BIOCHEMICAL JOURNAL LETTERS

Does fasting decrease the inhibitory effect of malonyl-CoA on hepatic β-oxidation?

The inhibitory effect of malonyl-CoA on both the oxidation of fatty acid (Cook *et al.*, 1980; Ontko & Johns, 1980; Veerkamp & Van Moerkerk, 1982) and the activity of CPT_1 in rat liver mitochondria (Saggerson & Carpenter, 1981*a*; Bremer, 1981; Robinson & Zammit, 1982) is decreased on fasting. However, McGarry & Foster (1981) have stated that this sensitivity change is of little or no physiological significance and have also criticised the experimental approach used in this laboratory. I should like to answer this criticism and demonstrate that previously published studies from the laboratory of McGarry and Foster support our conclusions.

McGarry & Foster (1981) infer that our assay of CPT_1 uses excessive palmitoyl-CoA/albumin ratios which will offset the effect of malonyl-CoA (resulting in a higher I_{50} for malonyl-CoA) and also, by an unspecified detergent action, increase the amount of CPT which is suppressible by malonyl-CoA. It is true that the I_{50} will be altered by changing the [palmitoyl-CoA], since the inhibitor alters the $K_{0.5}$ for this substrate without altering the $V_{max.}$, giving sigmoidal kinetics (Saggerson & Carpenter, 1981*b*). Other changes in experimental conditions, e.g. the presence of thiol group reagents (Saggerson & Carpenter, 1982) or changes in $[H^+]$ (E. D. Saggerson & C. A. Carpenter, unpublished work) also alter the sensitivity to malonyl-CoA. The fact remains that when CPT_1 is assayed under identical experimental conditions in mitochondria from fed and fasted rats, the enzyme shows substantially decreased sensitivity to malonyl-CoA after fasting (Saggerson & Carpenter, 1981*a*,*b*). Fig. 1 restates this, showing that after fasting CPT_1 is less sensitive to malonyl-CoA over a very wide range of enzyme activity induced by alteration of the palmitoyl-CoA/albumin ratio. Detergent effects of palmitoyl-CoA under the conditions used previously (Saggerson & Carpenter, 1981*a*) can be discounted since these cause no increase in leakage of citrate

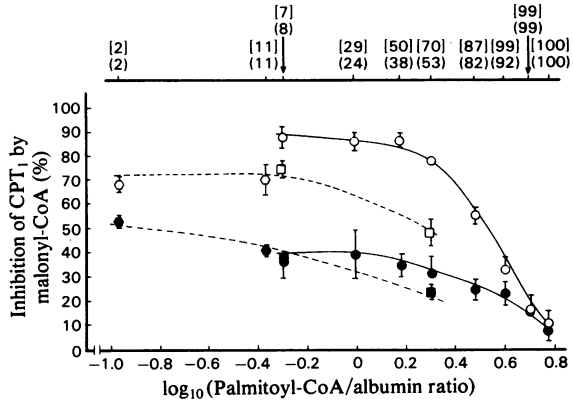

Fig. 1. *Effect of palmitoyl-CoA/albumin ratio on inhibition of CPT_1 by malonyl/CoA in liver mitochondria from fed and fasted (24 h) rats*

Open symbols, fed; closed symbols, fasted. Solid lines (○, ●); data derived from Fig. 1 of Saggerson & Carpenter (1981*b*). [Albumin] = 19.7 μM throughout. The values are means ± S.E.M. of four experiments. [Malonyl-CoA] = 50 μM. Broken lines; new data. The values are means of two experiments with the bars indicating the range of values. [Malonyl-CoA] = 10 μM. □, ■, [albumin] = 19.7 μM; ○, ●, [albumin] = 91 μM. The values in brackets and parentheses indicate the percentage of the $V_{max.}$ rate achieved in control assays without malonyl-CoA in the fed and fasted states respectively.

Abbreviations used: CPT, carnitine palmitoyltransferase (EC 2.3.1.21); CPT_1 and CPT_2, the overt and latent forms respectively of CPT; I_{50}, the concentration of malonyl-CoA required to suppress CPT_1 activity by 50%.

synthase (Saggerson & Carpenter, 1981*c*; Saggerson, 1982).

The reason for differences between ourselves and McGarry & Foster (1981) in the proportion of CPT activity that is suppressible by malonyl-CoA is attributable to differences in assay procedure. McGarry & Foster (1981) used an isotope exchange assay (assay I of McGarry *et al.*, 1978*a*). Isotope incorporation by this method is claimed to be due to the action of both CPT_1 (malonyl-CoA-sensitive) and CPT_2 (claimed to be malonyl-CoA-insensitive). We have used a 'unidirectional' assay (based on assay II of McGarry *et al.*, 1978*a*) which should only indicate CPT_1 activity. With intact mitochondria the proportion of the overt CPT that is malonyl-CoA suppressible should approach 100%, which is the case (Saggerson & Carpenter,

1981*a*). The fact that only approx. 60% of the overt transferase activity measured with octanoyl-CoA was malonyl-CoA-suppressible with a very low I_{50} (Saggerson & Carpenter, 1981*a*) could be explained by this activity being a composite of some carnitine acetyltransferase and a medium-chain-length transferase that is distinct from CPT. This overt carnitine octanoyltransferase activity clearly differs from CPT in a number of important respects (Saggerson & Carpenter, 1981*b*, 1982; Saggerson, 1982).

The use of the isotope exchange assay by McGarry & Foster (1981) could be problematical and could be the reason why they found little apparent change in malonyl-CoA sensitivity after fasting. The isotope exchange that is measured depends upon the initial generation of palmitoyl-CoA from palmitoylcarnitine. The rate of this generation may depend on the nutritional state (increased after fasting). Presumably the assay either eventually establishes a steady state [palmitoyl-CoA] or this continues to rise during the assay. Herein lies the problem, because the substrate and inhibitor effects of, respectively, palmitoyl-CoA and malonyl-CoA in this system are each influenced by the concentration of the other (Saggerson & Carpenter, 1981*b*).

Finally, Fig. 2, which pools experimental findings from the laboratory of McGarry and Foster, demonstrates that fasting substantially alters the relationship in hepatocytes between the rate of oleate oxidation and the content of malonyl-CoA. Unfortunately, no data are available for the rate of oxidation when the malonyl-CoA content is much below 1 nmol/g of cells. However, there are no experimental findings to suggest that inherent oxidative capacity in the absence of malonyl-CoA in the fasted state should be 2–3 times that of the fed state. I suggest that these findings support the contention that sensitivity to malonyl-CoA is considerably decreased in fasting and that this change is manifested within the cell.

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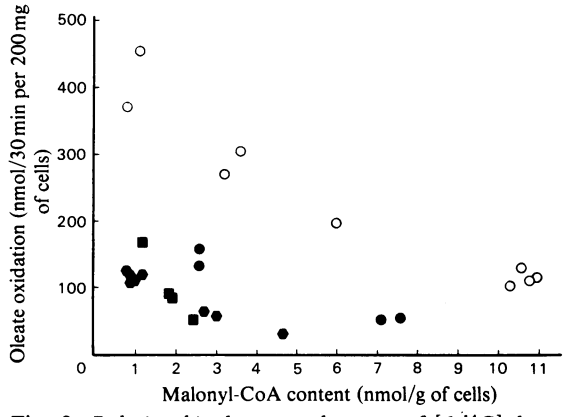

Fig. 2. *Relationship between the rate of [1-^{14}C]oleate conversion to total acid-soluble products + CO_2 and the content of malonyl-CoA in hepatocytes from fed and fasted (18 h) rats*

Open symbols, fasted; closed symbols, fed. The values are derived from the following: ○, from Fig. 4 and Table V of Boyd *et al.* (1981) ([oleate] = 0.1 mM); ●, from Table V of Boyd *et al.* (1981) ([oleate] = 0.1 mM); ■, from Table I of McGarry *et al.* (1978*b*) ([oleate] = 0.4 mM); ●, from Table I of McGarry & Foster (1979) ([oleate] = 0.4 mM).

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