High sensitivity of carnitine acyltransferase I to malonyl-CoA inhibition in liver of obese Zucker rats

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Received 25 January 1985

Carnitine acyltransferase of liver mitochondria prepared from obese Zucker rats has a higher sensitivity to inhibition by malonyl-CoA compared with carnitine acyltransferase of mitochondria prepared from lean Zucker rats.

Carnitine acyltransferase I *Malonyl-CoA* *Mitochondria* *Liver* *Zucker rat* *Obesity*

1. INTRODUCTION

The genetically obese Zucker rat shows an accumulation of triacylglycerols in the liver [1,2]. This steatosis can originate from an increased lipogenesis [3–5], or a decreased fatty acid oxidation, or both. The oxidation of fatty acids has been observed to be decreased in perfused liver [7], and in hepatocytes [8,9] of obese rats. By contrast, Brady and Hoppel [10] reported that liver mitochondria of obese and lean rats show the same capability for fatty acid oxidation. This discrepancy could be explained by the considerable loss, through the mitochondria isolation procedure, of metabolites known to regulate fatty acid oxidation in the intact cell [11–14]. Among these metabolites, malonyl-CoA is of particular interest because it strongly inhibits carnitine acyltransferase I (CAT I) (EC 2.3.1.21), the enzyme that catalyzes the synthesis of acylcarnitine and regulates the entry of fatty acids into mitochondria [15]. Moreover, CAT I has been suggested to respond differently to this inhibition, i.e., according to the nutritional and hormonal state [16–18].

The purpose of this work was, therefore, to determine whether mitochondrial CAT I of obese rats was more sensitive to the inhibitory effect of malonyl-CoA than in lean rats. This could explain the decreased oxidation of long chain fatty acids in the whole liver and in hepatocytes of obese Zucker rats, and thus, eventually, help in understanding the mechanism of lipid accumulation.

2. MATERIALS AND METHODS

11-week old male rats of the Zucker strain were from the Centre de Selection et d'Elevage d'Animaux de Laboratoire (CSEAL) du CNRS, Orléans-la-Source, France. The obese rats (fa/fa) and their lean littermates (Fa/?) weighed 420 ± 30 and 275 ± 15 g, respectively. They were killed by decapitation at 08:00 after 16 h food-deprivation. Livers were homogenized in a medium containing 0.25 M sucrose, 10 mM triethanolamine and 1 mM EDTA, at pH 7.4 (1:5, w/v), then centrifuged at $500 \times g$ for 10 min at 3°C. The sedimented pellet was resuspended and diluted with the same medium to the same volume as the initial homogenate, and recentrifuged at $500 \times g$ for 10 min. This operation was repeated three times. All the supernatants were eliminated except the last one that was centrifuged at $1300 \times g$ for 10 min. The pellet was resuspended in the same medium, EDTA excepted, and recentrifuged at $1300 \times g$ for 10 min. The final pellet gave a mitochondrial fraction only very slightly contaminated with peroxisomes, and good membrane integrity (unpublished). This fraction was used for the study of oleate oxidation. For the

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00145793/85/$3.30

estimation of CAT I activity, mitochondria were isolated by the same procedure as above except that the homogenizing medium was replaced by 0.25 M mannitol, 5 mM Hepes buffer (pH 7.4) and 1 mM EGTA as in [16], throughout the operation.

To study fatty acid oxidation, mitochondria were incubated in duplicate at 35°C in 2 ml of a medium containing 50 μM potassium [1-^{14}C]- or [10-^{14}C]oleate (CEA, Saclay, France) bound to 17.5 μM fatty acid-free bovine serum albumin (Sigma, St. Louis, MO), 20 mM potassium phosphate (pH 7.4), 50 mM KCl, 4 mM $MgCl_2$, 1 mM ATP, 50 μM CoA, 1 mM L-carnitine and 0.2 mM L-malate. The reaction was initiated by the addition of 1 mg of freshly prepared mitochondrial proteins. After 12 min the incubations were stopped by the addition of 10 ml of 10% perchloric acid, and the measurement of ^{14}C-labelled acid-soluble products (ASP) was performed as in [19]. The activity of the carnitine palmitoyltransferase I (EC 2.3.1.21) was measured in duplicate at 25°C according to Bremer [16] with slight modifications: 0.2 mg of freshly prepared mitochondrial proteins was added to 1 ml of medium containing 80 mM mannitol, 75 mM KCl, 25 mM Hepes buffer (pH 7.3), 0.2 mM EGTA, 45 μM palmitoyl-CoA, 0.5 mM dithiothreitol, 1% fatty acid-free bovine serum albumin, 2 mM KCN and increasing amounts of malonyl-CoA. After a 30 s preincubation, 0.7 mM L-[*methyl*-^{3}H]carnitine, about 1.4 μCi/μmol, was added. 6 min later, the reaction was stopped with 1 ml of 1 N HCl. The [^{3}H]palmitoylcarnitine was extracted with *n*-butanol as in [20] and then counted in Picofluor 30 (Packard Instrument Co) in a Packard 300C scintillation counter. The total carnitine content of homogenates was measured according to [21]. Mitochondrial proteins were estimated as in [22]. The triacylglycerols of the liver were estimated from their fatty acid content, as determined by gas-liquid chromatography [23]. Statistical significance was estimated by using Student's *t*-test.

3. RESULTS AND DISCUSSION

The liver of obese rats (420 ± 30 g) was significantly heavier ($p < 0.001$) than that of lean rats 275 ± 15 g), and had a higher content in triacylglycerol fatty acids (35.2 ± 6.4 vs 2.3 ± 0.7 mg/g, respectively, $p < 0.001$).

Table 1 shows that whatever the position of the labeled carbon atom in the oleate molecule, i.e., at the carboxyl end or in the middle of the chain, oleate oxidation was the same within each phenotype. This suggests that the oxidation of the oleate molecule was complete. Since an altered permeability of the mitochondrial membranes would be accompanied by an only partial oxidation of the fatty acid molecule [24], this provides evidence for the maintenance of mitochondria integrity in our experimental conditions. Furthermore, oleate oxidation was similar in obese and lean rats, which indicates that the oxidative capacity of the isolated liver mitochondria is not altered in obesity. This is in agreement with the previous findings of Brady and Hoppel [10].

The rate of fatty acid oxidation is related to the rate of entry of fatty acids into the mitochondria, and this entry is controlled by the activity of CAT I. Table 2 shows that CAT I activity was only

Table 1

Oxidation[a] of [1-^{14}C]- and [10-^{14}C]oleate by liver mitochondria from obese and lean Zucker rats

Substrate	Obese ($n = 4$)	Lean ($n = 4$)
[1-^{14}C]Oleate	54.8 ± 1.5[b] (NS)	56.5 ± 2.0
[10-^{14}C]Oleate	54.6 ± 2.1 (NS)	51.2 ± 1.9

[a] Results are given as nmol [^{14}C]oleic acid, the radioactivity of which was recovered as ^{14}C-labelled acid-soluble products

[b] Mean ± SE; NS, not significantly different from the lean ($p > 0.05$)

Table 2

Activity of CAT I and carnitine content in hepatic mitochondria from obese and lean Zucker rats

	Obese	Lean
CAT I[a] ($n = 5$)	2.87 ± 0.22[b]*	2.55 ± 0.13
Carnitine content[c] ($n = 6$)	87 ± 15**	138 ± 5

[a] The enzyme activity is expressed as 10^{-3} IU per mg mitochondrial protein

[b] Mean ± SE; * significantly different from the lean, $p < 0.05$; ** $p < 0.001$

[c] Results are given as nmol per g of mitochondrial protein

slightly higher in obese than in lean rats, and that the carnitine content was markedly lower in obese rats ($p<0.001$). A low carnitine content would depress fatty acid oxidation in isolated mitochondria, but the addition of carnitine in the medium suppresses this effect. In this condition, the almost identical CAT I activity might partly explain the similar fatty acid oxidation observed in mitochondria of obese and lean rats ([10], this study). On the other hand, in the cell, the low carnitine content of mitochondria might not constitute a limiting factor for fatty acid oxidation, as suggested from studies with hepatocytes in which the fatty acid oxidative activity was not enhanced by the addition of exogenous carnitine [9].

As shown in fig.1, in obese rats CAT I activity was extremely sensitive to the inhibitory effect of malonyl-CoA. At a high concentration (100 μM), this metabolite totally inhibited CAT I activity in obese rats, but only partially (80%) in lean rats. The concentration of malonyl-CoA that depressed CAT I activity by 50% was estimated at 1.4 ± 0.2 and 20 ± 6 μM in obese and lean rats, respectively. McGarry and Foster [25] and Beynen et al. [26] have reported that the level of hepatic malonyl-CoA is directly related to the rate of lipogenesis in several metabolic situations. Since in the liver of obese rats lipogenesis is enhanced, especially through the higher acetyl-CoA carboxylase activity [27], a high malonyl-CoA concentration might be expected. This high concentration, that still remains to be demonstrated, would strongly inhibit CAT I activity, and therefore fatty acid oxidation, in vivo. Nevertheless, as a lowering of malonyl-CoA might not be sufficient to explain the increased ketogenesis in starvation [28–30], so an increase of malonyl-CoA might not be sufficient to explain the lower fatty acid oxidation in obese rats. Actually, our results suggest that, even for an identical concentration, the fatty acid oxidation might be reduced in obesity because of the very high sensitivity of CAT I to malonyl-CoA inhibition. The transport of fatty acids across the mitochondrial inner membrane might therefore be a limiting step in fatty acid oxidation. However, studies by Triscari et al. [9] with hepatocytes from fasted obese

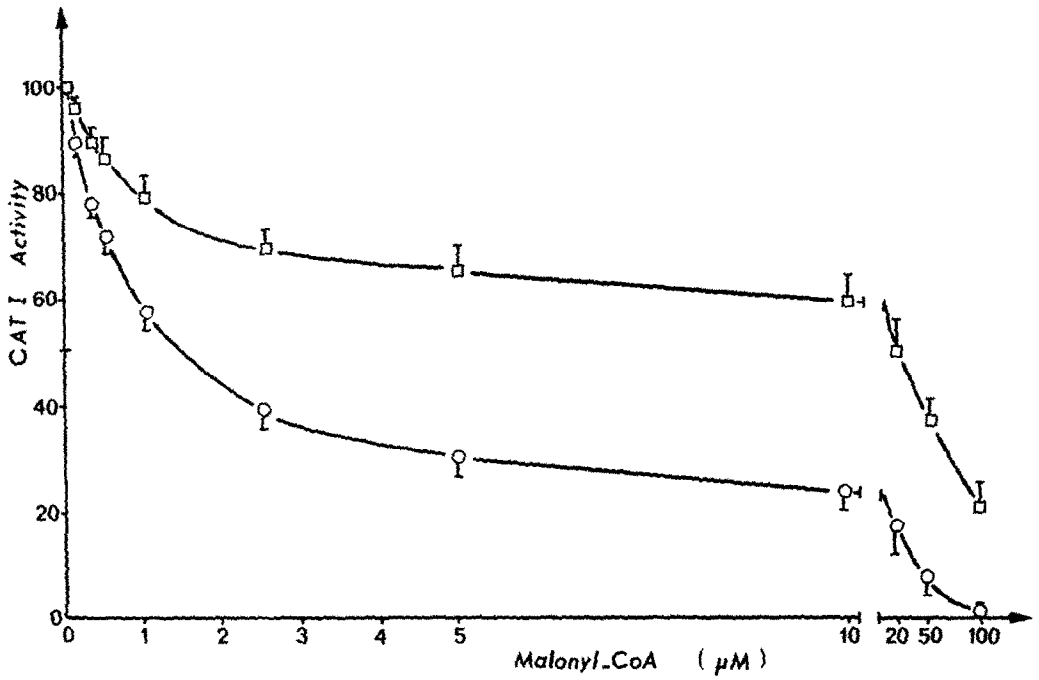

Fig. 1. Effect of increasing malonyl-CoA concentrations on the activity of CAT I in mitochondria isolated from livers of obese (○) and lean (□) Zucker rats. Results are expressed as % of CAT I activity, estimated in the absence of malonyl-CoA. Each point represents the mean of 5 duplicated determinations; each determination corresponds to one mitochondrial preparation from one rat. T-bars show SE.

rats indicate that the failure in ketogenesis might occur at a step following the entry of fatty acids into the mitochondria. This discrepancy concerning the site of the impairment could be explained by the fasted state of the rats used by these authors. Indeed, the mitochondrial CAT I sensitivity to malonyl-CoA inhibition was shown to be strongly reduced in this nutritional condition [16,17]. Moreover, the rats used by Triscari et al. [9] were different from ours in various aspects (female rats and identical lipid content of hepatocytes in both phenotypes).

In summary, in the liver of the obese rat, the very high sensitivity of CAT I to malonyl-CoA inhibition might, for the most part, explain the depressed fatty acid oxidation; this effect could be reinforced by a possible high malonyl-CoA concentration, and eventually by the low carnitine content of mitochrondria and of total tissue (unpublished). As a consequence, the fatty acids that do not enter the oxidative pathway should contribute to lipid accumulation. However, since malonyl-CoA is an intermediary metabolite in the synthesis of fatty acids, its inhibitory effect on fatty acid oxidation should be regarded rather as a consequence of the enhanced lipogenesis.

ACKNOWLEDGEMENTS

This work was supported in part by help from Laboratories Fournier, Dijon. We thank Dr. Groscolas for helpful discussion, Professor A.T. James for valuable advice, and Mr R. Brieda for excellent technical assistance.

REFERENCES

[1] Malewiak, M.I., Griglio, S., Mackay, S., Lemonnier, D. and Rosselin, G. (1977) Diab. Metab. 3, 81–89.
[2] Wang, D.S., Fukuda, N. and Ontko, J.A. (1984) J. Lipid. Res. 25, 571–579.
[3] Martin, R.J. (1974) Life Sci. 14, 1447–1453.
[4] Bloxham, D.P., Fitzsimons, J.T.R. and York, D.A. (1977) Horm. Metab. Res. 9, 304–309.
[5] Godbole, V. and York, D.A. (1978) Diabetologia 14, 191–197.
[6] Triscari, J., Ontko, J.A. and Sullivan, A.C. (1980) Nutr. Rep. Int. 21, 269–278.
[7] Fukuda, N., Azain, M. and Ontko, J.A. (1982) Fed. Proc. 41, 1216.
[8] McCune, S.A., Durant, P.J., Jenkins, P.A. and Harris, R.A. (1981) Metab. Clin. Exp. 30, 1170–1178.
[9] Triscari, J., Greenwood, M.R.C. and Sullivan, A.C. (1982) Metab. Clin. Exp. 31, 223–228.
[10] Brady, L.Y. and Hoppel, C.L. (1983) Am. J. Physiol. 245, E 239–245.
[11] McGarry, J.D., Mannaerts, G.P. and Foster, D.W. (1977) J. Clin. Invest. 60, 265–270.
[12] Hoppel, C.L. (1982) Fed. Proc. 41, 2853–2857.
[13] Morrow, F.D., Allen, C.E. and Martin, R.J. (1979) Fed. Proc. 38, 280.
[14] Lund, H., Borrebaek, B. and Bremer, J. (1980) Biochim. Biophys. Acta 620, 364–371.
[15] McGarry, J.D., Takabayashi, Y. and Foster, D.W. (1978) J. Biol. Chem. 253, 8294–8300.
[16] Bremer, J. (1981) Biochim. Biophys. Acta 665, 628–631.
[17] Robinson, I.N. and Zammit, V.A. (1982) Biochem. J. 206, 177–179.
[18] Stakkestad, J.A. and Bremer, J. (1983) Biochim. Biophys. Acta 750, 244–252.
[19] Clouet, P., Henninger, C. and Bézard, J. (1982) Biochimie 64, 899–906.
[20] Bremer, J. and Norum, K.R. (1967) Eur. J. Biochem. 1, 427–433.
[21] Ramsay, R.R. and Tubbs, P.K. (1975) FEBS Lett. 54, 21–25.
[22] Layne, E. (1957) Methods Enzymol. 3, 450–451.
[23] Bézard, J.A. and Ouedraogo, M.A. (1980) J. Chromatogr. 196, 279–293.
[24] Davidoff, F and Korn, E.D. (1965) J. Biol. Chem. 240, 1549–1558.
[25] McGarry, J.D. and Foster, D.W. (1979) J. Biol. Chem. 254, 8163–8168.
[26] Beynen, A.C., Vaartjes, W.J. and Geelen, M.J.H. (1979) Diabetes 28, 828–835.
[27] Taketomi, S., Ishikawa, E. and Iwatsuka, H. (1975) Horm. Matab. Res. 7, 242–246.
[28] Benito, M. and Williamson, D.H. (1978) Biochem. J. 176, 331–334.
[29] Ontko, J.A. and Johns, M.L. (1980) Biochem. J. 192, 959–962.
[30] Cook, G.A., Otto, D.A. and Cornell, N.W. (1980) Biochem. J. 192, 955–958.