Insulin-independent and extremely rapid switch in the partitioning of hepatic fatty acids from oxidation to esterification in starved-refed diabetic rats

Possible roles for changes in cell pH and volume

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The requirement for a normal insulin response in mediating the starved-to-refed transition, with respect to the partitioning of hepatic fatty acids between β-oxidation and esterification to glycerol, was studied. Diabetic rats were starved for 24 h and refed ad libitum for various periods of time. There was no increase in plasma insulin in response to the meal. However, the fatty acid oxidation:esterification ratio was very rapidly decreased from the starved to the fed value, most of the transition being achieved within the first hour of refeeding. There was a 2 h lag in the response of hepatic malonyl-CoA concentration, such that this rapid switch from oxidation to esterification could not be explained on the basis of changes in the absolute concentration of this inhibitor of carnitine palmitoyltransferase I (CPT I). Hepatic pyruvate and lactate concentrations both increased by several-fold upon refeeding and peaked after 1 h and 3 h, respectively. The hepatic lactate:pyruvate ratio increased 3.2-fold during the first 3 h of refeeding, suggesting that the cytosolic NAD^+–NADH couple became much more highly reduced during the lag-period between the onset of inhibition of flux of fatty acids towards oxidation and the rise in malonyl-CoA concentration. This may be indicative of a lowering of intracellular pH, which would amplify greatly the sensitivity of CPT I to the inhibitor. In view of the very rapid and high food intake by these diabetic rats, the possibility is also considered that portal concentrations of amino acids and other metabolites could give rise to an increase in liver cell-volume that would inhibit CPT I acutely by an as yet unknown mechanism [M. Guzman, G. Velasco, J. Castro and V. A. Zammit (1994) FEBS Lett. **344**, 239–241].

INTRODUCTION

The role of acute insulin action on hepatic fatty acid metabolism has been controversial. The observed direct effects of insulin on enzymes of fatty acid synthesis and oxidation are highly variable when tested either *in vivo* or in perfused liver and isolated hepatocyte preparations [1–5]. A consistent aspect that emerges from these studies, however, is that the reversal of the effects of insulin deficiency on the liver may require several hours of insulin treatment either *in vivo* or of preparations *in vitro*. In particular, treatment of streptozotocin-diabetic rats with insulin does not reverse the effects of insulin deficiency on the kinetic properties or maximal activity of mitochondrial overt carnitine palmitoyltransferase I (CPT I) until after several hours of treatment [6]. The consequences of this refractoriness of CPT I to insulin treatment in diabetic rat liver have recently been demonstrated directly by monitoring the time-course of changes in the partitioning of hepatic fatty acids *in vivo* [7]. Insulin treatment did not alter the relative partitioning of [^{14}C]oleate label between oxidation and esterification for at least 5 h after the start of insulin treatment. Thereafter, there were gradual, reciprocal changes in the proportion of [^{14}C]oleate label that was incorporated into oxidation products or glycerolipids. This lag-period coincided with the time-course for the reversal of the desensitized state of CPT I to its inhibitor, malonyl-CoA [6]. A similar refractoriness to changes in the partitioning of acyl-CoA between the two pathways was observed *in vivo* upon refeeding of 24 h-starved rats [8,9], such that it took more than 8 h from the start of refeeding to restore fully this parameter to its value in the fed, post-absorptive state. This was in marked contrast with the rapidity with which the switch from oxidation to esterification occurred in meal-fed animals during ingestion of their daily 3 h meal [10]. Because in the latter animal model, meal-induced insulin secretion is significantly greater than in normal 24 h-starved-refed rats [11,12], it was considered plausible that the higher portal insulin concentrations achieved in meal-fed rats could be central to their ability to switch off hepatic fatty acid oxidation rapidly [10]. Consequently, these observations raised the prospect that physiologically induced surges in portal insulin concentration could achieve changes in hepatic metabolism that cannot be mimicked by pharmacological doses of the hormone administered peripherally (as in [7]). Alternatively, the effects of food intake could occur independently of insulin action.

Therefore, in the present study we have sought to test the effect of a meal in severely diabetic animals, i.e. animals that could not respond to refeeding with increased insulin secretion. Such an approach was aimed at providing a model in which the other physiological responses to refeeding (e.g. increased hepatic portal concentration of metabolites) could be distinguished from the effects of insulin. We monitored the time-course of the partitioning of hepatic fatty acid metabolism between oxidation and esterification (using the technique of selective labelling of hepatic fatty acids *in vivo* [13]). We found that, even in the absence of an insulin response to refeeding, the liver of these diabetic animals underwent an extremely rapid switch from fatty acid oxidation to esterification (even faster than in meal-fed rats). However in contrast to the latter [10], the switch could not be explained on the basis of changes in hepatic malonyl-CoA concentrations.

MATERIALS AND METHODS

Animals

The experimental (lipoprotein-recipient) animals were female Wistar rats that weighed 200 g before induction of diabetes with

Abbreviations used: CPT, carnitine palmitoyltransferase; TAG, triacylglycerol; PDH, pyruvate dehydrogenase; ACC, acetyl-CoA carboxylase.

a single intraperitoneal injection of streptozotocin (80 mg/kg). They were given a chow diet ad libitum (BS&S, Edinburgh, UK). Its composition was 56% carbohydrate, 18.5% protein and 3.1% fat. The animals were given glucose in their drinking water (10%) for the first 24 h after streptozotocin injection. Thereafter, their glucosuria was monitored and on the 5th day after treatment they were fitted with an intrajugular catheter under halothane anaesthesia. They were used for experiments after a further 4 days, when their food intake had recovered to normal. Starved animals had their food removed for 24 h before the experiment. The average blood-glucose in the fed state was >35 mM on the day preceding the experiment. Lipoprotein-donor rats were males (>400 g body weight) that were given 10% fructose in drinking water 24 h prior to being exsanguinated (see [13]).

Use of cholesteryl [1-^{14}C]oleate-labelled lipoproteins to monitor hepatic fatty acid partitioning *in vivo*

The rationale behind the use of cholesteryl [1-^{14}C]oleate-labelled very low density lipoprotein and chylomicron-remnants to selectively label the fatty acids of the liver *in vivo* has been presented elsewhere [9,10,13]. Briefly, the ester was incorporated into the hydrophobic core of the remnants, obtained from plasma of heparin-treated, functionally hepatectomized male rats, by cholesteryl ester transfer protein-catalysed exchange *in vitro* [14]. The lipoproteins were then injected through the indwelling jugular catheter into the recipient rats, and the formation of labelled products of the metabolism of the [1-^{14}C]oleate, generated intrahepatically through the hydrolysis of the ester specifically taken up by the hepatocyte population, was monitored. The preparation and use of the labelled lipoproteins were exactly as described elsewhere [9,10,13] except that in the later experiments lipoproteins were separated from the labelling medium by gel filtration on Sephadex G-25, rather than by centrifugation followed by dialysis. This reduced by 24 h the time required from bleeding of the donor animals to injection into the recipient rats and helped to minimize the deterioration of the lipoproteins during preparation. The collection of $^{14}CO_2$ and analysis of hepatic and plasma ^{14}C-lipids (which accumulated owing to injection of Triton WR 1339, 15 min after injection of label [13]) were performed as in [13]. As described in [7], urine was collected while the animals were in the metabolic chambers and the bladder contents of the animals were emptied at the end of the experiments and added to the urine collected. The combined urine samples were counted for ^{14}C-radioactivity.

Other methods

Metabolites were assayed in perchloric acid extracts of either freeze-clamped liver samples or of freshly drawn aortic blood samples obtained from anaesthetized animals. D-3-hydroxybutyrate, acetoacetate, glycerol-3-phosphate, lactate and pyruvate were assayed using standard spectrophotometric assays. In addition, malonyl-CoA was measured as described previously [15] and commercially available kits were used to measure plasma triacylglycerols, (Sigma, Poole, Dorset, U.K.; cat. no. 337-10A) non-esterified fatty acids (NEFAC, Wako Chemicals, GmbH, Neuos, Germany) and glycerol (Sigma, 337-40A). Plasma insulin was measured by e.l.i.s.a.

Isolation of mitochondria and assay of CPT I activity

Mitochondria were isolated from livers of fed, 24 h-starved and 3 h-refed diabetic animals as described previously [9]. The activity of CPT I was measured using the assay mixture described therein, at 130 μM and 35 μM concentrations of palmitoyl-CoA. At the latter concentration, the activity was measured in the presence of a range of malonyl-CoA concentrations up to 100 μM. Quantification of the conversion of [^{3}H]carnitine into palmitoyl [^{3}H]carnitine was performed by butanol extraction [16].

Materials

The sources of all materials were as described in [9,10].

RESULTS AND DISCUSSION

The animals used in this study were severely diabetic for 10 days before being used (blood glucose in the fed state was 38.0±2.0 mM). The use of such chronically diabetic rats was necessary to avoid the overlap of the complications of the acute toxicity of streptozotocin (and associated inappetence) and the trauma of surgery. This resulted in a model that was particularly useful for the type of study we performed, as after this period of insulin deficiency the rats were essentially depleted of adipose tissue, such that, when starved they experienced a decrease in plasma NEFA concentration (from 0.48±06 mequiv. per litre in fed animals to 0.17±0.22 mequiv. per litre in the 24 h-starved animals). From these observations it can be inferred that in these animals a substantial proportion of plasma NEFA turnover in the fed state is due to lipolysis of chylomicron-triacylglycerol (TAG) (see [17]). This inference is supported by the fact that there was a 2-fold increase in plasma NEFA concentration during the first 3 h of refeeding of the starved rats (Figure 1), whereas in normal 24 h-starved animals, refeeding lowers plasma NEFA through the antilipolytic action of insulin on adipose tissue. As expected, blood glycerol concentration increased in parallel with that of NEFA (Figure 1a). It is noteworthy however,

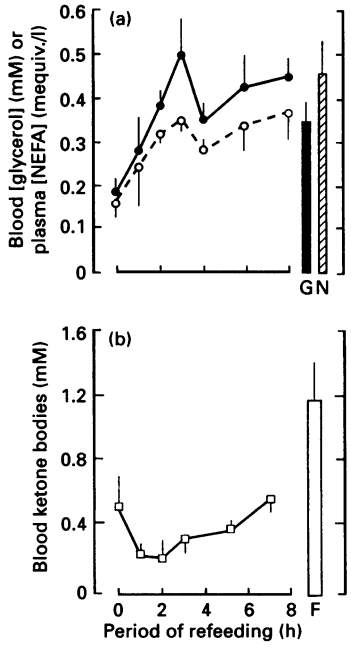

Figure 1 Time-courses for changes in the concentration of (a) blood glycerol (●) and plasma NEFA (○) and (b) blood ketone bodies in 24 h-starved diabetic rats (zero-time) and during refeeding

The values for the parameters in fed diabetic rats are given by the vertical bars (G, glycerol; N, NEFA; F, fed). Values are means±S.E.M. for 4–8 animals for each time-point.

Table 1 Food intake and plasma insulin concentrations in 24 h-starved diabetic rats refed chow ad libitum

The plasma insulin concentrations in fed (post-prandial) rats were 37.6 ± 4.5 and 2.4 ± 0.1 (n = 5) μ-units/ml for normal and diabetic rats respectively. None of the values observed during refeeding were statistically significantly different from either fed or starved diabetic rat values.

Period of refeeding (h)	Food intake (g)	Plasma insulin (μ-units/ml)
0	–	1.7 ± 1.2
1	2.9 ± 0.4	2.1 ± 1.6
3	7.9 ± 1.1	2.1 ± 1.6
6	11.8 ± 0.8	4.3 ± 1.8

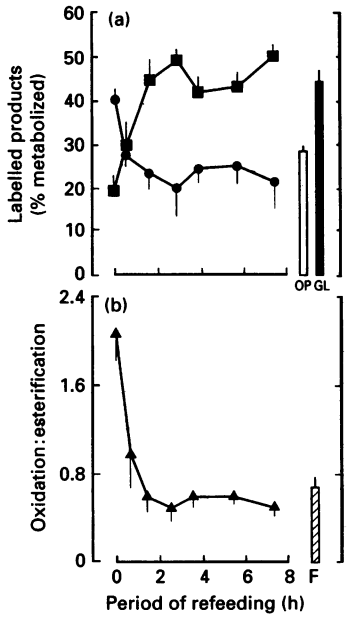

Figure 2 Effect of different periods of refeeding on the partitioning of hepatic fatty acids between oxidation and esterification in 24 h-starved diabetic rats

In (**a**) the recovery of ^{14}C-label in oxidation (●) and total esterification (■) products (liver plus plasma) during the initial 60 min period after injection of label is shown for successive 1 h periods during the refeeding of starved diabetic animals. In (**b**) the time-course of changes in the oxidation:esterification ratio are shown. In (**a**) and (**b**) the respective values for the parameters in fed diabetic animals are given by the vertical bars (OP, oxidation products; GL, glycerolipids; F, fed). Values are means (±S.E.M.) for 4–9 animals for each time-point.

that in spite of the doubling of plasma NEFA concentration, the blood concentration of ketone bodies not only failed to increase but tended to decrease (Figure 1b). This indicated that food intake resulted in the generation of a direct, antiketogenic signal within the liver. Moreover, this meal-induced signal was unrelated to insulin secretion, as no rise in plasma insulin concentration was observed in these animals in the prandial period (Table 1), as expected from their severely diabetic state.

Partitioning of hepatic fatty acids between oxidation and esterification

The selective targeting of cholesteryl [1-^{14}C]oleate to the liver within apoC-poor remnants allowed us to test this inference directly by monitoring the relative amounts of ^{14}C-glycerolipid and ^{14}C-oxidation products that were formed from the [1-^{14}C]oleate generated by intrahepatic hydrolysis of the cholesteryl [1-^{14}C]oleate over successive 1 h periods in fed, starved and starved-refed diabetic rats. The results are summarized in Figure 2. The total amount of ^{14}C in liver and plasma glycerolipids (which accumulated in plasma owing to the injection of Triton WR 1339 into the animals, see the Materials and methods section) are shown. The amounts of label in the CO_2 exhaled within the first 60 min as well as that excreted in the urine (presumed to have resulted from renal filtration of ketone bodies, cf. [7]) have been combined. It can be seen that, when diabetic rats were starved for 24 h, the already high proportion (29.0 ± 0.7%) of ^{14}C-fatty acid going to oxidation products, (compared with 14.9 ± 1.5% of the injected dose in fed normal animals [9]), was raised even higher (to 40.5 ± 1.5% of the injected dose). This observation suggests that the preferential oxidation of fatty acids observed in these severely diabetic rats in the fed state was still considerably below the maximum degree of diversion of acyl-CoA towards oxidation that can be achieved by the additional stimulus of starvation. As a result, the oxidation/esterification ratio in the starved diabetic animals was more than double that in the fed diabetic rats (Figure 1b).

Upon refeeding of the 24 h-starved diabetic rats, there was an immediate switch in the partitioning of [^{14}C]oleate from oxidation to esterification (Figure 2). This was apparent even within the first hour of refeeding (i.e. when label was injected just before the presentation of food) and was complete within the second hour. This was in complete contrast to the trends observed after refeeding of normal 24 h-starved rats [9] and occurred even faster than the switch that was observed for meal-fed rats during ingestion of their daily meal [10]. These data illustrate that whereas insulin secretion during the prandial phase may be involved in the response of normal animals to refeeding, it is certainly not necessary. Therefore, the rapidity of the switch between oxidation and esterification in meal-fed rats [10] is unlikely to be related to the extent to which insulin is raised in those animals, as starved-diabetic rats can achieve an even faster response in the absence of insulin secretion. It is also evident that this insulin-independent signal delivered to the liver during a meal is either of smaller magnitude or is efficiently counteracted in normal 24 h-starved-refed animals in which the intrahepatic counter-oxidative response is extremely slow [9].

Effects of refeeding on hepatic malonyl-CoA and glycerol-3-phosphate concentrations

Two candidates for such a signal were considered to be the hepatic concentrations of malonyl-CoA (which inhibits CPT I) and glycerol-3-phosphate (which is a substrate for glycerol-3-phosphate acyltransferase, which catalyses the first reaction that commits long-chain acyl-CoA to glycerolipid synthesis).

The malonyl-CoA concentration in the liver of starved diabetic rats was relatively variable (Figure 3a), but after 1 h of refeeding it stabilized at a value that was 80% lower ($P < 0.01$) than in the liver of fed diabetic animals. Moreover, there was no increase in malonyl-CoA concentration after a further 1 h of feeding (i.e. after 2 h in total). Thereafter, the concentration increased sharply to peak at 3 h, followed by a steady decrease to 'starved' levels over the next 4 h. The peak value attained at 3 h was only modestly (75%) higher than in the fed state. The 2 h lag in the response of malonyl-CoA concentration is noteworthy as during this period the switch from fatty acid oxidation to esterification had already fully occurred. This suggests that factors other than the absolute concentration of malonyl-CoA are involved in

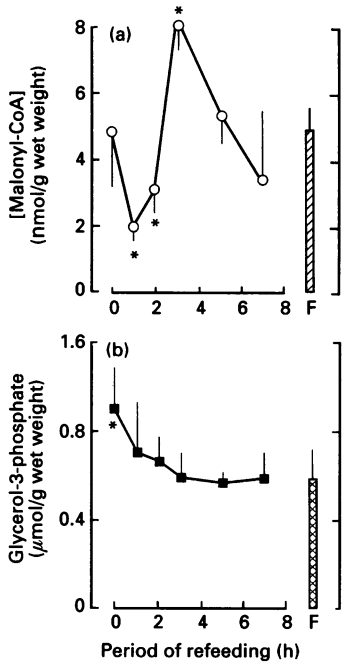

Figure 3 Time-courses for the changes in hepatic concentrations of (a) malonyl-CoA and (b) glycerol-3-phosphate in 24 h-starved diabetic rats refed for the periods indicated

The vertical bars indicate the values for the concentrations observed in fed animals. Values are means (±S.E.M.) for four separate animals for each time-point. Those that are statistically significantly different from 'fed' values are indicated by an asterisk [$P < 0.01$ in (**a**); $P < 0.05$ in (**b**)].

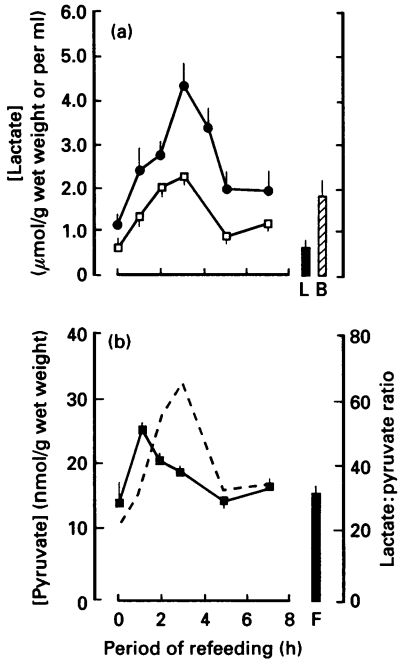

Figure 4 Time courses for changes in (a) hepatic (□) and blood (●) concentrations of lactate, and (b) hepatic concentration of pyruvate (■) and the lactate:pyruvate concentration ratio (broken line) during refeeding of 24 h-starved diabetic rats

The vertical bars indicate the values for the respective parameters observed in fed animals (L = liver; B = blood; F, fed). Values are means (±S.E.M.) for four separate animals for each time-point.

rapidly switching off the ability of acylcarnitine synthesis to compete successfully for cytosolic long-chain acyl-CoA.

The hepatic concentration of glycerol-3-phosphate in starved diabetic rats was only marginally significantly higher ($P < 0.05$) than that in fed animals (Figure 3b). This was in spite of the markedly lower blood glycerol concentration in the starved state (Figure 1). Similarly, the 3-fold increase in blood glycerol concentration that occurred upon refeeding did not result in any increase in hepatic glycerol-3-phosphate concentration. On the contrary, the latter declined towards the 'fed' value throughout the refeeding period. This suggests that the increased diversion of long-chain acyl-CoA towards esterification immediately upon the start of feeding was not due to a mass-action effect owing to increased availability of cytosolic glycerol-3-phosphate. The decline in the concentration of this metabolite, which was observed in spite of the increase in the concentration of its immediate precursor (blood glycerol), suggests that the increased diversion of acyl-CoA towards esterification resulted in a higher rate of utilization of glycerol-3-phosphate than could be met by the increased rate of delivery of glycerol to the liver. (Utilization of glycerol-3-phosphate would also be required for gluconeogenesis).

Sensitivity of CPT I to malonyl-CoA in isolated mitochondria

In view of the lag in the response of hepatic malonyl-CoA concentration to refeeding, the possibility was tested that the sensitivity of CPT I to malonyl-CoA might have changed rapidly upon refeeding of the starved diabetic animals, such that, even if the malonyl-CoA concentration was not elevated, CPT I would become inhibited. The activity of CPT I at different malonyl-CoA concentrations was tested for fed, 24 h-starved and starved-3 h-refed rats. We confirmed that CPT I becomes desensitized to malonyl-CoA in the diabetic rat liver [8]. Moreover, we could not detect any further decrease in sensitivity upon starvation of diabetic rats. However, refeeding of the animals for 3 h did not alter this degree of sensitivity (results not shown; cf. [9]).

Blood and liver concentrations of pyruvate and lactate

The delayed and phasic response of hepatic malonyl-CoA concentration to refeeding in diabetic rats implied that a transient increase in the flux through pyruvate dehydrogenase (PDH) and acetyl-CoA carboxylase (ACC) had occurred. The substrate for increased flux through ACC is likely to have been lactate. It is known to be the preferred substrate for lipogenesis in the liver [18,19] and it provides not only a source of C_3 substrate but also of activators of PDH and ACC. Thus, pyruvate inhibits PDH kinase [20] and favours PDH activation through net dephosphorylation. In turn, the formation of acetyl-CoA by the reaction catalysed by PDH results in the formation of cytosolic citrate, an allosteric activator of ACC [21] as well as an inhibitor of ACC phosphorylation [22]. In view of these considerations, the concentrations of lactate in whole blood and frozen liver samples (and of pyruvate in the latter) were measured to see if the time-course of changes in these concentrations could account for the rise in hepatic malonyl-CoA concentration (Figure 4). Blood and liver lactate concentrations were doubled within 1 h and peaked at 3 h at concentrations 4-fold higher than in the starved state. In addition, the intrahepatic pyruvate concentration peaked after only 1 h of refeeding (at 2.5 μmol/g). If the effective concentration of pyruvate achieved within the mitochondria

under these conditions is sufficient to inhibit PDH kinase [20], then net dephosphorylation of PDH could be an early event, although because of hysteretic behaviour of the enzyme due to multisite phosphorylation and increased PDH kinase expression in diabetes (see [23,24]), full activation of the enzyme could be delayed (cf. [25]). It is noteworthy that the decline of lactate concentrations in both blood and liver after reaching their respective peak values at 3 h, coincided with the time-course for the decline in malonyl-CoA concentration (Figure 3a).

Changes in the intrahepatic lactate:pyruvate ratio

The hepatic lactate:pyruvate concentration ratio was similar in livers of fed or fasted diabetic rats (approx. 20; see Figure 4). The asynchronous increases in lactate and pyruvate concentrations that occurred upon refeeding resulted in a steady increase in this ratio to reach a value of greater than 60 after 3 h. This occurred in spite of the fact that hepatic pyruvate concentrations peaked ahead of those of lactate, because, in relative terms, the increase in lactate (2.4-fold) was greater than that in pyruvate (1.8-fold) even after 1 h of refeeding. Moreover, after the 1 h time-point, hepatic lactate concentrations continued to increase for a further 2 h, whereas the pyruvate concentration gradually decreased to normal. These data indicate that, during the first 3 h of refeeding, there was a major shift in the equilibrium position of the NAD^+–NADH couple in the cytosol towards a much more reduced state.

The significance of this large change in cytosolic redox state may reside in its implication for cytosolic pH. It is recognized that a more reduced cytosolic redox state is associated with the increased production of H^+ [26]. Therefore, it is possible that the rapid increase in the state of reduction of the NAD^+–NADH redox couple may be reflected in a lowering of cytosolic pH if the capacity of the liver cells to compensate for the increased rate of H^+ production is overwhelmed. Any fall in intracellular pH would have a profound effect on the sensitivity of CPT I to malonyl-CoA, as the latter inhibits the enzyme much more strongly at the lower end of the physiological range of pH [27,28]. Therefore, such a lowering of pH would enable CPT I to be inhibited even at the low concentrations of malonyl-CoA observed during the first 2 h of refeeding. We tentatively suggest that the very rapid switch away from fatty acid oxidation that occurs upon refeeding of starved diabetic animals may be related to the experimentally observed changes in cytosolic redox state. There has been disagreement about the possible role of the strong pH-dependence of malonyl-CoA-inhibition of CPT I in limiting ketogenesis during diabetic ketoacidosis [see 27,28], although this property of the enzyme has been suggested to play a role in the inhibition of fatty acid oxidation in anoxic muscle [29]. The present results may represent the first, if indirect, experimental evidence that this striking kinetic characteristic of the enzyme may be important in modulating the partitioning of fatty acids in the liver under conditions of increased lactate delivery to the liver.

An alternative and/or complementary mechanism may involve the recently described malonyl-CoA-independent inhibition of CPT I activity by an increase in liver cell volume *in vitro* [30]. (Although such an increase in volume is known to activate protein phosphatase activity [31], this is not thought likely to have a direct effect on CPT I [32].) In this respect, it should be noted that the rate of food intake is much higher upon refeeding in starved diabetic and meal-fed rats given free access to food upon refeeding (Table 1) than it is in normal 24 h-starved-refed animals [10]. In addition, conditions characterized by hyperphagia or gorging are accompanied by adaptive changes in the gut that increase the rate of absorption of nutrients. It is likely that the portal concentrations of amino acids and other metabolites absorbed from the gut into the hepatic portal circulation may be sufficient to induce liver-cell swelling [33] in starved-refed diabetic and meal-fed rats. It is possible, therefore, that the rapidity with which the ability of acylcarnitine synthesis to compete for cytosolic long-chain acyl-CoA is switched off is related primarily to the rate of delivery of such substrates to the liver from the gut, in direct proportion to the rate of food intake and intestinal absorption upon refeeding. In non-diabetic animals, insulin would be expected to complement these effects through its contribution towards hepatocyte swelling [34] and by raising malonyl-CoA concentration, although the latter only occurs efficiently in meal-fed animals [9,10].

In particular, we would like to emphasize the very different response of the liver of diabetic animals to insulin treatment on the one hand [7] and to refeeding on the other (present study). From our previous study [7] it was clear that in diabetic rats treated with insulin the pattern of partitioning of hepatic fatty acid metabolism did not change for at least 5 h after the start of insulin repletion. This was in agreement with previous findings in which the capacity of the liver to oxidize fatty acids was studied using perfused livers isolated from insulin-deficient rats treated with insulin *in vivo* for various periods before isolation and perfusion of the liver [35] or in hepatocytes isolated from diabetic rats treated with insulin [36]. From our present and previous studies it is evident that, contrary to insulin treatment, food intake can deliver a potent and direct antiketogenic signal to the liver which is (1) independent of insulin secretion and (2) not mimicked by several hours of treatment by the hormone delivered peripherally (in fed diabetic rats [7]).

These inferences may have important implications for the management of diabetic ketosis in humans. The treatment of this condition would obviously benefit from a rapid inhibition of the ketogenic capacity of the liver. Our studies indicate that insulin treatment of such patients may not be sufficient to achieve this acutely. Current clinical practice involves low-dose insulin infusions as part of the treatment (see [37]). This would be expected to have an indirect antiketogenic effect due to the antilipolytic action of the hormone on adipose tissue. However, in the light of the present and previous [7] observations, such a course of treatment would not be expected to exert an immediate, direct antiketogenic effect on the liver. In future work, it will be important to ascertain whether other hormones are involved and which substrates, delivered to the liver via the portal circulation, are most efficient in providing the potent antiketogenic signal observed.

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