Biochem. J. (1996) **317**, 791–795 (Printed in Great Britain)



# Flux control exerted by mitochondrial outer membrane carnitine palmitoyltransferase over $\beta$-oxidation, ketogenesis and tricarboxylic acid cycle activity in hepatocytes isolated from rats in different metabolic states

Lesley DRYNAN*‡, Patti A. QUANT†§ and Victor A. ZAMMIT*‖

Hannah Research Institute, Ayr, Scotland KA6 5HL, U.K., and †Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, U.K.

The Flux Control Coefficients of mitochondrial outer membrane carnitine palmitoyltransferase (CPT I) with respect to the overall rates of $\beta$-oxidation, ketogenesis and tricarboxylic acid cycle activity were measured in hepatocytes isolated from rats in different metabolic states (fed, 24 h-starved, starved–refed and starved/insulin-treated). These conditions were chosen because there is controversy as to whether, when significant control ceases to be exerted by CPT I over the rate of fatty oxidation [Moir and Zammit (1994) Trends Biochem. Sci. **19**, 313–317], this is transferred to one or more steps proximal to acylcarnitine synthesis (e.g. decreased delivery of fatty acids to the liver) or to the reaction catalysed by mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase [Hegardt (1995) Biochem. Soc. Trans. **23**, 486–490]. Therefore isolated hepatocytes were used in the present study to exclude the involvement of changes in the rate of delivery of non-esterified fatty acids (NEFA) to the liver, such as occur *in vivo*, and to ascertain whether, under conditions of constant supply of NEFA, CPT I retains control over the relevant fluxes of fatty acid oxidation to ketones and carbon dioxide, or whether control is transferred to another (intrahepatocytic) site. The results clearly show that the Flux Control Coefficients of CPT I with respect to overall $\beta$-oxidation and ketogenesis are very high under all conditions investigated, indicating that control is not lost to another intrahepatic site during the metabolic transitions studied. The control of CPT I over tricarboxylic acid cycle activity was always very low. The significance of these findings for the integration of fatty acid and carbohydrate metabolism in the liver is discussed.

## INTRODUCTION

In common with other metabolic processes, mitochondrial fatty acid oxidation, which in the liver results either in complete oxidation to carbon dioxide or in partial oxidation to ketone bodies, is controlled at several sites [1,2]. The intrahepatic control sites can be grouped into those that occur in the cytosolic compartment and those within the mitochondrial matrix. The most important cytosolic step at which control is exerted is that catalysed by overt carnitine palmitoyltransferase (CPT I), which resides in the mitochondrial outer membrane and thus interacts with the cytosolic pools of substrates and effectors. Amongst the latter, malonyl-CoA is the most important, being a potent inhibitor of CPT I in competition with the acyl-CoA substrates of the enzyme [3]. The control of CPT I by malonyl-CoA is complicated by at least one other factor, namely that the sensitivity of the enzyme to the inhibitor changes with physiological state. Thus under (patho)physiological conditions characterized by increased rates of hepatic fatty acid oxidation, the $K_i$ of the enzyme for malonyl-CoA is markedly increased ([4–6]; see [1] for a review). After a lag period of several hours [7] there is also increased expression of the enzyme protein and activity [8]. These adaptations, coupled with the decrease in hepatic malonyl-CoA concentration, would be expected to facilitate acylcarnitine formation from long-chain acyl-CoA, thus increasing the rate of acyl chain transfer across the mitochondrial inner membrane and into the mitochondrial matrix for oxidation. These properties of CPT I suggest that the enzyme exerts major control over the mitochondrial oxidation of acyl-CoA during the onset of ketogenic episodes [7,9].

However, it has been clearly demonstrated that this is not the case during reversal of ketogenic states. Thus the kinetic characteristics of CPT I in starved rats are only restored very gradually (over a period of many hours) upon refeeding to those observed in the fed animal [10]. The same applies to insulin-treated diabetic rats [9]. Direct evidence for the shift of control away from CPT I during these transitions has been obtained by monitoring the time courses of changes in the partitioning of hepatic acyl-CoA between oxidation and esterification in the livers of rats *in vivo* [11–13].

The question arises as to the identity of the other step(s) to which control is transferred during these periods. It has been emphasized elsewhere [13] that the extrahepatic effects of insulin (i.e. through its anti-lipolytic action in adipose tissue) are crucial in determining the supply of non-esterified fatty acids (NEFA) to the liver, and provide a means through which control can be shifted to an extrahepatic site, i.e. fatty acid mobilization from adipose tissue. With respect to intrahepatic sites at which control could be exerted, studies on the expression of mitochondrial 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase in starved, diabetic and insulin-treated animals have shown that both the mRNA coding for the protein and the amount of

Abbreviations used: CPT I, mitochondrial outer membrane carnitine palmitoyltransferase; NEFA, non-esterified fatty acids; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; TDGA, tetradecylglycidic acid; TCA cycle, tricarboxylic acid cycle; ASP, acid-soluble products.

‡ Present address: MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.

§ Present address: Unit of Paediatric Surgery, Institute of Child Health and Great Ormond Street Hospital for Children NHS Trust, 30 Guilford Street, London WC1N 1EH, U.K.

‖ To whom correspondence should be addressed.

immunodetectable protein increase during the onset of ketogenic states, and these changes are rapidly reversed (1 h) by refeeding or insulin treatment. These observations have led to the suggestion that significant control over ketogenesis is exerted at the reaction catalysed by HMG-CoA synthase, especially during the reversal of ketogenic states [14–16]. The magnitude of these changes in expression ( > 100 %) would dominate the changes in overall activity of the enzyme, as the changes in the fraction of the enzyme in the active form (due to a succinylation–desuccinylation mechanism [17]) are rather small by comparison.

In order to study the role of intrahepatic mechanisms and to determine whether control is exerted primarily at extra- or intra-mitochondrial sites, it is necessary to bypass the extrahepatic control exerted by changes in the NEFA supply to the liver. In the present study this was achieved by using isolated hepatocytes incubated with a fixed concentration of fatty acids and in which the activity of CPT I was titrated (progressively inhibited) with increasing concentrations of a specific, irreversible, inhibitor (tetradecylglycidic acid, TDGA). This allowed the calculation of the Flux Control Coefficient for CPT I through the application of the method developed by Groen et al. [18]. Our data indicate that the control exerted by CPT I over total $\beta$-oxidation and ketogenesis is uniformly very high in all types of cells studied, irrespective of whether the animals from which the cells were obtained were fed or starved, and, after a period of starvation, whether they were refed or insulin-treated. However, the control of CPT I over tricarboxylic acid (TCA) cycle activity was always very low, especially in cells from starved–refed or starved/insulin-treated rats.

## MATERIALS AND METHODS

### Animals

Female Wistar rats (200–250 g) were used for preparation of hepatocytes. They were maintained under a 12 h light/12 h dark regime and were fed a standard laboratory chow diet (see [11]) *ad libitum*. Starved animals had food withdrawn 24 h prior to use. Starved–refed animals were given food for 2 h, during which period they consumed approx. 3 g of food. Insulin-treated 24 h-starved rats were injected with 2 units of crystalline porcine insulin delivered intraperitoneally in 200 $\mu$l of 0.9 % (w/v) NaCl [14]; in addition, they received 1 ml of 2 M glucose solution in saline, also intraperitoneally, to minimize the degree of hypoglycaemia.

### Preparation and incubation of hepatocytes

Hepatocytes were prepared by a modification of the method of Seglen [19], as described previously [20]. They were incubated at 37 °C, with shaking (in 25 ml conical flasks equipped with small rolls of filter paper in centre wells), at a density of $1.3 \times 10^6$ cells/ml in 3 ml of Krebs medium [21] containing 20 mM glucose, 2 % albumin (defatted) and 1 mM carnitine. The flasks were gassed with $O_2/CO_2$ (19:1), sealed and incubated for 30 min, after which 5 $\mu$l aliquots of DMSO, either alone (control) or containing appropriate amounts of TDGA to give the concentrations indicated, were added to each flask. After a further 10 min incubation, a 1 ml aliquot of prewarmed Krebs medium containing 2 $\mu$mol of sodium palmitate (final concentration 0.5 mM) and 0.4 $\mu$Ci of [$^{14}$C]palmitic acid were added. The tubes were gassed and sealed, and incubation was allowed to proceed for a further 60 min. Incubations were terminated by the addition to the incubation medium of 400 $\mu$l of 20 % perchloric acid, delivered to each flask with a syringe needle through the rubber cap. Aliquots (250 $\mu$l) of a mixture of $\beta$-phenylamine and methanol (1:1, v/v) were added to the centre wells with a syringe while the flasks were still sealed. The flasks were cooled to 10 °C and shaken for a further 60 min to collect the $^{14}CO_2$ formed on the alkali-saturated filter paper in the centre wells. The filter paper rolls were placed individually in scintillation vials, and the respective wells were rinsed with the same alkaline mixture and the wash transferred quantitatively to the respective scintillation vials. The acidified incubation medium was centrifuged at 2000 ***g*** for 10 min. An aliquot of the supernatant was used directly for measuring radioactivity ($^{14}$C-labelled acid-soluble products; [$^{14}$C]ASP) and the rest was frozen at −20 °C until used for assay of ketone body concentrations.

A duplicate set of cell incubations was performed to measure the effects of increasing the TDGA concentration on CPT activity in the same batch of cells. The flasks were treated in the same way except that the fatty acid substrate was not radiolabelled. At 40 min after the addition of TDGA, the contents of the flasks were added to 1 ml of digitonin solution in Krebs medium (1.5 mg/ml) to permeabilize the cells. After 10 s exactly, the mixture was diluted into 40 ml of ice-cold medium containing 150 mM KCl, 5 mM Tris and 1 mM EGTA (pH 7.4) and centrifuged at 1000 ***g*** for 3 min. The permeabilized cells were collected as a pellet and resuspended in 0.5 ml of a KCl medium. This cell ghost suspension was used to measure CPT activity.

### Assay of CPT activity and ketone body concentrations

These were performed by standard methods. The CPT assay for permeabilized cells was performed as described in [22,23] using 135 $\mu$M palmitoyl-CoA as substrate, in the presence of 1 % albumin. Activities were corrected for the recovery of cell material by dividing the activity by the protein content of the permeabilized cell suspensions. Acetoacetate and 3-hydroxybutyrate were assayed spectrophotometrically in perchloric acid extracts of cell incubation media after neutralization.

### Statistical analysis

Flux Control Coefficients were calculated from the slopes of straight lines fitted to data obtained at low ( $\leqslant 0.075\ \mu$M) concentrations of TDGA using linear least-squares regression, as described in [24]. The S.E.M.s for control strengths were calculated using analysis of variance for values obtained for different cell preparations (see S.E.M.s in Table 1). Incubations at these concentrations of inhibitor were carried out at least in triplicate for any one hepatocyte preparation.

### Materials

TDGA was a gift from McNiell Pharmaceuticals. [1-$^{14}$C]Palmitic acid was from Amersham International (Amersham, Bucks., U.K.). All other chemicals were from Sigma (Poole, Dorset, U.K.).

## RESULTS AND DISCUSSION

The well established changes in the activities of CPT I and mitochondrial HMG-CoA synthase that occur after starvation and refeeding or insulin treatment of starved rats (see the Introduction) were confirmed in the present study (results not shown).

The method for determining the Flux Control Coefficient of an enzyme by titrating (inhibiting) the activity with increasing concentrations of a specific inhibitor was developed by Groen et

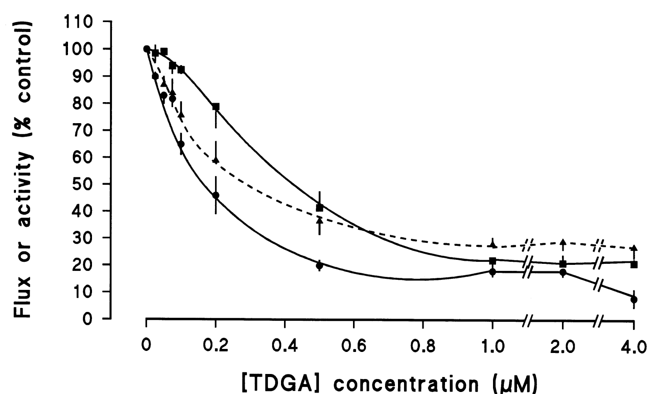


**Figure 1 Inhibition of CPT I activity (●), $^{14}CO_2$ formation (■) and [$^{14}$C]ASP formation (▲) by increasing concentrations of TDGA in incubations of hepatocytes isolated from fed rats**

Hepatocytes were incubated with the indicated concentrations of TDGA for 10 min before addition of sodium palmitate (see the Materials and methods section). Incubations were allowed to proceed for a further 60 min and the formation of products was quantified. Values are normalized by expressing them as a percentage of those obtained in the absence of inhibitor for each cell preparation. The absolute rates of [1-$^{14}$C]palmitate conversion into $^{14}CO_2$, [$^{14}$C]ASP and total $^{14}$C-labelled products in the absence of TDGA were $0.04 \pm 0.03$, $0.20 \pm 0.04$ and $0.26 \pm 0.06$ $\mu$mol/h per $10^6$ cells respectively (means $\pm$ S.E.M. from five separate cell preparations). The activity of CPT I in the absence of TDGA was $0.64 \pm 0.12$ nmol/min per mg of permeabilized cell protein at 37 °C.

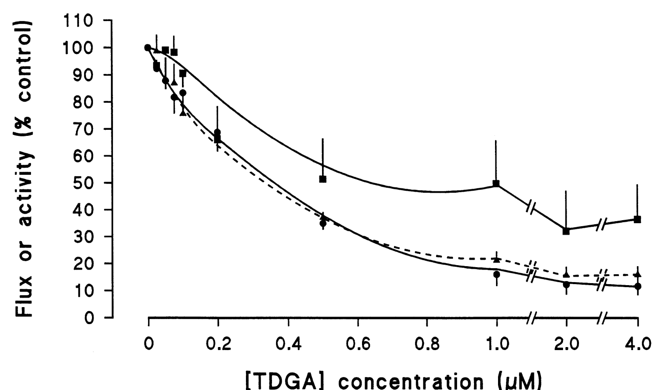


**Figure 2 Inhibition of CPT I activity (●), $^{14}CO_2$ formation (■) and [$^{14}$C]ASP formation (▲) by increasing concentrations of TDGA in incubations of hepatocytes isolated from 24 h-starved rats**

See the legend to Figure 1 for details. The absolute rates of [1-$^{14}$C]palmitate conversion into $^{14}CO_2$, [$^{14}$C]ASP and total $^{14}$C-labelled products in the absence of TDGA were $0.03 \pm 0.01$, $0.58 \pm 0.13$ and $0.69 \pm 0.09$ $\mu$mol/h per $10^6$ cells respectively (means $\pm$ S.E.M. for three separate cell preparations). The activity of CPT I in the absence of TDGA was $1.44 \pm 0.30$ nmol/min per mg of permeabilized cell protein at 37 °C.

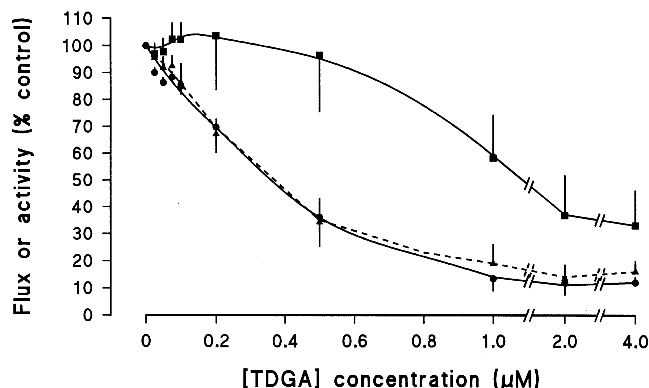


**Figure 3 Effect of increasing TDGA concentrations on CPT I activity (●), $^{14}CO_2$ formation (■) and [$^{14}$C]ASP formation (▲) in incubations of hepatocytes isolated from 24 h-starved/2 h-refed rats**

See the legend to Figure 1 for details. The absolute rates of [1-$^{14}$C]palmitate conversion into $^{14}CO_2$, [$^{14}$C]ASP and total $^{14}$C-labelled products in the absence of TDGA were $0.04 \pm 0.01$, $0.56 \pm 0.07$ and $0.59 \pm 0.11$ $\mu$mol/h per $10^6$ cells respectively (means $\pm$ S.E.M. for three separate cell preparations). The activity of CPT I in the absence of TDGA was $1.31 \pm 0.38$ nmol/min per mg of permeabilized cell protein at 37 °C.

al. [18]. In the present study, TDGA was used as a specific inhibitor of CPT I. It is known that the CoA ester of TDGA (and other oxirane ring-containing analogues of acyl-CoA substrates) binds irreversibly to CPT I and results in its inhibition [25]. In permeabilized cells, the carnitine acyltransferase activity measured using palmitoyl-CoA as substrate represents primarily mitochondrial CPT I, as extensively validated previously [26], even though carnitine acyltransferase activity is also present in peroxisomes [27] and microsomes [28]. This is thought to occur because, in the latter two membranes, overt transferase activity is more specific for medium-chain fatty acids [29]. Moreover, it has been suggested [26] that microsomal carnitine acyltransferase in permeabilized hepatocytes is not inhibited by the low concentrations of TDGA used in hepatocyte incubations. In preliminary experiments in which the time course of the TDGA-induced inhibition of CPT was monitored, we verified that this was maximal after 10 min of incubation and persisted for at least 90 min, as expected from the covalent, irreversible, nature of the interaction between the inhibitor and the enzyme.

Our experiments were designed to enable us to monitor the effects of increasing concentrations of TDGA on CPT I activity, and on the carbon fluxes from [$^{14}$C]palmitic acid to $^{14}CO_2$ and to [$^{14}$C]ASP, which are known to represent primarily ketone bodies [30]. Consequently, because [$^{14}$C]ASP determination can be performed much more accurately than the measurement of small amounts of ketone bodies, only the former was used for the computation of Control Coefficients [18], using the equation:

$$C^J_{\text{CPT}} = \left(\frac{\delta J}{J} \cdot \frac{v_{\text{CPT}}}{\delta v_{\text{CPT}}}\right)_{I=0}$$

where $C^J_{\text{CPT}}$ is the Flux Control Coefficient of CPT I over flux $J$ at zero inhibitor (TDGA) concentration ($I = 0$), and $v_{\text{CPT}}$ is the activity of CPT I. In this instance three sets of values for $J$ ($\beta$-oxidation, ASP formation and $CO_2$ formation) were measured and, consequently, three sets of Control Coefficients could be derived: $C^{\beta\text{OX}}_{\text{CPT}}$, $C^{\text{ASP}}_{\text{CPT}}$ and $C^{\text{CO2}}_{\text{CPT}}$ respectively.

### Control of CPT I over flux to ketone bodies

As shown in Figures 1–4, the inhibition curves for CPT activity and [$^{14}$C]ASP formation were very similar for all types of hepatocyte preparation studied. Accordingly, as expected from the above equation, the value for the Control Coefficient of CPT I with respect to ASP formation ($C^{\text{ASP}}_{\text{CPT}}$) was uniformly high for all types of cells tested (0.85, 0.84, 0.75 and 0.92 for cells from fed, starved, starved–refed and starved/insulin-treated animals; see Table 1). This observation indicates that CPT I exerts a high degree of control over ASP (mostly ketone body) formation in hepatocytes isolated from fed, starved and either starved–refed or starved/insulin-treated rats. As mentioned above, these

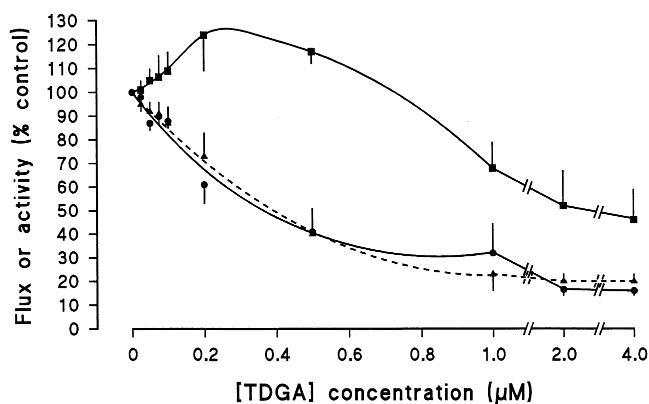


**Figure 4 Effect of increasing TDGA concentrations on CPT I activity (●), $^{14}CO_2$ formation (■) and [$^{14}$C]ASP formation (▲) in incubations of hepatocytes isolated from 24 h-starved/insulin-treated rats**

See the legend to Figure 1 for details. The absolute rates of [1-$^{14}$C]palmitate conversion into $^{14}CO_2$, [$^{14}$C]ASP and total $^{14}$C-labelled products in the absence of TDGA were $0.03 \pm 0.01$, $0.53 \pm 0.09$ and $0.57 \pm 0.10$ $\mu$mol/h per $10^6$ cells respectively (means ± S.E.M. for three separate cell preparations). The activity of CPT I in the absence of TDGA was $1.62 \pm 0.22$ nmol/min per mg of permeabilized cell protein at 37 °C.

**Table 1 Flux Control Coefficients for CPT I ($C_{CPT}$) over the rates of formation of [$^{14}$C]ASP and $^{14}CO_2$, and over the sum of these two parameters ($\beta$-oxidation)**

Values (means ± S.E.M.) indicated by an asterisk are statistically significantly different ($P < 0.05$) from those of the Control Coefficients calculated for the other parameters in the same type of cell preparation.

| Cells prepared from | $C_{CPT}^{CO2}$ | $C_{CPT}^{ASP}$ | $C_{CPT}^{\beta\text{-OX}}$ |
|---|---|---|---|
| Fed rats ($n = 5$) | $0.23 \pm 0.06$* | $0.85 \pm 0.20$ | $1.06 \pm 0.29$ |
| 24 h-starved rats ($n = 3$) | $0.24 \pm 0.13$* | $0.84 \pm 0.32$ | $0.92 \pm 0.13$ |
| 24 h-starved/2 h-refed rats ($n = 3$) | $-0.26 \pm 0.16$* | $0.75 \pm 0.12$ | $1.13 \pm 0.45$ |
| 24 h-starved/insulin-treated rats ($n = 3$) | $-0.75 \pm 0.70$* | $0.92 \pm 0.57$ | $0.90 \pm 0.56$ |

Coefficients were calculated for conditions in which hepatocytes had an equal supply of exogenous fatty acids, thus avoiding the changes in control exerted over ketone body formation by the changes in NEFA supply to the liver that occur *in vivo*. The results demonstrate that, when the fatty acid supply is not limiting (*in vitro*), CPT I continues to exert full control over ketone body formation even when, either by refeeding or by insulin treatment of starved rats, the maximal activity of mitochondrial HMG-CoA synthase is acutely down-regulated [14–16]. Consequently the inference drawn from previous studies that, because the mitochondrial HMG-CoA synthase mRNA and protein content in the liver show acute changes in response to nutritional state, this signifies that the HMG-CoA synthase acquires significant control under these conditions (e.g. during reversal of ketogenic states; see [14–16]) needs to be re-evaluated in the light of the present results. Our results are fully compatible with the conclusions drawn from experiments conducted *in vivo* [11,12], namely that, during the reversal of ketogenic states, flux control is shifted away from CPT I to a step proximal, rather than distal, to the step catalysed by the enzyme. Had control shifted to a step distal to CPT I, i.e. to an intramitochondrial reaction, this would have been detected in the present experiments.

When the Control Coefficients of CPT I with respect to the overall $\beta$-oxidation rate were calculated, these too were uniformly high, as would be expected from the fact that the proportion of overall palmitate oxidation utilized for $CO_2$ formation represents a relatively small fraction of the total oxidation rate (see below). In this respect, it is unlikely that the TCA cycle could have exerted significant negative control on ketogenic flux, because of the relatively low proportion of total oxidative flux that was accounted for by $^{14}CO_2$ formation in cells from animals in any of the conditions studied.

## Control over flux to $CO_2$ (TCA cycle activity)

In contrast to the uniformly high positive value of $C_{CPT}{}^{ASP}$, the value for the Control Coefficient for CPT I over $CO_2$ production ($C_{CPT}{}^{CO2}$) was much lower (Figures 1–4), and even became negative in cells from starved–refed and starved/insulin-treated rats. This latter phenomenon was particularly pronounced in starved/insulin-treated rats in which, initially, the progressive inhibition of CPT I and overall flux through $\beta$-oxidation was accompanied by an increase in the incorporation of label into $CO_2$ (see below). The simplest explanation for the low values of $C_{CPT}^{CO2}$ in hepatocytes from fed animals is that the $K_m$ of citrate synthase for acetyl-CoA formed from the $\beta$-oxidation of palmitate is lower than that of acetoacetyl-CoA thiolase [2], i.e. citrate formation is near-saturated with acetyl-CoA even when the overall flux through the $\beta$-oxidation pathway is substantially inhibited. Consequently, modest inhibition of CPT I by low concentrations of TDGA affects citrate synthesis to a much lesser extent than it affects ketone body formation. This inference agrees with that drawn from studies conducted on isolated rat liver mitochondria oxidizing fatty acids [31]. In those studies it was shown that the rate of citrate synthesis is near-maximal before any significant formation of ketone bodies occurs.

Interestingly, this relationship between the utilization of [$^{14}$C]acetyl-CoA for $^{14}CO_2$ or for [$^{14}$C]ASP formation persisted in hepatocytes isolated from starved animals, irrespective of whether or not they were refed. This indicates that partitioning of carbon flux at this branchpoint is not determined by the level of expression of enzymes (including mitochondrial HMG-CoA synthase) involved in the HMG-CoA cycle pathway of ketone body formation, and emphasizes the importance to the cell of the maintenance of flux through the TCA cycle (see below). This was evident in all the types of cells studied, in spite of the fact that the percentage of the overall flux through $\beta$-oxidation that could be accounted for by $^{14}CO_2$ formation decreased from $15.9 \pm 1.6$ % in cells from fed rats to approx. 5.5 % in cells from all the starved animals tested. In addition to the effects of the relative kinetic characteristics of citrate synthase and acetoacetyl-CoA thiolase, the possibility must also be considered that intramitochondrial acetyl-CoA may be compartmentalized, with a pool being channelled towards formation of citrate, as suggested previously [32].

The increase in $^{14}CO_2$ formation observed in cells from starved–refed or starved/insulin-treated rats when CPT I activity (and flux to [$^{14}$C]ASP) was inhibited (by as much as 60 %) suggests that, in these cells, inhibition of flux through $\beta$-oxidation results in the increased diversion of acetyl-CoA towards citrate synthesis. This is unlikely to have resulted from increased availability of intramitochondrial acetyl-CoA because the absolute rate of $\beta$-oxidation of palmitate in cells from starved animals was markedly higher (cf. CPT I activity) than in cells from fed animals (see legends to Figures). Consequently, even after substantial inhibition of flux through $\beta$-oxidation, the intramitochondrial concentration of acetyl-CoA would still be

sufficiently high to keep citrate synthase saturated with this substrate. Therefore the increased rate of formation of $^{14}CO_2$ under these conditions is likely to have been due to an increased intramitochondrial concentration of oxaloacetate. In support of this, it was found that the 3-hydroxybutyrate/acetoacetate ratio of the ketone bodies secreted into the medium was $8.14 \pm 1.22$ ($n = 4$) for cells from fed animals and $2.31 \pm 0.24$ ($n = 3$) in cells from starved–refed animals, when palmitate was oxidized in the absence of TDGA. In the latter case the ratio decreased further (to $1.18 \pm 0.07$) in the presence of 0.5 $\mu$M TDGA, indicating that the intramitochondrial $NADH/NAD^+$ couple became even less reduced. A lower intramitochondrial $NADH/NAD^+$ ratio would be expected to result in a shift of the equilibrium position of the reaction catalysed by malate dehydrogenase towards oxaloacetate, thus increasing the availability of this substrate for citrate formation. This would increase the rate of citrate formation because citrate synthase is not saturated with the metabolite at the concentration of oxaloacetate present in the mitochondrial matrix [30,31].

In conclusion, the present data show that, when extrahepatic factors are excluded, whereas the control exerted by CPT I over ketone body formation remains very high irrespective of the nutritional state of the animals, that exerted over the TCA cycle is very low and decreases even further during the initial stages of the reversal of this ketogenic state. The latter is likely to be an important strategy *in vivo*, through which, under conditions of decreased delivery of NEFA to the liver (e.g. during refeeding of starved animals), the flux of carbon to the TCA cycle is spared in order to ensure that enough energy is made available for continued gluconeogenic flux [33,34], in spite of the decreased overall rate of $\beta$-oxidation of fatty acids [1].

L.D. was supported through a BBSRC LINK research grant. We thank A. Caldwell for excellent assistance, and the Scottish Office Agricultural, Environment and Fisheries Department for additional support. P.A.Q. thanks St. John's College, University of Cambridge, for the tenure of a Senior Medical Research Fellowship for the duration of this work.

## REFERENCES

1 Zammit, V. A. (1981) Trends Biochem. Sci. **6**, 46–49
2 Zammit, V. A. (1995) Diabetes Rev. **2**, 132–155
3 McGarry, J. D., Mannaerts, G. P. and Foster, D. W. (1977) J. Clin. Invest. **60**, 265–270
4 Cook, G. A., Otto, D. A. and Cornell, N. W. (1980) Biochem. J. **192**, 955–958
5 Ontko, J. A. and Johns, M. L. (1980) Biochem. J. **192**, 959–962
6 Cook, G. A. (1984) J. Biol. Chem. **259**, 12030–12033
7 Drynan, L., Quant, P. A. and Zammit, V. A. (1996) Biochem. J., in the press
8 Kolodziej, M. P., Crilly, P. J., Corstorphine, C. G. and Zammit, V. A. (1992) Biochem. J. **282**, 415–421
9 Grantham, B. D. and Zammit, V. A. (1988) Biochem. J. **249**, 409–414
10 Grantham, B. D. and Zammit, V. A. (1986) Biochem. J. **239**, 485–488
11 Moir, A. M. B. and Zammit, V. A. (1993) Biochem. J. **289**, 49–55
12 Moir, A. M. B. and Zammit, V. A. (1994) Biochem. J. **304**, 177–182
13 Moir, A. M. B. and Zammit, V. A. (1994) Trends Biochem. Sci. **19**, 313–317
14 Casals, N., Roca, N., Guerrero, M., Gil-Gomez, G., Ayte, J., Ciudad, C. J. and Hegardt, F. G. (1992) Biochem. J. **283**, 261–264
15 Serra, D., Casals, N., Asino, G., Royo, T., Ciudad, C. J. and Hegardt, F. G. (1993) Arch. Biochem. Biophys. **307**, 40–45
16 Hegardt, F. G. (1995) Biochem. Soc. Trans. **23**, 486–490
17 Quant, P. A. (1990) Biochem. Soc. Trans. **18**, 994–995
18 Groen, A. K., van der Meer, R., Westerhoff, H. V., Wanders, R. J. A., Akerboom, T. P. M. and Tager, J. M. (1981) in Metabolic Compartmentation (Sies, H., ed.), pp. 9–37, Elsevier, Amsterdam
19 Seglen, P. O. (1973) Methods Cell Biol. **13**, 30–83
20 Zammit, V. A. (1995) Biochem. J. **312**, 57–62
21 Krebs, H. A. and Henseleit, K. (1932) Hoppe-Seylers Z. Physiol. Chem. **210**, 33–66
22 Boon, M. R. and Zammit, V. A. (1988) Biochem. J. **249**, 645–652
23 Guzman, M., Kolodziej, M. P., Caldwell, A. M., Corstorphine, C. G. and Zammit, V. A. (1994) Biochem. J. **300**, 693–699
24 Small, J. R. (1993) Biochem. J. **296**, 423–433
25 Kiorpes, T. C., Hoerr, D., Ho, W., Weaner, L. E., Inman, M. G. and Tutwiler, G. F. (1984) J. Biol. Chem. **259**, 9750–9755
26 Guzman, M. and Gellen, M. J. H. (1992) Biochem. J. **287**, 487–492
27 Chatterjee, B., Song, C. S., Kim, J. M. and Roy, A. K. (1988) Biochemistry **27**, 9000–9006
28 Lilly, K., Sugaisky, G. E., Umeda, P. K. and Bieber, L. L. (1990) Arch. Biochem. Biophys. **280**, 167–174
29 Broadway, N. M. and Saggerson, E. D. (1995) Biochem. Soc. Trans. **23**, 490–494
30 Garland, P. B., Shepherd, D., Nicholls, D. G. and Ontko, J. (1968) Adv. Enzyme Regul. **6**, 3–30
31 Lopes-Cardozo, M., Mulder, L., Van Vugt, F., Hermans, P. G. and van den Bergh, S. (1975) Mol. Cell. Biochem. **9**, 155–173
32 Des Rosier, C., David, F., Garneau, M. and Brunengraber, H. (1991) J. Biol. Chem. **266**, 1574–1578
33 Sugden, M. C., Watts, D. I., Palmer, T. N. and Myles, D. D. (1983) Biochem. Int. **7**, 329–337
34 Katz, J. and McGarry, J. D. (1984) J. Clin. Invest. **74**, 1901–1909

Received 9 February 1996/25 March 1996; accepted 29 March 1996