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Adipocyte Cholesterol Storage: Effect of Starvation (41188)

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Abstract. The content of free and esterified cholesterol in epididymal and subcutaneous adipocytes was measured during starvation in adult male rats in order to test the hypothesis that adipose tissue cholesterol esters are mobilized concomitantly with free cholesterol and triglyceride during starvation. Plasma and liver lipid concentrations were also determined as a function of time during food deprivation. Plasma triglyceride decreased significantly within 72 hr and liver triglyceride increased significantly after 144 hr of starvation. Plasma total cholesterol also decreased due to starvation, but only after 144 hr. No significant alteration in hepatic cholesterol was observed at any time. In epididymal adipose cells, free cholesterol was mobilized during fasting while the cholesterol ester pool remained constant. In marked contrast, cells from the subcutaneous depot showed no consistent changes in either free or esterified cholesterol despite the fact that cells lost about 55% of their triglyceride pool. These data provide another example of the metabolic heterogeneity of the various adipose tissue beds. Although neither cholesterol ester turnover nor hydrolase activity was directly measured, the results appear to be inconsistent with the hypothesis that an adipose tissue cholesterol ester hydrolase plays a role in the hydrolysis of stored esters during acute starvation, and further suggest that the turnover of cholesterol and cholesterol esters is probably different in adipose tissue.

Adipose tissue is a major cholesterol storage organ in the rat (1). This tissue also contains a neutral cholesterol ester hydrolase which is capable of hydrolyzing cholesterol esters *in vitro* (2, 3). Despite extensive attempts at purification of this enzyme (2–4), it has not been possible to separate it from hormone-sensitive lipase. Since cholesterol ester hydrolase is activated by a cAMP-dependent protein kinase and sensitive to lipolytic hormones, it has been postulated that this enzyme may function in the hydrolysis of adipose tissue cholesterol esters during triglyceride mobilization (2–4). In order to test this hypothesis *in vivo*, we examined the effect of acute starvation on the content of adipocyte-free and esterified cholesterol. Experiments were conducted using rats exceeding 400 g body wt in which at least 90% of adipose tissue cholesterol resides within fat cells and less than 10% in stromal–vascular elements (1, 5). In addition, both epididymal and subcutaneous fat depots were studied since previous work from this laboratory has suggested that epididymal tissue may not be representative of the entire adipose organ (6–8).

Materials and Methods. Male, Sprague–Dawley rats with a mean body weight of 464.9 ± 2.4 g (n = 16) previously fed laboratory rat chow (Rodent Laboratory Chow No. 5001, Ralston Purina Co., St. Louis, Mo.) and maintained on a regular 12-hr light–dark cycle, were divided into four groups. Animals in Group A were fed *ad libitum* while animals in Groups B, C, and D were fasted 24, 72, and 144 hr, respectively, in cages with wire-mesh floors to minimize coprophagia. All rats were sacrificed at 9 AM by decapitation. Epididymal and inguinal subcutaneous adipose tissues were removed (approximately 2 g of each depot) and incubated with collagenase to liberate fat cells, as previously described (6, 9). Cells were washed three times in Krebs–Ringer bicarbonate buffer. Aliquots of cell suspensions were extracted in an isopropranol–Zeolite system (10). Free and esterified cholesterol were separated using Sephadex LH-20 gel filtration (11). Pooled fractions from the columns were saponified prior to determination of cholesterol by an automated procedure (12). Triglyceride in the lipid extracts was also measured by an automated method (13). Fat cell size was then calculated from the mean cell diameter, determined microscopically as previously described (6–8). Cellular volume and

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TABLE I. BODY WEIGHTS AND ADIPOCYTE SIZES DURING STARVATION

Group	Initial body weight (g)	Final body weight (g)	Adipocyte sizes (μg triglyceride $\times 10^{-2}$/cell)	
			Epididymal	Subcutaneous
A	466 ± 5	—	22.8 ± 2.4	17.2 ± 2.6
B	461 ± 8	433 ± 6[a]	21.2 ± 1.9	14.4 ± 1.2
C	470 ± 3	405 ± 3[b]	16.2 ± 1.4[c]	11.3 ± 1.3
D	465 ± 5	367 ± 2[b]	12.9 ± 2.3[c]	7.8 ± 1.8[c]

Note. Group A rats were fed and Groups B–D were fasted 24, 72, and 144 hr, respectively. Values are mean ± SE, $n = 4$ for each group.

[a] Significantly less than initial value, $P < 0.05$.

[b] Significantly less than initial value, $P < 0.01$.

[c] Significantly less than Group A control value, $P < 0.05$.

triglyceride content were also calculated (6, 14). Plasma and liver lipids were quantitated using similar extraction and assay conditions. Student's t test (Table I) and analysis of variance followed by Tukey's multiple range test (Figs. 1–3) were employed to determine statistical differences between means (15).

Results and Discussion. Body weights at the beginning and throughout starvation, as well as final adipocyte sizes, are shown in Table I. The fast produced significant decreases in body weight in all groups (B–D). After 72 hr of food deprivation, epididymal and subcutaneous cell sizes decreased in size 28.7 and 34.2%, respectively, and by 56.6 and 43.7% after 144 hr. At both 72 and 144 hr the absolute amount of triglyceride lost per cell was slightly greater in the epididymal than in the subcutaneous adipocytes. This observation is consistent with the previous finding from this laboratory that in adult rats, cells with the largest initial size are affected to the greatest extent by starvation (8).

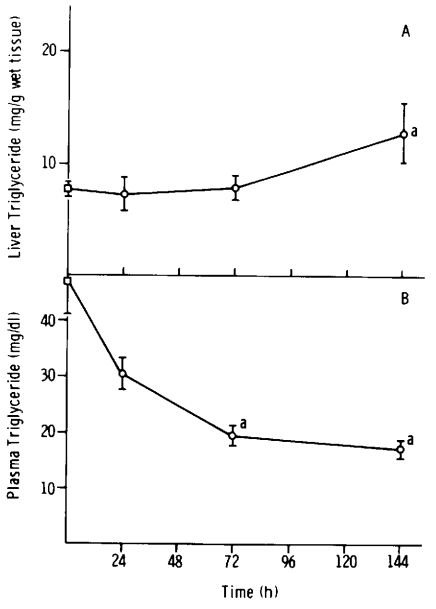

FIG. 1. Triglyceride concentrations in liver (A) and plasma (B) as a function of the length of the fast. Data are presented as the mean ± SE, $n = 4$. (a) Signifies values that are significantly different from the initial value at Day 0.

Figure 1 shows the triglyceride concentration in liver and plasma as a function of the duration of starvation. As shown by others (16), plasma triglyceride decreases significantly during starvation. Liver triglyceride was significantly increased over the initial value after 144 hr of starvation, possibly due to an influx of free fatty acid substrate. Plasma cholesterol, both free and esterified, decreased significantly only after prolonged fasting (144 hr, Fig. 2). Others have found increases (17, 18), no change (16, 19, 20), or decreases (21) in plasma total cholesterol with starvation. Variation in results among investigators may be related to differences in the age, sex, or strain of the rats. Liver-free and esterified cholesterol did not change significantly with time, but due to slight but nonsignificant decreases in free sterol and increases in esters (Fig. 2), the cholesterol ester content expressed as a percentage of the total cholesterol increased significantly from 41.4 to 87.6% following 144 hr of food deprivation. Increases in the percentage of total choles-

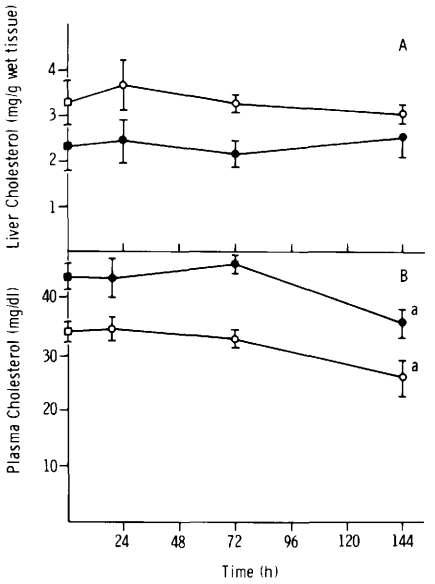

FIG. 2. Free (○) and ester (●) cholesterol concentration of liver (A) and plasma (B) as a function of the length of the fast. Data are presented as the mean ± SE, $n = 4$. (a) Signifies values that are significantly different from all other values.

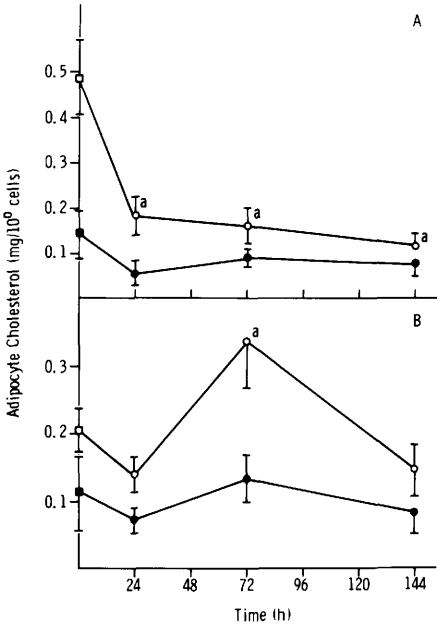

FIG. 3. Free (○) and ester (●) cholesterol content of adipocytes derived from the epididymal (A) and subcutaneous (B) adipocytes. Data are expressed as mean ± SE, $n = 4$. (a) Signifies values that are significantly different from the initial value at Time 0.

terol in the ester form also occur in rats fed fat-free or cholesterol-containing diets (7). The increased hepatic cholesterol levels reported in other starvation studies is probably due to ester accumulation (18).

As shown previously (7, 11), 70–80% of adipocyte cholesterol is in the free form in normal animals on a chow diet, with epididymal cells containing more cholesterol than subcutaneous cells (Fig. 3). Free cholesterol content (mg/10^6 cells) of epididymal adipocytes was significantly decreased after a 24 hr fast and continued to decrease slowly thereafter until reaching a value 23% of the initial value (144 hr, Fig. 3A). In marked contrast, cholesterol ester content of epididymal adipocytes did not change significantly as a result of starvation. The decreased content of free cholesterol and relative constancy of the ester pool in epididymal cells has been noted by one other laboratory after 60 hr of fasting in young rats (5). However, as shown in Fig. 3B, these trends were not manifest in cells from the largest adipose tissue depot, i.e., subcutaneous. In these cells, the cholesterol ester content, as in the epididymal depot, remained constant at all times during starvation. The free cholesterol content in subcutaneous cells, however, increased significantly at 72 hr and was at no time lower than the initial value.

Since cellular cholesterol depletion at the end of the entire 144-hr period was proportional to triglyceride (Table I), the concentration of cholesterol (mg/mg triglyceride) in epididymal cells was not different from that of fed controls (Group A). But in subcutaneous cells, the ratio of free cholesterol to triglyceride remained relatively constant after the 144-hr fast, despite a seemingly anomalous increase in free cholesterol content at 72 hr which was observed in repeated experiments. A similar observation has been reported in human subcutaneous adipose tissue after weight loss due to jejunoileal bypass (22). The cholesterol ester content did not change in either depot (Fig. 3) despite loss of cellular triglyceride (Table I), therefore the ester concentration (mg/mg triglyceride) in both depots increased due to starvation. Clearly, the time

at which adipocytes are isolated during starvation determines the resultant cholesterol/triglyceride ratio. In fact, evidence is presented for the mobilization of cholesterol independent from that of triglyceride in epididymal cells after only a 24-hr fast (Table I and Fig. 3).

Although rat epididymal adipose tissue contains an enzyme capable of hydrolyzing cholesterol esters *in vitro* (2–4), the present data are not consistent with the hypothesis that this enzyme plays a role in the mobilization of stored esters during triglyceride breakdown since the ester pool remained constant during starvation. The present data, however, cannot rule out an increased turnover of the cholesterol ester pool with no change in pool size. It has been further suggested that this enzyme may also be important in the hydrolysis of circulating cholesterol esters. Uptake of chylomicron cholesterol ester by adipose tissue has been demonstrated (23–27), and although this mechanism would not be operative in fasted animals, the hydrolysis of internalized chylomicron cholesterol ester may be mediated by adipose tissue cholesterol ester hydrolase in the fed state.

Adipose tissue of the rat may also possess low-density lipoprotein (LDL) receptors as in human adipose tissue (28, 29), and LDL cholesterol esters may be the substrate for the hydrolase enzyme via an extralysosomal pathway (29). Indeed, in the pig, adipose tissue accounts for a major uptake of LDL (30) and LDL binding and cholesterol ester hydrolase activity are both cyclic AMP dependent (29). LDL receptor-mediated uptake of cholesterol esters may be responsible in part for the accumulation of cholesterol esters in adipose tissues of hypercholesterolemic rats (7).

When expressed per unit protein or organ mass, adipose tissue contains more cholesterol than most other organs or membranes (1). We have previously shown in the whole animal that plasma cholesterol and adipocyte cholesterol storage can increase in parallel while fat cell size remains constant (7). In addition, we (6) and others (5, 22, 31, 32) have demonstrated a positive correlation between fat cell cholesterol content and fat cell size. Thus, the decrease in free cholesterol content during starvation in epididymal cells could be a reflection of decreased cell size (Table I). A previous study from this lab (7) demonstrated a direct relationship between plasma cholesterol and adipocyte cholesterol storage. Since in the present study epididymal-free cholesterol fell most rapidly in the first 24 hr of the fast during a time when plasma cholesterol was unchanged, the decreased adipocyte cholesterol cannot be explained simply on the basis of a reversed cell to plasma cholesterol gradient compared to our earlier work (7). Obviously, other mechanisms must be postulated for subcutaneous tissue, and the present experiments illustrate the potential error in assuming epididymal tissue to be representative of the entire adipose tissue organ.

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