Observations on Blood Lipid and Intermediary Metabolite Concentrations During Conventional Insulin Treatment or CSII

PAUL LAWSON, PHILIP D. HOME, AND RICHARD BERGENSTAL FOR THE KROC COLLABORATIVE STUDY GROUP

SUMMARY

An objective of The Kroc Collaborative Study Group trial of the feasibility of maintaining improved control of plasma glucose concentrations with continuous subcutaneous insulin infusion (CSII) was to test the nonglycemic aspects of metabolic control in relation to microvascular disease. Serum lipid levels were assessed in the 68 patients completing the 8-mo trial, before and after randomization to conventional insulin treatment (CIT) or CSII. During CSII, fasting serum cholesterol concentrations, normal at baseline (186 ± 7 mg/dl), were unchanged at 4 and 8 mo (183 ± 8 and 186 ± 10 mg/dl). Fasting serum triglyceride concentrations fell on treatment with CSII (baseline 90 ± 12 mg/dl, 8 mo 60 ± 7 mg/dl, $P < 0.01$), but were unchanged during CIT (baseline 88 ± 8 mg/dl, 8 mo 83 ± 10 mg/dl). Thirty-two patients in three centers had 24-h profiles of intermediary metabolites measured at baseline (0), 4, and 8 mo. Mean 24-h venous blood lactate levels fell during CSII (baseline 1.28 ± 0.12 mmol/L, 4 mo 0.99 ± 0.4 mmol/L, $P < 0.05$; 8 mo 1.05 ± 0.11 mmol/L), but blood alanine levels were unchanged. Venous blood 3-hydroxybutyrate fell from 0.12 ×/÷ 1.18 mmol/L at baseline to 0.06 ×/÷ 1.22 mmol/L at 8 mo during CSII ($P < 0.01$), mainly due to decreases at 0400 and 0600 h. Decreases in fasting serum triglyceride levels confirm previous investigations of insulin-dependent diabetic subjects treated with CSII; decreases of venous blood lactate and 3-hydroxybutyrate levels toward normal indicate that these metabolic effects of CSII recognized in short-term studies are sustained over an 8-mo period. DIABETES 1985; 34 (Suppl. 3):27–30.

In the therapy of diabetes mellitus, subcutaneous (s.c.) insulin injections are generally regarded as a means of controlling hyperglycemia in patients without endogenous insulin secretion. Although the plasma glucose concentration is clearly of physiologic importance in that it is normally a major regulator of insulin secretion, and is of clinical importance in causing symptoms through glycosuria, metabolically it is but one link in the network of biochemical pathways regulated by insulin.[1] It is possible to envisage that plasma glucose concentration per se could be an important factor in the pathogenesis of diabetic tissue damage, via nonenzymatic glycosylation for example,[2] but nearly all other mechanisms will involve abnormal substrate flux through the key metabolic pathways. This is most clearly the case for mechanisms of atherosclerosis.

It is therefore relevant to inquire as to whether other markers of metabolism, recognized often to be abnormal on conventional injection treatment (CIT), are improved during treatment with continuous subcutaneous insulin infusion (CSII). Periods of short-term treatment have suggested this to be the case,[3,4] but patient numbers were small, sampling infrequent, and the improvement often obtained on a baseline of very poor control on injection therapy. We have therefore measured blood intermediary metabolite and fasting lipid concentrations in patients taking part in the trial organized by The Kroc Collaborative Study Group.

MATERIALS AND METHODS

Patients. The protocol for selection, randomization into the two treatment groups, the clinical characteristics of the patients, and treatment procedures during the study have been given elsewhere[5] (see pages 5–12 and 13–16). Venous blood for fasting lipid levels was obtained at baseline (0), 4, and 8 mo before breakfast and after the bedtime snack in 68 patients. In addition, three of the clinical centers (UWO, Chicago, and Guy's) provided samples for the analysis of intermediary metabolite concentrations from a total of 32 patients, forming equal subgroups of each of the two treatment groups in the study (CSII and CIT). The glycemic characteristics of these patients, including the mean values from the in-hospital diurnal plasma glucose profiles, did not differ from those of the whole group[5] (see pages 22–26).

From the Department of Medicine, Royal Victoria Infirmary, Newcastle upon Tyne, United Kingdom (P.D.H.); Hammersmith Hospital, London, United Kingdom (P.L.); and University of Chicago, Chicago, Illinois (R.B.).
Address reprint requests to Dr. Philip Home, Department of Medicine, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP, United Kingdom.

TABLE 1
Fasting serum cholesterol and triglyceride concentrations in 68 patients with insulin-dependent diabetes mellitus randomized to CIT and CSII (mean ± SEM)

	Month*	CSII	CIT
Serum cholesterol (mg/dl)	0	187 ± 7	183 ± 9
	4	183 ± 8	185 ± 8
	8	186 ± 10	187 ± 9
Serum triglycerides (mg/dl)	0	90 ± 12	88 ± 8
	4	70 ± 7	75 ± 7
	8	60 ± 7†	83 ± 10

*Baseline (0 mo) observations were obtained before randomization.
†$P < 0.01$ compared with 0 mo.
Normal ranges: fasting serum cholesterol concentrations (<251 mg/dl) and fasting triglyceride concentrations (35–175 mg/dl).
Other definitions can be found in Table 2.

Protocol. Patients were admitted to hospital at baseline (0 mo) and at 4 and 8 mo of treatment. A fasting venous blood sample was obtained for lipid analysis. The serum was separated within an hour, and was stored at −20°C. Blood for intermediary metabolite concentrations was drawn before each of the three main meals and bedtime snack, 90 min after the three main meals, and twice-hourly from midnight to 0600 h. Blood samples (0.50 ml) were immediately deproteinized in 0.3 mmol/L perchloric acid (4°C) in water, and stored at 4°C until centrifugation within 24 h. The supernatant was then deep frozen (−20°C). Blood for plasma glucose analysis was drawn into fluoride-oxalate tubes at the same time points as for the metabolite estimations.

Analytic techniques. Frozen samples were air-freighted in dry ice to a central laboratory for analysis (Royal Victoria Infirmary, Newcastle upon Tyne, United Kingdom, see pages 17–21). Blood lactate, alanine, and 3-hydroxybutyrate concentrations were measured by standard fluorimetric autoanalyzer techniques,[6] with interassay coefficients of variation (CV) of 5.5%, 9.2%, and 6.0%, respectively, in the assay range used in this study. Plasma glucose was analyzed by a glucose-oxidase method (GOD-PAP, Boehringer Mannheim, Mannheim, FRG) (CV 1.9% at normal fasting levels), serum total cholesterol by a cholesterol esterase/oxidase method on a Technicon II autoanalyzer (CV 1.9%), and serum triglyceride as glycerol by a fully enzymatic method (Boehringer Mannheim, CV 12.6%) on the same system. Lipoprotein measurements were not made in this study.

Statistical analysis. Estimates of blood 3-hydroxybutyrate concentrations were log-transformed before analysis to correct skewed distributions. Statistical significance was sought using analysis of variance and Student's *t*-test, the latter without correction for multiple testing in view of the lack of independence of successive observations in each patient. Twenty-four-hour mean intermediary metabolite concentrations were calculated as the mean of the 11 estimations on each patient (cf., mean plasma glucose) with no weighting.[7]

RESULTS

Serum lipids. Fasting serum total cholesterol concentration was similar, and did not change, in the two treatment groups throughout the study (Table 1).

In contrast, fasting serum triglyceride concentrations, similar at baseline (Table 1), fell during treatment with CSII and were significantly lower by month 8 (0.7 ± 0.1 versus 1.0 ± 0.1 mmol/L, $P < 0.01$, Table 1).

Blood intermediary metabolites. The mean data for blood lactate, alanine, and 3-hydroxybutyrate are given in Table 2, with (to aid interpretation) the plasma glucose results as determined by the central laboratory for the 32 patients concerned. Differences in plasma glucose paralleled those of the whole study[5] (see pages 22–26). The mean of the diurnal

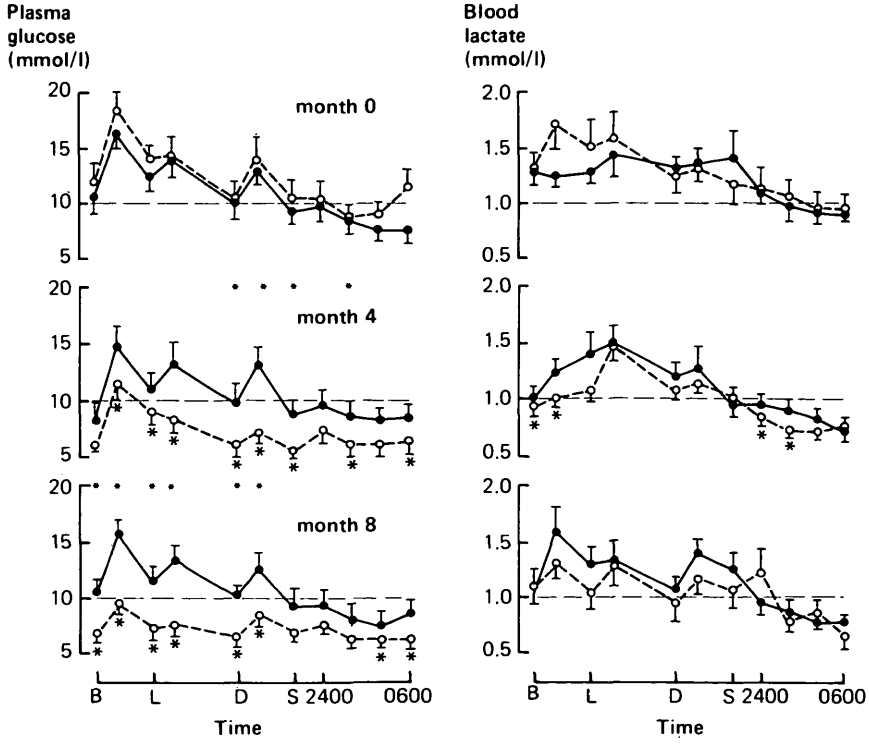

FIGURE 1. Plasma glucose (left) and blood lactate (right) concentrations at baseline (0 mo, upper panel) and after 4 and 8 mo (middle and lower panels) of treatment by conventional insulin treatment (CIT) (●——●) or CSII (○——○). Baseline observations were obtained before randomization. Observations were made before and 90 min after breakfast (B), lunch (L), and the main evening meal (D), before the evening snack (S), and at the times indicated during the night. Mean ± SEM. Difference ($P < 0.05$) between treatments is indicated by a small star (*); difference from baseline at month 4 or month 8 during CSII is indicated by a large star (★).

TABLE 2
Twenty-four-hour mean plasma glucose and blood lactate, alanine, and 3-hydroxybutyrate concentrations in 32 insulin-treated patients with insulin-dependent diabetes mellitus randomized to CIT and CSII (mean ± SEM)

	Month*	CSII	CIT
Plasma glucose (mg/dl)	0	221 ± 18	194 ± 16
	4	135 ± 9‡,¶	187 ± 14
	8	130 ± 5‡,#	189 ± 13
Lactate (mmol/L)	0	1.28 ± 0.12	1.21 ± 0.11
	4	0.99 ± 0.04‖	1.09 ± 0.9
	8	1.05 ± 0.11	1.11 ± 0.9
Alanine (mmol/L)	0	0.32 ± 0.02	0.32 ± 0.03
	4	0.33 ± 0.02	0.31 ± 0.02
	8	0.36 ± 0.02	0.35 ± 0.04
3-Hydroxybutyrate (mmol/L)†	0	0.12 ×/÷ 1.18	0.11 ×/÷ 1.14
	4	0.07 ×/÷ 1.26	0.08 ×/÷ 1.08§
	8	0.06 ×/÷ 1.22§	0.09 ×/÷ 1.14

*Baseline (0 mo) observations were obtained before randomization.
†3-Hydroxybutyrate concentrations were log-transformed before statistical analysis.
‡$P < 0.001$, §$P < 0.01$, and ‖$P < 0.05$ compared with month 0.
¶$P < 0.001$, and #$P < 0.01$ compared with CIT.
CSII, continuous subcutaneous insulin infusion; and CIT, conventional insulin treatment.

blood lactate estimations did not differ between the treatment groups at baseline (Table 2) but fell significantly by 4 mo during CSII (1.28 ± 0.1 to 0.99 ± 0.04 mmol/L, $P < 0.05$). This decrement, though maintained at 8 mo (1.05 ± 0.11 mmol/L) was not statistically different from baseline. None of the means at any single time point over the course of diurnal sampling during CSII was significantly lower than those obtained during CIT. The diurnal profiles of blood lactate concentrations, in contrast to those of plasma glucose (Figure 1) showed little change with treatment, the trend to improvement during CSII not conforming to any pattern in relation to sampling before and after meals, through the course of the night or in the fasting state.

Blood alanine levels did not differ between treatments at any time during the study (Table 2) and were not changed during CSII.

As one might expect, blood 3-hydroxybutyrate diurnal profiles at baseline were typical of patients treated with twice-daily injections (Figure 2).[8] At 4 mo there was little difference in values between the treatment groups, but at 8 mo the pattern observed at baseline had become reestablished during CIT so that values before and after breakfast, after lunch, and at 0600 h were significantly higher than those during CSII ($P < 0.05$). Indeed, at 8 mo, CSII was associated with a suppression of mean 3-hydroxybutyrate concentrations to below 0.1 mmol/L at all sampling times, so that the mean concentration over the 24-h period was significantly lower than at baseline ($P < 0.01$, Table 2).

DISCUSSION

Although control (nondiabetic) data were not collected as part of the Kroc Study[5] (see pages 5–12), it is clear that plasma glucose concentrations were not completely normalized in the majority of patients. Nevertheless, with only two patients in the whole study having elevated serum cholesterol concentrations at baseline, it is perhaps not surprising that mean concentrations remained well within the normal range and were unchanged on CSII. Serum triglycerides are more sensitive to poor diabetic control,[9] but again only five patients were above acceptable limits (160 mg/dl). Nevertheless, mean concentrations of 89 ± 12 mg/dl (Table 1) were unchanged on CIT, but clearly reduced by CSII.

Short-term studies have previously demonstrated the reduction of serum cholesterol and triglyceride concentrations with improved blood glucose control.[4,9] This reduction in serum lipids has been from much higher values than seen at baseline in the current study, however, and it has been pointed out that serum total cholesterol concentrations do not change significantly except in those patients with very poor control initially.[9] Triglyceride concentrations are more sensitive to disturbances of blood glucose control and can be demonstrated to fall even with quite small reductions in moderately elevated plasma glucose levels.[9] The present data indicate that the previously reported fall to normal concentrations[4] was maintained for at least 8 mo. It should, however, be recognized that the major part of fasting serum triglycerides is rapidly turning over as VLDL-triglycerides,[9] and that normal serum concentrations could still represent abnormal rates of lipoprotein metabolism.

The data on intermediary metabolites are more difficult to interpret. In the same assay, normal 24-h mean venous blood

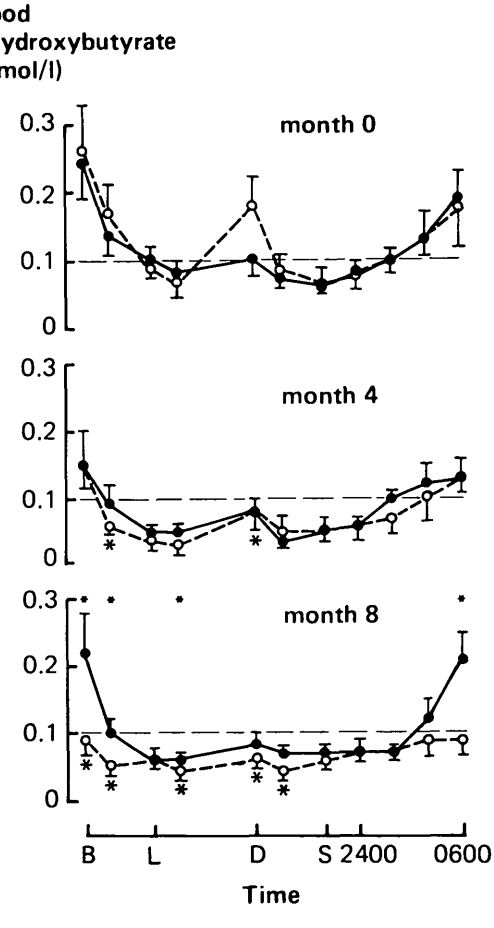

FIGURE 2. Blood 3-hydroxybutyrate concentrations at baseline (0 mo, upper panel) and after 4 and 8 mo (middle and lower panels) of treatment by CIT (●——●) or CSII (○——○). Other details and abbreviations are as for Figure 1.

lactate concentrations were 0.76 ± 0.4 mmol/L, as determined in a local population of mixed sex in the same age range as the study group.[8] Baseline blood concentrations in the Kroc Study subgroup of patients are clearly elevated therefore, and fall during CSII to levels previously reported after intensification of insulin injection therapy.[8,10] Although the scatter of observations between patients meant that concentrations were never significantly lower on CSII than those on CIT, this only underlines the continuing abnormality of lactate levels noted previously after CSII.[11] It has been suggested that this may be related to peripheral, rather than portal, insulin administration,[11,12] but until more physiologic plasma insulin profiles are achieved in the insulin-treated patient, it is perhaps unwise to ascribe the cause of peripheral hyperinsulinemia,[13] decreased insulin sensitivity,[14] and continued metabolic disturbance solely to the systemic route of insulin delivery.

Unlike blood lactate and branched-chain amino acids,[4] blood alanine concentrations are usually normal in patients with insulin-dependent diabetes considered to have average blood glucose control, and do not change on intensification of therapy or normalization of plasma glucose with a plasma glucose-controlled insulin infusion system (artificial pancreas).[8] The present study confirms these observations and shows them to be sustained over an 8-mo period of time.

Venous blood 3-hydroxybutyrate concentrations were significantly reduced toward normal (24-h mean 0.04 ×/÷ 1.31 mmol/L)[9] during CSII at 8 mo, although 0600-h and prebreakfast concentrations were not clearly different from CIT at 4 mo (Figure 2). At this time point, intermediate in the course of treatment phase, the rise in plasma glucose concentrations during CSII after breakfast (Figure 1) is similar to that seen on CIT, suggesting underinsulinization in the latter part of the night. Overall outpatient blood glucose control was, however, significantly different at this time.[5] Although the 3-hydroxybutyrate diurnal profile during CSII at 8 mo does not show the normal physiologic fall in the latter part of the night,[8] levels are much more stable than the rapidly fluctuating concentrations on CIT. During a short-term (24 h) normalization of plasma glucose, a plateau of 3-hydroxybutyrate concentrations is observed during the night[8] that is at much higher levels than reported here. It would, therefore, appear that longer periods of near-normoglycemia, such as we have achieved, are needed to correct ketone body metabolism as reflected by venous blood 3-hydroxybutyrate concentrations.

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