A circadian rhythm in lipid mobilization which is altered in IDDM

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Summary It is not clear how circadian lipolysis and circulating concentrations of non-esterified fatty acids (NEFA) are altered in intensively treated insulin-dependent diabetic (IDDM) patients. Ten IDDM patients on an intensive insulin regimen and eight healthy control subjects were investigated under ordinary living conditions for 27 h by microdialysis of subcutaneous adipose tissue. The true tissue glycerol concentration and adipose blood flow changes were monitored as an index of lipolysis. A circadian pattern in adipose tissue lipolysis was observed in both groups, decreasing during the day and increasing during evening-night. The daytime decrease was normal, but the evening-night rise was elevated in IDDM ($p = 0.03$). Circulating NEFA decreased during the day and increased at night. The latter increase was enhanced threefold in IDDM ($p = 0.003$) and

correlated with fasting glucose levels ($r = 0.77$). Nocturnal growth hormone (GH) was increased fivefold in IDDM and correlated to nocturnal lipolysis ($r = 0.83$). Adipose tissue blood flow increased during the night in a similar fashion in both groups. Near-normalization of glucose for 24 h in IDDM did not affect the nocturnal increases in NEFA, GH and lipolysis. In conclusion, a circadian rhythm in lipolysis was found. Increased lipolytic rates during evening-night may at least in part raise nocturnal circulating NEFA. Nocturnal NEFA and lipolysis are further enhanced in IDDM, maybe due to elevated GH, but not to insulinopenia or hyperglycaemia. [Diabetologia (1997) 40: 1070–1078]

Keywords Microdialysis, glycerol, non-esterified fatty acids, circadian rhythm, growth hormone.

Although insulin-dependent diabetes mellitus (IDDM) is accompanied by several metabolic abnormalities [1], metabolic control in clinical practice is focused on glycaemic control. Insulin influences the circulating non-esterified fatty acids (NEFA) via its antilipolytic action in adipose tissue. Circulating NEFA are elevated in untreated IDDM, a finding attributed to insulinopenia [2–4]. Insulin therapy

normalizes plasma NEFA [4–6], at least during the day. In normal and IDDM subjects NEFA increase at night [7–9]. The mechanisms underlying this and the influence of IDDM are not understood. Growth hormone (GH) may be involved, since it regulates nocturnal lipolysis [8, 10] and is elevated in IDDM [4, 11, 12]. An increase in NEFA may lead to a number of abnormalities, as discussed in detail elsewhere [13]. In many cases blood glucose levels are poorly controlled at night in IDDM [14]. This might also be influenced by NEFA, which compete with glucose in muscle metabolism and stimulate liver gluconeogenesis [15].

It is possible to monitor continuously the lipolysis end-product glycerol in adipose tissue with microdialysis [16, 17]. Small microdialysis tubes are placed in the interstitial space of subcutaneous adipose tissue and slowly perfused with a neutral solvent. The

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Abbreviations: IDDM, Insulin-dependent diabetes mellitus; NEFA, non-esterified fatty acids; GH, growth hormone; CV, coefficient of variation; AUC, area under the curve; ANOVA, analysis of variance.

Table 1. Clinical characteristics of patients and healthy control subjects

	IDDM	IDDM re-investigated	Control subjects
Sex (male/female)	5/5	3/4	3/5
Age (years)	31 ± 2 (20–49)	33 ± 3 (23–49)	41 ± 4 (28–66)
BMI (kg/m ²)	23.3 ± 0.5 (20.4–24.8)	23.4 ± 0.6 (20.4–24.8)	21.9 ± 1.0 (18.8–26.5)
Diabetes duration (years)	22 ± 3 (8–36)	22 ± 4 (10–36)	
Insulin dose (IU/kg × 24 h)	0.7 ± 0.05 (0.4–1.0)	0.6 ± 0.07 (0.4–1.0)	
HbA _{1c} (%) ^a	7.6 ± 0.2 (6.1–8.8)	7.6 ± 0.3 (6.1–8.8)	

Mean ± SEM. Range in parentheses

^a HbA_{1c} normal range 3.5–5 %

outgoing dialysate will reflect the composition of the interstitial fluid due to the diffusion of substances across the dialysis membrane. The microdialysis technique can also be used over a long period of time for continuous monitoring of the true metabolite concentration in the interstitial space of subcutaneous adipose tissue in ambulatory IDDM patients [18] and the variations in adipose tissue blood flow [19]. The latter is important for lipolysis since the local blood flow rate may influence the mobilization of lipids from adipose tissue [20]. Using microdialysis, in this study we have monitored the circadian adipose tissue glycerol and blood flow variations (lipolysis index) during ambulatory conditions in control and IDDM subjects and compared them to the circulating NEFA.

Subjects and methods

Subjects. Ten non-obese patients with IDDM and eight age-matched, non-obese, drug-free healthy subjects were studied (Table 1). Background retinopathy in nine patients and microalbuminuria in one were the only evident major diabetic complications. All patients received multiple insulin injection therapy (4–5 doses). They were otherwise healthy and took no other medication. The study was approved by the ethics committee at Huddinge Hospital. Informed consent was obtained.

Microdialysis device. The microdialysis catheter has been described in detail previously [21]. Briefly, a semi-permeable membrane (30 × 0.62 mm, molecular cut-off 20 000 kDa) is glued to the end of a double-lumen cannula. The perfusion fluid enters the device through the outer cannula, streams down to the microdialysis membrane and leaves through an inner cannula, from which it is collected. An exchange of substances in the extracellular fluid takes place over the membrane [16].

Study protocol. The study started at 07.30 hours on day 1. The subjects had breakfast and took morning insulin at home. A cubital vein catheter was inserted for blood sampling. Two microdialysis catheters were introduced percutaneously into the periumbilical subcutaneous adipose tissue and continuously perfused with sterile Ringer's solution [22] alone or with the addition of ethanol (50 mmol/l). Portable microinfusion pumps (MiniMed 504; Minimed Technologies, Sylmar, Calif., USA) at flow rates of 0.5 µl/min (Ringer catheter) and 5 µl/min (Ringer-ethanol catheter) were used. Fractions of dialysate were sampled for the analyses of glucose, glycerol and ethanol (1-h

samples from 08.00 hours to 21.00 hours and 2-h samples during the night in order to permit the subjects to sleep, despite sampling). The ratio of ethanol in the outgoing vs the ingoing solvent was calculated for the ethanol-Ringer catheter and reflects indirectly qualitative variations in adipose tissue blood flow [23]. Previous investigations have directly compared the microdialysis and Xenon techniques to measure blood flow variations in adipose tissue; an excellent correlation was observed [24].

Glucose, glycerol and NEFA were determined in venous blood obtained in the middle of each dialysate sampling period. Nighttime concentrations of plasma GH and serum free immunoreactive insulin were determined in the seven patients who were re-investigated (see below) with an intravenous insulin infusion and in six control subjects.

The study conditions were standardized for meals and snacks: lunch at 11.30 hours, afternoon snack at 14.00 hours, dinner at 16.30 hours, evening snack at 19.00 hours and breakfast day 2 at 07.30 hours. The portable pumps allowed the subjects to move freely between the sampling times, but physical activity was restricted to short walks inside the hospital. All subjects were at rest in bed from 22.00 hours and slept through the night.

Seven of the IDDM patients with clinical characteristics similar to those in the whole group (Table 1) were re-investigated 2–6 months after the first study. All had stable body weight and had remained at their previous level of physical activity. The patients reported to the diabetes unit at 20.00 hours on the day before the study. They received a variable intravenous insulin infusion (Actrapid; Novo Nordisk, Copenhagen, Denmark) which was adjusted to achieve euglycaemia (5–7 mmol/l). The microdialysis catheters and the venous plasma cannula were inserted and sampling and schedule for physical activity, meals and sleep were performed exactly as described above. According to the results of frequent venous blood glucose analyses (HemoCue; HemoCue AB, Ängelholm, Sweden) the intravenous insulin infusion was varied to obtain sustained circadian normoglycaemia throughout the study period. Hypoglycaemia was avoided in all patients.

Separate experiments were performed to calculate the adipose tissue interstitial glycerol recovery using 1-¹⁴C glycerol [25]. The fluxes of endogenous and isotopic glycerol over the membrane were assumed to be equal. Three healthy, normal-weight subjects were microdialysed with Ringer's solution, supplemented with 1500 cpm/µl of 1-¹⁴C glycerol (Amersham Sweden AB, Solna, Sweden). Dialysate was sampled in 45-min fractions during 270 min. The recovery (in%) was calculated as

$$\frac{100 \times (\text{cpm}_{\text{in}} - \text{cpm}_{\text{out}})}{\text{cpm}_{\text{in}}}$$

where cpm_{in} is the radioactivity in the ingoing perfusate and cpm_{out} is the radioactivity in the dialysate.

Chemical analyses. Plasma and dialysate glucose concentrations were determined by a glucose oxidase method [26] (coefficient of variation (CV) 5 % for both). The dialysate glycerol concentration was assessed by a commercial colorimetric method (Randox Laboratories Ltd, Ardmore, UK) (CV 5 %). The intraindividual CV for adipose tissue dialysate glycerol sampled with microdialysis was $6 \pm 2\%$ in previous experiments [27]. Plasma glycerol was determined by bioluminescence [28] (CV < 3.7 %), NEFA by an enzymatic colorimetric test-kit (Wako Chemicals, GmbH, Neuss, Germany) (CV < 2.7 %), and ethanol by an enzymatic, fluorometric method [29] (CV 2 %). Free insulin in serum was determined by a radioimmunoassay kit (Pharmacia, Uppsala, Sweden) after precipitation with polyethylene glycol. The lower limit of the assay was 12 pmol/l (all samples in subjects and patients were above this limit) and the CV was less than 6 %. Plasma GH was analysed by a commercial fluoroimmunoassay, Delfia (Wallac Oy, Turku, Finland), using international reference standard 80/505 and the CV was 6.7 %.

Statistical analysis. The circadian variations were evaluated by analysis of variance corrected for repeated measurements (ANOVA) for comparison throughout the circadian profiles, using one- or two-factor analysis, respectively, for comparison within the group or between the groups. Data were analysed at the following time intervals: 09.00 hours – 20.00 hours (day/diurnal), 20.00 hours – 08.00 hours (night/nocturnal), and 09.00 hours – 08.00 hours (circadian) unless stated otherwise. The area under the curve (AUC) was calculated by a trapezoidal method. Mean values and AUCs were compared using the Student's unpaired or paired *t*-test, when applicable. The respective increase in NEFA and dialysate glycerol during the afternoon was compared with Wilcoxon's signed rank-test. The GH concentrations were compared using the Mann-Whitney rank-test for non-paired values because the concentrations were not normally distributed. Linear correlation analysis by the method of least square was performed. Values are expressed as means $\pm$ SEM.

Results

Findings with metabolites. The circadian plasma glucose profiles for the two groups are shown in Figure 1A. Glucose was increased in the IDDM patients during the day and there was a further rise in glucose concentrations during the night in IDDM (one-factor ANOVA $F = 1.8$, $p = 0.03$). The concentrations of dialysate glucose were almost identical with the respective plasma concentrations throughout the experimental period in both groups (dialysate concentrations not shown). The tissue dialysate glucose concentration was $92 \pm 3\%$ of the respective plasma concentration confirming earlier results [18, 22] and indicating a complete or near-complete recovery of glucose in the dialysate.

Dialysate glycerol concentrations, are shown in Figure 1B. The mean calculated rate of recovery of glycerol obtained from the isotope experiments was $94.5 \pm 2.6\%$, indicating almost complete recovery.

Fig 1A–C. Circadian variations in plasma glucose, dialysate glycerol and serum NEFA. Microdialysis catheters were inserted in the abdominal subcutaneous tissue and were continuously perfused. Dialysate was sampled in 1–2 h fractions for the analysis of glucose (not shown) and glycerol. Blood sampling was performed in the middle of each dialysate fraction. The circulating glucose (A), dialysate glycerol (B) and serum NEFA (C) is shown. Values are mean $\pm$ SEM in 10 IDDM patients (□) and 8 healthy controls (●). Arrows indicate meals

The tissue dialysate glycerol in both groups decreased from noon to a nadir at 20.30 hours in the healthy control subjects and at 18.30 hours in the IDDM group. Thereafter, a gradual increase in the tissue glycerol was observed throughout the night. This circadian pattern differed significantly between the two groups ($F=7.2$, $p=0.0001$ by two-factor ANOVA). Dialysate glycerol began to increase earlier in the evening in IDDM as compared with the control state. The mean nocturnal tissue dialysate glycerol concentrations were higher in IDDM ($277 \pm 30 \mu\text{mol/l}$) than in the control state ($181 \pm 24 \mu\text{mol/l}$; $t=2.4$, $p=0.03$). However, the mean diurnal dialysate glycerol concentrations were similar in the two groups (IDDM 211 ± 25 vs control $238 \pm 31 \mu\text{mol/l}$, $t=0.7$, $p=0.49$). The plasma concentrations were one third to one sixth of the concentrations in the tissue dialysate confirming earlier results of microdialysis [27, 30, 31]. Increased nocturnal plasma glycerol levels were found in the IDDM patients but there was no difference between the groups during the day (two-factor ANOVA 20.00–08.00 hours $F=2.6$, $p=0.02$, 09.00–20.00 hours $F=0.52$, $p=0.88$).

The circadian patterns of circulating NEFA are shown in Figure 1C. During the day there were distinct preprandial increases and postprandial decreases in the concentrations of NEFA, which were similar in both groups (two-way ANOVA 09.00–20.00 hours $F=1.99$, $p=0.04$). The daytime NEFA pattern differed from the respective pattern of dialysate glycerol; in the afternoon (13.30–16.30 hours) the relative increase in circulating NEFA concentrations was larger than the respective change in dialysate glycerol concentrations (IDDM: NEFA $75 \pm 31\%$ vs dialysate glycerol $7 \pm 14\%$, $p=0.02$, control subjects: NEFA $154 \pm 64\%$ vs dialysate glycerol $-21 \pm 9\%$, $p=0.02$). The diurnal mean level of NEFA or the area under the NEFA diurnal curve did not differ significantly between the groups (values not shown). However, there were pronounced differences in nocturnal NEFA. In the IDDM group, NEFA increased continuously during the night whereas in the control group, NEFA peaked at 02.00 hours and then gradually decreased (two-factor ANOVA 20.00–08.00 hours $F=3.71$, $p=0.003$). The night-time mean NEFA was 0.41 ± 0.05 in IDDM vs $0.26 \pm 0.03 \text{ mmol/l}$ in control subjects ($t=2.44$, $p=0.03$). A similar difference was observed in night-time AUC for NEFA. The night-time AUC for NEFA and the dialysate glycerol were positively correlated in IDDM ($r=0.75$, $p=0.02$). The concentrations of the fasting NEFA and the AUC of night-time NEFA correlated positively to the concentrations of the fasting plasma glucose in IDDM ($r=0.77$, $p=0.01$ and 0.75 , $p=0.01$, respectively). No significant correlations between these measures were found in the control group.

In order to investigate the role of glucose control on lipolysis in IDDM the patients were divided into

two groups on the basis of HbA_{1c} (figure not shown). The dialysate glycerol concentrations did not differ between the patients with poor or average glycaemic control ($\text{HbA}_{1c} > 7.6\%$, $n=6$) and those with good glycaemic control ($\text{HbA}_{1c} < 7.6\%$, $n=4$) (ANOVA 2-factor repeated measurement $F=1.3$, $p=0.2$). Also the insulin requirements were similar in these two groups (0.72 ± 0.06 vs $0.63 \pm 0.20 \text{ IU/kg}$, $t=0.8$, $p=0.5$). Age and diabetes duration did not differ significantly between the groups (data not shown).

Seven IDDM patients were re-investigated during a 24-h intravenous insulin infusion (Fig. 2). The metabolic profiles in the first and second investigations were compared. With the intravenous insulin infusion, a marked improvement in the circadian glucose profile was achieved (circadian two-factor ANOVA $F=2.7$, $p=0.0005$) and the nocturnal hyperglycaemia observed in the first study was avoided (night-time two-way ANOVA $F=5.6$, $p=0.0001$). Plasma glucose is shown in Figure 2A, glucose values during intravenous insulin infusion were kept at 5.9–8.0 mmol/l. Similar results were obtained with dialysate glucose (figure not shown).

However, there was no significant difference in the overall dialysate glycerol pattern between the two investigations (circadian two-factor ANOVA $F=1.02$, $p=0.4$). Nor were there any significant differences in the dialysate glycerol between the two investigations when day- and night-time values were investigated separately. The circulating glycerol concentrations were also not affected by the intravenous insulin infusion (data not shown). Intravenous insulin administration lowered the concentrations of circulating NEFA during the daytime, as compared to the first study (two-factor ANOVA F -test 3.23, $p=0.003$). However, the night-time concentrations of NEFA were not affected by intravenous insulin (two-factor ANOVA $F=0.69$, $p=0.66$; Fig. 2C). Lastly, compared to control subjects mean nocturnal NEFA levels were still increased in the IDDM patients receiving intravenous insulin (0.26 ± 0.03 vs $0.34 \pm 0.02 \text{ mmol/l}$, $t=2.54$, $p=0.025$).

Findings with blood flow. Figure 3 shows the dialysate ethanol outflow/inflow ratio. In both groups, a significant decrease in the ratio, indicating an increased ethanol escape (blood flow) was observed during the night (circadian one-factor ANOVA control group $F=2.66$, $p=0.002$, IDDM group $F=4.92$, $p=0.0001$; this did not differ between the two groups ($F=0.71$, $p=0.79$ by two-factor ANOVA) (Fig. 3A). In the re-investigated IDDM patients, the ethanol escape increased markedly during the day (09.00–20.00 hours) when insulin was given intravenously, as judged by the reduction in the ethanol outflow/inflow ratio (mean ethanol ratio daytime, intravenous insulin 0.57 ± 0.03 vs subcutaneous insulin 0.70 ± 0.03 ,

Fig 2A-C. Circadian patterns of venous plasma glucose (A), adipose tissue glycerol (B) and serum concentrations of NEFA (C) are presented in seven IDDM patients receiving their regular s.c. insulin regimen (●) or a variable intravenous insulin infusion when re-investigated (○). Microdialysis sampling was performed as described in the legend to Figure 1

Fig 3A, B. Circadian variations in the ethanol escape in patients with IDDM (○) and healthy control subjects (●). Microdialysis sampling was performed as described in the legend to Figure 1 and the ethanol out/inflow ratio in all of the IDDM patients and in six of the control subjects is presented in A. The dialysis solvent was supplied with ethanol (50 mmol/l). Changes in the outflow/inflow ratio reflect variations in the local blood flow. Post-hoc analysis of the one-way ANOVA revealed a significant difference ($p < 0.05$) between the time intervals 09.00–20.00 hours and 03.00–07.00 hours in the IDDM patients and from 09.00–23.00 hours and 01.00–07.00 hours in the control group. In B, the circadian pattern of the ethanol out/inflow ratio in response to intravenous insulin (○) or s.c. insulin (●) is shown ($n = 7$)

$t = 5.54$, $p = 0.002$; Fig. 3B). The night-time decrease in ethanol ratio was maintained when insulin was administered intravenously (one-factor ANOVA intravenous insulin $F = 1.96$, $p = 0.03$). The mean night-time ethanol ratios in the two investigations did not differ significantly (intravenous insulin 0.52 ± 0.03 vs subcutaneous insulin 0.64 ± 0.04 , $p = 0.07$).

Findings with hormones. The free insulin concentrations in serum and plasma GH concentrations are shown in Figure 4. IDDM patients on subcutaneous insulin had lower free insulin concentrations than the control subjects in the early night hours, but the concentrations were normalized with intravenous insulin. GH concentrations were higher in the IDDM patients from 20.30–02.00 hours. ($p = 0.005–0.04$), than in the control subjects. This difference remained when insulin was given intravenously. In IDDM patients, the mean nocturnal GH (20.30–06.00 hours) correlated to the corresponding mean nocturnal dialysate glycerol ($r = 0.83$, $p = 0.04$) but not to HbA_{1c} or fasting glucose concentration.

Comparison of findings at 08.00–11.00 hours day 1 and 2. It is seen in Figure 1 that plasma glucose in diabetic subjects and dialysate glycerol in control and diabetic subjects had somewhat different kinetic profiles between 08.00 and 11.00 hours on days 1 and 2. This is most likely due to different procedures at these two occasions. On day 1 all subjects arrived at the hospital in a non-standardized fashion except fasting overnight. On day 2 they were standardized as regards time in bed during the night, wake-up and breakfast.

Discussion

In the present study, adipose tissue lipolysis has been investigated in vivo under normal living conditions. This was done with a relatively new technique, microdialysis, which permits continuous monitoring of glycerol and blood flow variations in subcutaneous adipose tissue [16, 17]. In theory, variations in glycerol in the interstitial space are determined by three major factors: local production, local utilization and removal by blood flow. Fat tissue has an insignificant re-utilization of glycerol. Thus, production by lipolysis and removal by blood flow are the only important factors controlling the level of glycerol in adipose tissue. The method used in this study determines only relative changes in lipolysis and blood flow. Alternative techniques exist for measuring true lipolysis and blood flow rates, such as isotope infusion or arteriovenous cannulation, in combination with Xenon clearance (for tissue blood flow). Unfortunately it is impossible to use such methods under present experimental conditions (ambulatory measurements for 24 h).

Our study shows a distinct circadian rhythm of lipolysis, which is altered in IDDM. When data for adipose tissue glycerol and ethanol ratio are considered together, it appears that the lipolytic activity gradually declines during the day and increases at night. The pattern is different for circulating NEFA, which involves a distinct meal-suppression and prandial

Fig 4A, B. Nocturnal concentrations of free insulin (A) and GH (B) in seven IDDM patients receiving their regular s.c. insulin regimen (●) or a variable i.v. insulin infusion when re-investigated (○) and in six healthy control subjects (▲). (A) IDDM patients on s.c. insulin vs control subjects; ANOVA $F = 4.1$, $p = 0.003$. (B) * $p < 0.05$, ** $p < 0.01$ for differences in GH between IDDM patients on s.c. insulin and the control subjects

increase in the day and an increase during the night. If we assume that adipose tissue is the major delivery source of NEFA, it seems reasonable to suggest that several factors control the circulating NEFA level in the day, whereas adipose tissue lipolysis is a major determinant at night. It should be noted that only one subcutaneous adipose tissue depot was investigated. Large regional variations are known to exist between various subcutaneous fat depots and between subcutaneous and inner (i.e. visceral) fat depots [32]. Unfortunately, the latter adipose tissue region cannot be investigated in a clinical setting. However, NEFA release from the presently investigated adipose tissue

region correlates strongly to mean arterial NEFA concentrations indicating that this fat depot is representative for the whole body [33]. Hence, regional variations may be of less importance than previously believed.

As noted by others [16, 30, 31], we found that the concentration of glycerol in adipose tissue was much higher than that in plasma. A gradual increase in adipose tissue blood flow occurred during the night, in accordance with findings in the subcutaneous tissue of the lower limb [34]. Increased blood flow accelerates removal of glycerol from the extracellular space of adipose tissue [19, 23]. Whether the same is true for NEFA remains to be established. As discussed in detail [23], water-insoluble NEFA may be even more sensitive to changes in blood flow than water soluble glycerol. Local re-esterification of NEFA could also influence NEFA mobilization, besides lipolysis and blood flow, although a recent study has shown that re-esterification does not occur to a significant extent in the presently investigated major fat depot [33]. Thus, increased rates of nocturnal lipolysis and blood flow in subcutaneous adipose tissue could together be responsible for elevated concentrations at NEFA at night. A constant rate of glycerol turnover in vivo during the night has been demonstrated in healthy subjects [9, 10]. Methodological factors may explain the divergence in results. Glycerol turnover in plasma indirectly measures the overall rate of lipolysis in all adipose tissue regions. We measured lipolysis directly in the major fat depot (abdominal subcutaneous fat).

Of clear clinical significance is the observation that the circadian lipolytic rhythm was markedly altered in IDDM. Although adipose glycerol concentrations and circulating NEFA concentrations were normal during the day, they were markedly elevated between 20.00 hours and 08.00 hours, which is half of the circadian period. Plasma glycerol was also elevated during the evening-night in IDDM. Since there was no significant difference between control and IDDM subjects in the nocturnal relative increase in the adipose tissue blood flow, it is likely that the increase in the rate of adipose lipolysis to some extent explains the evening and nocturnal increases in circulating NEFA in IDDM. However, other changes in NEFA turnover (e.g. the degree of re-esterification and regional variations in adipose tissue lipolysis) may also contribute since the night-time pattern of NEFA (but not of adipose tissue glycerol) differed between control and IDDM subjects. It seems unlikely that the microdialysis method fails to detect rapid changes in tissue glycerol. Previous experiments (exercise, insulin infusions) have shown an excellent correlation between temporal changes in adipose tissue and plasma glycerol [27, 35, 36].

The above findings clearly emphasize the importance of studying IDDM patients not only during the day. Normal lipolysis rates and circulating NEFA

concentrations in adequately controlled IDDM patients have been demonstrated in the daytime [5, 6]. Our patients, who also were fairly well controlled, also had normal values during the first half of the day.

An elevation in the circulating NEFA at night may impair glucose metabolism, through mechanisms discussed earlier in this paper. Indeed, we found a strong correlation ($r = 0.8$) between the fasting and night-time concentrations of NEFA and the fasting glucose concentrations in the IDDM subjects. This indicates that the nocturnal rise in NEFA could be partly responsible for the early morning hyperglycaemia. A strong temporal relationship between plasma NEFA levels and hepatic glucose output has previously been shown [37] as well as a reduction of nocturnal glucose production after pharmacological NEFA suppression [38].

Are disturbed lipolysis and NEFA levels at night in IDDM due to low insulin concentrations or hyperglycaemia? The fact that dialysate glycerol began to increase earlier in the evening in the IDDM patients as compared with control subjects (Fig. 1) strongly suggests this. However most of the other data in this study argues against a role for insulin and glucose. Similar results were obtained if subjects with good ($HbA_{1c} < 7.6\%$) and average or poor ($HbA_{1c} > 7.6\%$) glucose control were compared (although some caution must be exercised regarding this finding, since the number of subjects was small). Furthermore, the concentrations of free insulin in serum were normal late at night (they were slightly decreased in the evening). More important, nocturnal adipose tissue glycerol and circulating NEFA concentrations did not normalize during the 24 h of intravenous insulin infusion, which almost completely normalized the blood glucose and circulating insulin concentrations both day and night. The presently obtained circulating insulin concentrations should induce complete suppression of lipolysis, which is known to occur at insulin concentrations lower than those needed to influence glucose metabolism [39]. As regards the adipose tissue blood flow, the data are somewhat difficult to interpret since the overall ethanol ratio was decreased in the daytime during the insulin infusion. In spite of this, the adipose tissue concentrations of glycerol during the evening and night remained significantly elevated during the insulin infusion and a nocturnal increase in the relative blood flow measurement was maintained. We therefore conclude that insulinopenia or hyperglycaemia are not responsible for the abnormal nocturnal lipolysis in IDDM.

What causes the circadian rhythm in lipolysis and why does it change in IDDM? The present study was not designed to answer this question. However, there may be several causes. Growth hormone can regulate lipolysis and glucose levels at night [10, 14]. Thus, the elevated growth hormone concentration observed

during the night in IDDM might further increase the nocturnal lipolytic rate and cause hyperglycaemia at night. Increased concentrations of GH are present in poorly controlled IDDM patients [40, 41] and intensive insulin treatment may correct some [42, 43], but not all [11] abnormalities in GH secretion. Our patients were fairly well controlled and a 24-h insulin infusion failed to normalize nocturnal GH. It is quite possible that longer periods of insulin infusion might be needed to correct GH. GH-induced lipolysis does not account for the increased requirement for insulin late at night in GH-deficient IDDM patients [10]. In our IDDM patients, however, night-time GH, nocturnal lipolysis and fasting glucose were interrelated. This agrees with previously reported findings that when the nocturnal GH peak and the NEFA increase were abolished, *in vivo* glucose metabolism improved in IDDM [8]. Clearly, the relationship between lipolysis, GH and hyperglycaemia in IDDM must be investigated further, for example, by more frequent sampling of GH or investigations during longer periods than 24 h of near-normoglycaemia. Other factors affecting the lipolytic pattern could be the autonomic nervous system, which also shows a circadian rhythm [34], local substances, such as adenosine and prostaglandins, which influence lipolysis and circadian variations in the sensitivity of insulin [13].

In summary, this study shows a circadian rhythm in lipolysis under normal living conditions. An increased nocturnal rate of lipolysis may, at least in part, cause elevated circulating NEFA concentrations during the night. Night-time lipolysis and NEFA are further increased in IDDM. Since abnormally elevated NEFA may have several side effects, including hyperglycaemia, the present study shows the importance of including circadian variations in adipose tissue lipolysis when evaluating the metabolic control in IDDM patients. However, more direct methods should be developed for this purpose, allowing measurement of true lipolytic rates under ambulatory conditions.

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