GLYCEROLPHOSPHATE ACYLTRANSFERASE, DIHYDROXYACETONEPHOSPHATE ACYLTRANSFERASE AND CARNITINE PALMITOYLTRANSFERASE IN A GLYCOGEN STORAGE DISEASE (gsd/gsd) RAT

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1. Introduction

An inbred strain of rat (gsd/gsd) with a glycogen storage disease has been described in [1]. The defect in these animals appears to result from a deficiency of liver phosphorylase kinase [2]. Besides exhibiting mild hypoglycaemia in the fed state and inability to mobilise glycogen in the fasted state, these animals show abnormalities in lipid metabolism in that they have diminished rates of hepatic fatty acid and cholesterol synthesis and considerably lower concentrations of plasma triacylglycerols [3]. It is apparent that these animals may be a useful model for studies of equivalent forms of human glycogen storage diseases and it was therefore of interest to investigate some enzymatic aspects of lipid metabolism in this condition. Accordingly measurements have been made of hepatic glycerolphosphate acyltransferase (GPAT) activities (mitochondrial and microsomal) and dihydroxyacetonephosphate acyltransferase (DHAPAT) activity. These activities have been found to change in a number of physiological states and change in parallel with changes in hepatic triacylglycerol synthesis and secretion [4–7]. The activity of the overt form of carnitine palmitoyltransferase (CPT_1) has also been measured in liver mitochondria and the sensitivity of this enzyme to inhibition by malonyl CoA determined. This is thought to be an important factor in the regulation of hepatic fatty acid oxidation [8] and the sensitivity to this effector has been shown to change in at least one physiological state, namely fasting [9].

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2. Materials and methods

Coenzyme A thioesters were from PL-Biochemicals, L-carnitine from Sigma, radiochemicals from The Radiochemical Centre (Amersham) and other chemicals from Boehringer (Mannheim).

Adult male gsd/gsd (GSD(+)) and control (GSD(−)) rats, a substrain of the NZR/Gd line [1] were used. These were fed a standard diet with water ad libitum. The animals were anaesthetised with diethyl ether at 10:00–11:00 h and a 1–2 g portion of liver freeze-clamped in situ between metal blocks previously cooled in liquid nitrogen. Freeze-clamped pieces of liver were stored at −80°C. Portions of these were subsequently pulverised under liquid nitrogen in a mortar, transferred to a glass Potter-Elvejhem homogeniser and vigorously homogenised (25 strokes) with a motor-driven teflon pestle in an ice-cold sucrose medium consisting of 0.23 M sucrose, 10 mM Tris–HCl (pH 7.4), 10 mM KF, 1 mM EDTA, 1 mM EGTA and 0.25 mM dithiothreitol (60 mg liver/ml medium). The resulting homogenate was used directly for assay of GPAT and DHAPAT. A further 3–4 g portion of liver was removed from the animal and homogenised (4 strokes) in the same ice-cold sucrose medium and centrifuged to obtain mitochondria as in [10]. The mitochondria were washed with this buffer and finally resuspended in the same buffer at ~4 mg mitochondrial protein/ml. Protein was measured [11] using bovine serum albumin as a standard.

Aliquots (50 μl) of fresh whole mitochondria were assayed for CPT (EC 2.3.1.21) activity at 25°C in 1.0 ml containing 200 μM L-[^{3}H]carnitine, 40 μM palmitoyl CoA and defatted albumin (1.3 mg/ml) as in [9]. Aliquots (50 μl) of homogenised frozen liver were assayed at 30°C for GPAT (EC 2.3.1.15) and

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Table 1
GPAT activities in GSD(+) and GSD(−) rat livers

	Palmitoyl CoA (total activity)	Palmitoyl CoA (NEM-insensitive activity)	Palmitoyl CoA (NEM-sensitive activity)	Oleoyl CoA (total activity)
GSD(−)				
nmol . min^{-1} . g wet wt^{-1}	201 ± 23	102 ± 11	99 ± 12	120 ± 8
nmol . min^{-1} . liver^{-1}	2858 ± 246	1454 ± 136	1405 ± 126	1705 ± 61
GSD(+)				
nmol . min^{-1} . g wet wt^{-1}	144 ± 5[a]	67 ± 4[a]	77 ± 1	100 ± 2
nmol . min^{-1} . liver^{-1}	2250 ± 51[a]	1046 ± 17[a]	1204 ± 54	1561 ± 69

[a] Indicates $P < 0.05$ for GSD(+) *vs* GSD(−)

The activities are means ± SEM of 4 animals in each case. Assays were performed with palmitoyl CoA or oleoyl CoA as acyl substrate as indicated. Where present NEM was 10 mM

DHAPAT (EC 2.3.1.42) using the conditions in [6]. The GPAT assay contained 45 μM palmitoyl CoA or oleoyl CoA and 0.5 mM [U-^{14}C]glycerol phosphate. The DHAPAT assay contained 60 μM palmitoyl CoA and 0.5 mM [U-^{14}C]dihydroxyacetonephosphate (generated in situ from fructose bisphosphate [6]). Both assays contained defatted albumin (4 mg/ml).

Results are expressed as means ± SEM and statistical significance determined using Student's *t*-test.

3. Results and discussion

The mean wet weights of livers were: GSD(+) rats, 15.7 ± 0.8 g; GSD(−) rats, 14.4 ± 0.6 g (4 animals in each case). GPAT in microsomes differs from the activity in mitochondria in that the former is inhibited by thiol reagents whereas the latter is insensitive [12–14]. This difference may be exploited to obtain estimates of mitochondrial and microsomal GPAT activities in crude homogenates of freeze-clamped tissue without recourse to subcellular fractionation [6]. Accordingly, GPAT that is insensitive to 10 mM *N*-ethylmaleimide (NEM) refers to the mitochondrial form and the NEM-sensitive activity, calculated by difference, refers to the microsomal form [6]. An alternative estimate of microsomal GPAT is obtained by using an unsaturated acyl substrate, i.e., oleoyl CoA since liver mitochondrial GPAT has little activity with this substrate [13] and >95% of total homogenate GPAT activity with oleoyl CoA is NEM-sensitive [15]. Both forms of estimation are employed here (table 1).

Livers from GSD(+) rats had reduced GPAT activities (table 1). This was mainly due to a significant decrease in the NEM-insensitive activity rather than a change in the microsomal activity. It is noteworthy that in various other states, alterations in hepatic GPAT, that parallel changes in hepatic triacylglycerol synthesis are mainly confined to the mitochondrial form of the enzyme [4–6]. In the GSD(+) state there was also a significant decrease in the total hepatic DHAPAT activity (table 2). In percentage terms this was similar to the decrease in the mitochondrial GPAT (table 1). NEM stimulates DHAPAT in peroxisomal and mitochondrial fractions [6,16], but decreases that in microsomal fractions [6,17]. Assays performed in the presence of NEM should therefore estimate an activity

Table 2
DHAPAT activities in GSD(+) and GSD(−) rat livers

	Assayed without NEM	Assayed with NEM (10 mM)
GSD(−)		
nmol . min^{-1} . g wet wt^{-1}	37.5 ± 1.2	37.7 ± 2.1
nmol . min^{-1} . liver^{-1}	537 ± 15	540 ± 25
GSD(+)		
nmol . min^{-1} . g wet wt^{-1}	23.9 ± 0.1[b]	25.9 ± 1.1[a]
nmol . min^{-1} . liver^{-1}	375 ± 19[b]	404 ± 13[a]

[a,b] Indicate $P < 0.01$, < 0.001 for GSD(+) *vs* GSD(−)

The activities are means ± SEM of 4 animals in each case

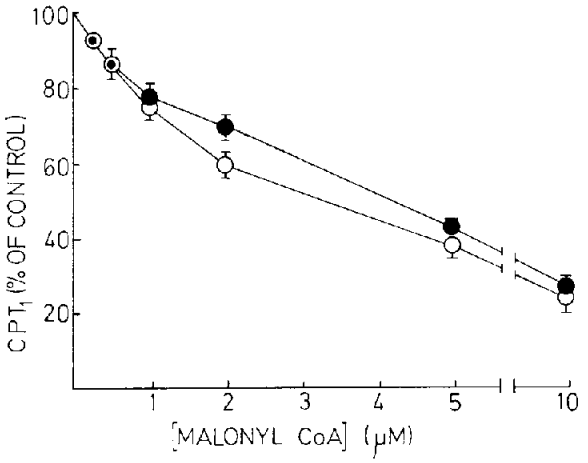

Fig.1. Effects of malonyl CoA on CPT_1 activity. The activities are means ± SEM of 3 expt and are expressed as percentages of the CPT_1 activity in the absence of malonyl CoA: (○) GSD(−); (●) GSD(+). CPT_1 activities in the absence of malonyl CoA were: GSD(+), 2.25 ± 0.09; GSD(−), 1.86 ± 0.04 (nmol . min^{-1} . mg $protein^{-1}$; $n = 4$; $P < 0.01$). Total CPT activities in the presence of Triton X-100 (0.4 mg/ml) were: GSD(+) 5.19 ± 0.11; GSD(−) 4.31 ± 0.08 (nmol . min^{-1} . mg $protein^{-1}$; $n = 4$; $P < 0.001$).

in which the contribution from a microsomal enzyme is small. Since a diminished DHAPAT activity was also seen in the GSD(+) livers in the presence of NEM, it is likely that it is non-microsomal DHAPAT activity which is decreased in this condition.

The mechanisms underlying the observed decreases in GPAT and DHAPAT activities are unknown at present. However, the freeze-stop and rapid assay techniques employed are likely to detect changes resulting both from covalent modification of activity as well as more persistant alterations in total enzyme protein.

Figure 1 shows that the sensitivity of CPT_1 to malonyl CoA inhibition was unaltered in the GSD(+) state with a mean concentration of malonyl CoA for 50% inhibition of ~4 μM. However, the activities of both CPT_1 (expressed per mg mitochondrial protein) and total CPT activity measured after disruption of mitochondria with Triton X-100 were increased by 20% (see legend to fig.1). These changes, and those in GPAT and DHAPAT, presumably reflect the initial lesion in glycogen metabolism. It has not yet been established whether the lowering of plasma triacylglycerols in the GSD(+) state [3] is due to a decrease in hepatic triacylglycerol synthesis. However, the increases in CPT and the diminished activities of DHAPAT and NEM-insensitive GPAT would tend to redirect hepatic fatty acid metabolism away from esterification and towards oxidation and also depress triacylglycerol secretion.

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