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Metabolic rhythms in adolescents with diabetes

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SUMMARY Metabolic rhythms were studied over 24 hours in eight adolescents with insulin dependent diabetes before and two months after attempting to improve diabetic control with home blood glucose monitoring. A significant improvement in blood glucose concentration was observed, although 24 hour mean concentrations remained grossly abnormal. This improvement was accompanied by significant falls in blood glycerol and total ketone bodies concentrations and a significant rise in blood lactate concentration. Without attention to other factors affecting diabetic control, the introduction of home blood glucose monitoring produces only a small improvement in control.

Control of diabetes in adolescence is influenced by a variety of physiological and psychological factors.[1] Continuous subcutaneous insulin infusion treatment improves control in insulin dependent diabetic patients[2 3] but experience in children and adolescents is limited, and initial reports suggest that this treatment is less acceptable to these age groups.[4] Most adolescent diabetics are still treated with subcutaneous insulin regimens supported by home blood glucose monitoring.

In practice, home blood glucose monitoring is often added to the treatment regimen without a major effort being made to reform diet or exercise, both of which have profound effects upon diabetic control.

We have attempted to simulate this situation in adolescent diabetic patients by studying the impact of home blood glucose monitoring upon control as assessed by diurnal rhythms of blood glucose and other intermediary metabolites and hormones which are known to be abnormal during treatment of insulin dependent diabetes.[5]

Patients and methods

Patients. Eight insulin dependent adolescent patients (three boys) were studied. 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). Patients were not included if, on clinical and biochemical evidence, they were in the 'honeymoon' period of diabetes, or in partial remission.[6] Six patients were taking twice daily mixtures of short and intermediate acting insulin (Velosulin and Insulatard, Nordisk, or Actrapid and Monotard, Novo Laboratories) and two were taking once daily mixtures of Actrapid and Monotard. At recruitment into the study none of the adolescents was using home blood glucose monitoring . The study was approved by the Ethical Committee of the Central Birmingham Health Authority.

Protocol. A 24 hour metabolic profile was performed at the start of the study, immediately after recruitment and before any attempt to improve control. After this intensive home blood glucose monitoring was done with seven point blood glucose estimations (before and two hours after main meals and before bed) using BM20–800 blood glucose strips (Boehringer) on at least one day per week. Insulin dosage was adjusted by a paediatrician or by the patient after discussion with the paediatrician on the basis of the results aimed at producing preprandial blood glucose concentrations of 4 mmol/l and concentrations of less than 7 mmol/l after meals. Changes were made in the dose of insulin but not in type of insulin or frequency of injections. Results reported by the patient were not checked by the physician. No attempts were made to influence directly dietary compliance or exercise patterns. Within eight weeks all patients reported blood glucose profiles with all values less than 10 mmol/l. A second metabolic profile was performed two months after the start of the study.

Patients were admitted to hospital the evening before the study. At 7.30 am a teflon cannula (22G Abbocath, Abbott Ireland) was inserted into an antecubital vein. Between samples the cannula was kept patent by flushing with saline (154 mmol/l). A fasting sample was taken at 8.00 am after which

insulin was given. Blood samples were taken hourly until 10 pm then two hourly to 6 am. Breakfast was eaten at 8.30 am, lunch at 12.15 pm, and dinner at 6.15 pm with snacks at 10.30 am, 3.30 pm, and 9.30 pm. Evening insulin was given at 5.45 pm. The carbohydrate content of the diet and individual meals was that prescribed for regular consumption at home and was the same for the two studies.

Patients were encouraged to be ambulant between blood samples, being free to leave the ward and move between floors of the hospital.

Blood (5 ml) was withdrawn after discarding 2 ml from the cannula. Whole blood (1 to 2 ml) was mixed with ice cold 5% (v/v) perchloric acid and refrigerated immediately. The remainder was placed in a plastic tube and after clotting, serum was separated and stored at −20°C for subsequent measurement of C peptide and growth hormone.

Glucose, lactate, pyruvate, 3-hydroxybutyrate, glycerol, and alanine concentrations were measured in the perchloric acid extract by continuous flow enzymatic fluorimetric techniques.[7] Acetoacetate was measured within 36 hours in the perchloric acid extract by a spectrophotometric method.[8] Serum growth hormone was measured by a double antibody radioimmunoassay.[9] Serum C peptide was measured by a single antibody method using antibody M1230 from a commercially available kit (Novo Research Institute, Copenhagen) after precipitation of proinsulin/insulin antibody complexes by polyethylene glycol.[10] Total glycosylated haemoglobin was measured in the fasting blood sample by electroendosmosis (Corning). The normal range in our laboratory is 5·8 to 8·5%.

Student's *t* test for paired samples was used to compare glycosylated haemoglobin concentration, insulin dose, and fasting concentrations of metabolites. Metabolic rhythm data were analysed using two way analysis of variance,[11] with data classified by study and time. For the missing hourly values during the night, the value for the previous hour was repeated. For numerical comparison of the 24 hour rhythm data in the studies an average of the 24 hour mean values was calculated. Total ketone bodies (the sum of 3-hydroxybutyrate and acetoacetate) was log transformed before analysis since the data did not fit a Gaussian distribution.[12]

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

Table 1 *Fasting concentrations (mean (SEM)) of blood metabolites (mmol/l) and ratios in eight adolescent diabetic patients before (study 1) and after (study 2) improvement in diabetic control*

	Glucose	*Lactate*	*Pyruvate*	*Lactate: pyruvate*	*3-Hydroxy-butyrate*	*Aceto-acetate*	*TKB*	*3-OHB: AcAc*	*Glycerol*	*Alanine*
Study 1	15·4 (1·5)	0·93 (0·12)	0·09 (0·01)	10·8 (0·5)	0·61 (0·22)	0·29 (0·12)	0·91 (0·34)	2·3 (0·2)	0·14 (0·01)	0·26 (0·02)
Study 2	11·7 (2·6)	0·87 (0·06)	0·07 (0·01)	12·0 (0·6)	0·52 (0·27)	0·26 (0·15)	0·78 (0·42)	2·7 (0·5)	0·11 (0·01)	0·23 (0·03)
P	<0·07	ns	ns	<0·05	ns	ns	ns	ns	<0·08	ns

Statistical analysis by Student's *t* test; ns=not significant.
TKB=total ketone bodies; 3-OHB/AcAc=3-hydroxybutyrate-acetoacetate.

changes in the proportion of insulin given in the morning (63% first study, 64% second study) nor in the proportion given as quick acting insulin (32% *v* 36%).

Fasting blood glucose value was lower for the second study ($P<0.07$; Table 1), and the mean for the group was consistently different during the day but at no time was it less than 10 mmol/l (Fig. 1). The average 24 hour mean blood glucose concentration 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 ($F=7.20$; $P<0.01$; Table 2).

Fasting blood lactate value was not significantly different between studies (Table 1). After breakfast and morning insulin a more noticeable increase in blood lactate occurred during the second study (Fig. 2). After the evening meal blood lactate concentration was similar. Overall blood lactate concentration was significantly higher during the second study ($F=7.42$; $P<0.01$; Table 2). Blood pyruvate followed a similar diurnal pattern to lactate, although over the 24 hours differences were not statistically significant. Both fasting lactate/pyruvate ratio (Table 1) and lactate/pyruvate ratio over the 24 hours increased significantly in the second study (Table 2).

Total ketone bodies concentration was raised at the start of both studies but fell after breakfast and insulin. Throughout the 24 hours the mean concentration was consistently lower during the second study ($F=17.70$; $P<0.001$). After 9 pm there was a gradual rise in mean blood total ketone bodies concentration, although this was less noticeable in the second study (Fig. 3). Fasting blood glycerol did not differ significantly between studies (Table 1). Throughout, the mean blood glycerol concentration was lower during the second study (Fig. 3) and the

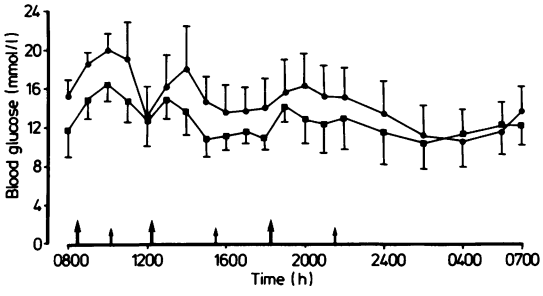

Fig. 1 *Blood glucose concentration (mean (SEM)) over 24 hours in eight adolescent diabetics before (●—●) and after (■—■) attempting to improve control with blood glucose monitoring. The large arrows indicate meals and the small arrows snacks.*

Table 2 *Average 24 hour mean (SEM) of blood metabolites (mmol/l) and ratios in eight adolescent diabetics before (study 1) and after (study 2) improvement in diabetic control*

	Glucose	*Lactate*	*Pyruvate*	*Lactate: pyruvate*	*3-Hydroxy-butyrate*	*Aceto-acetate*	*TKB*	*3-OHB: AcAc*	*Glycerol*	*Alanine*
Study 1	14·9 (2·4)	1·18 (0·05)	0·11 (0·01)	10·8 (0·5)	0·29 (0·09)	0·16 (0·05)	0·46 (0·14)	1·6 (0·2)	0·10 (0·01)	0·35 (0·02)
Study 2	12·6 (1·8)	1·39 (0·18)	0·12 (0·01)	11·6 (0·6)	0·17 (0·05)	0·12 (0·03)	0·29 (0·08)	1·4 (0·2)	0·09 (0·01)	0·36 (0·0)
F	7·02	7·42	3·29	10·20	12·38	9·26	17·70	1·72	13·67	3·58
P	<0·01	<0·01	ns	<0·01	<0·001	<0·01	<0·001	ns	<0·001	ns

Statistical analysis by 2-way analysis of variance (F=variance ratio; ns=non significant).
TKB=total ketone bodies; 3-OHB/AcAc=3-hydroxybutyrate-acetoacetate.

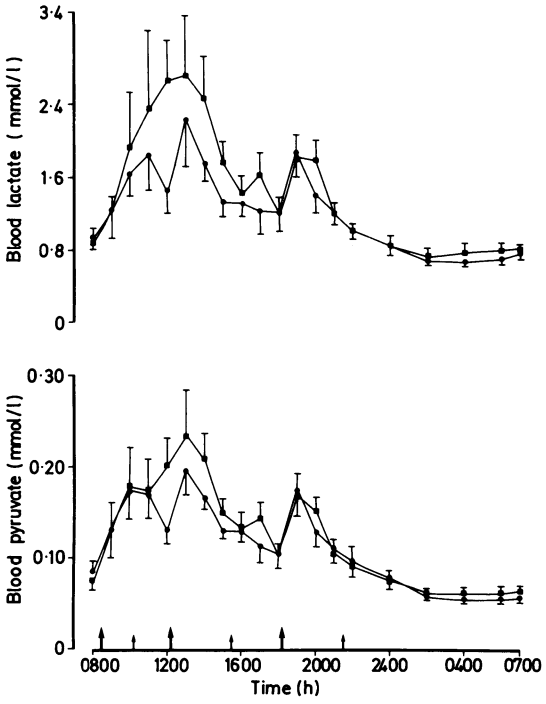

Fig. 2 *Blood lactate and pyruvate concentrations (mean (SEM)) over 24 hours in eight adolescent diabetics before (●—●) and after (■—■) attempting to improve control with blood glucose monitoring. The large arrows indicate meals and the small arrows snacks.*

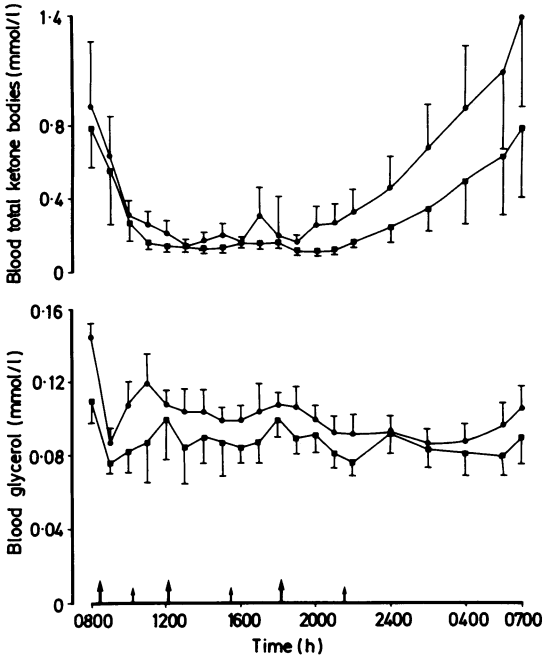

Fig. 3 *Blood total ketone bodies and glycerol concentrations (mean (SEM)) over 24 hours in eight adolescent diabetics before (●—●) and after (■—■) attempting to improve control with blood glucose monitoring. The large arrows indicate meals and the small arrows snacks.*

difference was statistically significant ($F=13{\cdot}67$; $P<0{\cdot}001$).

Neither fasting blood alanine nor the diurnal pattern differed significantly between studies.

One patient had no detectable C peptide during either study, and only one patient had a detectable concentration 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 four samples). With this variation within and between patients missing values make statistical analysis problematical. Consideration of either the 24 hour mean C peptide (for values obtained) or the sum of all C peptide values measured during a study did not suggest a consistent change. An increase in C peptide was observed in three patients and a decrease in four patients between the two studies.

No significant differences were found between the two studies for serum growth hormone. The average 24 hour mean concentration of growth hormone was 32·4 mU/l during the first study (range 11·5 to 64·8 mU/l) and 29·0 mU/l (range 9·3 to 45·6 mU/l) during the second study.

Discussion

Control of diabetes during adolescence is problematical, although home blood glucose monitoring has been used to good effect—albeit in the limited situation of a diabetic camp.[13] In the present study we observed an improvement in diabetic control as assessed by the glycosylated haemoglobin and 24 hour blood glucose concentrations, although normality was not obtained with the mean blood glucose concentration never less than 10 mmol/l. The discrepancy between the patients' tests of home blood glucose monitoring and observed blood glucose concentrations is of interest. Differences between home records of blood or urine tests and glycosylated haemoglobin in this age group have been reported[14] and attributed to deliberate manipulation. Indeed some degree of observer error may be implicated with patients' readings uninten-

tionally tending to favour lower values, although it cannot be ruled out that the performance of the study under hospital conditions may 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.[15]

Decreased activity is unlikely to explain the finding of appreciably raised fasting blood glucose concentration in both studies. Thereafter the pattern of blood glucose concentration was not grossly abnormal (Fig. 1), although the rise after breakfast was exaggerated and prolonged. A reduction in the fasting blood glucose value to normal concentrations in the present study may well have led to a near normal pattern of control.

The results for 24 hour mean blood glucose during the first study are similar to those obtained in diabetic children by Griffin *et al*[16] who also made no attempt to improve control before study. The same group, however, obtained better 24 hour mean blood glucose results after optimising blood glucose control on once or twice daily insulin injections.[15] Four of their 15 patients, however, had significant endogenous insulin secretion.[15 17]

In a previous study, Mann and colleagues[18] reported no significant differences 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.

Improved blood glucose control led to a significant increase in blood lactate concentration and a lesser and not significant increase in blood pyruvate concentration, with the major differences between the two studies occurring between 8 am and 6 pm. The combination of lower blood glucose with increased lactate suggests enhanced insulin stimulated glucose uptake and peripheral lactate production by the greater amount of insulin given in the second study.

The improvement in blood glucose control is reflected in the significant fall in blood total ketone bodies during the second study, although the diurnal pattern of blood total ketone bodies is not normal. Wildenhoff[19] has shown peaks in total ketone bodies occurring before meals and at 4 pm in normal subjects. In the present study, the rise in ketones before lunch was absent and blunted before dinner, and during the night there was a steady rise rather than a peak. Comparing the overnight patterns for glucose and total ketone bodies it would seem that the rise in blood total ketone bodies during the night is 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. Concentrations of glycerol obtained are within the normal range for young adults[5] despite high blood glucose concentrations. Further improvement of glucose through increased insulin administration might result in suppression of glycerol to abnormal values due to peripheral insulin administration.

In conclusion, blood glucose control was improved by a short period of home blood glucose monitoring in adolescent diabetic patients but normal values were not achieved. The fall in blood glucose concentration was accompanied by lower total ketone bodies and glycerol concentration but blood lactate concentration rose. The major stumbling block to normal blood glucose values 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 insulinisation. 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.

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References

1 Tattersall RB, Lowe J. Diabetes in adolescence. *Diabetologia* 1981;**20**:517–23.
2 Pickup JC, Keen H, Parsons JA, Alberti KGMM. Continuous subcutaneous insulin infusion: an approach to achieving normoglycaemia. *Br Med J* 1978;**i**:204–7.
3 Schiffrin A, Colle R, Belmonte M. Improved control in diabetes with continuous subcutaneous insulin infusion. *Diabetes Care* 1980;**3**:643–9.
4 Green SA. Pump therapy in children. *Diabetic Medicine* 1984;**1**:44–7.
5 Alberti KGMM, Nattrass M. Metabolic disorders of diabetes and their management. In: Besser M. *Advanced medicine 13*. Tunbridge Wells: Pitman Medical, 1977:173–89.
6 Ludvigsson J, Heding L, Lieden G, Marner B, Lernmark A. Plasmapheresis in the initial treatment of insulin-dependent diabetes mellitus in children. *Br Med J* 1983;**286**:176–8.
7 Lloyd B, Burrin J, Smythe P, Alberti KGMM. Enzymatic fluorimetric continuous-flow assays for blood glucose, lactate, pyruvate, alanine, glycerol and 3-hydroxybutyrate. *Clin Chem* 1978;**24**:1724–9.
8 Price CP, Lloyd B, Alberti KGMM. A kinetic spectrophotometric assay for rapid determination of acetoacetate in blood. *Clin Chem* 1977;**23**:1893–7.

[9] Hartog M, Gaafar MA, Meisser B, Fraser R. Immunoassay of serum growth hormone in acromegalic patients. *Br Med J* 1964;**ii:**1229–32.

[10] Heding LG. Radioimmunological determination of human C-peptide in serum. *Diabetologia* 1975;**11:**541–8.

[11] Armitage P. *Statistical methods in medical research*. Oxford: Blackwell Scientific, 1971:217–26.

[12] Foster KJ, Alberti KGMM, Hinks L, *et al*. Blood intermediary metabolite and insulin concentrations after an overnight fast: reference ranges for adults and interrelations. *Clin Chem* 1978;**24:**1568–72.

[13] Sonksen PH, Judd S, Lowy C. Home monitoring of blood glucose: new approach to management of insulin-dependent diabetic patients in Great Britain. *Diabetes Care* 1980;**3:**100–7.

[14] Citrin W, Ellis GJ, Skyler JS. Glycosylated haemoglobin: a tool in identifying psychological problems. *Diabetes Care* 1980; **3:**563–4.

[15] Werther GA, Jenkins PA, Turner RC, Baum JD. Twenty-four-hour metabolic profiles in diabetic children receiving insulin injections once or twice daily. *Br Med J* 1980;**281:**414–8.

[16] Griffin NK, Spanes A, Jenkins PA, Turner RC, Werther G, Baum JD. 24-hour metabolic profiles in diabetic children. *Arch Dis Child* 1980;**55:**112–7.

[17] Werther GA, Turner RC, Jenkins PA, Baum JD. Twenty-four-hour profiles of plasma C-peptide in type 1 (insulin dependent) diabetic children. *Diabetologia* 1982;**22:**245–9.

[18] Mann NP, Noronha JL, Johnston DI. A prospective study to evaluate the benefits of long-term self-monitoring of blood glucose in diabetic children. *Diabetes Care* 1984;**7:**322–6.

[19] Wildenhoff KE. Diurnal variations in the concentrations of blood acetoacetate, 3 hydroxybutyrate and glucose in normal persons. *Acta Med Scand* 1972;**191:**303–6.

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