total Daily Insulin u/kg	Morning Insulin %	Morning Insulin Soluble %	Evening Insulin Soluble %	Morning Insulin		Evening Insulin	
1	1.26	67%	50%	50%	30IN		15IN	
2	1.71	54%	40%	24%	16V	24I	8V	26I
3	1.28	66%	20%	19%	8A	33M	4A	17M
4	0.65	57%	33%	67%	8V	16I	12V	6I
5	0.88	100%	34%		12A	23M		
6	1.52	49%	63%	24%	25V	15I	10V	32I
7	0.72	100%	0%			28M		
8	1.67	54%	15%	23%	4A	22M	5A	17M
9	0.68	57%	33%	44%	8V	16I	8V	10I
10	1.12	83%	16%	100%	8A	42M	10A	
11	0.80	100%	42%		20S	28M		
12	1.37	62%	50%	50%	52IN		32IN	
13	0.98	62%	50%	50%	16IN		10IN	
14	1.37	61%	50%	50%	46IN		29IN	
15	0.89	64%	43%	38%	12V	16I	6V	10I
16	0.74	64%	22%	0%	4A	14M		10M
17	1.00	100%	31%		24S	54M		
18	0.89	58%	50%	50%	14V	14I	10V	10I
19	1.06	71%	50%	50%	40IN		16IN	
20	0.47	75%	56%	0%	10A	8M		6M
21	1.38	47%	50%	50%	25IN		28IN	
22	1.84	70%	50%	50%	52IN		22IN	
23	1.02	65%	29%	50%	10A	24M	4A	14M
24	1.18	71%	31%	17%	9V	20I	2V	10I
25	1.13	63%	46%	33%	19V	22I	8V	16I
26	0.71	70%	50%	50%	28IN		12IN	
27	0.88	55%	25%	0%	6V	18I		20I
28	1.29	61%	50%	50%	52IN		33IN	
Mean	1.09	68%	39%	39%	21	23	14	15
Maximum	1.84	100%	63%	100%				
Minimum	0.47	47%	0%	0%				

Table 2.3
Changes in height, weight and insulin dose in patients studied twice.

Patient Number	Height One cm	Height Two cm	Change Height cm	Weight One kg	Weight Two kg	Change Weight kg	Insulin One u/kg	Insulin Two u/kg	Change Insulin u/kg
1	145.0	147.5	2.5	35.7	38.1	2.4	1.26	1.47	0.21
2	141.0	145.0	4.0	43.4	45.0	1.6	1.71	1.67	-0.04
3	148.0	147.8	-0.2	48.6	49.7	1.1	1.28	1.33	0.05
4	159.2	159.2	0.0	64.6	65.9	1.3	0.65	0.94	0.29
5	144.7	145.6	0.9	39.9	40.6	0.7	0.88	0.99	0.11
6	167.2	167.9	0.7	54.0	54.6	0.6	1.52	1.54	0.02
7	143.5	144.0	0.5	38.8	40.5	1.7	0.72	0.84	0.12
8	130.9	131.9	1.0	28.7	29.4	0.7	1.67	2.04	0.37
9	174.1	176.8	2.7	62.1	67.9	5.8	0.68	1.06	0.38
10	168.0	168.8	0.8	53.5	53.3	-0.2	1.12	1.20	0.08
11	164.6	165.1	0.5	60.1	59.6	-0.5	0.80	1.04	0.24
12	161.6	163.1	1.5	61.2	61.8	0.6	1.37	1.68	0.31
13	133.5	134.6	1.1	26.5	28.2	1.7	0.98	1.13	0.15
14	168.5	170.1	1.6	54.6	58.6	4.0	1.37	1.50	0.13
15	160.1	161.5	1.4	49.3	52.2	2.9	0.89	1.15	0.26
16	152.6	152.5	-0.1	37.6	37.9	0.3	0.74	0.79	0.05
MEAN	153.9	155.4	2.0	47.4	49.0	1.5	1.10	1.27	0.17
MAX	174.1	176.8	4.0	64.6	67.9	5.8	1.71	2.04	0.34
MIN	130.9	131.9	-0.2	26.5	28.2	-0.5	0.65	0.79	-0.04

Table 2.4
Details of Insulin Treatment in patients 1 to 16 studied twice.

Numbers - units of insulin; IN - Initard; V - Velosulin; I - Insulotard;
A - Actrapid; M - Monotard; S - Semitard; HS - Human Soluble; HI - Human Isophane

PtNo	FIRST PROFILE Morning Insulin	FIRST PROFILE Evening Insulin	SECOND PROFILE Morning Insulin	SECOND PROFILE Evening Insulin
1	30IN	15IN	16V 22I	10V 8I
2	16V 24I	8V 26I	16V 24I	5V 30I
3	8A 33M	4A 17M	8A 34M	6A 18M
4	8V 16I	12V 6I	18V 20I	14V 10I
5	12A 23M		15A 25M	
6	25V 15I	10V 32I	25V 17I	10V 32I
7	28M		4A 30M	
8	4A 22M	5A 17M	10A 22M	14A 14M
9	8V 16I	8V 10I	16HS 24HI	16HS 16HI
10	8A 42M	10A	10HS 34HI	10HS 10HI
11	20S 28M		14HS 22HI	8HS 18HI
12	52IN	32IN	28HS 34HI	18HS 24HI
13	16IN	10IN	10HS 10HI	6HS 6HI
14	46IN	29IN	30HS 24HI	14HS 20HI
15	12V 16I	6V 10I	20HS 20HI	6HS 14HI
16	4A 14M	10M	6HS 14HI	10HI

Ireland Ltd, Sligo, Republic of Ireland) was inserted into a forearm vein. Between samples the cannula was kept patent by flushing with saline (154mmol/l).

The first sample was drawn at 0800hrs which was fasting and before insulin. The usual morning insulin was given at 0810hrs. Meals were taken at standard times, breakfast at 0830hrs, lunch at 1215hrs and dinner at 1815hrs with snacks at 1030hrs, 1530hrs, and 2130hrs. Evening insulin was given at 1745hrs.

The diet was selected from a standard menu and for those patients undergoing two studies was the same on each occasion. The carbohydrate content was the same as that prescribed for regular consumption at home.

Patients were encouraged to be ambulant during the day. They were free to move between the floors of the hospital and, if accompanied, go outside the hospital grounds. They were encouraged to climb the stairs to the ward at least once an hour but were at rest when the blood sample was taken.

Blood was drawn hourly on the hour during the day. Between 2200hrs and 0600hrs samples were taken every two hours. The last sample for metabolites was taken at 0700hrs. Most of the patients did not wake while the sampling was done at night, only two woke on every occasion.

After the metabolic profile was completed at 0700hrs a further fasting sample of blood was taken for Hba1 and insulin antibodies. Finally, a glucagon stimulation test for

C-peptide was performed (Faber and Binder, 1977). A sample of blood was taken immediately before 1mg of glucagon (Eli Lilly, Basingstoke, England) was injected. Further samples were taken 6 and 10 minutes later.

2.4. BLOOD SAMPLING

Blood was withdrawn from the cannula. The first 1-2ml was discarded and a further sample of 5ml was taken with a fresh syringe. Whole blood (1-2ml) was mixed with 5ml of ice-cold 5% (v/v) perchloric acid (BDH Chemicals Ltd., Poole, England) in a pre-weighed glass tube and stored at four degrees centigrade. At the completion of the study the tubes were reweighed and centrifuged at four degrees centigrade. The perchloric acid extract was separated and stored at minus twenty degrees centigrade.

The remainder of the blood was placed in a plastic tube and after clotting, serum was separated and stored at minus twenty degrees centigrade for subsequent measurement of C-peptide and growth hormone. Blood from the glucagon test was treated similarly.

The sample for Hba1 was taken into a plastic EDTA tube.

2.5 ASSAY METHODS

2.5.1.Metabolites

Blood glucose, lactate, pyruvate, 3-hydroxybutyrate, glycerol and alanine concentrations were measured in the perchloric acid extract by continuous flow enzymatic fluorometric techniques (Lloyd, Burrin, Smythe, Alberti,

1978). The assays utilize the property of reduced nicotinamide adenine dinucleotide (NADH) to exhibit fluorescence at 340nm while the oxidised (NAD) form does not. It was necessary to use "blanks" in the calculations for pyruvate, alanine, glycerol and 3-hydroxybutyrate to allow for the low level of fluorescence not due to enzymatic activity or NADH. This was not done for the much higher concentrations of glucose and lactate because the contribution to the calculation was negligible.

Blood acetoacetate concentration was measured in the perchloric acid extract by a kinetic spectrophotometric assay (Price, Lloyd, Alberti, 1977). The method measures the absorbance changes during one minute of the reaction catalysed by 3-hydroxybutyrate dehydrogenase with NADH as co-enzyme. The analysis was done within 36hrs because the concentration decreases with storage.

The precision of intermediary metabolite assays is given in Table 2.5.

2.5.2.Hormones

Serum C-peptide was measured by a single antibody radioimmunoassay method (Heding, 1975) using antibody M1230 from a commercially available kit (Novo Research Institute, Bagsvaerd, Copenhagen, Denmark). The assay was done after precipitation of proinsulin/insulin antibody complexes using polyethylene glycol (Kuzuya, Blix, Horwitz, Steiner, Rubenstein, 1977). The limit of detection was 0.025pmol/ml. Most of the measurements for a single patient were done in

Table 2.5
Precision of intermediary metabolism assays.

Metabolite	Within Batch		Between Batch	
	Mean Conc mmol/l	CV %	Mean Conc mmol/l	CV %
Glucose	1.90	3.0	1.99	3.7
	3.02	1.2	3.00	4.4
Lactate	0.35	1.1	0.36	3.0
	0.97	0.6	0.93	4.0
Pyruvate	0.021	2.1	0.026	5.2
	0.033	1.2	0.037	4.4
Alanine	0.138	0.6	0.128	4.0
	0.203	0.9	0.200	3.3
Glycerol	0.033	1.1	0.028	9.0
	0.089	0.9	0.083	7.5
3-hydroxy-	0.014	2.2	–	–
butyrate	0.069	0.7	0.060	4.3

Mean absorbance change/min

| Acetoacetate | 0.0083 | 7.9 | – | – |
| | 0.0960 | 1.2 | – | – |

one assay. The between assay coefficient of variation was less than 12% at a concentration of 0.13pmol/ml.

Serum growth hormone was measured by a double antibody radioimmunoassay method (Hartog, Gaafar, Meisser, Fraser 1964) using a locally raised antibody. Intra-assay coefficient of variation was less than 10%.

2.5.3.Glycosylated haemoglobin and insulin antibodies

Total glycosylated haemoglobin was measured by electroendosmosis (Corning Ltd., Halstead, Essex, England). Insulin antibodies were measured by Dr. T. Wilkin using an ELISA assay (Wilkin, Nicholson and Casey, 1985).

2.6 STATISTICAL METHODS

2.6.1.Calculations and equipment

Most of the calculations were done on the Birmingham University Honeywell DPS-8/70M mainframe computer with a Multics operating system. The programs used were the Statistical Package for the Social Sciences (Stanford University, USA), Minitab (The Pennsylvania State University, USA) and BMDP Biomedical Computer Programs (University of California, Berkley, USA).

Standard formulae were used for the calculation of means, standard deviations, standard error of the mean, Student's t-test and Spearman rank correlation (Armitage 1971). These formulae were used as indicated in the text to analyse all but the metabolic rhythm data (vide infra).

2.6.2.Missing data

Missing data was handled as follows. On only six sampling occasions was insufficient blood obtained and on a further two occasions laboratory problems lead to the loss of single measurements. The missing values were replaced by the mean of the previous and subsequent measurements. In all this amounted to less than 0.7% of the metabolic and hormone data.

2.6.3.Overnight samples.

Between 2200hrs and 0600hrs samples were taken 2hrly. For the "missing" hours (1100, 0100, 0300 and 0500) the value of the previous hour was repeated in order to avoid under representation the night data. This manoeuvre was completed before any of the analyses.

2.6.4.Analysis of metabolic rhythms.

The fasting values of the metabolic rhythms were compared using Student's t-test. Analysis of the rest of the metabolic data presented two main problems. Some of the metabolite concentrations were not normally distributed. To compound the problem the degree of skew varied during the day so that a manipulation at one time point may not affect another time point in the same way. Fortunately, the statistical methods are tolerant to a certain amount of skew particularly as it was in the same direction at the same time points in the different profiles. However, the ketone

data were widely skewed and were log transformed prior to analysis. In the tables antilogged values are given.

The second problem was the statistical method to use to compare two metabolic profiles. This has been discussed by Nattrass (Nattrass, 1982). The choice is between:

a) Student's t-test comparing means at each time point. This may show statically significant differences at specific time points but not show whether there was an overall difference between the profiles, although if most of the individual time points were different it would be more likely that the whole profile was different. Also the biological significance of a statistical difference at one or two time points during a day is difficult to assess.

b) A twenty four hour mean this may be calculated for each patient in a group and these values then compared between groups. The average of the twenty four hour means in each group has been calculated and is presented in tables in this thesis because it gives an idea of the size of the differences. Because the ketone data was skewed the individual patient 24hr means were calculated on log transformed data. However, to use twenty four hour means to compare the profiles under-utilizes the available data and effectively reduces the twenty measurements to one.

c) Two-way analysis of variance with metabolite concentration classified by group and time (Armitage, 1971). It is possible with this method to compare the differences in the variance between two groups removing the variance due

to the time of day the sample were taken. Each sample value is used in this method rather than a number derived from a number of samples. The significance of the difference between groups was calculated from the variance ratio using F-tables.

The ideal method of comparing data that has a diurnal rhythm such as this has probably yet to be developed. Meanwhile the last method seems to be the best and is the one used in this study.

Chapter 3 <u>METABOLIC PROFILES</u>

3.1. INTRODUCTION

3.2. METHODS

3.3. RESULTS.

    3.3.1. Glucose

    3.3.2. Lactate and Pyruvate

    3.3.3. Lactate/Pyruvate Ratio

    3.3.4. Alanine

    3.3.4. Ketone Bodies

    3.3.5. Glycerol

3.4. DISCUSSION

3.1 INTRODUCTION

It is well recognised that insulin has many effects but the study of intermediate metabolism in diabetes has concentrated on glucose. Studies in apparently well controlled adult patients (Alberti, Dornhorst and Rowe, 1975b) have shown widespread abnormalities. Adolescent patients have not been studied in such detail and there is little information over the full twenty-four hours in patients of any age group. The purpose of this chapter is to document the range and character of the metabolic abnormality in a group of insulin dependent adolescent patients by an analysis of the first metabolic profile performed.

3.2 METHODS

The data used in this analysis was from the first metabolic rhythm done on all the 28 patients after recruitment and before any specific attempt to improve diabetic control or change insulin type.

Recruitment and full details of the patients are given in Section 2.2. and Tables 2.1. and 2.2. and are briefly summarised here.

The age of the patients ranged from 10.8yrs to 17.4yrs (mean 13.8yrs) and ten of the twenty-eight were male. They had been diagnosed for between 2.1yrs and 12.9yrs (mean 6.7yrs). The patients were selected to be past their initial

partial remission phase of diabetes and slightly more were recruited from those less than six years since diagnosis.

The mean insulin dose was 1.09u/kg (range 0.47u/kg to 1.84u/kg). Four were on once daily insulin. Eighteen were treated with either Initard or Velosulin and Insulatard (Nordisk), seven with a combination of Actrapid and Monotard, two with Semitard and Monotard and one with Monotard (Novo) alone. About two thirds of the insulin was given in the morning and about two fifths of the insulin was soluble. However, the variation between the patients was considerable (Table 2.2).

The metabolic rhythms and assays were carried out in the way described in Sections 2.3, 2.4 and 2.5.

The BMDP statistical program was used to find the range, 25th and 75th quartiles and median of each metabolite at each time point. The results were plotted graphically using the Tell-a-graf (ISSCO) computer program.

3.3 .RESULTS.

The fasting values and 24 hr mean for each metabolite are shown in Tables 3.1 and 3.2. respectively. In each case the mean and standard error of the mean have been calculated for the whole group.

3.3.1. Glucose

The fasting values of blood glucose were all above normal (Table 3.1). The mean blood glucose was elevated at

Table 3.1
Fasting concentrations of blood metabolites (mmol/l) and ratios from all 28 patients.
SEM - standard error of mean: TKB - total ketone bodies;
3-OHB/AcAc - 3-hydroxybutyrate/acetoacetate.

Pt No	Glucose	Lactate	Pyruvate	Lactate Pyruvate Ratio	Alanine	3-hydroxy -butyrate	Aceto- Acetate	3-OHB AcAc Ratio	TBK	Glycerol
1	18.9	1.12	0.12	9.2	0.35	0.46	0.21	2.2	0.67	0.18
2	13.3	0.88	0.08	10.5	0.33	0.19	0.10	1.9	0.29	0.15
3	11.4	0.64	0.07	9.0	0.26	0.15	0.04	3.4	0.19	0.14
4	22.7	0.78	0.07	11.0	0.17	1.96	1.09	1.8	3.05	0.13
5	16.2	0.77	0.07	10.5	0.22	1.07	0.43	2.5	1.50	0.11
6	18.1	0.94	0.09	10.3	0.27	0.45	0.17	2.7	0.62	0.17
7	9.9	0.59	0.05	12.3	0.19	0.16	0.14	1.2	0.30	0.11
8	12.4	1.69	0.13	13.3	0.29	0.45	0.17	2.7	0.62	0.20
9	15.9	0.73	0.08	9.2	0.21	0.63	0.25	2.6	0.87	0.10
10	10.4	0.62	0.07	8.6	0.25	0.08	0.03	2.3	0.13	0.11
11	10.9	0.98	0.10	10.3	0.18	0.97	0.23	4.2	1.20	0.17
12	11.6	1.07	0.10	10.7	0.35	0.22	0.11	2.0	0.33	0.22
13	20.2	0.82	0.06	13.2	0.18	1.28	0.57	2.3	1.84	0.17
14	25.7	0.89	0.07	12.0	0.20	1.32	0.59	2.3	1.91	0.19
15	17.3	0.81	0.07	12.5	0.18	0.87	0.29	3.0	1.16	0.15
16	11.1	0.89	0.08	11.9	0.26	0.16	0.14	1.2	0.30	0.10
17	10.0	0.87	0.07	12.4	0.31	0.19	0.10	2.0	0.29	0.07
18	17.4	1.08	0.09	12.4	0.40	0.41	0.16	2.5	0.57	0.23
19	25.0	0.77	0.07	10.5	0.27	0.61	0.31	2.0	0.92	0.15
20	23.0	0.85	0.08	10.5	0.26	1.36	0.69	2.0	2.05	0.14
21	18.8	0.97	0.09	10.3	0.26	0.73	0.22	3.3	0.95	0.17
22	23.1	0.96	0.08	11.4	0.31	1.40	0.43	3.3	1.83	0.14
23	8.3	0.96	0.09	10.4	0.21	0.39	0.14	2.8	0.54	0.14
24	16.1	0.99	0.08	12.1	0.21	1.50	0.46	3.3	1.95	0.19
25	16.4	0.74	0.07	10.4	0.24	0.41	0.20	2.1	0.61	0.15
26	16.2	0.82	0.07	12.4	0.17	2.27	0.72	3.2	3.00	0.10
27	7.5	0.96	0.09	10.3	0.27	0.23	0.09	2.7	0.31	0.16
28	24.0	0.82	0.07	11.9	0.32	0.71	0.20	3.6	0.91	0.22
Mean	16.1	0.89	0.08	11.1	0.25	0.52	0.22	2.5	0.75	0.15
SEM	1.0	0.39	0.01	0.2	0.01	0.46	0.43	0.1	0.44	0.01

Table 3.2
Average of 24hr means of blood metabolite concentrations and ratios from all 28 patients
SEM - standard error of mean; TKB - total ketone bodies;
3-OHB/AcAc - 3-hydroxybutryate/acetoacetate. Note ketone data logged for analysis.

Pt No	Glucose	Lactate	Pyruvate	Lactate Pyruvate Ratio	Alanine	3-hydroxy -butyrate	Aceto- Acetate	3-OHB AcAc Ratio	TBK	Glycerol
1	10.9	1.06	0.11	9.4	0.40	0.10	0.10	1.1	0.21	0.10
2	7.9	1.28	0.13	10.2	0.41	0.07	0.06	1.3	0.12	0.09
3	7.9	0.98	0.10	9.3	0.37	0.06	0.05	1.2	0.11	0.07
4	16.4	1.28	0.12	10.7	0.22	0.53	0.33	1.6	0.85	0.13
5	20.0	1.11	0.10	11.2	0.37	0.25	0.20	1.4	0.47	0.11
6	9.6	1.12	0.11	10.7	0.40	0.07	0.07	1.2	0.15	0.08
7	26.9	1.26	0.10	12.3	0.32	0.39	0.23	1.7	0.62	0.11
8	17.2	1.36	0.10	13.6	0.31	0.10	0.07	1.7	0.17	0.10
9	15.5	0.87	0.09	9.3	0.29	0.16	0.13	1.3	0.29	0.09
10	7.9	0.86	0.09	10.3	0.34	0.05	0.05	1.1	0.10	0.08
11	18.8	0.88	0.09	9.9	0.23	0.59	0.28	2.1	0.87	0.10
12	7.3	1.27	0.12	10.8	0.47	0.04	0.06	0.8	0.10	0.11
13	15.4	1.53	0.13	12.3	0.41	0.13	0.12	1.2	0.26	0.11
14	12.0	1.88	0.16	12.3	0.41	0.14	0.12	1.3	0.26	0.10
15	12.7	1.49	0.13	11.3	0.37	0.09	0.08	1.2	0.17	0.07
16	13.1	0.84	0.08	11.4	0.33	0.06	0.06	0.9	0.12	0.05
17	12.7	0.99	0.08	11.8	0.39	0.09	0.08	1.2	0.18	0.07
18	13.1	1.89	0.18	10.1	0.58	0.07	0.06	1.2	0.13	0.09
19	13.3	1.10	0.12	9.7	0.37	0.10	0.09	1.2	0.19	0.09
20	17.0	1.75	0.17	10.0	0.45	0.15	0.08	1.9	0.24	0.13
21	14.0	1.40	0.12	12.2	0.38	0.13	0.07	2.0	0.20	0.12
22	13.9	1.27	0.13	10.4	0.43	0.13	0.08	1.8	0.21	0.09
23	17.1	0.96	0.10	9.5	0.33	0.12	0.09	1.4	0.21	0.10
24	8.1	1.33	0.12	10.8	0.43	0.09	0.08	1.2	0.18	0.09
25	13.5	0.98	0.11	9.2	0.31	0.12	0.11	1.2	0.23	0.08
26	16.6	0.90	0.08	12.0	0.20	1.09	0.56	2.0	1.66	0.10
27	9.3	0.72	0.08	9.4	0.39	0.04	0.05	1.0	0.09	0.07
28	8.9	1.63	0.16	10.6	0.45	0.10	0.08	1.5	0.20	0.14
Mean	13.5	1.20	0.11	10.7	0.37	0.18	0.12	1.4	0.31	0.10
SEM	0.3	0.03	<0.01	0.1	0.01	0.04	0.02	<0.1	0.06	0.01

16.1 mmol/l and rose after breakfast and insulin until mid-morning (Fig 3.1). The poorest control of blood glucose for the whole group was seen in the morning and the range was also greatest then. At 1100hrs one patient had the highest value recorded (38.0mmol/l) while an hour later the lowest value in the group was only 3.1mmol/l, a concentration not reached again by any patient until the early hours of the morning.

After lunch there was another general rise, not as great as that after breakfast, followed by a slow decline until the early hours of the morning. At 0200 and 0400hrs the mean concentration was most stable and only changed from 8.6mmol/l (the lowest median concentration) to 8.7mmol/l. At 0400hrs the range was at its least - 3.0 to 20.8mmol/l. By 0600hrs the blood glucose had started to rise and this continued in the next hour.

3.3.2. Lactate and Pyruvate.

From normal fasting concentrations (Table 3.1) blood lactate and pyruvate rose rapidly after breakfast (Fig 3.2 and Fig 3.3). The other main meals of the day have a similar effect but the concentrations began to fall within a few hours of the last meal and were stable over night. However there were some grossly elevated values particularly in the morning. Blood lactate is rarely above 2 mmol/l at rest.

3.3.3. Lactate/Pyruvate Ratio.

There was little variation in the median

Figure 3.1 Diurnal rhythm of blood glucose concentration (median, 25th and 75th centiles, range) in 28 adolescent diabetic patients. Arrows indicate meal and snack times.

Figure 3.2 Diurnal rhythm of blood lactate concentration (median
25th and 75th centiles, range) in 28 adolescent diabetic patients.
Arrows indicate meal and snack times.

Figure 3.3 Diurnal rhythm of blood pyruvate concentration (median, 25th and 75th centiles, range) in 28 adolescent diabetic patients. Arrows indicate meal and snack times.

lactate/pyruvate ratio over the 24hrs and most of the values were about 10 (Table 3.2 and Fig 3.4). There was a small fall after breakfast and insulin and then a slow rise over the day.

3.3.4. Alanine.

Like lactate and pyruvate the fasting concentration was normal (Table 3.1). It was just possible to see an effect of meals on the profile and alanine concentration built to a peak in the early afternoon. Changes were slow and did not reach stable levels again until 0200hrs (Fig 3.5).

3.3.4. Ketone Bodies.

The profiles for 3-hydroxybutyrate, acetoacetate and their sum, "total" ketone bodies are shown in Figs 3.6, 3.7 and 3.8. Fasting concentrations (Table 3.1) were grossly elevated in the majority of patients but fell rapidly to normal values. A slight rise occurred in the late afternoon but then fell again. After midnight there was a steady rise in total ketone bodies. The ketone data was clearly skewed and this was not fully corrected, though improved, by logarithmic transformation. The ratio of the two metabolites was fairly constant (Table 3.2, Fig 3.9) apart from the first sample of the day when the total ketones were at the highest value and there was relatively more 3-hydroxybutyrate (Table 3.1).
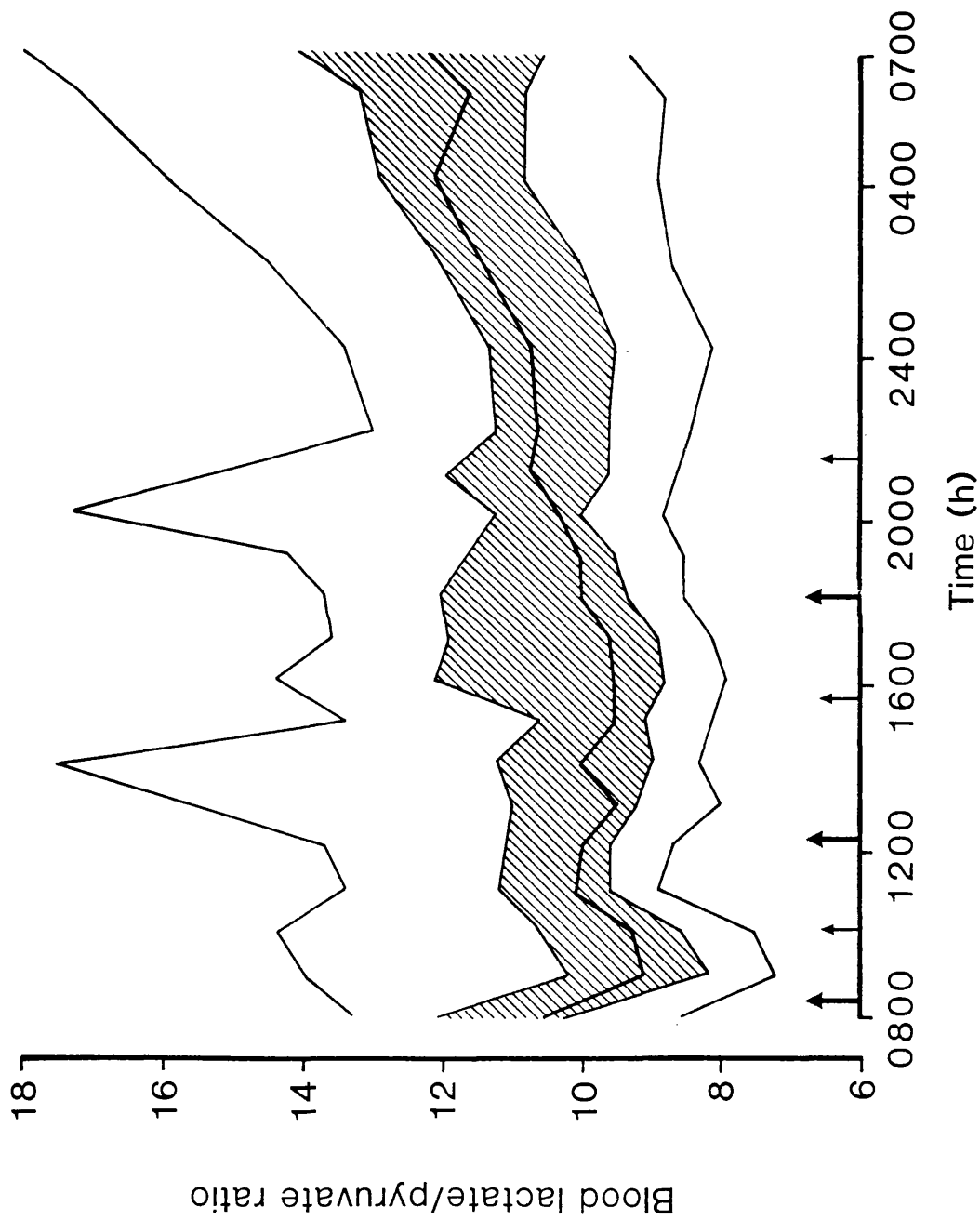
Figure 3.4   Diurnal rhythm of the ratio between blood lactate and blood pyruvate (median, 25th and 75th centiles, range) in 28 adolescent diabetic patients.   Arrows indicate meal and snack times.
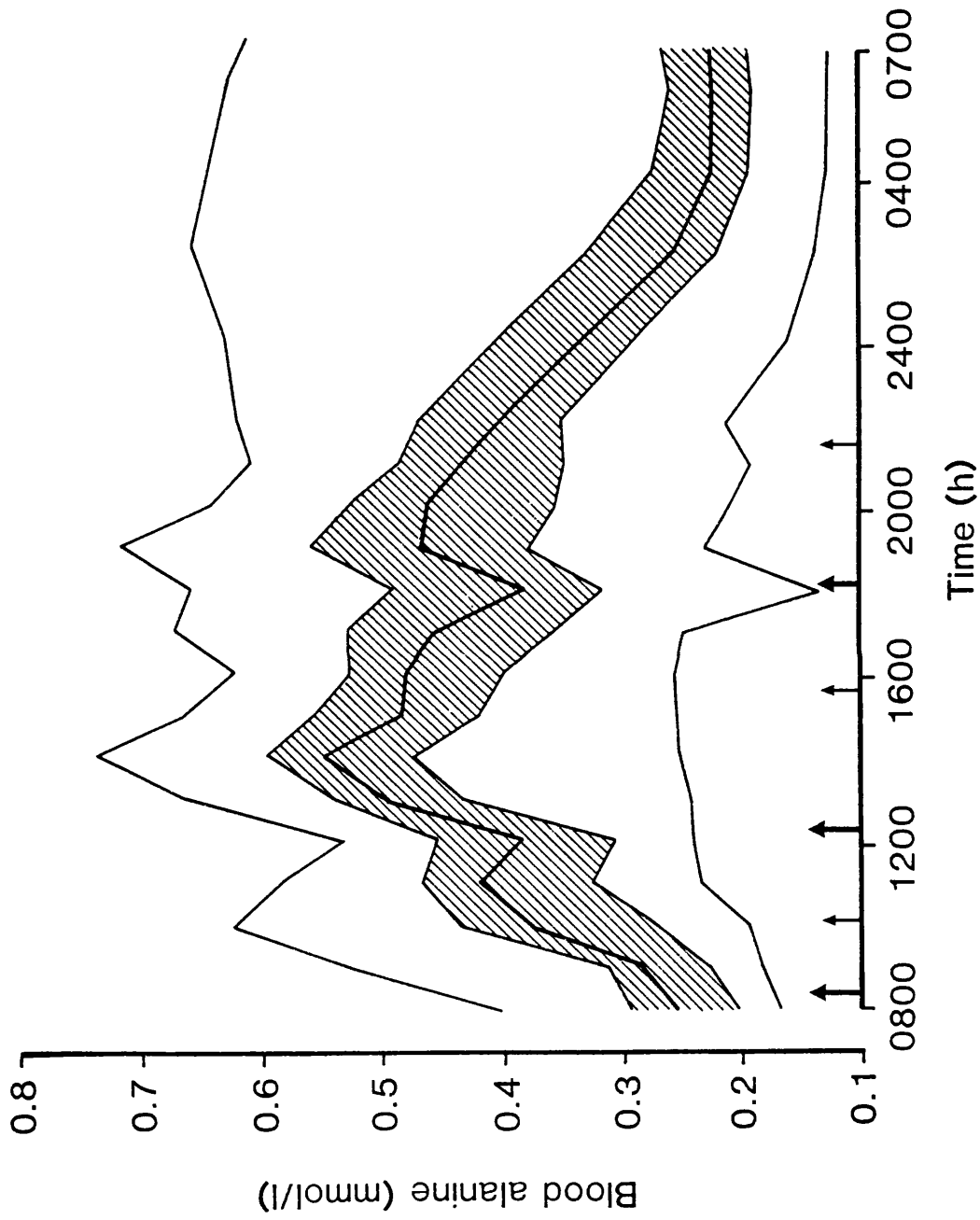
Figure 3.5   Diurnal rhythm of blood alanine concentration (median, 25th and 75th centiles, range) in 28 adolescent diabetic patients. Arrows indicate meal and snack times.
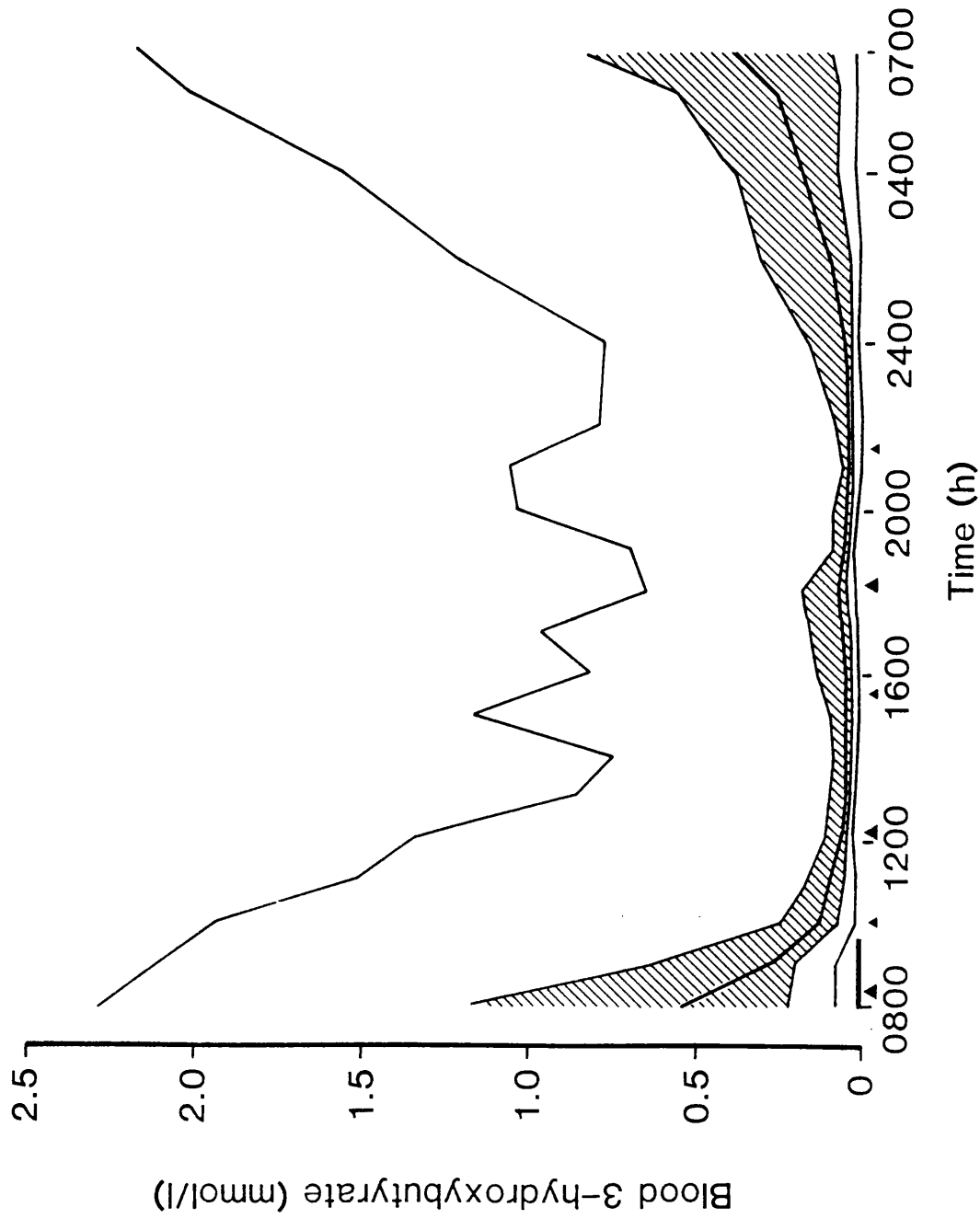
Figure 3.6 Diurnal rhythm of blood 3-hydroxybutryate concentration (median, 25th and 75th centiles, range) in 28 adolescent diabetic patients. Arrows indicate meal and snack times.
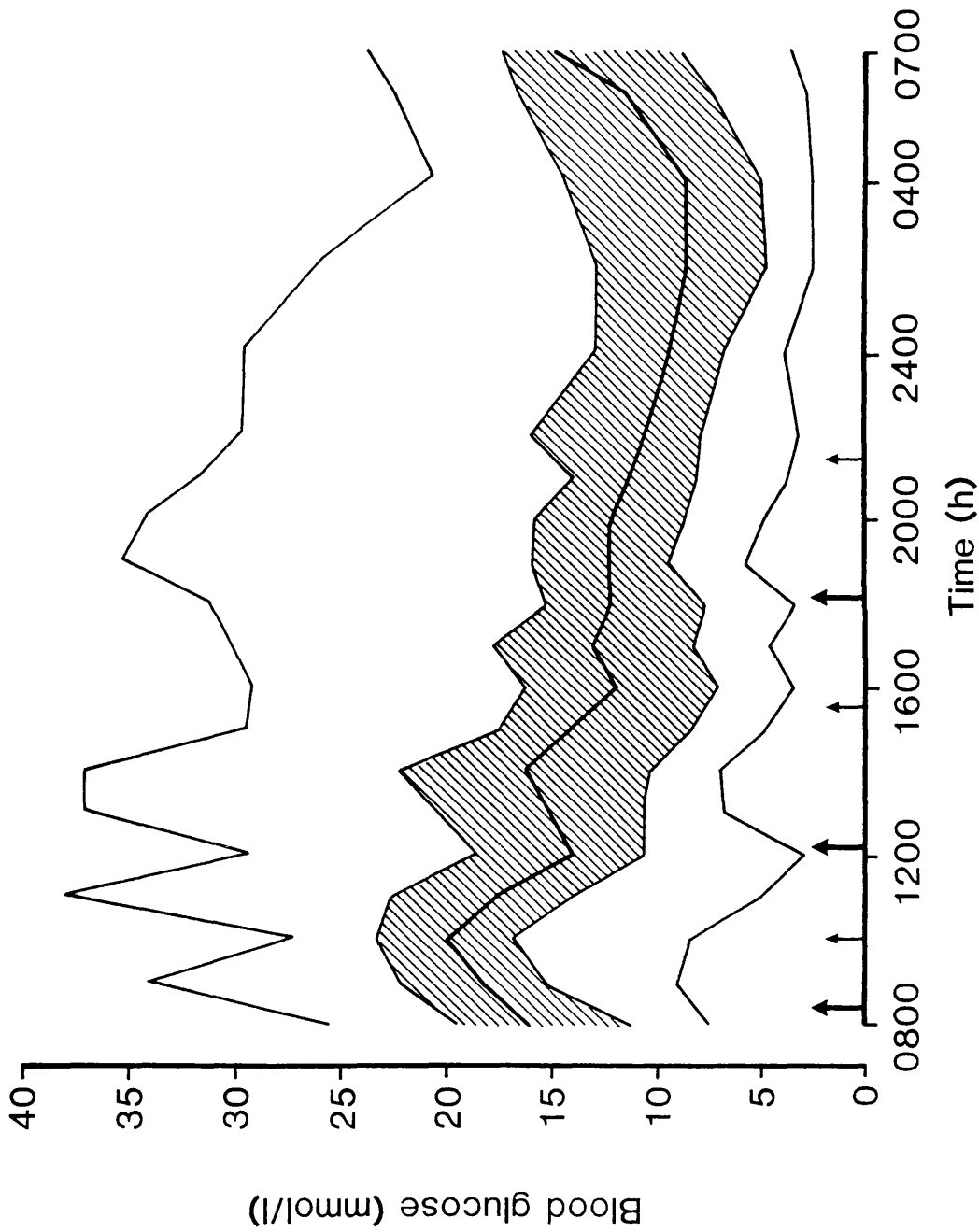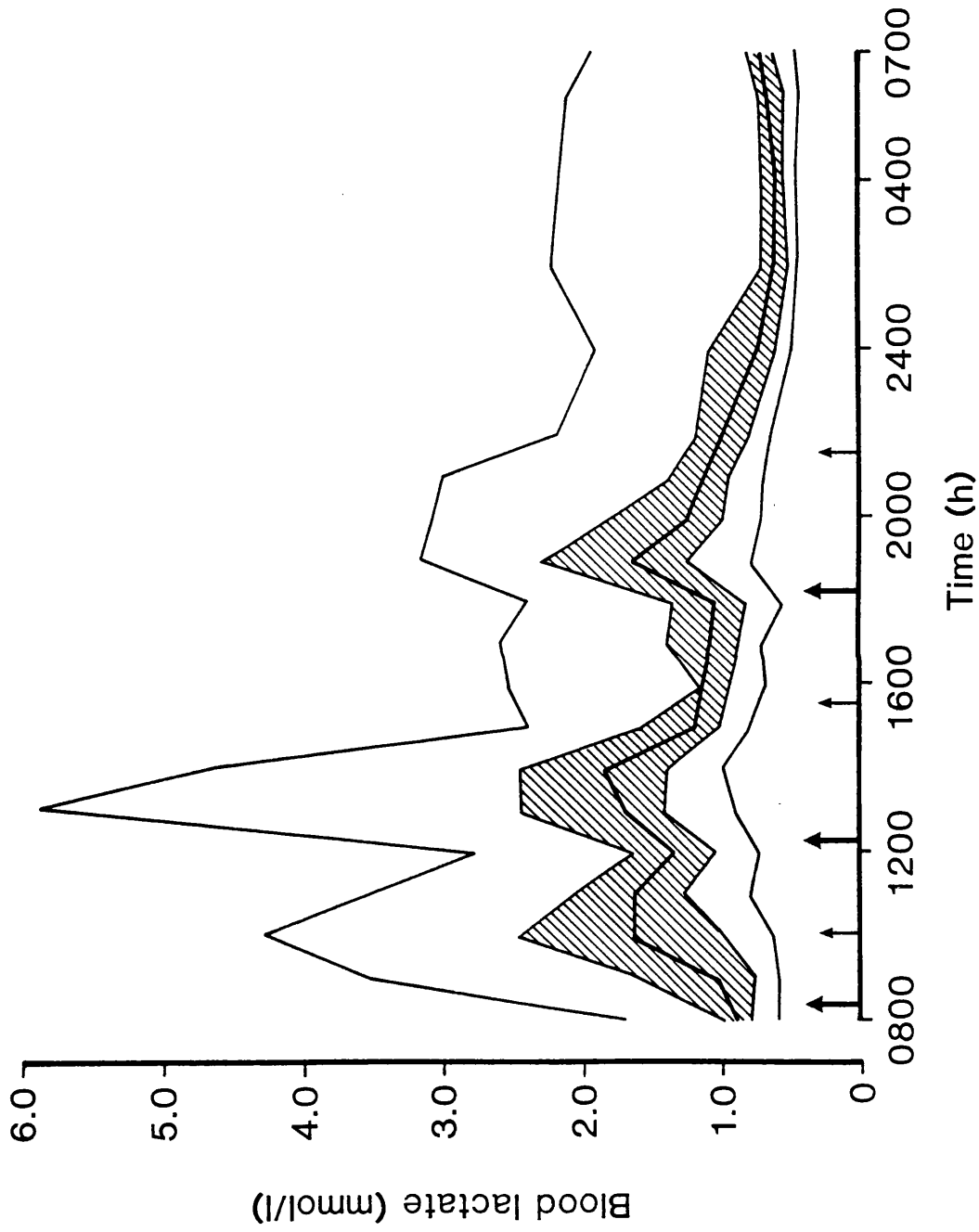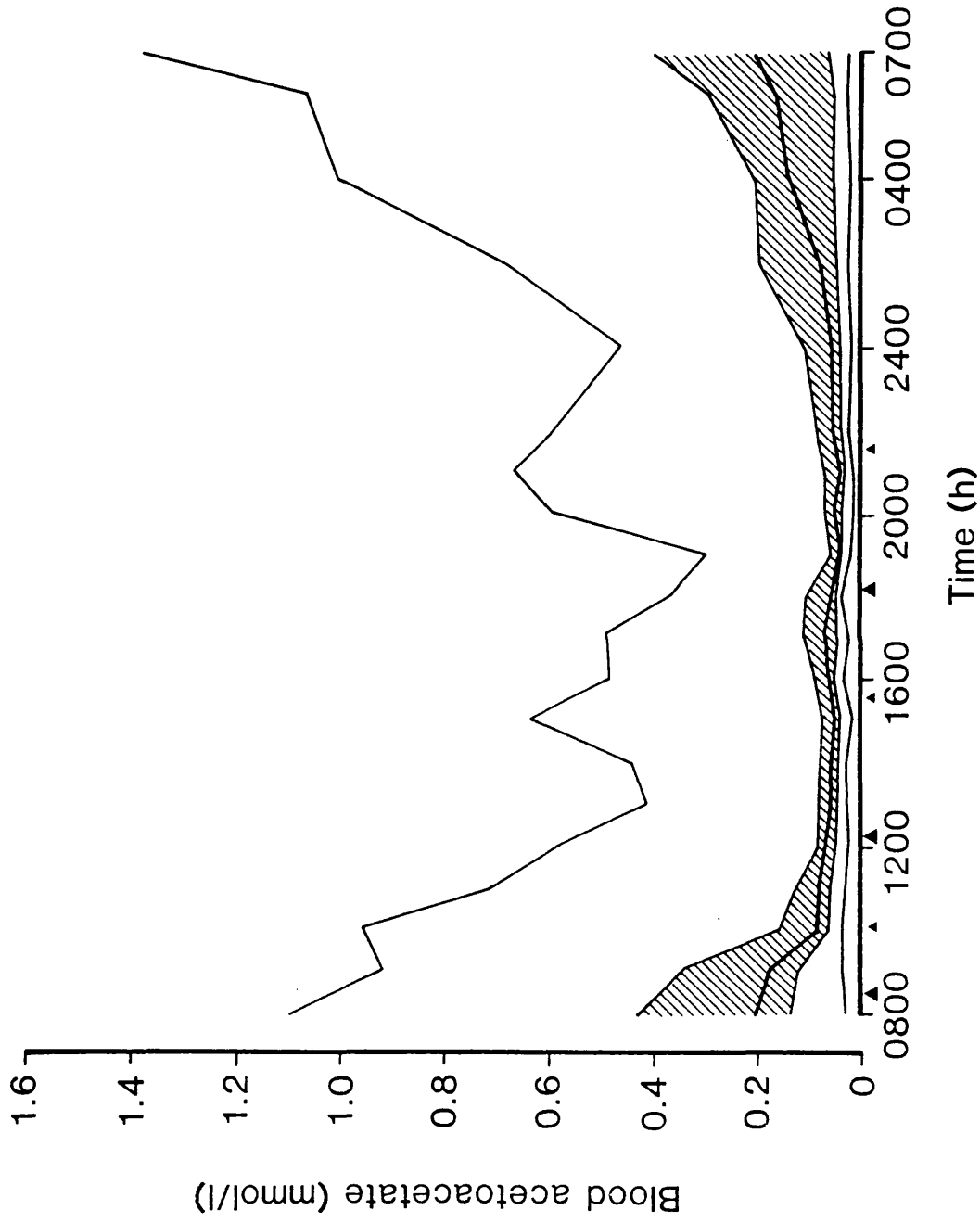
Figure 3.7 Diurnal rhythm of blood acetoacetate concentration (median, 25th and 75th centiles, range) in 28 adolescent diabetic patients. Arrows indicate meal and snack times.
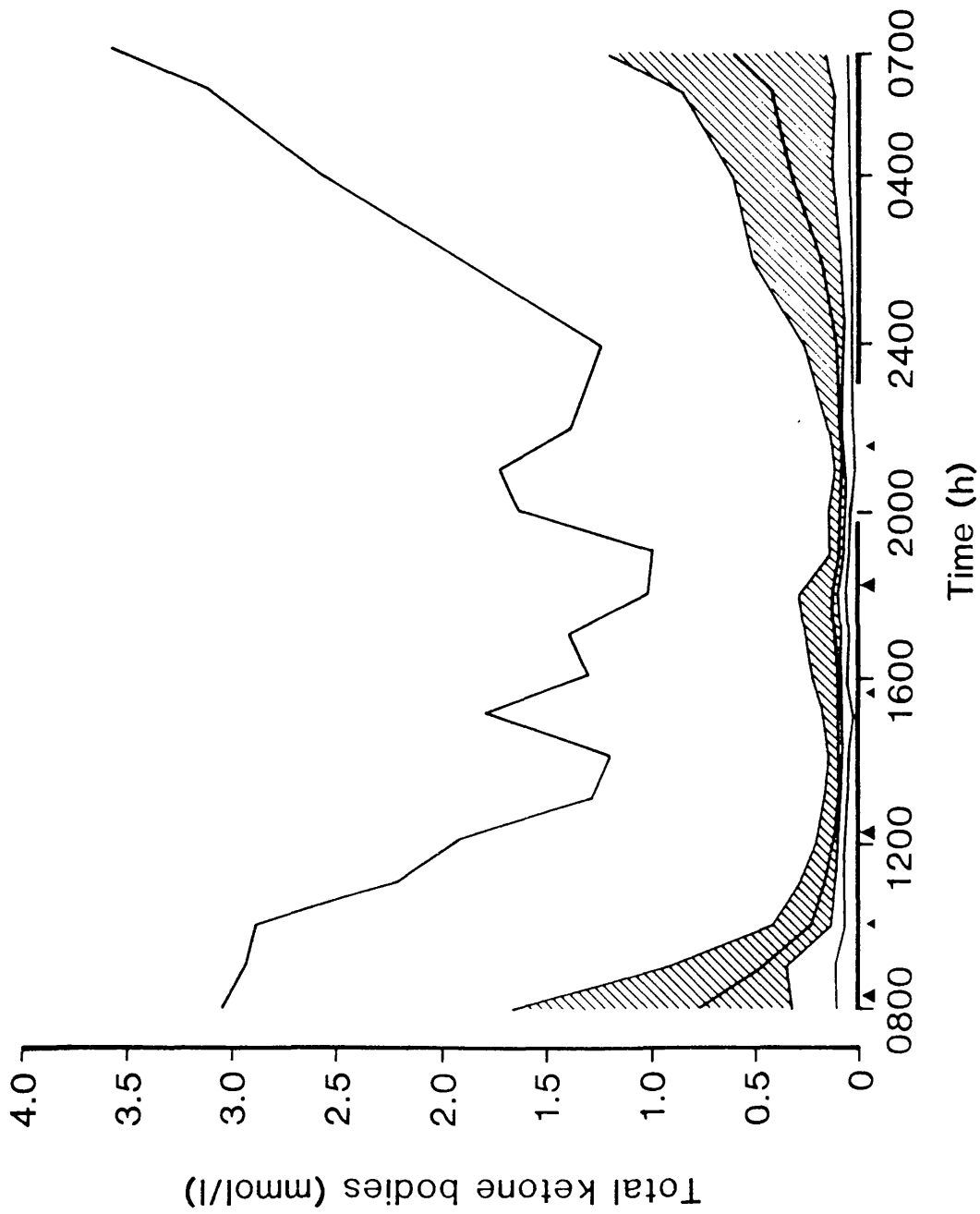
Figure 3.8 Diurnal rhythm of blood total ketone body concentration (median, 25th and 75th centiles, range) in 28 adolescent diabetic patients. Arrows indicate meal and snack times.
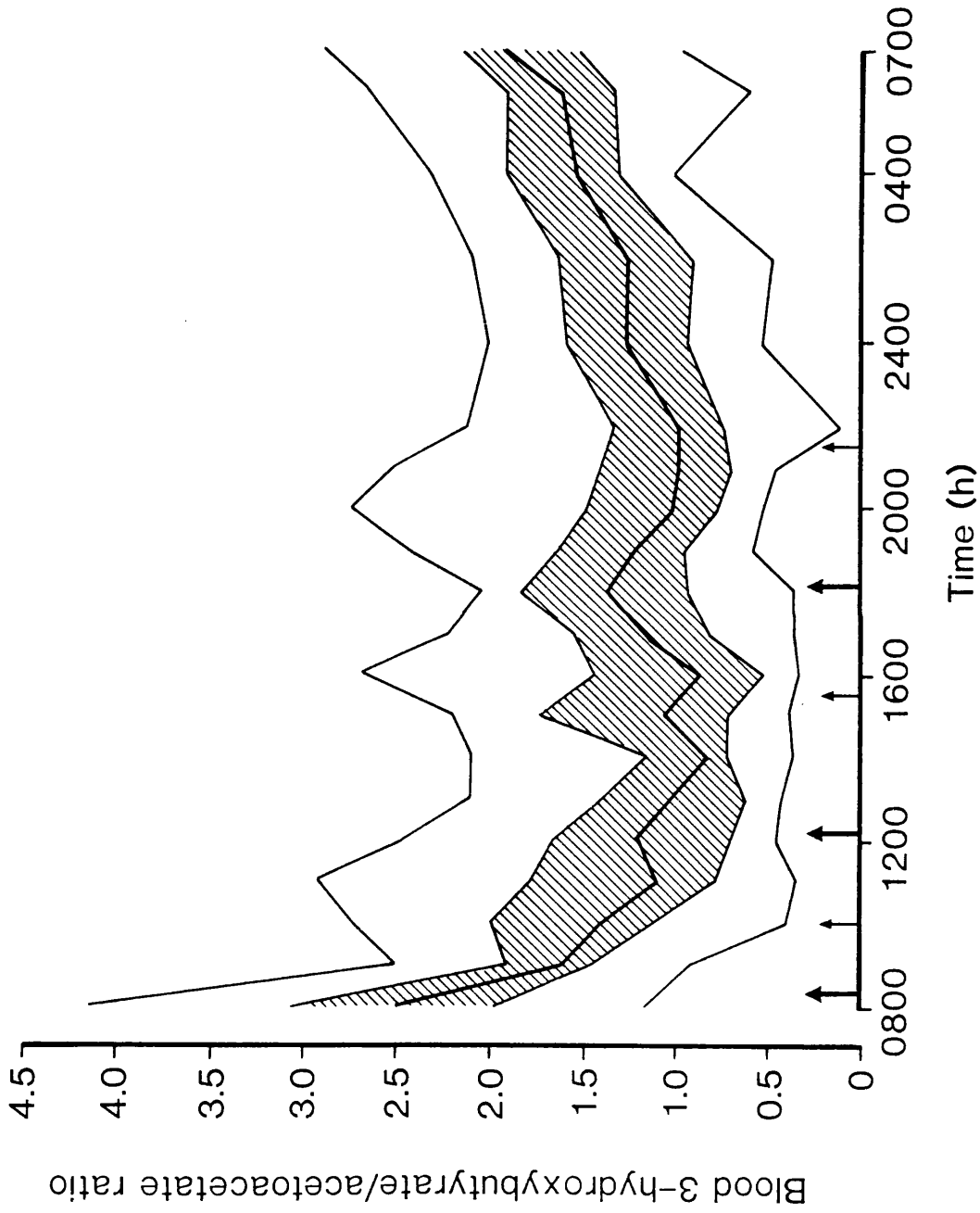
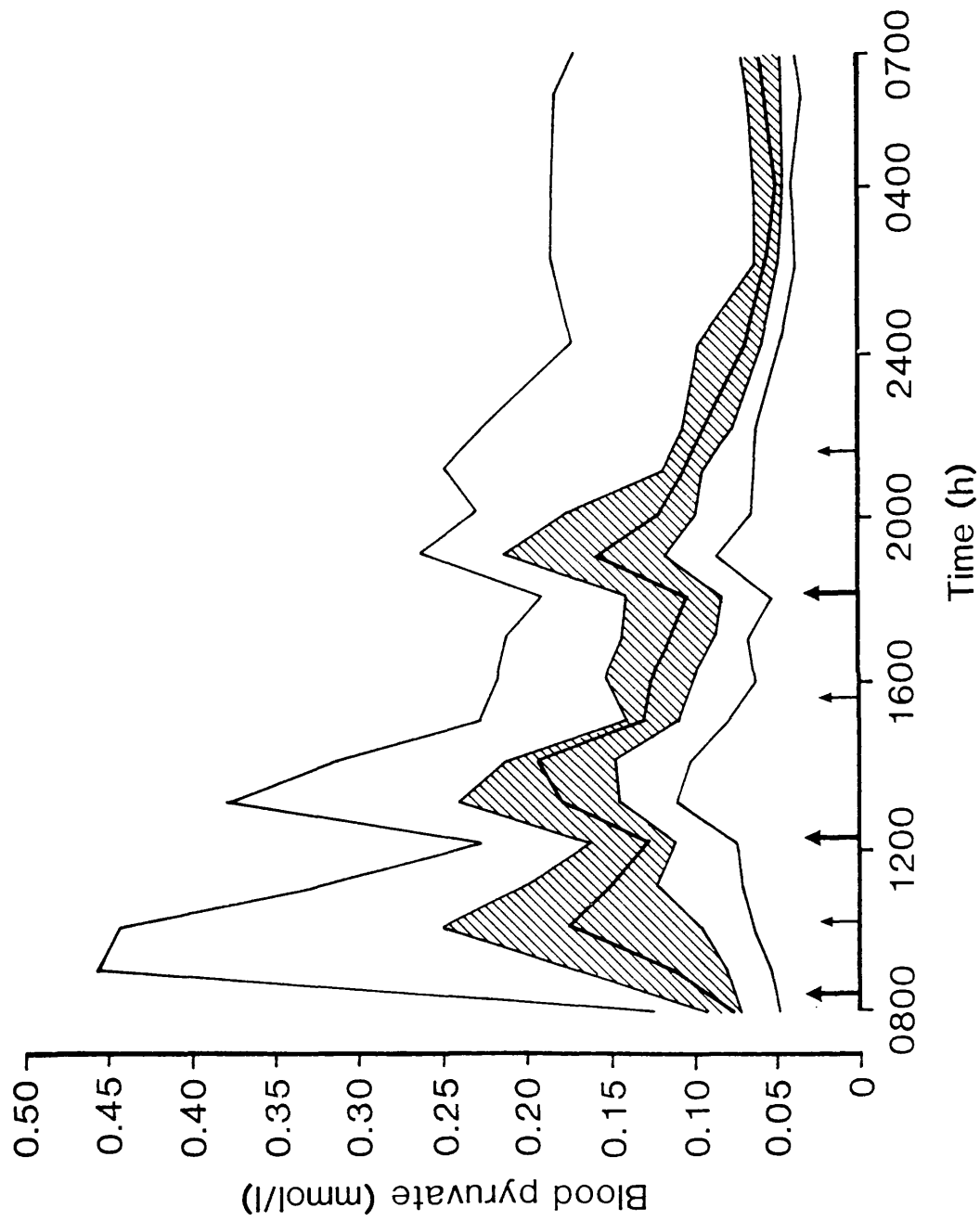Figure 3.9 Diurnal rhythm of the ratio between blood
3-hydroxybutryate and blood acetoacetate (median, 25th and 75th
centiles, range) in 28 adolescent diabetic patients. Arrows
indicate meal and snack times.

3.3.5. Glycerol

Most of the patients had elevated fasting blood glycerol (Table 3.1). There was a consistent fall after insulin and breakfast but throughout the rest of the day little change (Table 3.2, Fig 3.10). Of all the metabolites glycerol showed least change over the 24hrs.

3.3.7. Growth hormone

The graph of growth hormone (Fig 3.11) demonstrates the wide fluctuations seen with this hormone. The generally higher growth hormone levels expected in the early part of the night were seen.

3.3.6. C-peptide

Graphical representation and analysis of C-peptide was complicated by many patients with values below the detection limit of the assay. Fig 3.12 shows all the measurements made and the number of values unrecordable at each sampling time. Most of the maximum values were from a single patient who had had diabetes for ten years but whose control was not particularly good (patient number 18, see Section 4.3). Apart from this patient, there was very little change over the 24hrs.

3.4. Discussion

There are little data on normal metabolic profiles. Alberti's group (Alberti, Dornhorst and Rowe, 1975a) has studied a wide age range of subjects and shown that there
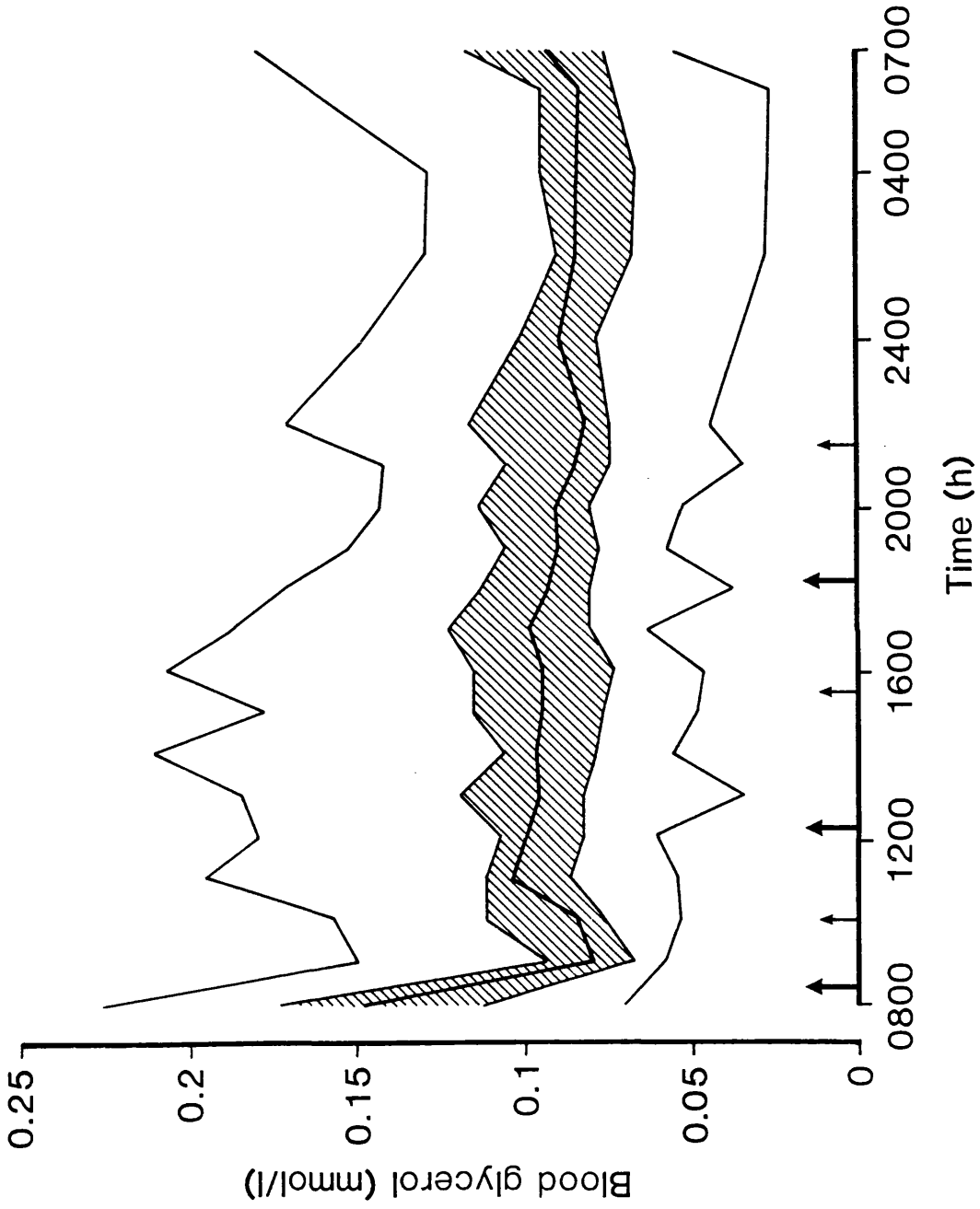
Figure 3.10 Diurnal rhythm of blood glycerol concentration (median, 25th and 75th centiles, range) in 28 adolescent patients. Arrows indicate meal and snack times.
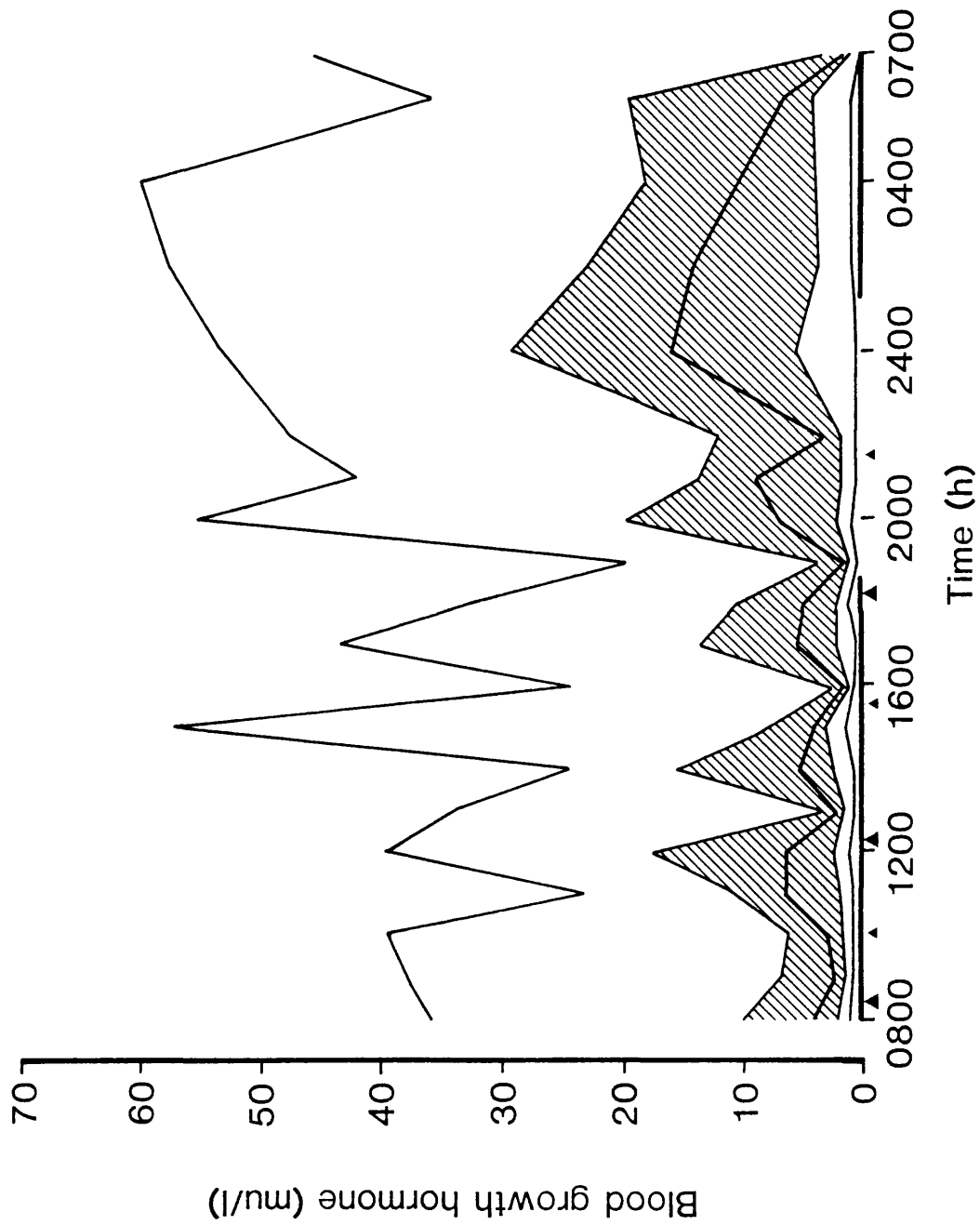
Figure 3.11 Diurnal rhythm of blood growth hormone concentration (median, 25th and 75th centiles, range) in 28 adolescent patients. Arrows indicate meal and snack times.
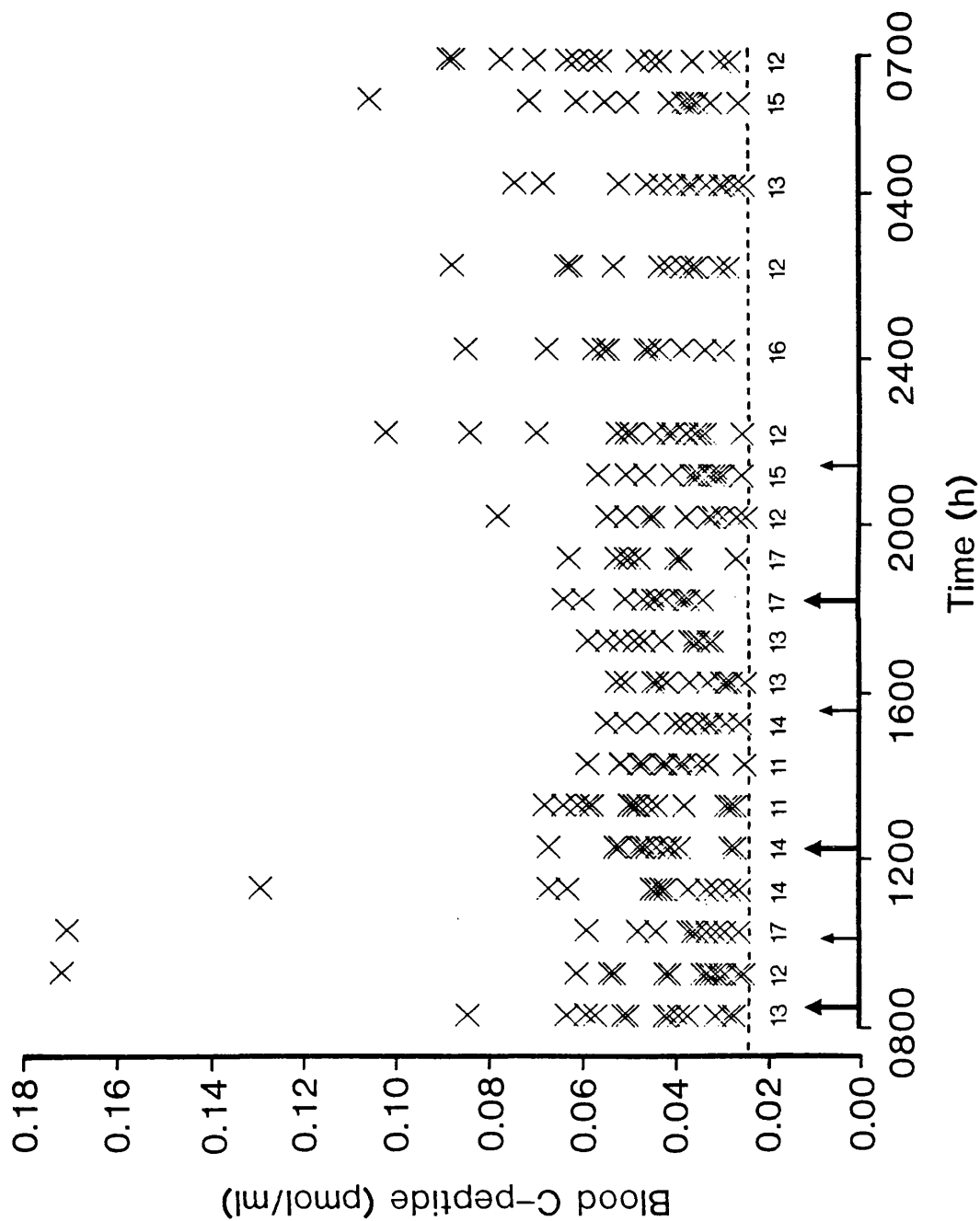
Figure 3.12 Diurnal rhythm of blood C-peptide concentration in 28 adolescent diabetic patients. Crosses indicate one value measured; line indicates detection limit of assay; numbers are numbers of measurements below detection limit. Arrows indicate meal and snack times.

are small changes in metabolite concentration with age with the tendency for an increase in concentration with increasing age particularly in glucose, total ketone and glycerol concentrations. Nattrass (Nattrass, 1982) also studied normal subjects. Adult normal fasting values of metabolites have been published (Foster, Alberti, Hinks, Lloyd, Postle, Smythe, Turnell and Walton, 1978). There have been no studies specifically of normal adolescents or children. It may be hazardous to compare diabetic adolescent data with normal adult data because the adolescent is in the endocrinological turmoil of puberty and clearly anabolic during the growth spurt. Even with these constraints in mind, it was quite clear that there was a profound abnormality of the metabolic profiles in these patients.

Glucose control was particularly abnormal with many patients never achieving a single normal value. There have been a large number of studies that measured blood glucose but only a few have used similar techniques to this study. Alberti (Alberti, Dornhorst and Rowe, 1975b) studied adult patients after admission to improve control and found glucose to be elevated but not as much as in these patients.

The mean 24hr blood glucose is similar to those obtained in diabetic children by Griffin et al (Griffin, Spanos, Jenkins, Turner, Werther and Baum, 1980) who, as in this study, made no prior attempt to improve control.

Conducting the study under hospital conditions may have affected the results because hospital life is very different from the childs' home life. In previous rhythm studies,

however, a good correlation has been found between blood glucose measurements at home and during the hospital study (Werther, Jenkins, Turner and Baum, 1980).

The fasting lactate and pyruvate were within normal limits but during normal activity the lactate is usually below 2 mmol/l and pyruvate usually below 0.2 mmol/l (Nattrass, 1982; Alberti et al, 1975a and 1975b). The normal rise in these metabolites after meals was preserved but the magnitude was greater especially in the morning. Blood lactate and pyruvate may rise in response to decreased hepatic uptake for gluconeogenesis or increased production. Initially, following insulin, hepatic uptake does not change but gluconeogenic precursors are diverted into glycogen (Radziuk, 1981) and it seems likely therefore that the increase in lactate and pyruvate indicates increased production of these metabolites, which probably occurs in the splanchnic area. The average of the 24hr means was similar to the values given for diabetic patients by Alberti and his group (Alberti et al, 1975b). Both were elevated. The ratio of the metabolites remained normal and changed little during the day.

Fasting alanine levels were normal but the concentration rose more quickly and higher than normal subjects reported in the literature. In Alberti's study the diabetic patients' alanine concentration rose more quickly but not further than the normal individuals. These effects on blood alanine may be secondary to changes in pyruvate metabolism (Pozefsky and Tancredi, 1972).

The mean of the fasting total ketone bodies was similar to the other studies and showed that many start the day with elevated total ketones, the upper limit of normal being about 0.5mmol/l. As would be expected from the sensitivity of lipolysis to insulin, the total ketone concentration fell rapidly much sooner than glucose concentration.

Wildenhoff (1972) studied normal subjects and showed peaks in total ketone bodies occurring before meals and at 1600hrs. The rise before lunch was absent in these patients and that before the evening meal was blunted. Some individual patients also had abnormally high concentrations of total ketone bodies during the day, levels above 0.5mmol/l are not usually seen.

In most subjects (non-diabetic) studied by Wildenhoff there was a rise around midnight followed by a fall maximal at about 0430hrs while those studied by Alberti (Alberti et al, 1975b) showed little change overnight. In the present study there was a rise around midnight but the concentration of total ketones continued to rise during the rest of the night. This contrasts with the diabetics in the Alberti et al study and the diabetic children studied by Werther et al in whom the rise was later in the night. However in these two studies patients had been subjected to recent attempts to improve their control and a few of Werther's patients were in partial remission.

Comparing the overnight patterns for glucose and total ketones bodies it would appear that the rise in blood total ketones bodies during the night was an earlier indication of

too little insulin than the change in blood glucose. The mean total ketone body concentration was considerably elevated (about five times) when compared with normal young subjects in Alberti's group but they did not include any pubertal children. It is possible that the energy requirements of growth and puberty may influence concentrations of metabolites involved in energy production.

The ratio of 3-hydroxybutyrate to acetoacetate changes little over the day. The patient's fasting mean was slightly elevated compared to the normal mean (Foster, Alberti, Hinks, Lloyd, Postle, Smythe, Turnell and Walton, 1978) but all fasting values were within the normal range.

The mean fasting glycerol was elevated but fell rapidly after insulin and breakfast. Apart from this, the profile was similar to normal subjects (Nattrass, 1982). The average of the 24hr mean values was higher than young subjects but similar to old subjects (Alberti et al, 1975a).

Endogenous insulin as measured by C-peptide did not change with meals or snacks. Whether it is metabolically important is discussed in the next two chapters.

Growth hormone is normally secreted in a low basal amount supplemented by short lived peaks. To record these peaks requires sampling every 15 to 20 minuets. The purpose of this study was not primarily to investigate the effect of growth hormone secretion on metabolic profiles in diabetes when sampling of that frequency would be required, but to exclude major changes in growth hormone which might affect

metabolic profiles. The nature of growth hormone secretion makes analysis difficult and graphical representation somewhat artificial. By its nature, Fig 3.11 over represents the peaks especially during the day. However, the peaks in growth hormone secretion commonly seen in the night, especially in the early part, were preserved.

In summary, this chapter shows the degree of the metabolic abnormality in a group of adolescent diabetic children. The abnormality is most marked in glucose, lactate, pyruvate and total ketone concentrations. Good control of blood glucose was never achieved and although blood glucose did not begin to rise until the early hours of the morning, the ketone concentration began to increase around midnight. The large bolus of insulin in the morning, necessary to regain control, succeeded in inhibiting ketone production quickly but lactate and pyruvate concentrations rose above normal.

Essentially, these results are similar to previous studies. In some respects the results are worse but this may be due to the age of the patients or the lack of specific attempts to improve control before study. By the same token, these results may more accurately reflect the metabolic control achieved in many diabetic adolescents.

Chapter 4 <u>MEASUREMENTS OF C-PEPTIDE</u>

4.1. INTRODUCTION

4.2. METHODS

4.3. RESULTS

4.4. DISCUSSION

4.1. INTRODUCTION

Surveys of endogenous insulin production in children have suggested that it is fairly common and beneficial (Ludvigsson, Heding, Larsson, Leander, 1977). Few detailed studies have been done on adolescents (Griffin, Spanos, Jenkins, Turner, Werther and Baum, 1980). Adolescents have a reputation for poor control (Mann and Johnston, 1982) but one might expect a number to have endogenous insulin, and therefore better control, because relatively more will have suffered from their disease for a short period of time.

The status of endogenous insulin secretion has been assessed by measurement of C-peptide for some years. These measurements have been made in a variety of ways and in this chapter they are reviewed. Few studies have made multiple C-peptide measurements over 24hrs. Values derived from these data and some of the earlier methods have been used in an attempt to correlate degrees of endogenous insulin secretion with methods of assessing glucose control.

4.2. METHODS

All the patients were entered into this study. Only the first metabolic rhythm and glucagon test was considered from those that were studied twice. Details of the patients and the procedure for conducting the studies were given in Chapter 2.

The Hba1, C-peptide and glucose data (including mean 24hr blood glucose) were used.

(see Sections 2.5 and 2.6 for methods). To estimate residual endogenous insulin five measures of C-peptide were analysed:

1) 24hr total C-peptide - the sum of all the measurements made over the 24hrs including a correction for the few hours when results were not available (see Section 2.6).

2) 24hr peak C-peptide - the highest value recorded during the 24hrs.

3) Glucagon test t=0 - the C-peptide value at the start of the glucagon test.

4) Glucagon test peak - the highest C-peptide value recorded after the glucagon.

5) Glucagon test rise - the difference between 3) and 4).If no C-peptide was detected at t=0 the detection limit of the assay (0.025pmol/ml) was used to calculate the rise.

The data were analysed in three ways.

Firstly, correlations were sought between the measures of glycaemic control - mean 24hr blood glucose and Hba1 - and the measures of residual endogenous insulin (Tables 4.1 and 4.2). These data were not normally distributed so Spearman's correlation coefficient was used for the analysis.

Secondly, a correlation was sought between simultaneous C-peptide and blood glucose measurements during the 24hr

study. Only those patients with C-peptide measured on more than half the sampling occasions were entered into this part of the study, and only the pairs of measurements where the C-peptide concentration was above the detection limit were used (Table 4.3).

Finally, because one of the commonest methods of detecting significant endogenous insulin status is to measure C-peptide in a single early morning fasting sample using the assay detection limit as the cut off point, the early morning data was evaluated to see if this analysis would give consistent results. In the metabolic rhythms described here four measurements of C-peptide were made fasting and early in the morning: 0600hrs, 0700hrs, 0800hrs and immediately before the injection of glucagon (between 0710hrs and 0740hrs at the end of the study). These values were compared using the detection limit of the assay as the cut off point to determine whether patients were consistently classified as C-peptide 'positive' or 'negative'. This part of the study tested whether the time of blood sampling had a significant effect on the classification (Table 4.4).

4.3. RESULTS

There was no correlation between any of the measures of C-peptide and of diabetic control (Table 4.2).

Ten patients showed a measurable rise after glucagon (0.01pmol/ml or more) but only six were amongst the ten that secreted the most C-peptide over the day. The average of the

Table 4.1
Details of measures of glucose control and C-peptide in all patients.
ND - Not Detected.

Patient Number	24hr Mean Glucose mmol/l	Hba1 %	C-PEPTIDE (pmol/ml)		Glucagon test		
			24hr total	24hr peak	t=0	peak	rise
1	10.9	12.7	0.909	0.059	0.086	0.137	0.051
2	7.9	10.2	ND	ND	ND	ND	ND
3	7.9	13.2	1.464	0.102	ND	ND	ND
4	16.4	14.1	1.059	0.064	ND	ND	ND
5	20.0	13.4	0.993	0.068	0.031	0.061	0.030
6	9.7	12.7	1.020	0.084	0.035	0.048	0.013
7	26.8	15.0	0.565	0.048	0.026	ND	-0.001
8	19.5	11.3	0.362	0.063	ND	0.040	0.015
9	15.5	15.9	0.187	0.042	0.034	0.041	0.007
10	8.1	12.1	1.264	0.069	ND	ND	ND
11	18.8	17.8	0.377	0.050	0.035	0.036	0.001
12	7.3	9.0	0.676	0.049	ND	0.030	0.005
13	15.2	16.4	0.750	0.061	0.037	ND	-0.012
14	12.0	10.9	0.969	0.088	0.053	0.052	-0.001
15	12.7	12.2	1.238	0.085	0.027	0.037	0.010
16	13.1	10.7	0.182	0.084	ND	0.073	0.048
17	12.7	11.8	0.134	0.034	ND	ND	ND
18	13.1	13.9	1.239	0.171	0.050	0.132	0.082
19	13.3	13.8	0.039	0.039	ND	ND	ND
20	17.0	17.3	ND	ND	ND	ND	ND
21	14.0	14.9	ND	ND	0.026	0.031	0.006
22	13.9	9.9	ND	ND	ND	0.032	0.007
23	17.1	18.2	ND	ND	0.045	0.050	0.005
24	8.1	7.9	ND	ND	0.071	0.074	0.003
25	13.5	12.9	0.846	0.062	0.038	0.056	0.018
26	16.6	17.4	0.118	0.059	0.039	0.057	0.018
27	9.3	13.1	0.708	0.064	ND	0.040	0.015
28	8.9	14.8	0.589	0.053	0.025	0.031	0.006

Table 4.2

Correlation coefficient between measures of glucose
control and endogenous insulin.

	Mean 24hr Glucose	Hba1
Total C-peptide	-0.24	0.05
Peak C-peptide	-0.18	0.05
Glucagon Test		
t=0	0.14	0.25
Peak	0.06	-0.12
Rise	0.01	-0.20

Table 4.3

Correlation coefficient between
Glucose and simultanious C-peptide.

(Only patients with C-peptide measured on
more than 50% of sampling occasions.)

Patient Number	Correlation Coefficient (r value)	Significance (p value)
1	0.64	<0.001
3	0.17	NS
4	-0.39	NS
5	-0.10	NS
6	-0.37	NS
10	-0.55	<0.01
12	-0.03	NS
13	0.26	NS
14	0.18	NS
15	-0.44	<0.02
18	0.65	<0.01
25	-0.04	NS
27	-0.36	NS
28	-0.21	NS

mean 24hr blood glucose for the patients that showed a measurable rise after glucagon (13.8mmol/l) was similar to the patients that did not (13.3mmol/l). Hba1 was also very similar (13.0% and 13.5%).

Only two patients (numbers 1 and 18) showed a significant positive correlation between blood glucose and C-peptide measurement (r=0.64 and 0.65, p<0.01; Table 4.3). These two patients had the highest measurements after glucagon stimulation and ranked third and ninth for 24hr secretion but their control was not that good ranking ninth and fourteenth for 24hr mean blood glucose and eleventh and eighteenth for Hba1. Two patients (numbers 10 and 15) had a significant negative correlation between C-peptide and blood glucose.

The four early morning fasting samples gave inconsistent results for individual patients (Table 4.4). Only 12 of the 28 patients had consistently positive (7 patients) or consistently negative (5 patients) results. However, the number of patients who had C-peptide levels above the detection limit at each time point was similar; 13 patients at 0600hrs, 16 patients at 0700hrs, 15 patients at 0800hrs and 16 patients before the glucagon was given.

4.4. DISCUSSION

The first studies using C-peptide as a measure of endogenous insulin production (Block, Mako, Steiner, and Rubenstein, 1972) used C-peptide reactivity in which the proinsulin/insulin antibodies were not removed prior to

Table 4.4
To show variation in 'fasting' sample results.

"+" or "-" Above or below assay detection limit
"Y" - Yes, consistant result at the four sampling times.
"N" - No, inconsistant result at the four sampling times.

Patient Number	0600hr	Fasting 0700hr	Samples 0800hr	t=0	Consistent Result
1	+	+	+	+	Y
2	-	-	-	-	Y
3	+	+	+	-	N
4	+	+	+	-	N
5	-	+	+	+	N
6	+	+	+	+	Y
7	+	+	+	+	Y
8	+	-	-	-	N
9	-	-	-	+	N
10	+	+	+	-	N
11	-	-	+	+	N
12	-	+	-	-	N
13	+	+	+	+	Y
14	+	+	+	+	Y
15	+	+	-	+	N
16	-	-	+	-	N
17	-	-	-	-	Y
18	+	+	+	+	Y
19	-	-	-	-	Y
20	-	-	-	-	Y
21	-	-	-	+	N
22	-	-	-	-	Y
23	-	-	-	+	N
24	-	-	-	+	N
25	-	+	+	+	N
26	-	+	-	+	N
27	+	+	+	-	N
28	+	+	+	+	Y

Number
Positive   13   16   15   16
at each hr

Number consistently positive or negative (i.e. "Y")   12

C-peptide analysis. C-peptide forms part of the proinsulin molecule so antibody to it can crossreact and give a falsely high result. The affinity is only about 10% that of the C-peptide in modern assays but the amount of proinsulin may be significantly increased by the formation of proinsulin/insulin antibody complexes. This is the reason for the pre-treatment PEG step (see Section 2.5) and is why these results are not directly comparable to those earlier studies.

Comparison of different C-peptide assays showed that different assays give different results (Faber, Binder, Markussen, Heding, Naithani, Kuzuya, Blix, Horwitz, and Rubenstein, 1978). The difficulty in comparison may be compounded by using of the detection limit of the assay, to determine the C-peptide status of the patient.

It seems likely that the degree to which type 1 diabetic patients can secrete endogenous insulin is a continuum and presumably extends below the detection limit of the assays.

C-peptide has been measured in a variety of circumstances. The most simple test is to use a single sample either random (Hackett, Court, McCowen and Parkin, 1986) or postprandial (Hinde and Johnston, 1986; Knip, Sakkinen, Huttunen, Kaar, Lankela, Mustonen and Akerblom, 1982). In some of the patients studied here there were wide fluctuations and a single estimation may have as much relevance as a single blood glucose.

Fasting C-peptide has been used as a measure of endogenous insulin production (Ludvigsson, Heding, Larsson and Leander, 1977; Aurbach-Klipper, Sharph-dor, Heding, Karp and Laron, 1983). The problem is to decide what time "fasting" refers to. To define it as the time in the morning that the study starts or the time that the patient or the investigator gets to work seems somewhat arbitrary. As is discussed elsewhere in this work, the metabolic rhythm is continuous and, in the diabetic at least, the early morning is a time of great change rather than a physiological and biochemical starting point.

Only 12 of the 28 were consistently in the C-peptide positive or negative groups when the four "fasting" samples between 0600hrs and 0800hrs were considered, but a fairly constant number (13 to 16) were found to be positive at each time point. If one was looking for a "C-peptide positive" group one would find approximately the same proportion what ever time one chose but the actual patients selected would be different. Indeed other studies which have surveyed a similar population have found a similar percentage of patients with C-peptide secretion (Ludvigsson et al, 1977; Madsbad, Faber, Binder, McNair, Christiansen and Transbol, 1978).

Response to a standard dose of glucose is an attractive idea but the beta cell of the diabetic may not respond to glucose while it may respond to other stimuli (Heinze, Beisher, Keller, Winkler, Teller and Pfeiffer, 1978). A standard meal is even more attractive because it is more

"physiological" but the international definition is difficult, especially in children of considerably different sizes and tastes. A standard meal would also be easier to interpret if the patients were in reasonable control at the start of the meal. As stated earlier these patients were often very uncontrolled first thing in the morning. Clarson et al (Clarson, Daneman, Drash, Becker and Ehrlich) used a standard mixed liquid meal in a dose per kilogram body weight and showed that the test gave a similar result on repeat testing demonstrating its reproducibility. However, blood glucose was high at the start and rose further. The study included many patients in the partial remission period of diabetes who clearly had more endogenous insulin than those studied here.

The glucagon stimulation test also was originally standardised on patients who had more C-peptide than these patients. It was found to correlate with the C-peptide produced in response to another standard meal (Faber and Binder, 1977). Because our patients have much less C-peptide it may have no meaning in them.

In this study the patients' glucagon test was done at a time when exogenous insulin would be at its lowest - just before the morning insulin - so most of the endogenous reserves of insulin may have been used up. The most likely explanation, however, for the poor response to glucagon is that the patients simply do not have the capacity to produce more endogenous insulin. Whatever the reason even those who

did respond to the test did not have better control than those who did not respond.

The glucagon test has gained wide acceptance but Mirel et al (Mirel, Ginsberg-Feller, Horwitz and Rayfield, 1980) showed that individual patients may respond to one stimulus (glucose, glucagon or tolbutamide) but not another. Provocation test are therefore beset with problems. Their standardisation on patients with more endogenous insulin then these patients may make their application here particularly inappropriate.

A few studies have made multiple C-peptide measurements in a similar way to this study. One of these (Werther, Turner, Jenkins and Baum, 1982) has shown that the level of C-peptide may correlate with the concurrent blood glucose but other intermediary metabolites were not measured. The significance of endogenous insulin to glucose and other metabolites is discussed further in Chapter 5. Finally, C-peptide has been measured in urine where about 5% of the pancreatic secretion is excreted unchanged (Horwitz, Rubenstein and Katz, 1977). This has the obvious advantages of painless sampling but suffers the usual problem of the collection of complete urine samples especially in children.

This study does not support some of the accepted methods of assessing endogenous insulin secretion. A single measurement of fasting C-peptide may mislead. The glucagon stimulation test was not originally used in patients with so little endogenous insulin and even in those patients who

showed a response, albeit small, it did not seem to make any difference to blood glucose control.

Very few studies have measured C-peptide over 24hrs. Adult normal values have only recently been published (Kruszynska, Home, Hanning and Alberti, 1987). The sum of the measurements made over 24hrs is the most commonly used estimation of endogenous insulin activity in this thesis. It has been chosen as the most appropriate measure for metabolic profile work as it measures what is actually secreted rather than projecting what might be secreted at the time the metabolite is sampled.

However, there are two major problems with this approach. Firstly, endogenous insulin may be suppressed by low blood glucose as in the non-diabetic so that a well controlled patient may need to call on their endogenous insulin less often than one that is less well controlled. This argument assumes that the beta cell responds to glucose uniformly in every patient but this is known not to be the case (Mirel, Ginsberg-Feller, Horwitz and Rayfield, 1980). Indeed the beta cell does not seem to be very responsive to glucose concentration and over most of the day these patients' blood glucose was so high that the beta cell may have been maximally stimulated.

Secondly, it has been suggested that exogenous insulin may have an inhibitory effect on endogenous insulin secretion (Faber, Wahren, Ferrannini, Binder and Defronzo, 1978). Figure 3.12 suggests that this effect, if present, is small and short lived with only a small drop after the

morning insulin and no effect in the evening. Clarson et al (Clarson, Daneman, Drash, Becker and Ehrlich, 1987) could not show any effect of exogenous insulin on the C-peptide response to a standard meal.

It is widely accepted that during the partial remission phase of diabetes endogenous insulin is responsible for smoothing out the imperfect control of exogenous insulin. This study failed to show a similar effect in those patients secreting C-peptide but who were past their partial remission phase. This could be because the secretion is not in response to blood glucose (Heinze et al, 1978). In only two of the patients was there a significant correlation between C-peptide and blood glucose and in these the control was not particularly good suggesting that the response was "set" too high.

The lack of effect of endogenous insulin on control of diabetes in these children contrasts with other studies (Ludvigsson et al, 1977; Knip et al, 1982; Binder and Faber, 1978). This may be because a particularly poorly controlled group of patients was studied. Alternatively, it may be that the adolescent diabetic, as well as apparently requiring more exogenous insulin (Mann and Johnston, 1984), needs more endogenous insulin to show a measurable effect on control. Madsbad et al (Madsbad, Faber, Binder, McNair, Christiansen and Transbol, 1978) showed that the C-peptide secretion declined more slowly in those patients diagnosed later in life so the surveys that include older patients may have more patients with significant endogenous insulin.

This study shows that endogenous insulin secretion was·
not an important determinant in overall blood glucose
control in adolescent diabetic patients past their partial
remission phase. The following chapter looks at this problem
more closely by an analysis of the metabolic rhythms of
those patients with the most and the least endogenous
insulin.

Chapter 5 <u>METABOLIC EFFECTS OF ENDOGENOUS INSULIN</u>

5.1. INTRODUCTION

5.2. METHODS

5.3. RESULTS

5.4. DISCUSSION

5.1. INTRODUCTION

The previous chapter (Chapter 4.0) showed how this study failed to demonstrate a correlation between control as measured by mean 24hr blood glucose or glycosylated haemoglobin and various measures of endogenous insulin. In this chapter the metabolic profiles are studied to see if there are subtle differences in glucose profiles missed by these general measurements. In addition the other metabolites are also studied because the possible improved insulinization of the liver in a patient with endogenous insulin may affect other metabolites as well as glucose metabolism.

5.2. METHODS

In an effort to make full use of the available data a different approach to the previous chapter was taken. Again the first 24hr metabolic rhythms were examined but two groups were selected from the twenty eight patients.

The patients were ranked in order of total C-peptide measured over the 24hr profile. The ten patients in whom the most C-peptide was measured formed group one and the ten in whom the least was measured formed group two. C-peptide was above the detection limit of the assay on a mean of 18 of the possible 20 times in group one (range 15 to 20) but only on a mean of one occasion in group two (range 0 to 3). The patients in group one were numbers 1, 3, 4, 5, 6, 10, 14, 15, 18, 25, in Table 2.1 and group two were numbers 2, 16, 17, 19, 20, 21, 22, 23, 24 and 26. Three patients in each

group were male. Full details can be found in Tables 2.1 and 2.2. A summary is given in Table 5.1. Details of the C-peptide secretion of each patient are given in Table 5.2.

Student's t-test was used to compare age, duration of diabetes, insulin dose (units/kg), percentage of the dose given in the morning, percentage of the dose given as short acting insulin and glycosylated haemoglobin (Table 5.1). All patients were treated with either Velosulin and Insulatard (Nordisk) or Actrapid and Monotard (Novo) except one patient treated with Semitard and Monotard (Novo). One patient in each group was treated with one injection of insulin a day, all the rest were treated with two.

The details of the procedure and methods of the metabolic rhythm are given in Chapter 2. Student's t-test was used to compare the fasting data and analysis of variance to compare the profiles (see Section 2.6).

5.3. RESULTS

There were no statistical differences between the groups with respect to age or details of insulin dose or treatment (Table 5.1). The duration of diabetes was significantly longer in group two by a mean of 3.2 years.

There were no statistical differences between the groups in the fasting metabolite concentrations (Table 5.3).

Fasting blood glucose was poor in both groups and slightly higher in group one. Glucose rose to a peak mid-morning (Fig 5.1) and then fell more swiftly and further

Table 5.1
Summary patient details group one and group two.

	Age yrs	Duration Diabetes yrs	Insulin Dose u/kg	Total Dose Soluble %	Morning Soluble %	Evening Soluble %
Group One	13.7	5.3	1.09	66	41	49
	11.7-15.5	2.4-10.0	0.65-1.52	49-100	16-63	19-100
Group Two	14.3	8.5	1.13	6.9	41	29
	12.0-17.0	4.6-11.6	0.47-1.84	47-100	22-56	0-50
t-test p	NS	<0.001	NS	NS	NS	NS

Table 5.2
C-peptide details group one and group two.
ND - Not Detected

Patient No		C-PEPTIDE (pmol/ml) 24hr total	24hr mean
GROUP ONE			
3	¦	1.464	0.061
10	¦	1.264	0.053
18	¦	1.239	0.052
15	¦	1.238	0.052
4	¦	1.059	0.044
6	¦	1.020	0.043
5	¦	0.993	0.041
14	¦	0.969	0.040
1	¦	0.909	0.038
25	¦	0.846	0.035
GROUP TWO			
16	¦	0.182	0.008
17	¦	0.134	0.006
26	¦	0.118	0.005
19	¦	0.039	0.001
2	¦	ND	ND
20	¦	ND	ND
21	¦	ND	ND
22	¦	ND	ND
23	¦	ND	ND
24	¦	ND	ND

Table 5.3
Fasting metabolite concentrations group one and group two: mean and SEM of blood metabolites (mmol/l) and ratios. Group one: high C-peptide, Group two: low C-peptide. Statistical analysis by Student's t-test for paired samples (NS - not significant; TKB - Total Ketone Bodies; 3OHB/AcAc - 3-hydroxybutryate/acetoacetate ratio). Note ketone data logged for analysis.

	Glucose	Lactate	Pyruvate	Lactate Pyruvate Ratio	Alanine	3-hydroxy-butyrate	Aceto-Acetate	3-OHB AcAc Ratio	TBK	Glycerol
Group one										
Mean	17.5	0.84	0.08	10.5	0.25	0.50	0.20	2.5	0.72	0.15
SEM	1.4	0.05	0.01	0.4	0.02	0.84	0.96	0.1	0.84	0.01
Group two										
Mean	16.5	0.90	0.08	11.2	0.26	0.60	0.26	2.5	0.87	0.13
SEM	1.9	0.02	0.01	0.3	0.02	0.84	0.68	0.2	0.78	0.01
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 5.4
Average 24hr mean metabolite concentration group one and group two: Mean and SEM of blood metabolites (mmol/l) and ratios. Group one: high C-peptide, Group two: low C-peptide. Statistical analysis by two way analysis of variance (F - variance ratio; NS - not significant; TKB - Total Ketone Bodies; 3OHB/AcAc - 3-hydroxybutryate/acetoacetate ratio)

	Glucose	Lactate	Pyruvate	Lactate Pyruvate Ratio	Alanine	3-hydroxy-butyrate	Aceto-Acetate	3-OHB AcAc Ratio	TBK	Glycerol
Group one										
Mean	12.4	1.27	0.12	10.3	0.38	0.15	0.12	1.4	0.27	0.09
SEM	1.2	0.12	0.01	0.3	0.03	0.05	0.03	0.1	0.07	0.01
Group two										
Mean	13.4	1.18	0.11	10.6	0.37	0.10	0.12	1.6	0.23	0.09
SEM	1.0	0.09	0.01	0.3	0.02	0.03	0.05	0.1	0.05	0.01
F	3.9	2.68	4.50	5.70	0.3	0.67	0.26	33.97	0.36	0.18
p	<0.05	NS	<0.05	<0.05	NS	NS	NS	<0.01	NS	NS

in group one patients. The greatest difference between the groups was during the afternoon. There was little difference during the evening and slightly higher glucose in group one from the middle of the night. Group one patients have a small but significantly lower blood glucose (p<0.05, Table 5.4) on testing by analysis of variance. Haemoglobin A1 was not significantly different on t-test (mean group one 12.8%, group two 13.2%).

The rapid fall in blood glucose in the morning in group one was associated with a peak in the pyruvate measurement (Fig 5.2). In group two there was a smaller peak and the difference at this time seemed to be largely responsible for the significant difference between the groups (p<0.05, Table 5.4). Lactate followed a similar time course to pyruvate (Fig 5.2) but the relative increase was not so great and was not significant (Table 5.4). These changes were reflected in the ratio between lactate and pyruvate concentrations (Table 5.4).

There was little difference in concentrations of alanine between the two groups over the 24hrs (Table 5.4). Like the other intermediary metabolites the general shape of the profile was similar to the profiles discussed in Chapter 3.

The total ketone body concentration was high in the fasting samples of both groups (Table 5.3, Figure 5.3). The fall in group one was steeper and the concentration lower for most of the day but after midnight the concentration was

Figure 5.1 Mean (SEM) 24 hour blood glucose concentrations in group one, 10 adolescent diabetic patients with some circulating C-peptide (closed circles) and group two, 10 adolescent diabetic patients without appreciable circulating C-peptide (open circles). Arrows indicate meal and snack times.
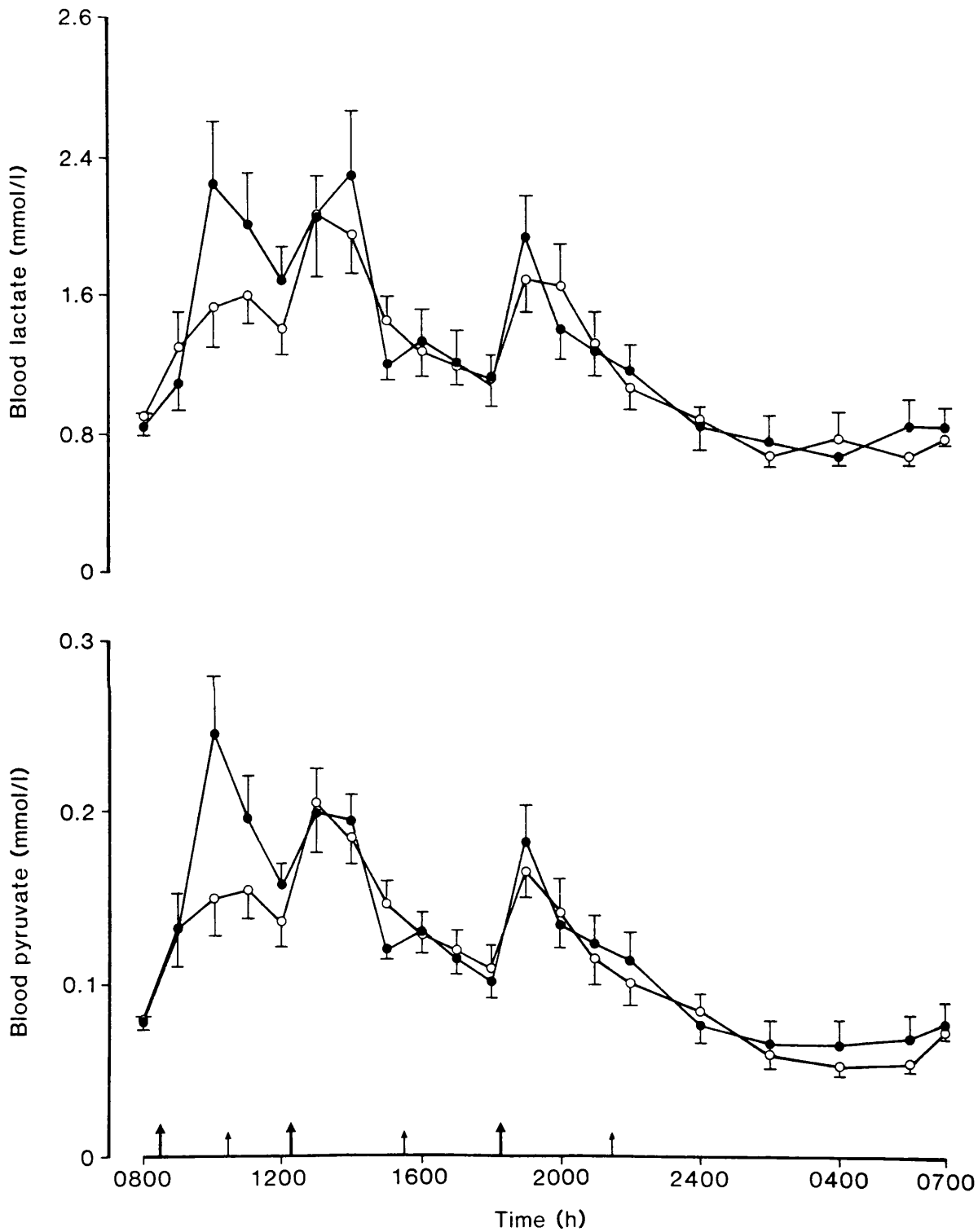
Figure 5.2 Mean (SEM) 24 hour blood lactate and blood
pyruvate concentrations in group one, 10 adolescent diabetic
patients with some circulating C-peptide (closed circles)
and group two, 10 adolescent diabetic patients without
appreciable circulating C-peptide (open circles). Arrows
indicate meal and snack times.
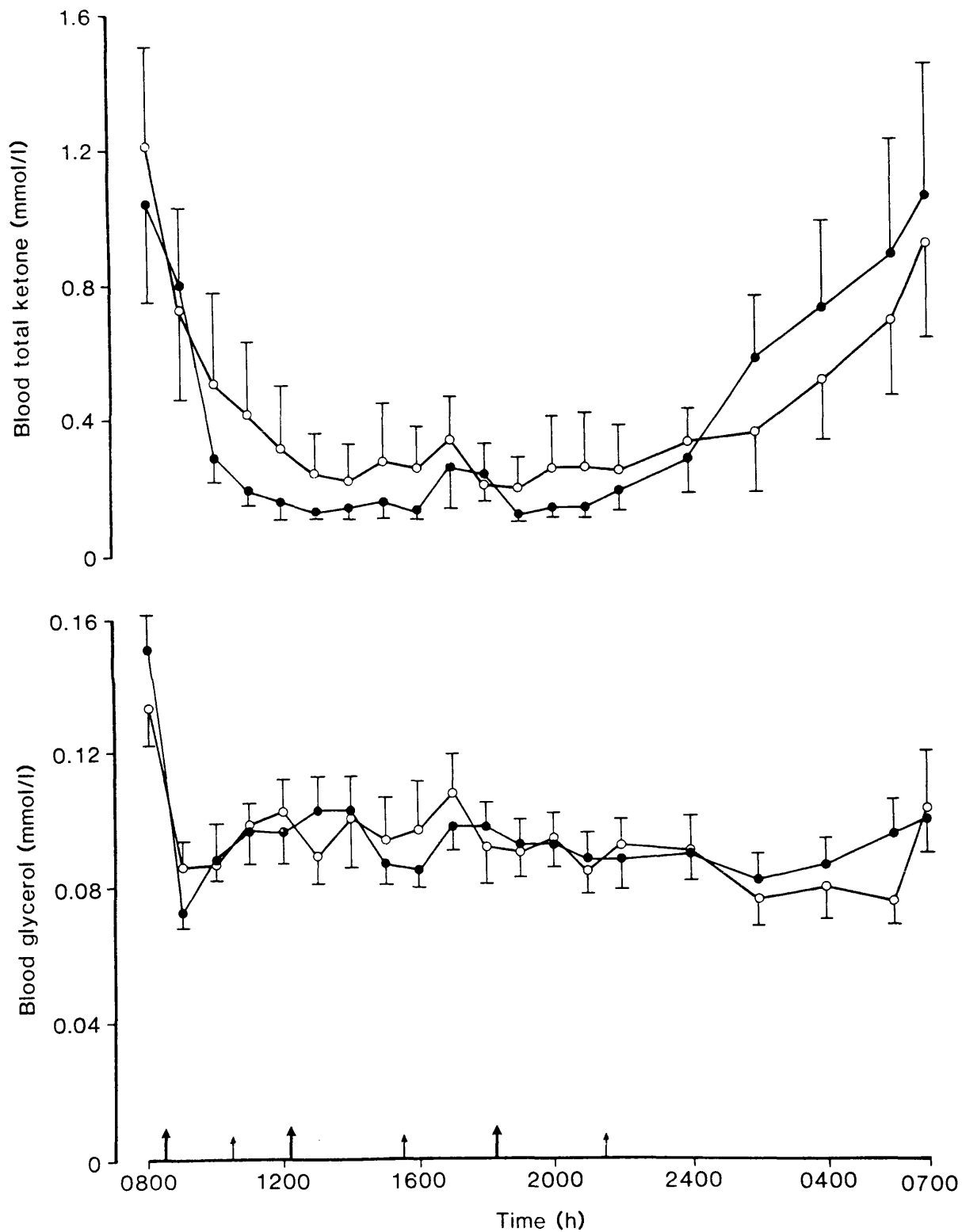
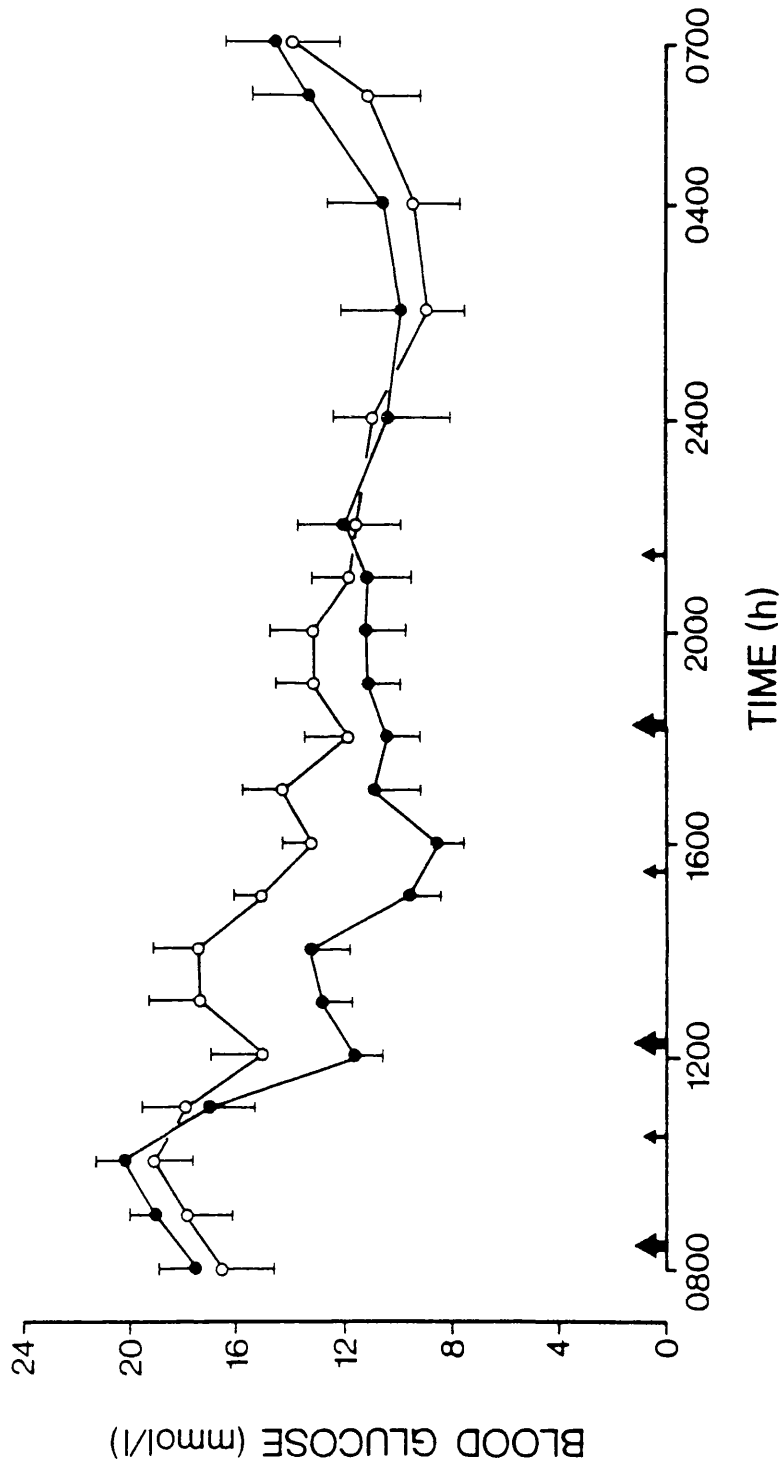Figure 5.3 Mean (SEM) 24 hour blood total ketone bodies and blood glycerol concentrations in group one, 10 adolescent diabetic patients with some circulating C-peptide (closed circles) and group two, 10 adolescent diabetic patients without appreciable circulating C-peptide (open circles). Arrows indicate meal and snack times.

higher in group one than group two. When considered over all by analysis of variance there was no difference between the two groups (Table 5.4).

There was a significant difference in the 3-hydroxybutyrate acetoacetate ratio. This appeared to be due to a higher 3-hydroxybutyrate in group one (Table 5.4). Glycerol concentrations were very similar throughout the day (Fig 5.3).

There were insufficient data points in group two to make a reasonable graph of C-peptide. Figure 5.4 shows the maximum and median C-peptide concentration in group one patients. (Since group one included the higher C-peptide producers selected from the original group of 28, the data in this graph was very similar to that used for Figure 3.12.)

5.4. DISCUSSION

The purpose of this study was to examine the effect of low levels of endogenous insulin secretion on metabolic profiles. The previous chapter discusses the different ways of studying C-peptide in diabetics with this degree of endogenous insulin. The 24hr total C-peptide was chosen as the most appropriate and has the advantage that the patients are divided on the grounds of a measure of the substance we are interested in recorded at the same time as the metabolites. Selecting the top ten and the bottom ten C-peptide secretors to study gave reasonably sized groups which had a significantly different C-peptide status.
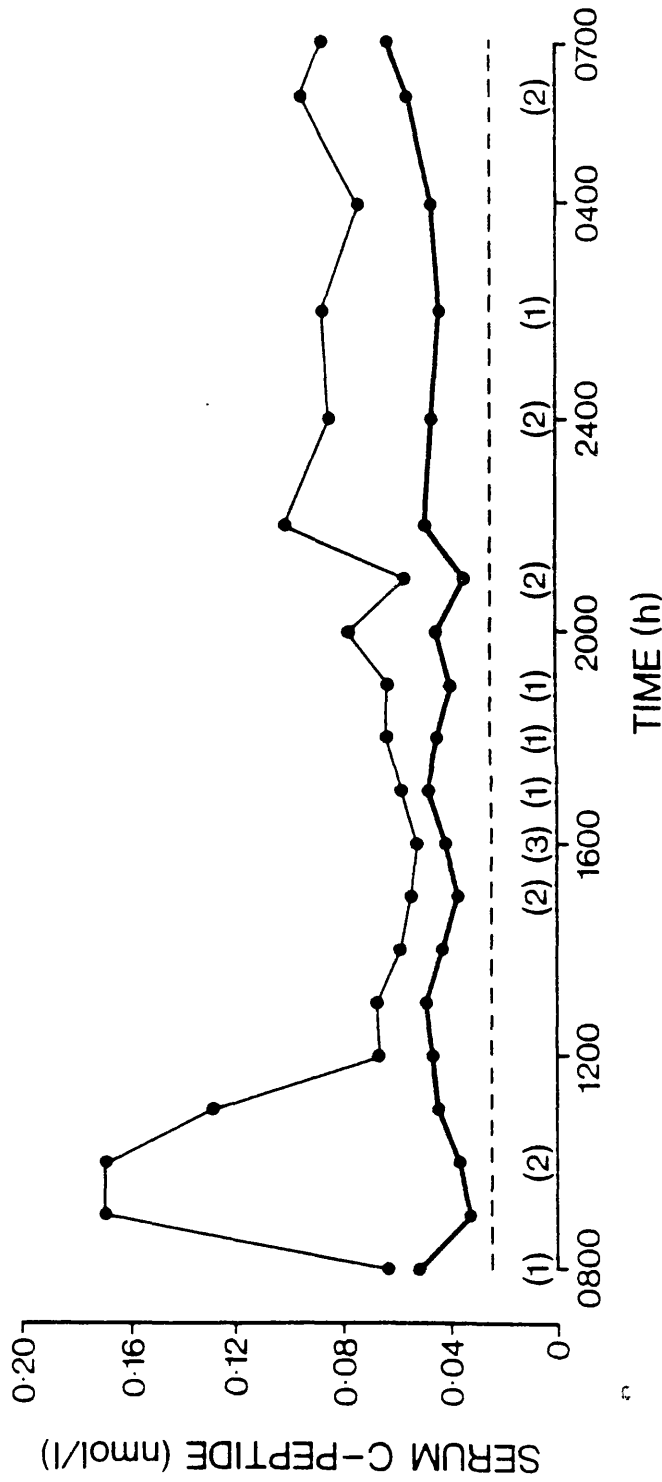
Figure 5.4 Maximum (top line) and median (bottom line) C-peptide concentration in Group one patients. Dotted line detection limit of assay. Numbers in parenthesis refers to number of patients with undetectable C-peptide at specific times.

However, the selection is to an extent arbitrary rather than scientific and really two groups of adolescent patients with very low levels of endogenous insulin were compared.

The groups did differ in the duration the patients had suffered from diabetes. This was to be expected from the work of Madsbad et al (Madsbad, Faber, Binder, McNair, Christiansen and Transbol, 1978). The study assumes that the response to insulin, endogenous or exogenous, is the same however long it has been since diagnosis. There is no evidence to suggest that this is not so. The groups do not differ significantly in age which in adolescence is important because of increased insulin requirement in this age group (Mann and Johnston, 1982). This increase may be an effect of pubertal status which was not recorded in these patients.

The fasting samples show that the groups started the day in equally poor control. Clearly any help that may be given by a small amount of endogenous insulin was overwhelmed by the metabolic abnormality at this time of day. It is well known that the morning is the time of most difficult diabetic control (Alberti, Dornhorst and Rowe, 1975b).

The small but significant effect on blood glucose was most obvious in the afternoon. The mechanism of action of the endogenous insulin is most likely to be on the liver, inhibiting glucose production. A small amount is unlikely to have an effect on peripheral uptake of glucose because it would be diluted in the peripheral circulation and the

concentration needed for this action of insulin is higher than that required for inhibition of hepatic glucose production. The small difference in blood glucose was not reflected in a significantly lower glycosylated haemoglobin.

The peak in the pyruvate concentration occurred as the glucose was still rising. As explained in Chapter 1 this sort of study gives information about blood concentrations and not directly about fluxes of metabolites. The rate of metabolism of glucose in group one may already have been increased at 1000hrs but not reflected in the blood glucose concentration until the subsequent hours. The higher pyruvate in group one at this time could have been due to a faster rate of glycolysis. Like the blood glucose profile the difference was small but significant. There was very little difference over the rest of the profile. As expected the blood lactate profile was similar to blood pyruvate but the difference between groups did not reach significance. The changes described were reflected in the lactate pyruvate ratio.

Lypolysis is very sensitive to low concentrations of insulin (Zierler and Rabinowitz, 1964). It is rather surprising then to record an effect on glucose but not on ketones. Overall the ketones were higher in group one (Table 5.4) but not significantly so. If the day until midnight is considered (Fig 5.3) the expected lower ketones could be demonstrated but after midnight the trend was reversed. It is very difficult to interpret the 3-hydroxybutyrate acetoacetate ratio data in the absence of a significant

difference in its component parts. The ketone data was skewed and although this was improved by log transformation it was not corrected entirely. A small difference in 3-hydroxybutyrate, acetoacetate or total ketone bodies may therefore be more difficult to show. This lack of effect on fat metabolism was confirmed by the lack of a significant difference in glycerol concentrations.

If a patient had a lot of endogenous insulin, it is reasonable to suppose that the liver could be adequately insulinized by the first passage of portal blood through the liver. The remaining insulin may then be sufficient to suppress peripheral lipolysis. As the endogenous insulin fails it is also reasonable to suppose that there could still be an effect on the liver but not on the fat cell. The liver will extract a proportion of the endogenous insulin and insufficient may remain to make a significant difference in the periphery especially when the small amount is diluted in the blood volume. Other studies in adults have investigated an effect of endogenous insulin on metabolites. Madsbad et al (Madsbad, Faber, Binder, Alberti and Lloyd, 1981) measured C-peptide and metabolites over part of the day and only showed an effect on blood glucose. The patients were divided on a glucagon test and almost all were secreting more insulin than these patients. A significant effect on blood glucose but not the other metabolites was shown.

In a similar study Asplin et al (Asplin, Hartog, Goldie, Alberti, Binder and Faber, 1978) divided patients

simply on the presence of detectable C-peptide at all sample times. Blood was drawn for hormone and metabolites over a 14hr period (no night samples). Correlations with glucose, lactate and total ketones were found but the patients selected for this study were either on very low or very high doses of insulin.

Gerbitz et al (Gerbitz, Kemmler, Edelmann, Summer, Mehnert and Wieland, 1979) also showed a beneficial effect of endogenous insulin with C-peptide concentration following fluctuations in glucose concentration but some of the patients were clearly in partial remission.

This study shows that endogenous insulin secretion may be metabolically active at least in glucose and pyruvate metabolism at lower concentrations than previously described. In comparison with some published accounts (Madsbad, McNair, Faber, Binder, Christiansen and Transbol, 1980) these patients would be considered to have insignificant amounts of endogenous insulin. The effect is small and from a clinical point of view probably irrelevant. It is certainly less important than the effects of insulin dose, diet and exercise. However, from a scientific point of view careful studies have to take the possible effects of even very small amounts of endogenous insulin into consideration.

Chapter 6 <u>METABOLIC EFFECTS OF IMPROVED CONTROL</u>

6.1. INTRODUCTION

6.2. METHODS

6.3. RESULTS

6.4. DISCUSSION

6.1. INTRODUCTION

Control of diabetes in childhood and adolescence is influenced by a variety of physiological and psychological factors (Baum and Kilmoth, 1985; Tattersall and Lowe, 1981). However, traditional methods for assessing and maintaining control of diabetes in children have been shown to be inaccurate (Malone, Hellrung, Malphus, Rosenbloom, Grgie and Weber, 1976; Drash, 1976; Griffin, Smith, Jenkins, Werther and Baum, 1976).

Recently, methods for continuous subcutaneous infusion of insulin by small pumps worn by the patient have been introduced. The machine delivers a basal supply of insulin and can be operated to give boosts before meals. It has been shown that this form of therapy improves control in insulin-dependent diabetic patients (Pickup, Keen, Parsons and Alberti, 1978; Tamborlane, Sherwin, Genel and Felig, 1979; Schiffrin, Colle and Belmonte, 1980; Champion, Shepherd, Rodger and Dupre, 1980) but experience in children is limited. Initial reports suggested that the pump is less acceptable to these age groups (Greene 1984; Knight, Boulton and Ward, 1984). The early reports of an increased risk of ketoacidosis and injection site infection have lessened with increased experience of the treatment.

In practice most adolescent patients are still treated with subcutaneous insulin regimes supported by home blood glucose monitoring (Sonksen, Judd and Lowy, 1978; Walford, Allison, Gale and Tattersall, 1978).

Home blood glucose monitoring is often added to the treatment regimen in an effort to improve diabetic control without a major effort being made to reform diet or exercise, both of which also have profound effects.

In assessing the effects of treatments on control emphasis has been placed on blood glucose concentration although abnormalities of other intermediary metabolites (Alberti and Nattrass, 1977) and hormones (Johansen and Hansen, 1971; Drejer, Hendriksen, Nielsen, Binder, Hagen and Kehlet, 1977) are known to occur during treatment of insulin-dependent diabetic patients.

The design of the experiment in this chapter attempts to study the impact of home blood glucose monitoring on control as assessed by blood glucose and other intermediate metabolites and hormones during metabolic rhythms in adolescent diabetic patients. This may simulate the situation in an adolescent clinic.

6.2. METHODS

The patients were selected from those that had been diagnosed for between 2 and 7 years. For details of patient recruitment and selection see Section 2.2. They were patients numbers 1 to 8 in the tables in chapter 2 summarised in Table 6.1. Their ages ranged from 10.8 to 15.5 years (mean 12.7 years) and duration of diabetes was from 3.8 to 6.3 years (mean 4.9 years). Six patients were treated with twice daily mixtures of short and intermediate acting insulin (Velosulin and Insulatard, Nordisk Limited, or

Table 6.1
Summary of patient details pork insulin group.

Patient Number	Age	Sex	Duration diabetes	Insulin One	Insulin Two	Change Insulin
	yrs		yrs	u/kg	u/kg	u/kg
1	12.6	M	4.8	1.26	1.47	0.21
2	12.0	F	5.3	1.71	1.67	-0.04
3	13.1	F	4.0	1.28	1.33	0.05
4	15.8	F	5.1	0.65	0.94	0.29
5	11.7	F	3.8	0.88	0.99	0.11
6	12.7	F	6.3	1.52	1.54	0.02
7	12.3	M	5.2	0.72	0.84	0.12
8	10.8	M	5.0	1.67	2.04	0.37
Mean	12.7		4.9	1.20	1.40	0.20
Min	10.8		3.8	0.65	0.84	-0.04
Max	15.5		6.3	1.71	2.04	0.37

Actrapid and Monotard, Novo Laboratories Limited,) and two were treated with once daily mixtures of Actrapid and Monotard.

No special effort was made before the study to improve control. The patients routine methods of monitoring their diabetes were with urine testing by Clinitest and Ketostix (Ames, Miles Laboratories Ltd, Stoke Court, Stoke Poges, Slough). Some of the patients had used reagent strips to monitor blood glucose for specific problems but none were using them regularly.

A 24hr metabolic profile was performed at the start of the study (see Section 2.3). Following this intensive home blood glucose monitoring was performed. Seven point blood glucose estimations (before and 2hrs after meals and before bed) were done using BM20-800 blood glucose strips (Boeringer Corporation, Lewes, East Sussex) on at least one day a week. Insulin was adjusted on the basis of the results aimed at producing pre-meal concentrations of 4mmol/l and post-meal blood glucose concentrations of less than 7mmol/l. Changes were made in the dose of insulin but not the type of insulin or frequency of injections. The results reported were not checked by laboratory methods. Within eight weeks all patients reported blood glucose profiles less than 10mmol/l. A second metabolic profile was then performed two months after the first study.

Samples were taken, treated and analysed as described in Sections 2.4 and 2.5.

Student's t-test for paired samples was used to compare glycosylated haemoglobin concentration, insulin dose, and fasting concentrations of metabolites. For the numerical comparison of the 24hr rhythm data the average of the 24hr mean values were calculated. The significance of the difference between the metabolic rhythm data was analysed using two way analysis of variance with data classified by study and time. Total ketone bodies (the sum of 3-hydroxybutyrate and acetoacetate) was log transformed before analysis. For further details of statistical methods see Section 2.6.

6.3. RESULTS

Total glycosylated haemoglobin concentration (mean ± SEM) decreased from 13.2 ± 0.4% to 10.8 ± 0.7% (p<0.05). Daily insulin dose increased in all patients. The mean ± SEM insulin dose for the first study was 1.2 ± 0.2 u/kg and for the second study 1.4 ± 0.2 u/kg (p<0.05) There were no significant changes in the proportions of insulin given in the morning (63% - first study, 64% - second study) nor in the proportion given as quick-acting insulin (32% versus 36%).

Fasting blood glucose was lower for the second study (Table 6.2; p<0.01) and the mean for the group was consistently lower during the day but at no time was it less than 10 mmol/l (Fig 6.1). The average 24hr mean blood glucose fell from 14.6 mmol/l in the first study to 12.6 mmol/l during the second study. Over the 24 hours there was

a significant improvement between studies (Table 6.3; F=7.02; p<0.01).

Fasting blood lactate was not significantly lower at the start of the second study (Table 6.2). Following breakfast and morning insulin a more marked increase in blood lactate occurred during the second study (Fig 6.2).After the evening meal blood lactate concentration was similar in the two studies. Overall blood lactate concentration was significantly higher during the second study (Table 6.3; F=7.42; p<0.01). Similar changes between the studies were seen for blood pyruvate concentration (Fig 6.2) although the differences in the fasting blood pyruvate and the 24hr profile were not statistically significant (Table 6.2; Table 6.3; F=3.29; p NS). Both fasting lactate/pyruvate ratio (Table 6.2; p<0.05) and the lactate/pyruvate ratio over the 24hr (Table 6.3; F=10.20; p<0.01) increased significantly in the second study.

Total ketone bodies concentration was raised at the start of both studies but fell following breakfast and insulin. Throughout the 24hrs the mean concentration was consistently lower during the second study (Fig 6.3; Table 6.3; F=17.70; p<0.001) and the difference was significant for total ketone bodies and its constituent parts. After 2100hr there was a gradual rise in mean blood total ketone bodies concentration although this was less marked in the second study. Fasting blood glycerol like fasting blood total ketone bodies did not differ significantly between the studies (Table 6.2). Throughout the 24hrs, the mean blood

Table 6.2
Fasting metabolite concentrations pork insulin group: mean and SEM of blood metabolites (mmol/l) and ratios before (study one) and after (study two) improvement in diabetic control.
p value - Student's t-test for paired samples; NS - not significant; TKB - total ketone bodies; 3OHB/AcAc - 3-hydroxybutyrate/acetoacetate. Note ketone data logged for analysis.

Pt No	Glucose	Lactate	Pyruvate	Lactate Pyruvate Ratio	Alanine	3-hydroxy -butyrate	Aceto- Acetate	3-OHB AcAc Ratio	TBK	Glycerol
Study One										
1	18.9	1.12	0.12	9.2	0.35	0.46	0.21	2.2	0.67	0.18
2	13.3	0.88	0.08	10.5	0.33	0.19	0.10	1.9	0.29	0.15
3	11.4	0.64	0.07	9.0	0.26	0.15	0.04	3.4	0.19	0.14
4	22.7	0.78	0.07	11.0	0.17	1.96	1.09	1.8	3.05	0.13
5	16.2	0.77	0.07	10.5	0.22	1.07	0.43	2.5	1.50	0.11
6	18.1	0.94	0.09	10.3	0.27	0.45	0.17	2.7	0.62	0.17
7	9.9	0.59	0.05	12.3	0.19	0.16	0.14	1.2	0.30	0.11
8	12.4	1.69	0.13	13.3	0.29	0.45	0.17	2.7	0.62	0.20
Mean	15.4	0.93	0.09	10.8	0.26	0.42	0.19	2.3	0.61	0.14
SEM	1.5	0.12	0.01	0.5	0.02	1.38	1.41	0.2	1.38	0.01
Study Two										
1	7.4	0.82	0.07	11.9	0.23	0.39	0.18	2.2	0.57	0.14
2	13.8	0.93	0.10	9.6	0.33	0.12	0.02	5.8	0.14	0.12
3	3.5	0.58	0.05	11.4	0.14	0.20	0.07	2.9	0.26	0.07
4	26.5	0.95	0.07	13.8	0.19	2.34	1.30	1.8	3.64	0.16
5	11.5	0.84	0.07	12.2	0.21	0.55	0.15	3.6	0.70	0.13
6	16.9	1.14	0.11	10.6	0.35	0.09	0.06	1.6	0.15	0.09
7	5.6	0.73	0.06	11.8	0.14	0.30	0.14	2.2	0.44	0.07
8	8.0	0.99	0.07	15.0	0.13	0.17	0.13	1.3	0.30	0.09
Mean	11.7	0.87	0.07	12.0	0.23	0.29	0.12	2.7	0.42	0.11
SEM	2.6	0.06	0.01	0.6	0.03	1.44	1.52	0.5	1.45	0.01
p	<0.07	NS	NS	<0.05	NS	NS	NS	NS	NS	NS

Table 6.3
Average 24hr mean metabolite concentrations pork insulin group: mean and SEM of blood metabolites
(mmol/l) and ratios before (study one) and after study two) improvement in diabetic control.
F - variance ratio (2-way analysis of variance); NS - not significant; TKB - total ketone bodies;
3OHB/AcAc - 3-hydroxybutyrate/acetoacetate. Note ketone data logged for analysis.

Pt No	Glucose	Lactate	Pyruvate	Lactate Pyruvate Ratio	Alanine	3-hydroxy -butyrate	Aceto- Acetate	3-OHB AcAc Ratio	TBK	Glycerol
Study One										
1	10.9	1.06	0.11	9.4	0.40	0.10	0.10	1.1	0.21	0.10
2	7.9	1.28	0.13	10.2	0.41	0.07	0.05	1.3	0.12	0.09
3	7.9	0.98	0.10	9.3	0.37	0.06	0.05	1.2	0.11	0.07
4	16.4	1.28	0.12	10.7	0.22	0.53	0.33	1.6	0.85	0.13
5	20.0	1.11	0.10	11.2	0.37	0.25	0.20	1.4	0.47	0.11
6	9.6	1.12	0.11	10.7	0.40	0.07	0.07	1.2	0.15	0.08
7	26.9	1.26	0.10	12.3	0.32	0.39	0.23	1.7	0.62	0.11
8	17.2	1.36	0.10	13.6	0.31	0.10	0.07	1.7	0.17	0.10
Mean	14.9	1.18	0.11	10.8	0.35	0.19	0.14	1.6	0.34	0.10
SEM	2.4	0.05	0.01	0.5	0.02	0.06	0.04	0.2	0.10	0.01
Study Two										
1	7.4	1.20	0.10	12.4	0.42	0.06	0.08	0.9	0.14	0.08
2	8.5	0.95	0.11	8.4	0.36	0.08	0.03	2.8	0.11	0.07
3	6.8	1.13	0.10	11.6	0.32	0.06	0.06	1.2	0.12	0.06
4	14.2	2.58	0.20	12.6	0.34	0.18	0.28	1.2	0.52	0.13
5	18.7	1.18	0.10	11.5	0.40	0.18	0.14	1.5	0.33	0.08
6	10.1	1.30	0.12	10.6	0.39	0.07	0.07	1.0	0.14	0.05
7	16.4	1.35	0.10	12.7	0.34	0.12	0.10	1.2	0.22	0.05
8	18.5	1.45	0.12	12.7	0.34	0.06	0.09	0.7	0.15	0.07
Mean	12.6	1.39	0.12	11.6	0.36	0.10	0.10	1.4	0.22	0.09
SEM	1.8	0.18	0.01	0.6	0.01	0.02	0.03	0.2	0.05	0.01
F	7.02	7.42	3.29	10.20	3.58	12.38	9.26	1.72	17.70	13.67
p	<0.01	<0.01	NS	<0.01	NS	<0.001	<0.01	NS	<0.001	<0.001

Figure 6.1 Mean (SEM) 24 hour blood glucose concentrations in eight adolescent diabetic patients before (circles) and after (squares) attempting to improve control with blood glucose monitoring. Arrows indicate meal and snack times.

Figure 6.2 Mean (SEM) 24 hour blood lactate and blood pyruvate concentrations in eight adolescent diabetic patients before (circles) and after (squares) attempting to improve control with blood glucose monitoring. Arrows indicate meal and snack times.

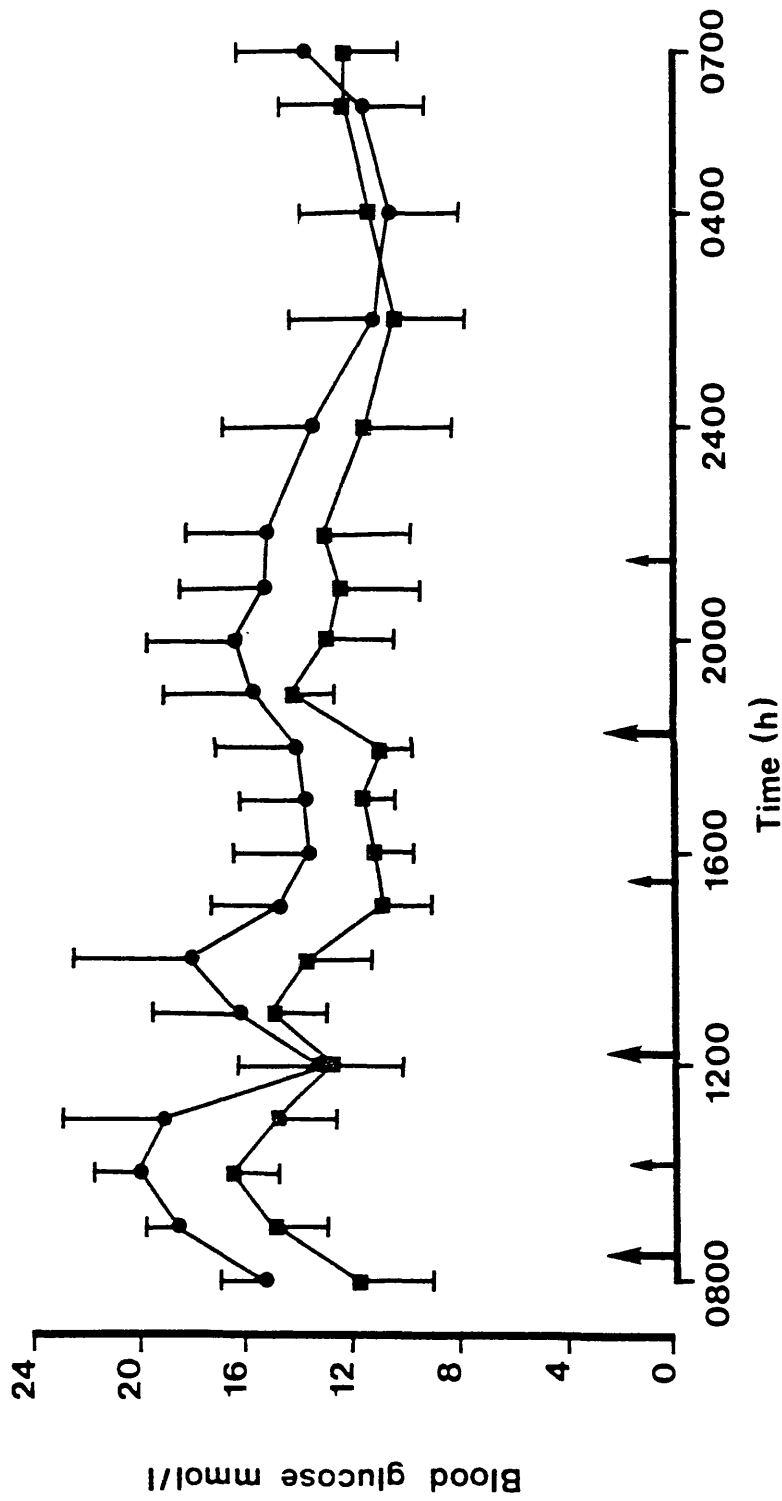Figure 6.3 Mean (SEM) 24 hour blood total ketone bodies and blood glycerol concentration in eight adolescent diabetic patients before (circles) and after (squares) attempting to improve control with blood glucose monitoring. Arrows indicate meal and snack times.
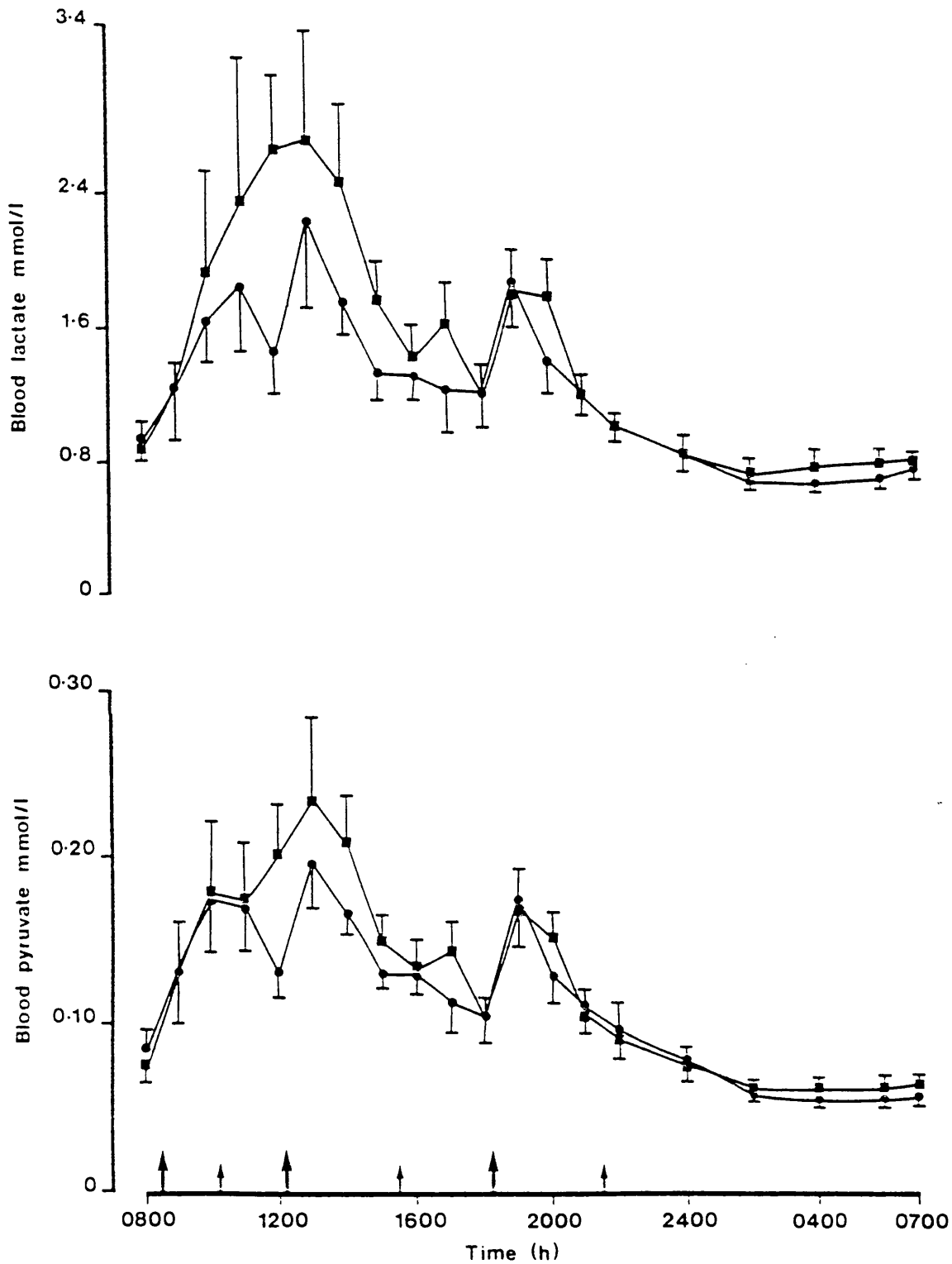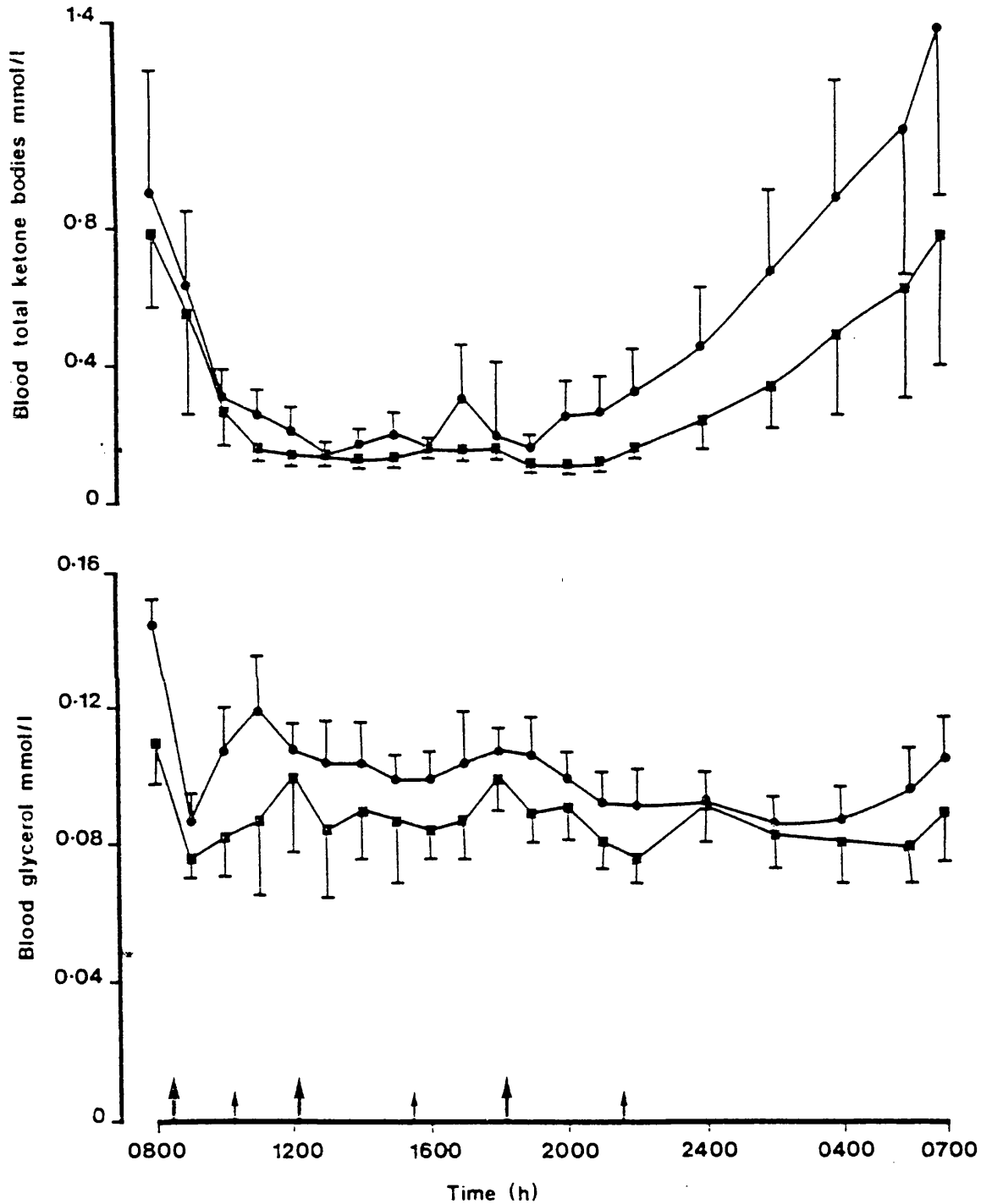
glycerol was lower during the second study and the difference was statistically significant (Fig 6.3; Table 6.3; F=13.67; p<0.001).

Neither fasting blood alanine nor the diurnal pattern differed significantly between studies (Table 6.2; Table 6.3; F=3.58; p NS).

One patient had no detectable C-peptide during either study and only one patient had a detectable level of C-peptide in every blood sample. In the remaining six patients C-peptide was detected in a similar number of samples during each study (maximum difference 4 samples). With this biological variation within and between patients missing values below the detection limit of the assay make statistical analysis problematical. Consideration of either the 24hr mean C-peptide (for values obtained) or the sum of all C-peptide values measured during a study by Student's paired t-test did not suggest a significant change. An increase in detectable C-peptide was observed in 3 patients and a decrease in 4 patients between studies. However, linear regression of the mean values (y=0.41x + 33; r=0.18) and total values (y=0.84x + 197; r=0.52) indicates slightly higher C-peptide levels in the second study.

No significant differences were found between the two studies for serum growth hormone. The average 24hr mean concentration of growth hormone was 8.4 mu/l during the first study (range 2.1-18.2 mu/l) and 9.7 mu/l (range 3.1-15.2 mu/l) during the second study. There was very little change in the insulin antibody status. The average

human, bovine and porcine insulin antibody was 25%, 19% and 23% respectively at the time of the first study and 28%, 20% and 26% at the time of the second.

6.4. DISCUSSION

Control of diabetes during adolescence is difficult (Mann and Johnston, 1982; Tattersall and Lowe, 1981). The method used here to improve control - home blood glucose monitoring - has been used to good effect in the limited situation of a diabetic camp (Sonksen, Judd and Lowy, 1980). In this study there was an improvement in diabetic control in the short term with glycosylated haemoglobin falling from 13.2 ± 0.4% to 10.8 ± 0.7%. A significant but more modest improvement in mean 24hr blood glucose concentration was observed but normality was not obtained with mean blood glucose never less than 10mmol/l.

The discrepancy between tests of home blood glucose monitoring and blood glucose concentrations observed is of interest. Differences between home records of blood and urine tests and glycosylated haemoglobin in this age group have been reported (Citrin, Ellis and Skyler, 1980) and attributed to deliberate manipulation. Indeed some degree of observer error may be implicated with patients' readings unintentionally tending to favour lower values. The performance of the study under hospital conditions may also have affected the results by contributing to a decrease in daily activity. In previous rhythm studies, however, a good correlation has been found between blood glucose

measurements at home and during the hospital study (Werther, Jenkins, Turner and Baum, 1980).

Decreased activity is unlikely to explain the finding of markedly elevated fasting blood glucose concentrations in both studies. The rise following breakfast was exaggerated and prolonged. Attention has previously been drawn to the importance of fasting blood glucose concentration in diabetes (Holman and Turner, 1977) and a reduction in fasting blood glucose to normal levels may well have led to much better control later in the day.

The results for the 24hr mean blood glucose during the first study are similar to those obtained in diabetic children by Griffin et al (Griffin, Spanos, Jenkins, Turner, Werther and Baum, 1980) who made no attempt to improve control prior to study. The same group, however, obtained better blood glucose control after improving control with a mean value of 8.2mmol/l compared with 11.7 mmol/l in this study. Four of their 15 patients had significant endogenous insulin secretion and these had significantly better control of blood glucose than the remaining eleven (Werther et al, 1980; Werther, Turner, Jenkins and Baum, 1982).

Mann and his colleagues (Mann, Noronha and Johnston, 1984) studied intensive education and home blood glucose monitoring in diabetic children over an 18 month period. Haemoglobin A1c levels were significantly improved but remained grossly abnormal (14.3%). A non-significant improvement in 24hr mean blood glucose concentration to 10.9 ± 0.4mmol/l (mean ± SEM) was observed but while these

results are better than the present report it should be noted that the 24hr mean was based on only pre-meal values. No significant differences were observed between children who received intensive education and those who received the same degree of education and did home blood glucose monitoring. In the present study it cannot be ruled out that the improvement in diabetic control was due to increased supervision of the adolescents rather than a direct effect of blood glucose monitoring.

In the second rhythm the improved blood glucose control led a significant increase in blood lactate concentration and a lesser and not significant increase in blood pyruvate concentration. The major differences between the two studies occurs between 0800hr and 1800hrs. Blood lactate and pyruvate may rise in response to decreased hepatic uptake for gluconeogenesis or increased peripheral production. Following insulin hepatic uptake does not change but gluconeogenic precursors are diverted into glycogen (Radziuk, 1981) and it seems likely therefore that the increase in lactate is due to enhanced peripheral production which may reflect peripheral administration of insulin by subcutaneous injections and therefore relative peripheral hyperinsulinism.

There is a significant fall in blood total ketone bodies during the second study although the diurnal pattern of total ketone bodies is not normal and remains similar to the first study. Even after improving control, comparing the overnight patterns for glucose and total ketones bodies, the

rise in blood total ketones bodies during the night is still an earlier and clearer indication of too little insulin than the change in blood glucose.

That the lower total ketone bodies concentration is due to a decrease in lipolysis during the second study is supported by the significant fall in blood glycerol. Levels of glycerol obtained are within the normal range for young adults (Alberti, Dornhorst and Rowe, 1975a) despite high blood glucose concentrations. Further improvement of blood glucose through increased insulin administration might result in suppression of glycerol levels to abnormal values due to peripheral insulin administration and therefore relative peripheral hyperinsulinism (Nosadini, Noy, Nattrass, Alberti, Johnston, Home and Orskov, 1982).

Alanine concentrations were not different between the two studies despite the changes in total ketones bodies. An inverse relationship between alanine and ketone bodies has been postulated (Sherwin, Hendler and Felig, 1975) although this has also been disputed (Fery and Balasse, 1980).

Madsbad et al (Madsbad, Krarup, Regeur, Faber and Binder, 1981) studied the effect of improved control, achieved by one weeks inpatient treatment with multiple insulin injections a day, on endogenous insulin production. An improvement in the C-peptide response to a test meal measured immediately before and immediately after the week was shown but two weeks later, after most had returned to their pre-study insulin, there was no difference from the initial test. No information was given about changes in the

endogenous insulin secretion over the day. The failure in this study to show any change may be because the improvement in control was too modest or that there was insufficient C-peptide reserve to improve, that is, the endogenous insulin was maximally stimulated on both study days.

Higher growth hormone concentrations are found in association with poor control (Vigneri, Squatrito, Pezzino, Filetti, Branca and Polosa, 1976). The changes in growth hormone observed in this study were small and not significant but the trend is in the expected direction. Again this may be due to the only small improvements in control.

In conclusion blood glucose control was improved by a short period of home blood glucose monitoring in adolescent diabetic patients but normalization was not achieved. The fall in blood glucose concentration was accompanied by lower total ketone bodies and glycerol concentration but blood lactate concentration rose. Improvement in control did not change endogenous insulin so it was unlikely that changes in endogenous insulin played any part in these differences.

The major stumbling block to normalization of blood glucose was difficulty in obtaining a normal fasting blood glucose. Measurement of blood total ketone bodies during the night might give a clearer indication of under-insulinization. Without attention to other factors that affect diabetic control, particularly diet and exercise, home blood glucose monitoring produces only a

modest improvement in control and does not restore metabolic

normality.

Chapter 7 <u>METABOLIC EFFECTS OF CHANGING INSULIN SPECIES</u>

7.1. INTRODUCTION

7.2. METHODS

7.3. RESULTS

7.4. DISCUSSION

7.1. INTRODUCTION

For many years insulin from cattle was the most widely available insulin. Structurally it differs from human insulin by alanine and valine substitution at positions eight and ten on the A chain and an alanine at position thirty on the end of the B chain. These differences make it more immunogenic than porcine insulin which has an identical A chain but the same alanine substitution on the B chain (Schlichkrull, Pingel, Heding, Brange and Jorgensen, 1975). Significant clinical problems with immunogenicity were unusual but beef insulin has largely dropped out of clinical use in paediatrics although some of these patients had used it in the past. Recently, insulins with an identical aminoacid sequence to human insulin have become available.

There are two methods of manufacturing human insulins. One method involves the chemical alteration of the porcine molecule (Markussen, 1984) the other to modify the plasmid DNA of bacteria in such a way that it makes insulin with the human aminoacid sequence (Chance, Kroeff, Hoffman and Frank, 1981).

A case has been made for the routine use of human insulins for newly diagnosed children with diabetes. Ludvigsson (1984) showed that in children treated with monocomponent porcine insulin from diagnosis those with antibodies to insulin had a poorer remission although the levels of antibodies were very low. Heding et al (Heding, Marshall, Persson, Dahlquist, Thalme, Lindgren, Akerblom, Rilva, Knip, Ludvigsson, Stenhammar, Stromberg, Sovik,

Baevre, Wefring, Vidnes, Kjaergard, Bro and Kaad, 1984) have shown that human monocomponent insulin has a lower immunogenicity than porcine insulin in newly diagnosed diabetic children.

The advantage for an established diabetic child is less clear and the use of human insulin has been advocated (Greene, Smith, Cartwright and Baum, 1983) and questioned (Mann, Johnston, Reeves and Murphy, 1983).

Keen et al (Keen, Glynne, Pickup, Viberti, Bilous, Jarrett and Marsden, 1980) showed no differences in the metabolic responses to intravenous porcine or human insulin but minor differences have been observed in onset, peak, and duration of action of intermediate and long-acting preparations (Clarke, Adeniyi-Jones, Knight, Leiper, Wiles, Jones, Keen, MacCuish, Ward, Watkins, Cauldwell, Glynne and Scotton, 1982).

It is clearly important to document the changes in glucose and other intermediary metabolites which may occur on changing patients from porcine to human insulin. In this chapter the metabolic profiles of a group of adolescent diabetic children are compared before and after changing to human insulin.

7.2. METHODS

Eight further patients (5 males) were studied. Their ages ranged from 11.1 to 15.5 years (mean 13.5 years). As before they were selected from those with a duration of diabetes 2 to 7 years (mean 4.2 years) and were past their

partial remission phase (see Section 2.2). Details of the patients are shown in Table 7.1.

Seven patients were treated with twice daily mixtures of short and intermediate acting insulin (Velosulin and Insulatard, Nordisk Limited, or Actrapid and Monotard, Novo Laboratories Limited) and one patient was treated with once daily mixtures of Semitard (Novo) and Monotard in the morning.

No special effort was made before the study to improve control. The patients routine methods of monitoring their diabetes were with urine testing by Clinitest and Ketostix (Ames). Some of the patients had used reagent strips to monitor blood glucose for specific problems but none were using them regularly.

A 24hr metabolic profile was performed at the start of the study as described in Section 2.3. Immediately after this the patients were changed to twice daily soluble and isophane human insulin (Humulin crb, Eli Lilly, Basingstoke, Hampshire) in similar proportions to their previous treatment.

Over the next two months home blood glucose monitoring was performed in addition to the usual urine analysis. Seven point blood glucose estimations (before and 2hrs after meals and before bed) were done using BM20-800 blood glucose strips (Boeringer) on at least one day a week. Insulin was adjusted, after telephone consultation or visit, on the

Table 7.1
Summary of patient details human insulin group.

Patient Number	Age	Sex	Duration diabetes	Insulin One	Insulin Two	Change Insulin
	yrs		yrs	u/kg	u/kg	u/kg
9	14.0	M	2.1	0.68	1.06	0.38
10	15.0	F	2.4	1.12	1.20	0.08
11	15.5	F	5.5	0.80	1.04	0.24
12	12.3	F	4.1	1.37	1.68	0.31
13	11.1	M	6.0	0.98	1.13	0.15
14	15.0	M	3.6	1.37	1.50	0.13
15	12.1	M	5.3	0.89	1.15	0.26
16	12.9	M	4.6	0.74	0.79	0.05
Mean	13.5		4.2	1.00	1.20	0.02
Min	15.5		2.1	0.68	0.79	0.05
Max	11.1		6.0	1.37	1.68	0.38

basis of the results aimed at producing pre-meal concentrations of 4mmol/l and post-meal blood glucose concentrations of less than 7mmol/l. The results reported were not checked by laboratory methods. Within eight weeks all patients reported blood glucose profiles less than 10mmol/l.

A second metabolic profile was then performed two months after the first study. Samples were taken, treated and analysed as described in Sections 2.4 and 2.5. As is evident these patients were treated in the same way as those in Chapter 6 except for the change in insulin type.

Student's t-test for paired samples was used to compare glycosylated haemoglobin concentration, insulin dose, and fasting concentrations of metabolites. For the numerical comparison of the 24hr rhythm data the average of the 24hr mean values were calculated. The significance of the difference between the metabolic rhythm data was analysed using two way analysis of variance with data classified by study and time. Total ketone bodies (the sum of 3-hydroxybutyrate and acetoacetate) was log transformed before analysis. For further details of the analysis see Section 2.6.

7.3. RESULTS

Insulin dose increased in all patients from a mean of 1.0 ± 0.1U/kg (mean $\pm$ SEM) during treatment with porcine insulin to 1.2 ± 0.1U/kg during treatment with human insulin ($p < 0.01$) However, there were no significant differences in

the proportion of insulin given in the morning (69% vs 63%) nor in the proportion given as quick acting insulin (39% vs 40%). Despite the increase in insulin dosage total glycosylated haemoglobin concentration did not change significantly (13.1 ± 1.1% vs 12.4% ± 0.7%).

Fasting blood glucose was higher during treatment with human insulin although this did not reach statistical significance (Table 7.2). Average 24hr mean blood glucose concentration was similar during both studies (Table 7.3; F=0.24; NS). Blood glucose concentration was poor during both studies (Fig 7.1) with higher mean concentrations during the day with porcine insulin and higher mean concentrations during the night with human insulin.

Fasting blood lactate was not different between studies (Table 7.2). Following breakfast and morning human insulin there was a more pronounced rise in blood lactate (Fig 7.2) and the mean concentration was also greater after the evening insulin and meal. The higher concentrations persisted throughout the night. Over the 24hr blood lactate was significantly higher during treatment with human insulin (Table 7.3; F=7.86; p<0.01).

Fasting blood pyruvate was significantly lower during human insulin treatment (Table 7.2; p<0.5). The diurnal pattern was similar to that observed for lactate (Fig 7.2) but differences between the two studies were small and not statistically significant (Table 7.3; F=3.14; NS). The lactate/pyruvate ratio was significantly higher during human

Table 7.2
Fasting metabolite concentrations human group: mean and SEM of blood metabolites (mmol/l) and ratios during treatment with porcine or human insulin.
p value - Student's t-test for paired samples; NS - not significant; TKB - total ketone bodies; 3OHB/AcAc - 3-hydroxybutyrate/acetoacetate. Note ketone data logged for analysis.

Pt No	Glucose	Lactate	Pyruvate	Lactate Pyruvate Ratio	Alanine	3-hydroxy -butyrate	Aceto- Acetate	3-OHB AcAc Ratio	TBK	Glycerol
Porcine Insulin										
9	15.9	0.73	0.08	9.2	0.21	0.63	0.25	2.6	0.87	0.10
10	10.4	0.62	0.07	8.6	0.25	0.08	0.03	2.3	0.13	0.11
11	10.9	0.98	0.10	10.3	0.18	0.97	0.23	4.2	1.20	0.17
12	11.6	1.07	0.10	10.7	0.35	0.22	0.11	2.0	0.33	0.22
13	20.2	0.82	0.06	13.2	0.18	1.28	0.57	2.3	1.84	0.17
14	25.7	0.89	0.07	12.0	0.20	1.32	0.59	2.3	1.91	0.19
15	17.3	0.81	0.07	12.5	0.18	0.87	0.29	3.0	1.16	0.15
16	11.1	0.89	0.08	11.9	0.26	0.16	0.14	1.2	0.30	0.10
Mean	15.4	0.85	0.08	11.1	0.23	0.47	0.20	2.5	0.70	0.15
SEM	2.0	0.05	0.01	0.6	0.02	1.46	1.41	0.3	1.41	0.02
Human Insulin										
9	10.1	0.81	0.07	12.1	0.23	0.40	0.23	1.7	0.64	0.11
10	16.4	0.65	0.05	12.0	0.18	0.25	0.18	1.4	0.42	0.12
11	25.6	0.85	0.07	12.0	0.74	1.74	0.74	2.4	2.48	0.14
12	25.4	1.10	0.10	11.0	0.40	0.79	0.40	2.0	1.19	0.10
13	20.2	0.96	0.06	15.0	0.67	1.78	0.67	2.7	2.46	0.16
14	22.8	0.84	0.07	12.0	0.32	0.62	0.32	2.0	0.93	0.13
15	15.2	0.85	0.06	13.2	0.20	0.69	0.24	2.8	0.94	0.17
16	11.1	0.65	0.06	11.8	0.20	0.17	0.20	0.9	0.36	0.08
Mean	18.4	0.84	0.07	12.4	0.20	0.60	0.33	2.0	0.94	0.12
SEM	2.0	0.05	0.01	0.4	0.01	1.36	1.21	0.2	1.29	0.01
p	NS	NS	<0.05	<0.05	NS	NS	NS	NS	NS	NS

Table 7.3

Average 24hr mean metabolite concentrations human insulin group: mean and SEM of blood metabolites (mmol/l) and ratios during treatment with porcine or human insulin.
F - variance ratio (2-way analysis of variance); NS - not significant; TKB - total ketone bodies; 3OHB/AcAc - 3-hydroxybutyrate/acetoacetate. Note ketone data logged for analysis.

| Pt No	Glucose	Lactate	Pyruvate	Lactate Pyruvate Ratio	Alanine	3-hydroxy -butyrate	Aceto- Acetate	3-OHB AcAc Ratio	TBK	Glycerol
Porcine Insulin										
9	15.5	0.87	0.09	9.3	0.29	0.16	0.13	1.3	0.29	0.09
10	7.9	0.86	0.09	10.3	0.34	0.05	0.05	1.1	0.10	0.08
11	18.8	0.88	0.09	9.9	0.23	0.59	0.28	2.1	0.87	0.10
12	7.3	1.27	0.12	10.8	0.47	0.04	0.06	0.8	0.10	0.11
13	15.4	1.53	0.13	12.3	0.41	0.13	0.12	1.2	0.26	0.11
14	12.0	1.88	0.16	12.3	0.41	0.14	0.12	1.3	0.26	0.10
15	12.7	1.49	0.13	11.3	0.37	0.09	0.08	1.2	0.17	0.07
16	13.1	0.84	0.08	11.4	0.33	0.06	0.06	0.9	0.12	0.05
Mean	12.8	1.21	0.11	10.8	0.36	0.16	0.11	1.4	0.27	0.09
SEM	1.4	0.14	0.01	0.4	0.03	0.06	0.03	0.2	0.09	0.01
Human Insulin										
9	9.0	1.12	0.09	12.7	0.34	0.05	0.07	0.8	0.12	0.07
10	10.6	0.97	0.09	10.5	0.31	0.04	0.09	0.9	0.09	0.05
11	13.3	1.70	0.14	12.0	0.26	0.14	0.12	1.3	0.27	0.09
12	9.7	2.26	0.20	11.6	0.42	0.07	0.04	2.1	0.12	0.09
13	16.1	2.03	0.15	13.2	0.44	0.19	0.12	1.1	0.25	0.07
14	9.7	1.43	0.13	12.0	0.36	0.08	0.10	0.9	0.19	0.07
15	14.1	1.02	0.09	12.4	0.33	0.06	0.08	0.9	0.14	0.06
16	17.8	0.94	0.07	12.5	0.33	0.08	0.14	0.6	0.18	0.05
Mean	12.6	1.43	0.12	11.8	0.35	0.08	0.09	1.2	0.17	0.08
SEM	1.1	0.18	0.02	0.3	0.02	0.01	0.01	0.1	0.02	0.01
F	0.24	7.86	3.14	36.20	0.85	11.00	12.17	3.82	13.03	10.28
p	NS	<0.01	NS	<0.001	NS	<0.01	<0.001	<0.05	<0.001	<0.01
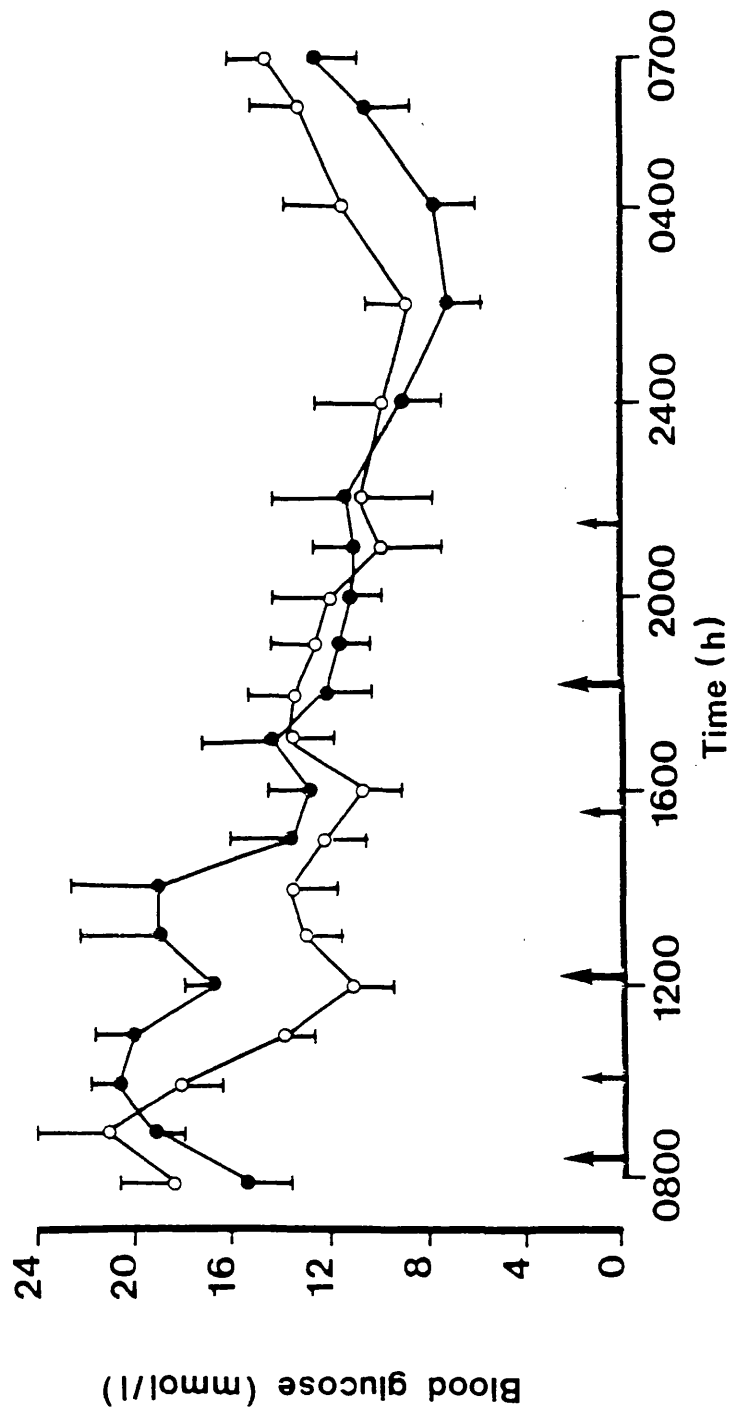
Figure 7.1   Mean (SEM) 24 hour blood glucose concentrations in eight adolescent diabetic patients during treatment with porcine (closed circles) or human (open circles) insulin. Arrows indicate meal and snack times.
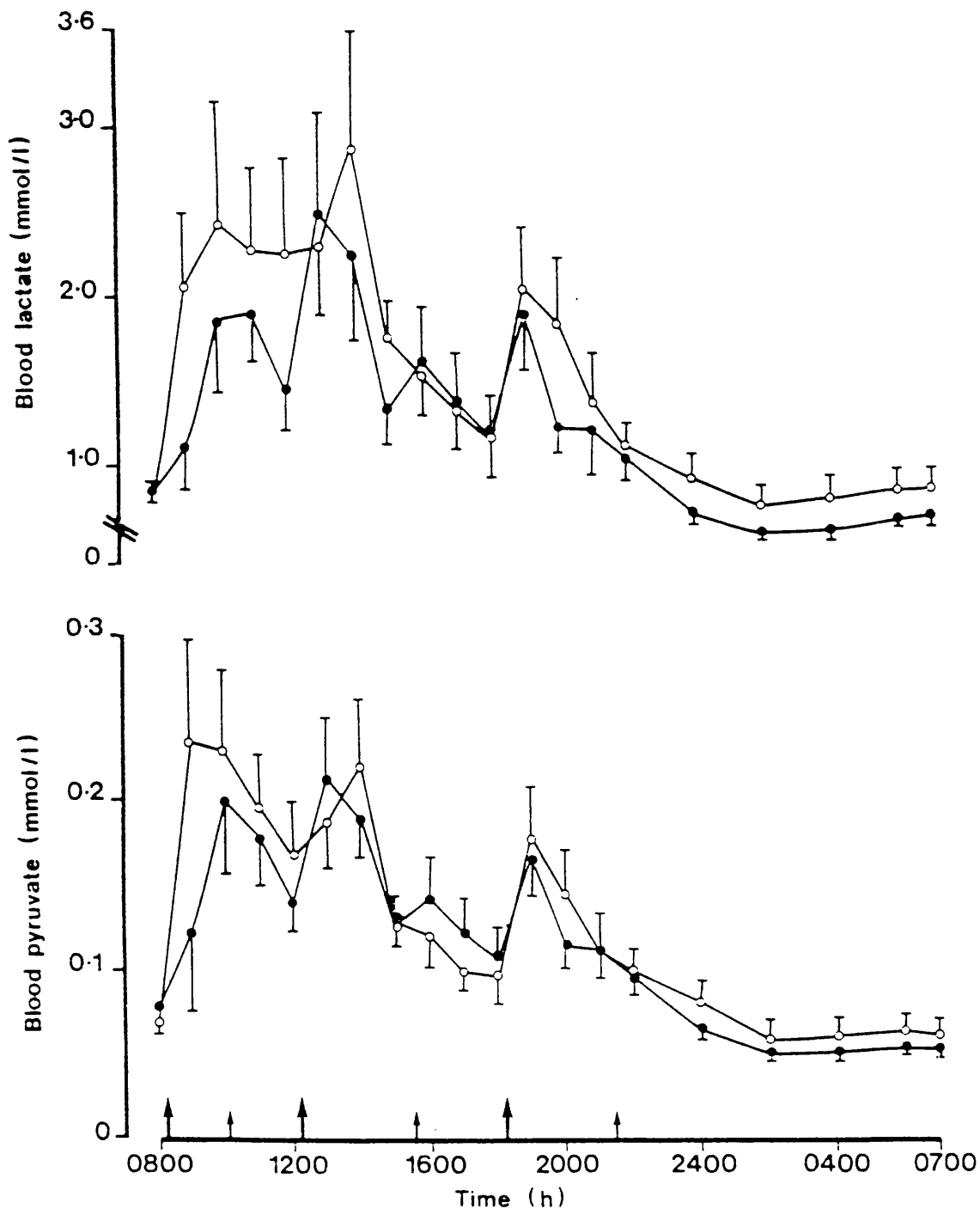
Figure 7.2 Mean (SEM) 24 hour blood lactate and blood
pyruvate concentrations in eight adolescent diabetic
patients during treatment with porcine (closed circles)
or human (open circles) insulin. Arrows indicate meal
and snack times.

treatment both fasting (Table 7.2; p<0.5) and over 24hrs (Table 7.3; F=36.2; p<0.001).

Total ketone bodies were not significantly different in fasting blood between studies (Table 7.2). The decrease following breakfast and morning insulin was most pronounced with human insulin (Fig 7.3) and the mean concentrations over 24hrs were significantly lower (Table 7.3; F=13.03; p<0.001). Blood glycerol concentration (Fig 7.3) was also significantly lower during human insulin treatment over the 24hrs (Table 7.3; F=10.28; p<0.01) but not fasting (Table 7.2). Blood alanine concentration did not differ significantly between studies (Table 7.2; Table7.3 F=0.85; NS).

Only one patient had measurable C-peptide in all blood samples. In the remaining patients C-peptide was detected in a similar number of samples in each study (maximum difference 5 samples). As stated in chapter 6, these within and between patient differences makes statistical analysis of the data difficult. Student's paired t-test on the mean C-peptide of the values measured or total C-peptide (the sum of values over 24hr) did not reveal significant differences between studies. The group tended to have higher C-peptide levels during the second study however as is shown by linear regression of C-peptide during human insulin versus porcine insulin (for mean C-peptide y=0.86x + 30.8, r=0.84; p<0.001; for total C-peptide y=0.80x + 63, r=0.83; p<0.001).

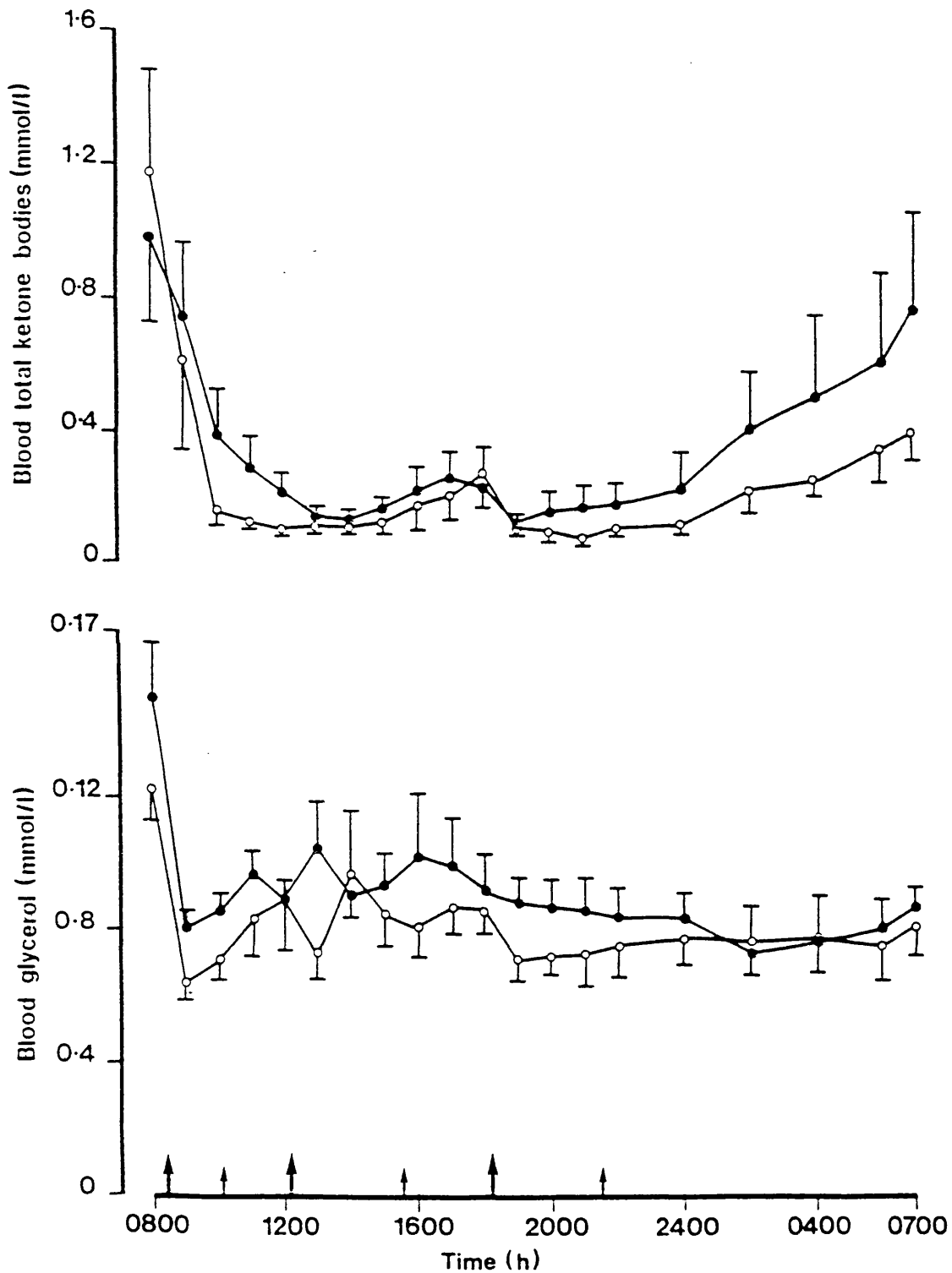Serum growth hormone was not significantly different

Figure 7.3   Mean (SEM) 24 hour blood total ketone bodies
and blood glycerol concentrations in eight adolescent
diabetic patients during treatment with porcine (closed
circles) or human (open circles) insulin.   Arrows indicate
meal and snack times.

between studies. Average 24hr mean concentration was 12.3mu/l (range 5.2-17.1mu/l) during porcine insulin and 11.3mu/l (range 6.2-18.2mu/l) during human insulin. Mean antibodies to human, bovine or pork insulin did not change significantly. During porcine treatment they were 17, 14 and 17 iu/l respectively and during human treatment 15, 12 and 16 iu/l.

7.4. Discussion

The production of human insulin is a major technological achievement and it may have manufacturing advantages. Clinical advantages or disadvantages however, have to be proved. This study was designed to reflect the sort of procedure a diabetic clinic might follow after a decision to change to human insulin, that is, the change is likely to involve a period of more intensive monitoring to adjust the insulin dose but may not set out to change diet or exercise patterns.

There was a significant increase in insulin dose from a mean of 1.0 U/kg (range 0.7-1.4 U/kg) on treatment with porcine insulin to 1.2 U/kg (range 0.8-2.0 U/kg) on treatment with human insulin. Despite this increase no improvement was observed in total glycosylated haemoglobin, fasting blood glucose,or mean 24hr blood glucose concentrations. A change in the diurnal blood glucose pattern was found with concentrations lower after breakfast and higher during the night with treatment with human insulin. The amount of insulin given as intermediate acting insulin in the evening rose from a mean (SD) 0.17 (0.11)

U/kg of porcine insulin to 0.28 (0.07) U/kg of human insulin, and the findings are thus consistent with reports of a shorter duration of action of human intermediate acting insulins (Clarke et al, 1982).

The more pronounced fall in blood glucose concentrations after breakfast and morning human insulin led to greater rises in pyruvate and lactate concentrations at this time. Over 24 hours blood lactate concentration was significantly higher with human insulin consistent with the increased insulin dose enhancing peripheral lactate production.

Total ketone bodies were significantly lower during the human insulin study. Rates of hepatic ketone body production are determined largely by the rate of supply of non-esterified fatty acids to the liver and by alterations in the intrahepatic fate of fatty acids by hormones, particularly insulin (McGarry, 1979). Decreased fatty acid supply to the liver during the second study is supported by the significant fall in glycerol that occurred, reflecting a decrease in lypolysis. This effect is likely to be due to an increased insulin dose rather than a different type of insulin. Even on the higher dose of insulin the diurnal pattern of total ketone bodies remains abnormal especially over night (Wildenhoff, 1972).

In conclusion, changing adolescent insulin dependent diabetic patients from porcine to human insulin (crb) did not improve blood glucose control despite an increase in dose. Significantly lower total ketone bodies and glycerol

concentrations were found while blood lactate concentration was higher. These metabolic changes were probably due to the increased insulin dose rather than any species difference in insulin. The study also shows significant differences in intermediate metabolites without significant differences in blood glucose control.

Chapter 8 <u>COMPARISON OF METABOLIC EFFECTS OF INSULIN SPECIES</u>

8.1. INTRODUCTION

8.2. METHODS

8.3. RESULTS

8.4. DISCUSSION

8.1. INTRODUCTION.

Intensive attention improves glycaemic control in the setting of an adult clinic (Worth, Home, Johnston, Anderson, Ashworth, Burrin, Appleton, Binder and Alberti, 1982) so simply recruiting patients to a study may alter metabolic rhythms.

Chapter 6 described changes in a group of patients subjected to a home blood glucose monitoring regime but no change in insulin species. Chapter 7 described changes which occurred in patients who were changed from pork to human insulin, in a way that may be undertaken in a diabetic clinic, and showed that this manoeuvre in itself failed to improve glycaemic control.

It could be argued that the process of stabilization of the patients on the human insulin should have lead to an improvement in blood glucose control because of the increased attention paid to them during this stabilization. Since no difference was shown there may be true worse control on human insulin hidden by improved control due to increased attention.

In order to determine more accurately the effect of a change of insulin species on metabolic profiles the study was designed so that the two groups studied in the last two chapters could be compared, one having stayed on their original insulin and the other changed to human insulin.

8.2 METHODS.

The patients in Chapters 6 and 7 were recruited from the same population (see Section 2.2). Immediately after the first profile was performed the patients were randomly allocated to one of two groups; either to stay on their previous insulin (the pork group) or to change immediately to the human insulin (the human group). The allocation was random but not blind. The conduct of the study after the randomisation has been described in the previous chapters. The second profile performed two months after the first with an intervening period of improved control, was then studied to look for differences between the two groups. At the time of the second metabolic profile six patients in the pork group were taking twice daily mixtures of short and intermediate acting insulin and two once daily mixtures. All the patients in the human group were being treated with twice daily mixtures of short and intermediate acting insulin. Full details of the patients and their treatments are given in Chapter 2, pork group patient numbers 1 to 8, human group patient numbers 9 to 16.

The metabolic profiles were performed and the data analysed as described in Chapters 2, 6 and 7.

8.3. RESULTS.

There were no significant differences between the groups with respect to age (mean pork group 12.7 yrs, human group 13.5 yrs) or duration of diabetes (mean pork group 4.9 yrs, human group 4.2 yrs). Insulin dose in the pork group

was higher 1.4 ± 0.2U/kg (mean ± SEM) versus 1.2 ± 0.1U/kg for the human group but was not significantly. The proportion of insulin given in the morning was not different between the groups (69 ± 7% pork; 62 ± 2% human). The proportion of insulin given as soluble in the morning was also similar (36 ± 5% pork; 43 ± 4% human).

There were no significant differences in any of the measures of glycaemic control. The glycosylated haemoglobin and fasting blood glucose were lower in the pork group: 10.8 ± 0.7% vs 12.4 ± 0.7% and 11.3 ± 2.6mmol/l vs 18.4 ± 2.0mmol/l respectively. Figure 8.1 shows that the lower glucose in the morning in the pork group was brief and that for much of the day there was little difference between the two groups. The average of the 24hrs means (Table 8.2) were the same 12.6mmol/l and the variance ratio was zero which shows that analysis of variance could not detect any difference between the groups.

Fasting blood lactate did not differ between the groups (Table 8.1). In the human group, after breakfast and lunch, the blood lactate concentration rose more quickly to give two peaks. The rise was slower in the pork group and there was only one peak. During the rest of the day the profiles were very similar with another peak after evening insulin and meal. The differences between the groups were not significant (Table 8.2; F=0.20; NS).

Fasting blood pyruvate was similar between the groups (Table 8.1) and the shape of the profiles were the same as

Table 8.1
Fasting metabolite concentrations second study: mean and SEM of blood metabolites (mmol/l) and
ratios after control using either porcine or human insulins.
p value - Student's t-test for paired samples; NS - not significant; TKB - total ketone bodies;
3OHB/AcAc - 3-hydroxybutyrate/acetoacetate. Note ketone data logged for analysis.

Pt No	Glucose	Lactate	Pyruvate	Lactate Pyruvate Ratio	Alanine	3-hydroxy -butyrate	Aceto- Acetate	3-OHB AcAc Ratio	TBK	Glycerol
Porcine Insulin										
Mean	11.7	0.87	0.07	12.0	0.23	0.29	0.12	2.7	0.42	0.11
SEM	2.6	0.06	0.01	0.6	0.03	1.44	1.52	0.5	1.45	0.01
Human Insulin										
Mean	18.4	0.84	0.07	12.4	0.20	0.60	0.33	2.0	0.94	0.12
SEM	2.0	0.05	0.01	0.4	0.01	1.36	1.21	0.2	1.29	0.01
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 8.2
Average 24hr mean metabolite concentrations second study: mean and SEM of blood metabolites (mmol/l) and ratios after improved control using either porcine or human insulins.
F - variance ratio (2-way analysis of variance); NS - not significant; TKB - total ketone bodies; 3OHB/AcAc - 3-hydroxybutyrate/acetoacetate. Note ketone data logged for analysis.

| Pt No	Glucose	Lactate	Pyruvate	Lactate Pyruvate Ratio	Alanine	3-hydroxy -butyrate	Aceto- Acetate	3-OHB AcAc Ratio	TBK	Glycerol
Porcine Insulin										
Mean	12.6	1.39	0.12	11.6	0.36	0.10	0.10	1.4	0.21	0.09
SEM	1.8	0.18	0.01	0.6	0.01	0.02	0.03	0.2	0.05	0.01
Human Insulin										
Mean	12.6	1.43	0.12	11.8	0.35	0.08	0.09	1.2	0.17	0.08
SEM	1.1	0.18	0.02	0.3	0.02	0.01	0.01	0.1	0.02	0.01
F	0.0	0.20	0.07	5.92	4.26	2.05	0.66	6.57	1.49	3.48
p	NS	NS	NS	<0.05	<0.05	NS	NS	<0.05	NS	NS
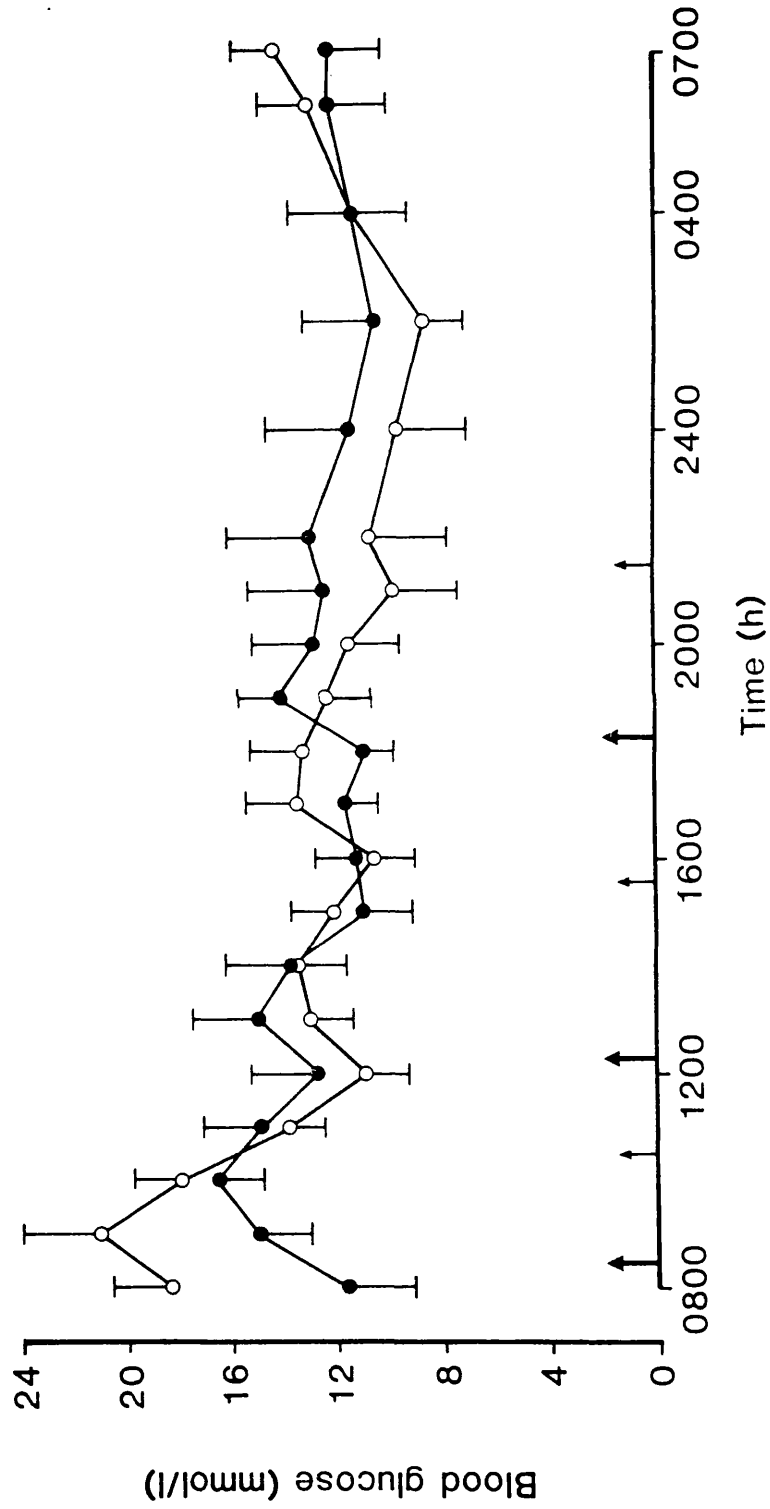
Figure 8.1 Mean (SEM) 24 hour blood glucose concentrations in sixteen adolescent diabetic patients after attempting to improve control, eight treated with porcine (closed circles) and eight with human (open circles) insulin. Arrows indicate meal and snack times.
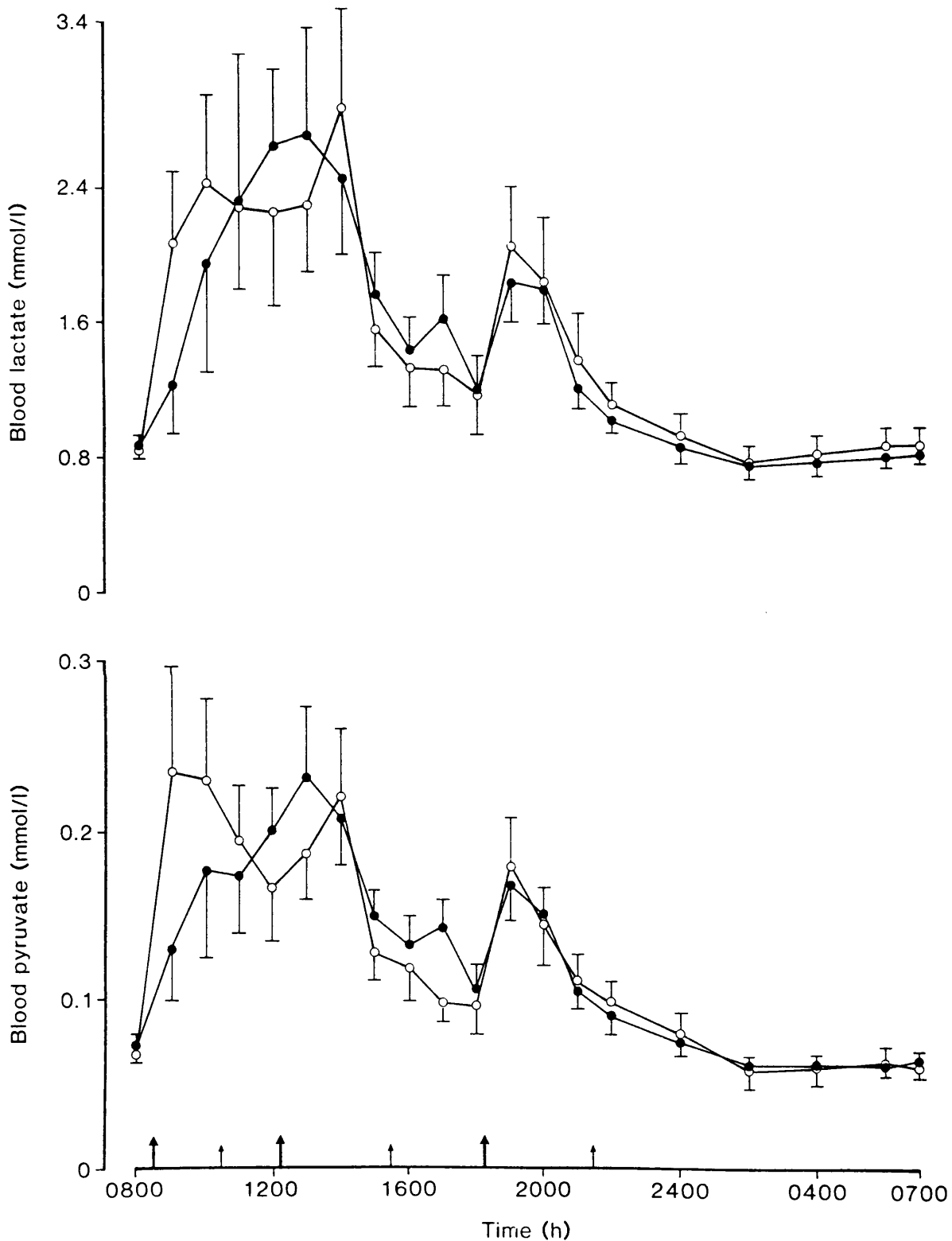
Figure 8.2 Mean (SEM) 24 hour blood lactate and blood pyruvate concentrations in sixteen adolescent diabetic patients after attempting to improve control, eight treated with porcine (closed circles) and eight with human (open circles) insulin. Arrows indicate meal and snack times.

those for lactate (Fig 8.2). The average 24h means were both 0.12mmol/1 (Table 8.2) and the variance ratio not significantly different.

The ratio of lactate to pyruvate (Fig 8.5) changed little over the day. The greatest change was immediately after morning insulin and breakfast. The ratio was just significantly higher during the human study (Table 8.2, F=5.92; p <0.05).

Total ketone bodies concentration was not significantly different between groups in the fasting blood (Table 8.1). It was higher in the blood from the from the human group but fell more steeply after insulin and breakfast (Fig 8.4). During the night the total ketone bodies concentration rose more quickly in the pork group but these differences were not significant overall (Table 8.2; F=1.49; p NS). However, the difference in the ratio of the 3-hydroxybutyrate to acetoacetate just reaches significance (Fig 8.5; Table 8.2; F=6.57; p<0.05) and was higher in the porcine group. Glycerol was not significantly different either fasting (Table 8.2) or over the day (Fig 8.4; Table 8.2; F=3.48; p NS).

Fasting alanine was similar in both groups (Table 8.1). The peaks after meals were more evident in the human group and overall the concentration was slightly but significantly lower in this group (Fig 8.3; Table 8.2; F=4.26; p <0.05).
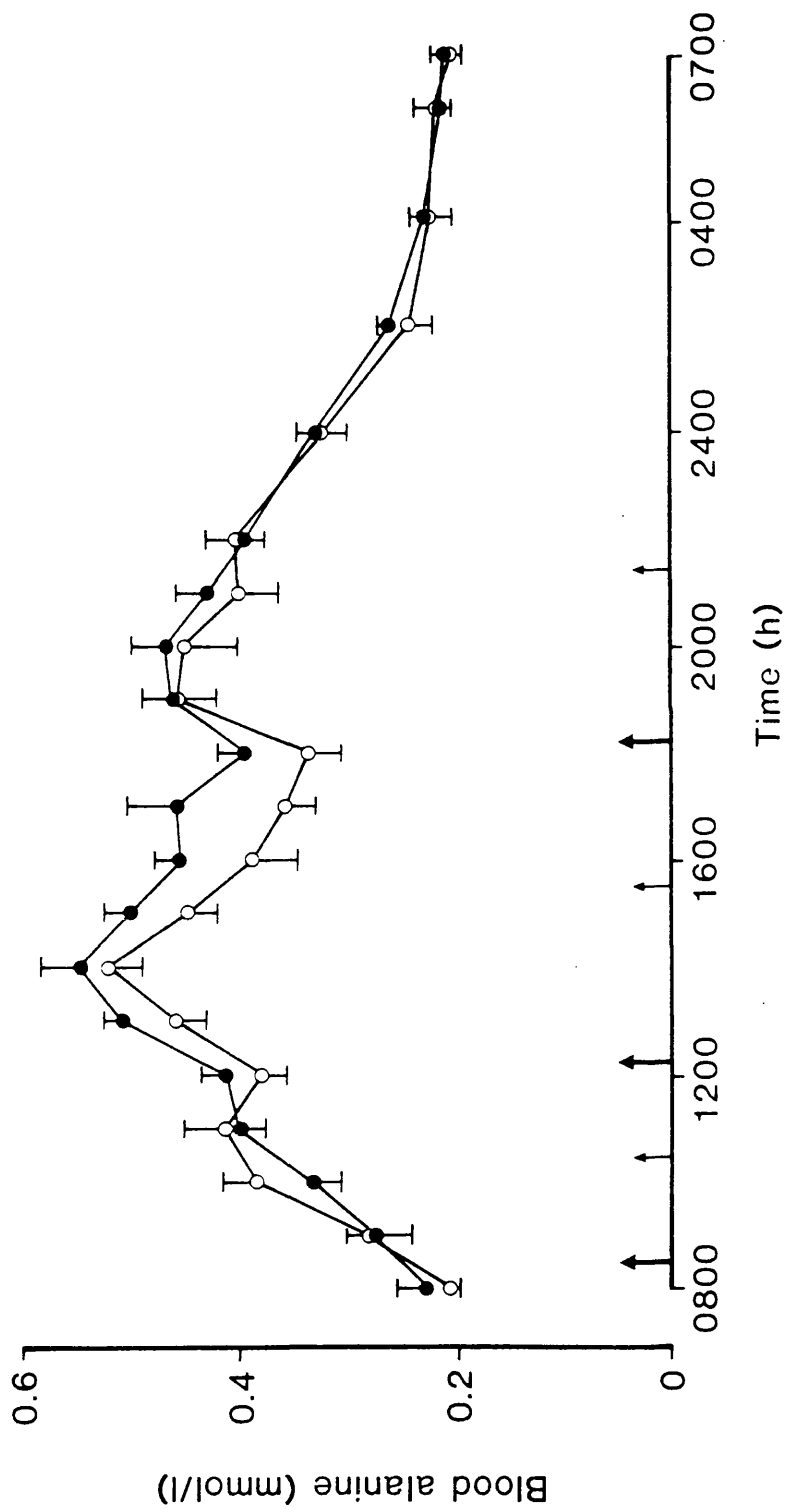
Figure 8.3   Mean (SEM) 24 hour blood alanine concentrations in sixteen adolescent diabetic patients after attempting to improve control, eight treated with porcine (closed circles) and eight with human (open circles) insulin.   Arrows indicate meal and snack times.
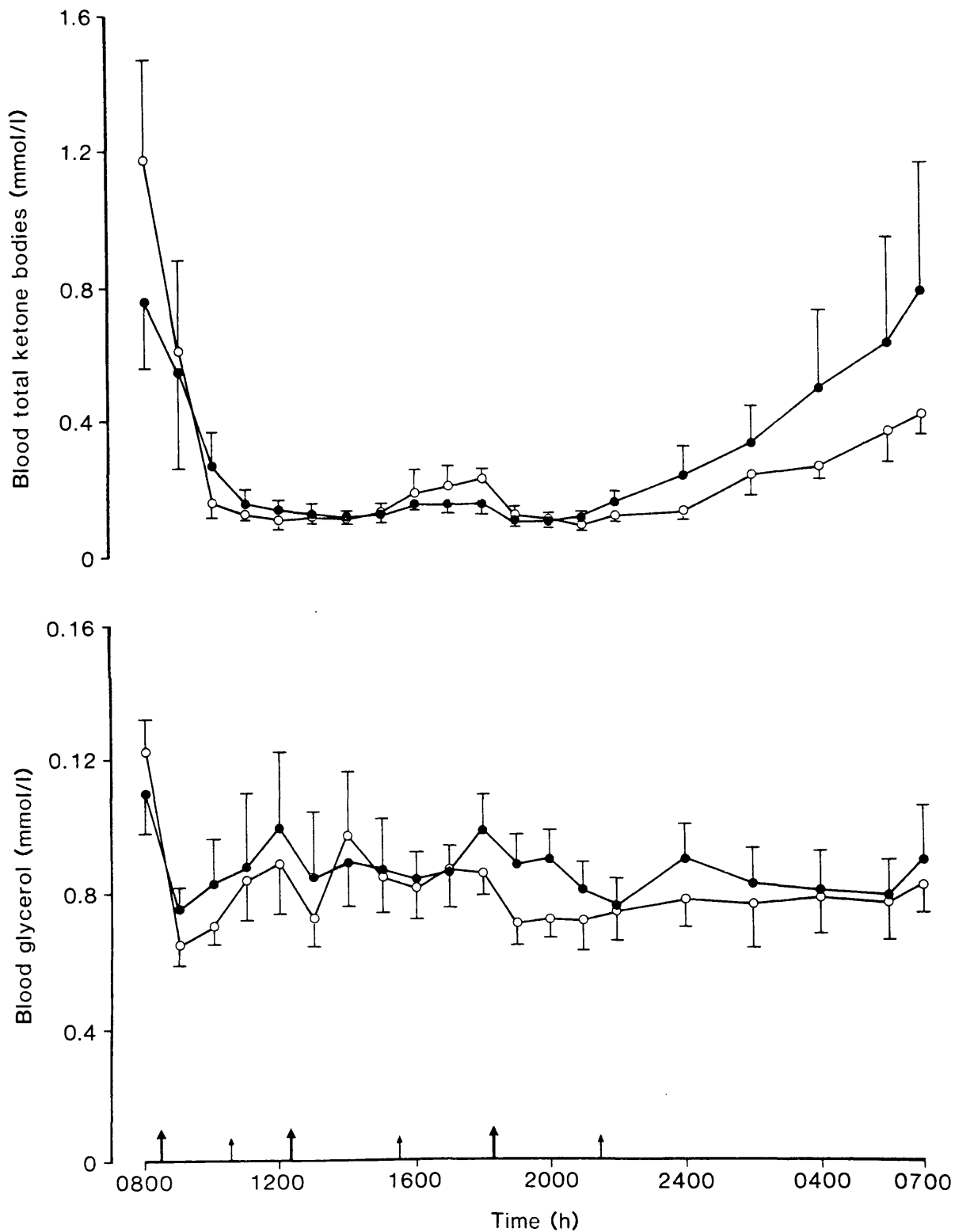
Figure 8.4 Mean (SEM) 24 hour blood total ketone bodies and blood glycerol concentrations in sixteen adolescent diabetic patients after attempting to improve control, eight treated with porcine (closed circles) and eight with human (open circles) insulin. Arrows indicate meal and snack times.

Figure 8.5 Mean (SEM) 24 hour of lactate/pyruvate and
3-hydroxybutryate/acetoacetate ratios in sixteen adolescent
diabetic patients after attempting to improve control, eight
treated with porcine (closed circles) and eight with human
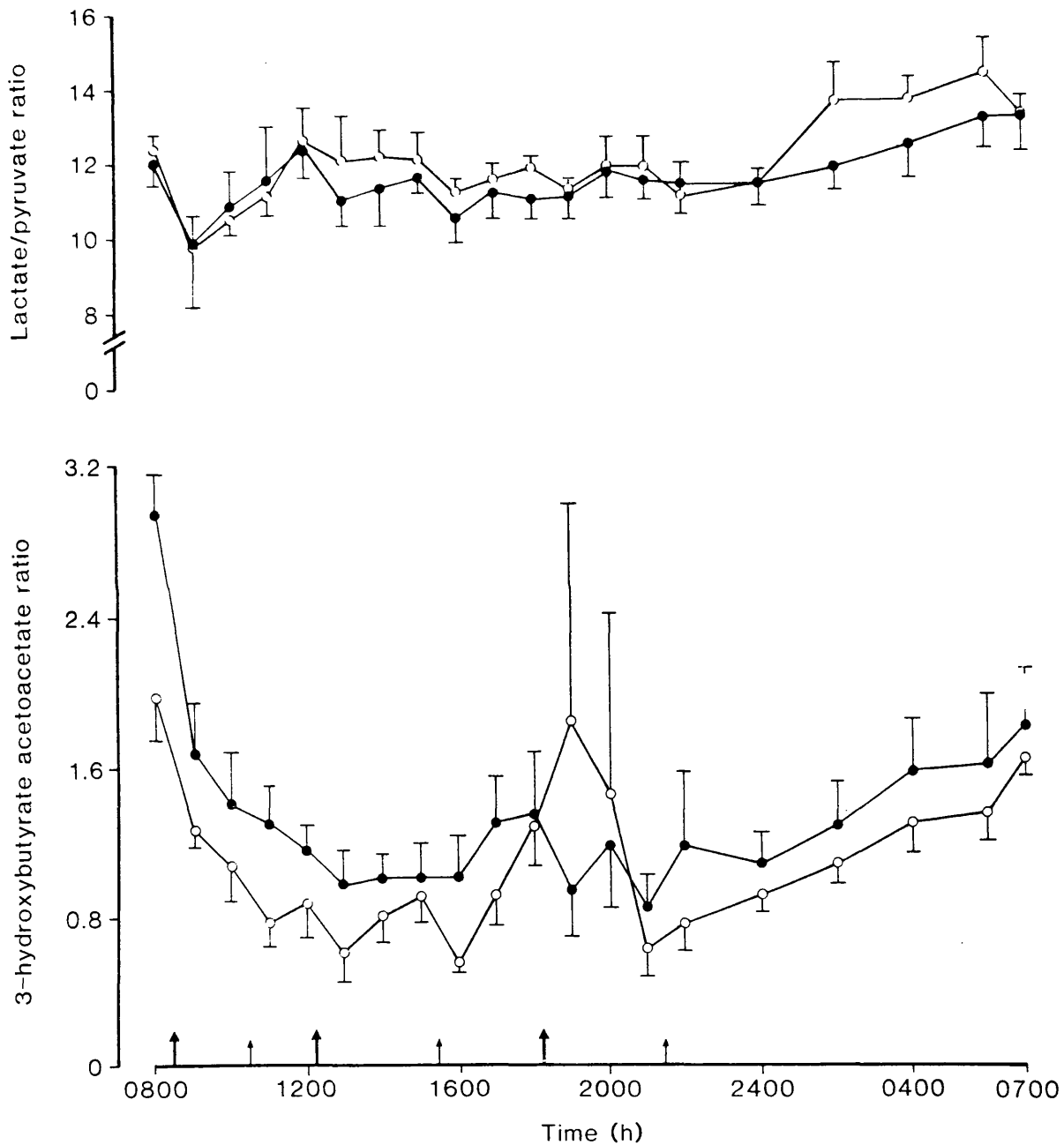(open circles) insulin. Arrows indicate meal and snack times.

Potentially, changes in endogenous insulin production, growth hormone or insulin antibody status could have affected the metabolic profiles but no significant differences were found between the two groups (Table 8.3).

8.4. Discussion.

The differences in the metabolite ratios in this study are small. The ratio between lactate and pyruvate are said to reflect the redox state of the hepatic cytosol and the ratio between the 3-hydroxybutyrate and acetoacetate the redox state of the hepatic mitochondria. The changes are slight and the biological significance obscure. Seeking this sort of change may be an over interpretation of the data.

The slightly lower blood alanine concentration in the human group cannot be explained by the suggested suppression of blood alanine concentrations by blood ketone concentrations (Sherwin, Hendler and Felig, 1975) because the total blood ketone concentration was also lower in the human group though not significantly so. This association has been questioned (Fery and Balasse, 1980).

There was remarkably little difference in the blood glucose control between the two groups but Fig. 8.1 shows that the profiles are not the same and that the poor control in the human group in the morning is balanced by better control later in the day. This is probably because the intermediate acting human insulin used has a quicker but shorter action. It is also clear that in both groups blood glucose concentration is poorly controlled.

Table 8.3
C-peptide, Growth hormone and Insulin antibodies in Pork and Human groups. Mean and SEM.

	Mean C-peptide pmol/ml	Total C-peptide pmol/ml	Average Growth Hormone mu/l	Insulin Antibodies		
				Human %	Bovine %	Porcine %
Pork Group						
Mean	43.8	845	9.73	27.5	19.9	25.5
SEM	25.2	640	4.57	22.7	12.2	19.7
Human Group						
Mean	46.3	785	11.33	15.3	12.4	15.5
SEM	10.7	425	4.58	10.1	8.6	10.8
p value	NS	NS	NS	NS	NS	NS

The insulin used after randomisation in this study was known to both doctor and patient so the study was not 'blind'. Since the start of this study two randomisation studies in children have been reported.

Mann et al (Mann, Johnston, Reeves and Murphy, 1983) used a double blind crossover technique to compare human and porcine insulins and found control worse on human insulin, Hba1 of 14.4% versus 13.8%. However, the ratio of short to intermediate acting insulin was kept constant and the dosage of insulin was almost identical on the two insulins.

Greene et al (Greene, Smith, Cartwright and Baum, 1983) in a similar double blind crossover study found no difference in Hba1 on human or porcine insulin. Slightly more insulin was used in the human insulin periods but not in significant amounts and the ratios were not fixed.

In both the studies described in Chapters 6 and 7 the insulin dose was increased by, on average, a similar amount (0.2 U/kg). In the analysis in this chapter the porcine group were using more insulin (but not significantly) than the human group. However, the porcine group were using a higher dose of insulin at recruitment.

These studies and the papers quoted indicate that to achieve similar blood glucose control in children on porcine insulin requires at least as much and probably more human insulin. Also this study shows little evidence for gross changes in other intermediate metabolites in patients treated with human insulin. The major stumbling block to

achieving good blood glucose control - the poor early morning control - may be worse with human insulin as used in these formulations.

Chapter 9 <u>GENERAL DISCUSSION AND CONCLUSIONS</u>

This thesis is primarily concerned with two areas of adolescent diabetes. Firstly, the measurement and metabolic effect of endogenous insulin in adolescent patients past the partial remission phase of their disease. Secondly, the use of the technique of 24hr metabolic profiles to help define the degree of the metabolic abnormality and to try to assess the effect of endogenous insulin, modest attempts to improve control and a change in insulin species on these profiles.

Before discussing the influences of a hormone in a biological system two problems of measurement must be reflected upon. The first is primarily a biochemical one which is whether the value that is finally recorded is a true reflection of the concentration of the hormone that is under investigation. This has been discussed in the introductory chapter and this thesis does not address it further.

The second problem in hormone measurement is the variation in the concentration of the hormone over time, the presumption being that this is biologically important. If there is no change over time then the answer is simple and a sample taken at any time would accurately measure the hormone in the biological system. If the difference in the hormone concentration between two groups under study was so great that the changes over time were relatively small in comparison then these changes would be irrelevant to the study and a random sample would again be sufficient. An example of this could be a study of endogenous insulin in a

population of established insulin dependent diabetic patients compared with a non-diabetic population.

If neither of these considerations apply there are two possibilities. One is to project what will happen to the hormone by a provocative test and the other is simply to make frequent measurements of the hormone.

The most commonly used provocative test, the glucagon stimulation test did not predict good control nor correlate with the C-peptide measured over the day in this study. This test was developed in patients with much more endogenous insulin than these patients so the use of the test in these circumstances may not be appropriate. Frequent measurement of the hormone was the method chosen in this work as the most appropriate for the study of this group of patients.

This immediately poses the problem of how often is frequent. The ideal must be continuous measurement but this has other consequences not least exsanguination. To some extent then, less frequent sampling is a compromise though possibly a reasonable one. A second problem is that this study does not show the potential for the hormone, that is, the amount in reserve, but if it does not show that potential over the period of study it could be argued that it is not relevant to the study.

The two areas of interest in this work overlap in the study of the metabolic effect of the small amounts of endogenous insulin found in these patients. The finding of a difference albeit small is rather against the published

opinion. That this difference is shown in glucose metabolism and not ketone metabolism is interesting and may be because of its portal secretion.

The descriptive chapter on the metabolic profiles of a group of adolescent diabetic patients is depressing in that it shows that despite treatment there remain large metabolic abnormalities. The particularly disturbed metabolism in *the* morning is documented but the seeds of this disturbance are sown during the night. The night is a fruitful area of study in diabetes and rather neglected. This study has demonstrated that the problems start early in the night with a rise in total ketone concentration. It is possible that this is because these patients are adolescents and therefore growing and in a metabolically anabolic state compared with an adult population. Normalization of the nocturnal hyperketonaemia, if it were possible, may not be of benefit with regard to growth. It is clear though that an improvement in nocturnal metabolic profiles would be likely if the evening insulin was delayed until later.

Improving control usually means increasing insulin dose. To the adolescent patient the penalties of too much insulin are immediate whilst those of too little are a long way off. However it is easy to make too much of these psychological effects and there was no reluctance amongst these patients to increase their dose of insulin although some of their parents were not so sure. Adolescent diabetic patients seem to need more insulin like their non-diabetic peers, but quite when and how much remains to be determined.

The increase in insulin had some effect but the metabolite concentrations were far from normalised. Even the 'target' metabolite, glucose, does not fair that well. In that the experiment was designed around a therapeutic manoeuvre that many diabetic clinics have made - the introduction of regular home blood glucose monitoring to current insulin treatment - it is somewhat depressing that the effect was not more profound.

It is also important to note that an improvement in glucose control may be associated with a deterioration in the 'control' of another metabolite. In this respect lactate concentration is an example. It is far from clear that if the complications of diabetes are due to persistent metabolic abnormality, they are solely due to the raised glucose. This work demonstrates that there are considerable abnormalities in other metabolites and that these may be increased by attempts to improve glucose control with higher subcutaneous insulin doses.

The development of industrial processes that are capable of producing large quantities of human insulin is a remarkable achievement. However, the product, though 'natural', is delivered into a depot in an area in the systemic circulation rather than trickled in carefully servo-controlled amounts into the portal circulation. This study shows that the basic metabolic abnormalities remain when human insulin is used and that insulin species is not the cause of these problems, at least when porcine and human insulin are considered.

It is interesting to note how similar the overall glucose control was when the two groups were compared after similar management for two months. Perhaps these values demonstrate the best control one can achieve in these circumstances. It would appear that insulin species is irrelevant to the argument but the profiles clearly demonstrate that the insulins are not identical in their effects. The human insulins appear to have an earlier onset and a shorter duration of action, not only in glucose metabolism but also in the metabolism of other substrates. Thus when chang ing patients to human insulin adjustments may need to be made in dose, formulation and mixture to achieve similar profiles.

This work has a few practical conclusions. In the clinical situation the doctor is sometimes presented with an adolescent whose diabetes is in good control. It would seem to be quite in order to congratulate the adolescent with enthusiasm on his or her good efforts without tempering that enthusiasm with scientific doubts that to some extent he or she is benefiting from some persistent endogenous insulin. There is little evidence that that secretion, however measured, has a practical metabolic effect past the partial remission period.

Conventional treatment resulted in poor metabolic control and on largely twice daily insulin only modest improvements were achieved with little difference between either porcine or human insulin. The metabolic profiles

suggest that in this age group the origins of the morning metabolic abnormality lie in the nighttime control. An improved glucose and ketone profile may be achieved by better insulinization overnight but the cost may be increased abnormality in the profile of another metabolite such as lactate.

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Retrospective Chapter.

This thesis was examined in June 1990. The examiners suggested a brief retrospective chapter commenting on four specific points raised by them and the novelty, clinical and scientific interest of the work.

a) Lack of control group.

The examiners in their report recognised the ethical problems of research on normal children. This is indeed the reason why, though scientifically desirable, we were unable to include normal metabolic rhythm data. Sometimes "normal" data on children becomes available when extra samples are taken during investigations for unrelated conditions which the child turns out not to have. However, they must have a symptom to warrant investigation in the first place and so cannot be truly normal. No such opportunity arose.

The lack of a control group reflects mostly strongly on the descriptive chapter 3 where the data are compared with published diabetic and normal young adult data. Our diabetic data do not differ greatly except in the rise in total ketone bodies early in the night. In the other chapters either i) a group of diabetic patients are compared with another group of diabetic patients or ii) a group of diabetic patients are compared with themselves before and after a manoeuvre. The lack of a control group in these studies is less important to the conclusions.

b) Reproducibility of metabolic testing.

We did not test the reproducibility from day to day because we felt that the metabolic rhythm technique was established in adult research and would therefore question the need to re-establish it in paediatrics. Secondly, we had difficulties in subject acceptance of a longer study. An early protocol of this research involved a longer initial admission but too many subjects dissolved into tears during the first evening so it was abandoned. Our impression is that, in this age group, 24 hrs is the maximum acceptable time for reliable compliance with this sort of study.

The reproducibility between home and hospital is discussed in chapter 3. Published work in children suggests that, at least for glucose, there is agreement between home and hospital measurements. A study sampling the children by following them around during their normal day is an attractive idea. Our enthusiasm was tempered by experience. The cannula cap of one of our subjects became accidentally dislodged in a local Boots and while the patient and accompanying nurse coped admirably, the shop girl did not look well. Careful consideration would need to be given to the safety of such a study in the sometimes clumsy, sometimes rough normal life-style of normal adolescents.

c) Failure to stage the children with regard to puberty and hence inadequate consideration of important physiological changes.

The study did consider puberty. It was designed to minimalise intrasubject pubertal effects by repeating the study after only two months when pubertal changes would be minimal. In the subjects studied twice increase in height was measured and the mean for the two groups was the same. A potential confounding pubertal hormone, growth hormone, was measured in some detail but did not reveal any significant effects.

There were three difficulties to consider if puberty was to be addressed further.

1) Adolescents may go through a phase of extreme shyness and are often reluctant to be examined for pubertal staging. It was important for the study to keep their full cooperation.

2) One of the aims of the research was to study the endogenous insulin production after the remission period. If we had to stratify by pubertal stage as well it would have been impossible to recruit sufficient numbers even from our large adolescent clinics.

3) Tanner staging is the only available tool. Whilst useful in some clinical situations it is not an exact scale. Considerable subjective judgement is required in applying the scale. Those that are used to marvelling at the variety of the normal naked human form when they view it on the examination couch should not be surprised that in the process of its formation their is even greater normal variation. Tanner stages three different morphological

features and while there is some correlation between them they are not necessarily a reflection of the same process. For example thearchy in the girls is due to oestrogens secreted by the ovary whilst adrenarchy is due to secretions from a different organ, the adrenal, facilitated by gonadotrophins but not controlled by them. There cannot, of course, be any "correlation" between the scale used for boys and that for girls (except a spurious one) because they are scales of different processes. Tanner staging does not identify other important effects of puberty notably the emotional changes and the growth spurt. Some attempt has been made to address the later as detailed above because this seemed the most likely confounding variable.

d) Inadequate consideration of exogenous insulin in particular lack of free insulin measurements.

Full details have been given of the insulin treatment. Free insulin was not measured for two reasons. At the time the study was being set up in 1982 the assay was not available to us. Secondly, one 24 hr study required 140-150 mls of blood. Our smallest subjects were about one third adult size. We self imposed this arbitrary maximum blood sampling volume on each study because it was equivalent to a single adult blood donation for our smallest patients. Since we have shown the lack of growth hormone and C-peptide effects future studies could include this measurement instead.

This thesis gives an account of studies on a novel group of diabetic patients. Adolescents are renown for their poor control for which they are usually unjustly blamed. There are three principle conclusions.

1) Using metabolic profiles, a more sensitive measure of metabolic control than simply blood glucose, I have failed to show that endogenous insulin has a significant effect after the partial remission phase of diabetes. While it is difficult to prove a negative this must temper the enthusiasm for endogenous insulin after the remission period.

2) The metabolic profiles have demonstrated that improvement in one metabolite may lead to a deterioration in another and that an overall improved average may hide increased fluctuations. The adolescents required a high dose of insulin and the human insulin formulation had a shorter duration of action.

3) Finally, it has been clearly demonstrated that there is a rise in total blood ketone bodies early in the night. This is evidence of insufficient insulinization before it is reflected in the blood glucose. Increasing insulin dose did not correct this problem.

The last two statements provide good evidence for a further study investigating the effect of delaying the intermediate acting insulin until bed time. This is clearly more important as human insulins become the standard insulins in use.

Parts of this work have been previously published.

Hocking MD, Rayner PHW, Nattrass M 1987.

Residual insulin secretion in adolescent diabetics after remission.

Archives of Disease in Childhood, 62: 1144-1147.

Hocking MD, Rayner PHW, Nattrass M 1986.

Metabolic rhythms in adolescents with diabetes.

Archives of Disease in Childhood, 61: 124-129.

Hocking MD, Crase J, Rayner PHW, Nattrass M 1986.

Metabolic rhythms in adolescents with diabetes during treatment with porcine or human insulin.

Archives of Disease in Childhood, 61: 341-345.

These papers were drawn from chapters 5, 6 and 7 respectively.