EFFECTS OF STARVATION AND ADRENALINE ON GLYCEROPHOSPHATE ACYLTRANSFERASE AND DIHYDROXYACETONE PHOSPHATE ACYLTRANSFERASE ACTIVITIES IN RAT ADIPOCYTES

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1. Introduction

It is now recognised that glycerolipid synthesis can be initiated by the acylation of both glycerol phosphate and dihydroxyacetone phosphate. Glycerol phosphate acylation is slightly decreased in extracts of rat epididymal fat pads after fasting [1,2]. In the short-term, treatment of rat adipocytes with adrenaline in vitro also decreases glycerol phosphate acyltransferase (GPAT) activity [3,4]. The response of dihydroxyacetone phosphate acyltransferase (DHAPAT) to these treatments in adipocytes or epididymal fat pads does not appear to have been reported.

In this study it was initially found that GPAT activity in isolated adipocytes was decreased by starvation (48 h) to a larger extent than found previously in epididymal fat pads. Consequently it was decided to investigate the effect of adrenaline in vitro on GPAT activity in adipocytes from fed and starved rats in order to establish whether or not the actions of adrenaline and starvation were independent of each other, or were mutually exclusive. In the latter case a common mechanism of regulation of this enzyme might be implied.

It has been claimed that GPAT and DHAPAT activities in rat adipocyte microsomes reside in the same enzyme [5]. If this is the case, GPAT and DHAPAT activities in adipocyte, where the majority of these activities is found to be microsomal [5], should be very similarly affected, in the long-term, by starvation and, in the short-term, by adrenaline treatment in vitro. This has been tested and found to be so.

2. Materials and methods

Adipocytes were prepared for the epididymal fat pads of male Sprague-Dawley rats by the method in [6]. The animals weighed 160–190 g 48 h before the start of the experiments when they were either fed or starved with unrestricted access to water. Adipocytes were incubated with or without adrenaline (0.63 μM), freeze-stopped and homogenised as in [4]. GPAT (EC 2.3.1.15) was assayed in centrifuged homogenates as in [3] using 0.5 mM [U-^{14}C]glycerol phosphate unless otherwise stated. DHAPAT (EC 2.3.1.42) was assayed by the method in [7] using 0.45 mM dihydroxy[^{14}C]acetone phosphate generated in situ from 0.5 mM [U-^{14}C]fructose 1,6-diphosphate by the action of excess aldolase and triosephosphate isomerase. Lactase dehydrogenase (EC 1.1.1.27) was assayed as in [3]. GPAT and DHAPAT activities in homogenates prepared from incubated adipocytes were generally expressed as nmol/min per unit of lactate dehydrogenase (LDH) activity to correct for any incomplete recovery of cells from incubations or incomplete homogenisation of cells. Adipocyte protein and DNA were measured as in [8] and [9], respectively.

3. Results and discussion

In initial experiments, adipocytes were prepared from fed and 48 h starved rats on the same day. Starvation decreased the GPAT activity from 19.4 ± 2.2 nmol/min per 100 μg DNA to 11.4 ± 1.7 nmol/min per 100 μg DNA ($P < 0.05$, 4 measurements). Starvation also decreased LDH activity from 5.8 ±

Table 1
Effect of starvation and adrenaline treatment on GPAT and DHAPAT activities in rat adipocytes

Addition to incubation	Dietary status	GPAT activity		DHAPAT activity	
		nmol/min per unit of LDH	nmol/min per 100 μg DNA	nmol/min per unit of LDH	nmol/min per 100 μg DNA
None	fed	4.19 ± 0.31	18.9 ± 1.6	0.67 ± 0.06	3.04 ± 0.31
	starved (48 h)	3.43 ± 0.16	14.6 ± 1.6	0.53 ± 0.04	2.22 ± 0.35
Adrenaline (0.63 μm)	fed	3.19 ± 0.17[b] (−23 ± 3%)[b]	14.8 ± 1.2	0.50 ± 0.04[b] (−23 ± 4%)[b]	2.35 ± 0.25
	starved (48 h)	2.36 ± 0.25[a] (−31 ± 8%)[b]	9.8 ± 0.7[c]	0.33 ± 0.02[a] (−36 ± 7%)[b]	1.36 ± 0.15[c]

[a,b] Indicate $P < 0.02$ and $P < 0.01$, respectively, for the effects of adrenaline on a paired-difference basis

[c] Indicates $P < 0.01$ for the effect of starvation versus the fed control

Adipocytes were prepared from fed or starved rats and incubated for 1 h in Krebs-Ringer bicarbonate containing fatty acid-poor albumin (32.5 mg/ml) and, where indicated, adrenaline. The results are means ± SEM of 8 and 6 experiments, respectively for the fed and starved state. The mean fat cell DNA was 5.3 and 3.5 μg per ml of flask contents for the fed and starved state respectively. The values in parentheses indicate the percentage effects of adrenaline

0.6 μmol/min per 100 μg DNA to 4.0 ± 0.6 μmol/min per 100 μg DNA. The GPAT/LDH activity ratio (see section 2) was decreased from 3.4 ± 0.4 to 2.9 ± 0.4 by starvation.

Table 1 shows that incubation for 1 h with 0.63 μM adrenaline significantly decreased GPAT/LDH and DHAPAT/LDH activity ratios in adipocytes from both fed and starved rats. Expressed per 100 μg DNA both enzyme activities were lowered by starvation although it should be noted that cell preparations from fed and starved animals were not made in parallel on the same day in these experiments. Table 1 also shows that the percentage effect of adrenaline on both activities was similar and that prior starvation did not reduce the effect of the hormone on the cells. In fact, a slightly larger response was seen in starved cells although this was not significantly different from that seen in the fed state.

Figure 1 shows the scatter diagram that is obtained when 58 measurements of GPAT and DHAPAT activities obtained from fed and starved cells are plotted against each other. A linear relationship is clearly seen ($r = 0.838, P < 0.001$) although there is a positive intercept on the ordinate. It appears therefore, within

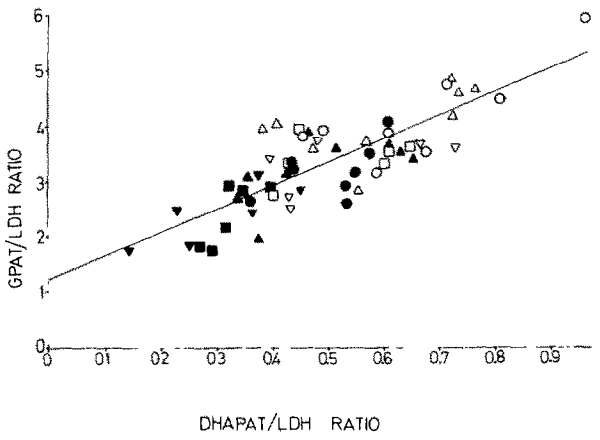

Fig.1. Relationship between adipocyte GPAT and DHAPAT activities. All the individual values from table 1 are included together with measurements made in cells incubated in the presence of glucose (5 mM) with or without adrenaline (0.63 μM). Cells from fed rats: (○) no addition; (●) with adrenaline; (△) with glucose; (▲) with glucose + adrenaline. Cells from starved rats: (□) no addition; (■) with adrenaline; (▽) with glucose; (▼) with glucose + adrenaline.

the range of conditions generated by the present treatment, that DHAPAT activity and the major proportion of the GPAT activity are closely linked. The conclusion [5] that adipocyte microsomal GPAT and DHAPAT activities reside in the same enzyme is not at variance with this finding. On the other hand changes in GPAT and DHAPAT activities in whole epididymal fat pad show no significant correlation in response to carbohydrate and fat feeding [10]. However, this latter study may be complicated by the presence of non-adipocyte cells in the preparation.

In conclusion, the long-term effects of starvation and the short-term effect of adrenaline on adipocyte GPAT and DHAPAT appear to be independent of each other. The experimental findings also lend support to the proposal that adipocyte GPAT and DHAPAT activities are closely related.

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