Hepatic mitochondrial inner-membrane properties, β -oxidation and carnitine palmitoyltransferases A and B

Effects of genetic obesity and starvation

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Hepatic mitochondrial carnitine palmitoyltransferase (CPT) properties, β -oxidation of palmitoyl-CoA and membrane polarization were measured in lean and obese Zucker rats. The $V_{\max}$ of the 'outer' carnitine palmitoyltransferase ('CPT-A') increased with starvation, with no change in the K_m for either carnitine or palmitoyl-CoA. The K_i for malonyl-CoA increased with starvation in lean rats, but not in obese rats. The $V_{\max}$ of the 'inner' enzyme ('CPT-B'), as measured by using inverted submitochondrial vesicles, increased with starvation in obese rats only, with no change in the K_m for either carnitine or palmitoyl-CoA. The K_i for malonyl-CoA was 2–5-fold higher in inverted vesicles than in intact mitochondria, and showed no alteration with starvation. The activities of both enzymes correlated positively with each other and with β -oxidation, and inversely with membrane polarization. Malonyl-CoA had little effect on gross membrane fluidity in the Zucker rat, as reflected by diphenylhexatriene fluorescence polarization. The results indicate that both enzymes are related and respond similarly to alterations in membrane fluidity. Membrane fluidity may provide a mechanism for co-ordinated control of CPT activity on both sides of the mitochondrial inner membrane.

INTRODUCTION

Previous studies using intact isolated mitochondria have indicated that total CPT activity in hepatic mitochondria is higher in obese adult Zucker rats than in their lean counterparts, and that total CPT activity increases with starvation in both lean and obese rats (Brady & Hoppel, 1983a). However, hepatic fatty-acid-oxidative capacity was not increased in obese rats, and did not increase with starvation. CPT-A, located on the outer surface of the mitochondrial inner membrane, has been proposed as the rate-limiting enzyme of hepatic mitochondrial β -oxidation of fatty acids (McGarry *et al.*, 1978; McGarry & Foster, 1980). Specific increases in CPT-A have been reported (reviewed by Hoppel & Brady, 1985) in starvation (Bremer, 1981; Cook, 1984; Saggerson *et al.*, 1984), in diabetes (Harano *et al.*, 1972; Cook *et al.*, 1984) and with hormonal alterations (Harano *et al.*, 1982; Stakkestad & Bremer, 1983) in the rat. However, these conditions have not led to increases in β -oxidative rates (DiMarco & Hoppel, 1975; Brady & Hoppel, 1983a,b).

The present study examines the relationship of CPT-A to hepatic mitochondrial β -oxidation under the same physiological conditions in Zucker rats. Since CPT-A and CPT-B (the activity of the inner surface of the mitochondrial inner membrane) are both membrane-associated, the question arises as to whether increases in enzyme activity are the result of synthesis or of membrane alterations. Many membrane enzymes have been shown to be influenced by membrane fluidity and protein–lipid interactions (Sandermann, 1978). Enzymes located in the

mitochondrial inner membrane have been shown to respond differently to changes in lipid composition (McMurchie *et al.*, 1983a), and the explanation has been offered that membrane 'microenvironment' rather than bulk membrane fluidity may control individual enzymes. Other membrane enzymes have also been shown to respond to nutritional (McMurchie *et al.*, 1983a,b,c), hormonal (Clejan *et al.*, 1980) and pharmacological states (Gordon *et al.*, 1980).

Although the hepatic mitochondrial-membrane characteristics of lean and obese Zucker rats have not been studied, those of lean and obese (*ob/ob*) mice have. York *et al.* (1982) found that polarization measured by 1,6-diphenylhexa-1,3,5-triene was depressed in obese mice relative to lean controls. This is consistent with changes in fatty acid composition of membrane phospholipids.

The purposes of the present studies were: (1) to determine kinetics, malonyl-CoA sensitivity and temperature-dependence of CPT-A and CPT-B *in situ* in Zucker rats; (2) to correlate CPT-A activity with hepatic mitochondrial β -oxidation in Zucker rats; and (3) to examine membrane characteristics under the same conditions.

EXPERIMENTAL

Animals

Zucker rats were obtained from Indiana University at 6 weeks of age, and maintained on a semi-synthetic diet

Abbreviations used: CPT, carnitine palmitoyltransferase (EC 2.3.1.21); CPT-A and CPT-B, CPT activity of the outer and the inner surface of the mitochondrial inner membrane respectively (nomenclature of Hoppel, 1982).

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(Brady & Hoppel, 1983a). The rats were housed in group cages, with access to food and water *ad libitum* for 2–4 weeks before use. In the starvation studies, food was withdrawn for 72 h.

Mitochondrial isolation and incubation

This procedure has been described in detail (Hoppel *et al.*, 1979). In brief, rats were killed by decapitation, and livers placed into ice-cold buffer (220 mM-mannitol, 70 mM-sucrose, 5 mM-Mops, 2 mM-EDTA, pH 7.4). Livers were rinsed, blotted, weighed, minced and homogenized with a loose-fitting Teflon pestle. Mitochondria were then isolated by differential centrifugation. The mitochondrial pellet was washed twice, and diluted to a final concentration of approx. 50 mg of protein/ml (Gornall *et al.*, 1949).

Inverted submitochondrial vesicles

These were prepared from the fresh mitochondria by using nitrogen compression/decompression as described by Fleischer *et al.* (1974). The inverted vesicles sedimented as brownish translucent pellets. The pellet was resuspended and used immediately. Success of the inversions has been previously determined in Sprague-Dawley rats (Brady *et al.*, 1985) by measuring the ratio of succinate oxidase to succinate-cytochrome *c* reductase, and by determination of D- β -hydroxybutyrate dehydrogenase (Vidal *et al.*, 1983). As measured by the succinate oxidase/succinate-cytochrome *c* reductase ratios, approx. 90% efficiency of inversion was obtained. The β -hydroxybutyrate dehydrogenase activity was undetectable in intact mitochondria in iso-osmotic buffer; in inverted vesicles at 30 °C, the activity was similar to that observed by Vidal *et al.* (1983). Further, we have observed no effect of starvation or diabetes on the efficiency of inversion (Brady *et al.*, 1985). There was no obvious difference in the characteristics of inverted vesicles prepared from lean and obese, or fed and starved, Zucker rats.

Assay methods

CPT activity was measured in the direction of palmitoylcarnitine formation with L-[methyl- 14 C]carnitine as substrate (Hoppel & Tomec, 1972). The CPT assay, pH 7.0, contained, in a final volume of 0.25 ml: 80 mM-KCl, 50 mM-Mops, 1 mM-EGTA, 1 mg of dialysed, defatted bovine serum albumin/ml, 4 mg of sodium tetrathionate/ml, 5–60 μ M-palmitoyl-CoA, 0.01–2.0 mM total L-carnitine (unlabelled plus 14 C-labelled). The reaction was initiated by the addition of 50 μ g of mitochondrial protein, and the reaction allowed to proceed for 2 min at 30 °C for the kinetic determinations unless otherwise noted. The specific radioactivity of the L-[methyl- 14 C]carnitine was adjusted as necessary to provide adequate sensitivity. The assay was linear for up to 4 min with 100 μ g of protein. Kinetic parameters were measured by varying both palmitoyl-CoA and carnitine (0.01–2 mM) concentrations (Segel, 1975; Brady *et al.*, 1985; Brady & Brady, 1985). Although for most of experiments only two palmitoyl-CoA concentrations were used (10 and 40 μ M), initial studies with three to five palmitoyl-CoA concentrations indicated good linearity of the replots. The two concentrations routinely used yielded comparable estimates of kinetic parameters. Dixon plots were used to estimate the malonyl-CoA sensitivity of the preparations (Segel, 1975). Malonyl-CoA was added to the assay over the range 0–50 μ M. Under the conditions employed, velocity was constant within the time and concentration used. Also, the Dixon plots showed statistically significant linear correlation over the malonyl-CoA concentrations used.

Arrhenius plots were generated over a temperature range of 6–50 °C, with 40 μ M-palmitoyl-CoA and 0.5 mM-carnitine in the standard assay mixture. Energy of activation, E_a , was calculated from the slope of the plot of $\ln(\text{activity})$ versus $1/T$, where slope = E_a/R (Williams & Williams, 1972). We have previously determined that, within this range of temperature, K_m values for carnitine

Table 1. Kinetics and malonyl-CoA-sensitivity of hepatic carnitine palmitoyltransferase activities from fed and starved lean and obese Zucker rats

Pairs of Zucker lean and obese rats were used at 8–9 weeks of age. Rats were either fed or starved for 72 h. Mitochondria and inverted submitochondrial vesicles were prepared as described in the Experimental section. CPT activity was assayed at pH 7.0 at 30 °C, in a mixture (0.25 ml) containing 80 mM-KCl, 50 mM-Mops, 1 mM-EGTA, 4 mg of sodium tetrathionate/ml and 1 mg of bovine serum albumin/ml. Palmitoyl-CoA concentrations were 10 and 40 μ M. L-Carnitine was present in the concentration range 0.01–0.5 mM, and malonyl-CoA in the range 0–50 μ M. The reaction was initiated by the addition of 50 μ g of fresh mitochondrial or vesicle protein, and allowed to proceed for 2 min. Each value is the mean for three to six animals: the S.E.M. is derived from the analysis of variance.

	Lean		Obese		S.E.M.
	Fed	Starved	Fed	Starved	
Intact mitochondria					
$V_{\max}$, (nmol/min per mg)	6.2	19.0	4.8	14.7	1.4
K_m for carnitine (mM)	0.4	0.2	0.2	0.3	0.08
K_m for palmitoyl-CoA (μ M)	31.6	32.3	42.3	30.2	8.6
K_i for malonyl-CoA (μ M)	11.6	41.3	23.9	18.2	5.1
Inverted mitochondria					
$V_{\max}$, (nmol/min per mg)	20.5	25.4	9.4	24.7	2.7
K_m for carnitine (mM)	0.3	0.2	0.2	0.3	0.03
K_m for palmitoyl-CoA (μ M)	50.0	47.0	19.0	50.1	6.0
K_i for malonyl-CoA (μ M)	58.3	89.1	69.3	109.0	14.5

Fig. 1. Relationship of CPT activities of intact mitochondria and inverted submitochondrial vesicles in Zucker rats

Intact mitochondria and inverted vesicles were prepared from lean and obese, fed and starved (72 h) female Zucker rats as described in the Experimental section. CPT activity was determined with 0.5 mM-L-carnitine and 40 μ M-palmitoyl-CoA over the temperature range 6–50 °C. Activity (v) is expressed as nmol of palmitoylcarnitine formed/min per mg of protein. Treatments are designated as follows: ●, lean fed; ■, obese fed; ○, lean starved; □, obese starved.

and for palmitoyl-CoA are not altered (L. J. Brady & P. S. Brady, unpublished work).

Fluorescence polarization was determined with 1,6-diphenylhexa-1,3,5-triene as probe (Prendergast *et al.*, 1981; Stubbs *et al.*, 1984). The concentration of mitochondrial or vesicle protein was adjusted to optimize the signal-to-noise ratio (5–10 mg of protein/ml). Polarization was determined spectrophotofluorimetrically (Perkin-Elmer model MFP-3L) with 366 nm excitation and 426 nm emission wavelengths, by using polarizing filters in both excitation and emission planes.

Materials

L-Carnitine was given by Sigma Tau, Rome, Italy. The L-[methyl- 14 C]carnitine was synthesized by the method of Ingalls *et al.* (1982), with the modification that demethylcarnitine was concentrated by freeze-drying, which provides adequate dryness to attain crystals. Palmitoyl-CoA was synthesized as described by Seubert (1960). Sodium tetrathionate was obtained from K & K Laboratories, Plainview, NY, U.S.A. Bovine serum albumin was prepared by defatting (Chen, 1967) and dialysing (Hanson & Ballard, 1968) commercially

available fraction V albumin. 1,6-Diphenylhexa-1,3,5-triene was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Malonyl-CoA was obtained from P-L Biochemicals, Piscataway, NJ, U.S.A. All other reagents were reagent grade or better.

RESULTS AND DISCUSSION

The kinetic properties and malonyl-CoA sensitivity of the CPT-A (intact mitochondria) and -B (inverted submitochondrial vesicles) activities from Zucker rats are presented in Table 1. Although no effect of genotype was observed for the calculated $V_{\max}$ of CPT-A, starvation increased $V_{\max}$ by 3-fold. The K_m values for L-carnitine and palmitoyl-CoA were not affected by genotype or starvation. We have previously found a 2-fold increase in the $V_{\max}$ of CPT-A in Sprague-Dawley rats with starvation and with diabetes (Brady *et al.*, 1985), and

Fig. 2. Relationship of hepatic mitochondrial β -oxidation and CPT-A activity in Zucker rats

CPT-A activity (v) and β -oxidation were measured in lean and obese fed and starved (72 h) Zucker rats as described in the Experimental section. In (a), CPT activity (nmol/min per mg of protein) and β -oxidation (ng-atoms of O/min per mg of protein) were varied by varying [palmitoyl-CoA], 2.5–40 μ M, and [L-carnitine], 0.05–0.5 mM. In (b), activity and β -oxidation were altered by changing temperature over the range 10–40 °C. Substrate concentrations were: ADP, 2 mM; malate, 2.5 mM; L-carnitine, 2 mM; palmitoyl-CoA, 40 μ M. Treatments are designated as follows: (a) ●, lean; ■, obese; (b) ●—●, lean fed; ■—■, obese fed; ○—○, lean starved; □—□, obese starved.

Fig. 3. Relationship of hepatic mitochondrial CPT activities to membrane fluorescence polarization in Zucker rats

Intact mitochondria (a) and inverted submitochondrial vesicles (b) were isolated from Zucker rats, and CPT activity was measured as described in the Experimental section. Fluorescence polarization was measured with 1,6-diphenylhexa-1,3,5-triene as a probe and correlated with CPT activities (v ; nmol/min per mg of protein) over the temperature range 10–40 °C. Treatments are designated as follows: ●—●, lean fed; ■—■, obese fed; ○····○, lean starved; □—□, obese starved.

others have also noted increased activity of CPT-A in starvation (Bremer, 1981; Saggerson *et al.*, 1982, 1984; Stakkestad & Bremer, 1983; Cook, 1984). The $V_{\max}$ of the inverted vesicles (CPT-B activity) was higher than that observed in intact mitochondria. The CPT velocity *in situ* in the intact and inverted mitochondria correlated linearly in the present study (Fig. 1), as we have previously observed in fed, starved and diabetic rats (Brady *et al.*, 1985; Brady & Brady, 1985). The correlation indicates that enzyme activity on both sides of the membrane responds similarly to perturbation, but in no way proves that they are the same enzyme protein. Bergstrom & Reitz (1980) and Clarke & Bieber (1981) have supported the concept that the CPT activity on both sides of the mitochondrial inner membrane is a single protein, and Bieber & Farrell (1983) suggested that membrane interaction may be important in regulation. In the present study, the CPT activities retain a constant ratio with temperature perturbation. This is certainly consistent with a single CPT protein. If, indeed, CPT-A and -B are the same protein, then it will be important to determine how the enzyme is allocated to the inner and the outer membrane surface. In particular, in the current

study, CPT-A activity increased 3-fold in lean rats with starvation, whereas the putative CPT-B activity in the same rats showed little change. At the same time, the obese rats maintained a constant ratio of CPT-A to -B with starvation. Thus there appear to be circumstances where the ratio of activities do shift.

The K_m values for carnitine and palmitoyl-CoA were unaffected by genotype or starvation in the inverted vesicles. The K_m values for carnitine were comparable with those observed in intact mitochondria; but, except for fed obese rats, the K_m values for palmitoyl-CoA tended to be higher in the inverted vesicles.

In the intact mitochondria, lean rats showed an almost 4-fold increase in the K_i for malonyl-CoA with starvation, but no change was observed in the obese rats. The pattern of response in the lean rats is similar to that observed for Sprague-Dawley and Wistar rats (Cook, 1984; Saggerson *et al.*, 1982, 1984). Clouet *et al.* (1985) have studied malonyl-CoA inhibition of CPT-A in 11-week-old male Zucker rats after 16 h of starvation. They found that mitochondria from obese rats were more sensitive to malonyl-CoA, although CPT-A activity was the same in mitochondria from lean and obese rats. The results are not strictly comparable with our own, since different assay conditions and periods of starvation were used. In the present study, there was no difference in CPT $V_{\max}$ in mitochondria from lean and obese rats. In fed female Zucker rats, the K_i for malonyl-CoA was higher in mitochondria from obese than from lean rats, but in 72 h-starved rats the K_i was lower in the mitochondria from obese rats. The K_i for malonyl-CoA in the inverted vesicles was 2–5 times that observed in intact mitochondria, and failed to show a significant effect of either genotype or starvation. The values were consistent with values that we have previously observed in starved and diabetic rats (Brady *et al.*, 1985). There is little question that the inverted vesicles do exhibit some malonyl-CoA sensitivity. Even if it is argued that contamination with right-side-out particles caused underestimation of the K_i for malonyl-CoA, the degree of contamination that would be necessary to yield the observed values in the face of a postulated totally malonyl-CoA-insensitive inverted vesicle becomes unreasonably large, and inconsistent with the reported efficiency of this method. It can also be argued that the K_i for malonyl-CoA that is observed in the inverted vesicles is too high to have physiological significance. The K_i for malonyl-CoA is increased if cytochrome *c*-Sepharose affinity chromatography is used to purify the inverted submitochondrial vesicles further (P. S. Brady & L. J. Brady, unpublished work). This indicates that inverted vesicles are quite insensitive to malonyl-CoA.

Malonyl-CoA-sensitivity of the outer enzyme has been used as evidence that there are two enzymes. However, Mills *et al.* (1984) suggest that the malonyl-CoA-sensitivity may be mediated primarily by a receptor located on the outer surface of the inner membrane. The present data suggest that, *in situ*, CPT-B may express some sensitivity to malonyl-CoA. Still, if there is a malonyl-CoA receptor, we cannot rule out the possibility that, in the preparation of the inverted vesicle, the receptor becomes exposed on the inside-out surface.

CPT-A has been proposed as the rate-limiting enzyme of mitochondrial fatty acid oxidation. If CPT-A were, in fact, rate-limiting, we would expect it to be induced in situations favouring fatty acid oxidation. One such

Table 2. Response of mitochondrial membrane fluorescence polarization to pH and malonyl-CoA in lean and obese fed and starved Zucker rats

Pairs of Zucker lean and obese rats were used at 8–9 weeks of age. Rats were either fed or starved for 72 h. Mitochondria were prepared as described in the Experimental section. 1,6-Diphenylhexa-1,3,5-triene fluorescence polarization was determined with 5–10 mg of mitochondrial protein/ml in 80 mM-KCl/50 mM-Mops/1 mM-EGTA adjusted to the desired pH with KOH at 25 °C. Each value is the mean of three observations: S.E.M., calculated from the analysis of variance, was 0.032.

pH	Genotype	[Malonyl-CoA] (μ M)...	Fluorescence polarization			
			Fed		Starved	
			0	100	0	100
6.6	Lean		0.177	0.181	0.189	0.172
	Obese		0.170	0.156	0.170	0.191
6.8	Lean		0.163	0.178	0.186	0.179
	Obese		0.187	0.160	0.174	0.159
7.0	Lean		0.163	0.170	0.180	0.170
	Obese		0.160	0.172	0.174	0.177
7.2	Lean		0.173	0.162	0.162	0.156
	Obese		0.149	0.136	0.150	0.162
7.4	Lean		0.145	0.149	0.158	0.170
	Obese		0.152	0.148	0.148	0.149

situation would be starvation. Starvation in the Zucker rat led to a 3-fold increase in CPT-A activity measured with 40 μ M-palmitoyl-CoA and 0.5 mM-L-carnitine. Under the same conditions, the mitochondrial state-3 oxidative rate for palmitoyl-CoA + carnitine (ng-atoms of O/min per mg of mitochondrial protein) did not increase. This confirmed our previous observations in starved Zucker rats (Brady & Hoppel, 1983a). We investigated this further by measuring CPT-A activity and mitochondrial oxygen consumption under a variety of conditions (Fig. 2a). CPT-A activity and β -oxidation were linearly related. Analysis of the relationship indicated that it could be described equally well by linear and quadratic functions. Since these experiments did not, then, resolve the relationship, we examined the effect of varying the temperature (10–40 °C) on CPT-A activity and β -oxidation (Fig. 2b). Under these conditions, CPT-A and CPT-B activities exhibited a strong linear correlation with oxidative rate.

Membrane fluidity is reciprocally related to polarization, and has been used as an indication of the physical state of the membrane lipids (Sandermann, 1978). Through specific choice of fluorescent probe, a variety of environments can be sampled. The 1,6-diphenylhexa-1,3,5-triene probe used in our studies has been used extensively to monitor changes in the deep, hydrophobic, regions of the membrane bilayer (Prendergast *et al.*, 1981; Stubbs *et al.*, 1984). The relationship of CPT-A and -B activities to membrane polarization, when temperature was used to perturb the membrane, is presented (Fig. 3). This relationship was linear, and was not altered by either genotype or starvation. Together with the data presented in Fig. 1, these results indicate that inner and outer CPT activities are correlated and respond similarly to changes in membrane fluidity (i.e. increased fluidity is associated with increased activity). We have previously noted that CPT activity increases with increasing membrane fluidity, as measured with 1,6-diphenylhexa-1,3,5-triene in the Sprague–Dawley rat (Brady *et al.*, 1985).

We have also reported that malonyl-CoA increases membrane rigidity in fresh intact hepatic mitochondria from fed, but not from starved or diabetic, Sprague–Dawley rats (Brady *et al.*, 1985). When the same experiments were performed with Zucker rats, we found that the response of fed lean rats was diminished compared with that of Sprague–Dawley rats, and that the response was totally absent in the lean starved and obese starved and fed rats (Table 2). We have postulated that the effect of malonyl-CoA could be mediated through alterations in membrane fluidity. Co-ordinated control of both CPT activities would be possible, then, through interaction of the malonyl-CoA with only the outer membrane surface. The lean Zucker rat, even in the fed state, is less sensitive to malonyl-CoA inhibition than is the Sprague–Dawley rat (Brady *et al.*, 1985). Still, in the lean Zucker rat the K_i for malonyl-CoA increases 4-fold with starvation, concurrent with a decrease in the membrane response malonyl-CoA. In the obese rats, sensitivity to malonyl-CoA inhibition is initially low in the fed state, and is not altered by starvation. Again, concurrent with decreased sensitivity to malonyl-CoA is decreased membrane response to malonyl-CoA.

The relationship of palmitoyl-CoA oxidation to membrane polarization is presented in Fig. 4. Over the temperature range studied, the relationship was linear, as it was for the enzyme activities with polarization. This is not surprising, in view of the relationship of the two CPT activities (Fig. 1) and their relationship to palmitoyl-CoA oxidation (Fig. 2). These data indicate that the physical state of the mitochondrial inner membrane may be important to the expression of CPT activity, and to malonyl-CoA inhibition, as well as to the broader process of mitochondrial β -oxidation. Factors altering the physical state of the membrane offer the potential for co-ordinated changes in activity of a number of enzymes, as opposed to the more classical interaction of specific effector with specific enzyme. CPT-A has been proposed as the rate-limiting enzyme of mitochondrial fatty acid

Fig. 4. Relationship of hepatic mitochondrial palmitoyl-CoA oxidation and membrane fluorescence polarization in Zucker rats

Mitochondrial palmitoyl-CoA oxidation (ng-atoms of O/min per mg of protein) was determined with 40 μ M-palmitoyl-CoA, 2 mM-L-carnitine, 2.5 mM-malate and 2 mM-ADP with a Clark-type oxygen electrode. Fluorescence polarization was determined with a 1,6-diphenylhexa-1,3,5-triene probe. Temperature was varied (10–40 °C). Treatments are designated as follows: ●—●, lean fed; ■—■, obese fed; ○····○, lean starved; □—□, obese starved.

oxidation. Yet it is only part of a complex system of fatty acid oxidation, involving mitochondria, peroxisomes and microsomal fractions. Although it is clear that the control exerted by malonyl-CoA on CPT-A could restrict long-chain acyl-CoA oxidation by mitochondria, such restriction might divert fatty acyl-CoA through peroxisomal oxidation, yielding medium-chain acylcarnitines, which are no longer subject to regulation by CPT-A as they enter the mitochondrion. If, however, malonyl-CoA control of mitochondrial fatty acid oxidation were exerted via changes in the mitochondrial membrane, then co-ordinate control of a number of membrane-bound enzymes of fatty acid oxidation could be effected. We do not know clearly that this does occur. We do know that, under certain conditions (Brady *et al.*, 1985), malonyl-CoA results in a decrease in the bulk mitochondrial membrane fluidity, which decreases CPT-A and -B activities. Bulk fluidity measurements proved less fruitful in the Zucker rats than we have observed in Sprague-Dawley rats, and this may point up the limitations of bulk measurements. What is clear from the present studies is that measurements of CPT activity *in situ* are necessary to

understand the relationship of this activity to the membrane and vice versa. We believe that the inverted vesicle may provide a useful means of probing the CPT-B activity *in situ*, which has heretofore not been explored.

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