Metabolism of isolated fat cells from various tissue sites in the rat: influence of hemorrhagic hypotension

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Abstract The in vitro lipolytic response to norepinephrine by rat adipocytes from epididymal, subcutaneous, perirenal, mesenteric, and omental tissue sites was studied in control and hypotensive animals. Lipolysis per millimole of triglyceride was found to be three to four times higher in mesenteric and omental fat cells than in adipocytes of the other sites sampled. The high lipolytic activity of mesenteric and omental adipocytes was partly attributable to their smaller cell size; however, lipolysis per cell was also higher. Hemorrhagic hypotension caused a 50–60% decrease in lipolytic activity at four of the five sites studied. Adipocytes of omental origin maintained their lipolytic activity at the prehypotensive level, however, indicating that the metabolic adjustments brought about by hemorrhagic hypotension are not uniform at all adipose tissue sites.

Supplementary key words norepinephrine-induced lipolysis

After hemorrhage there is an elevation of blood catecholamine levels (1) and an increase in sympathetic nervous activity (2). These factors are important in influencing blood flow and free fatty acid release by adipose tissue (3), for both norepinephrine and epinephrine cause vasoconstriction as well as stimulation of lipolysis.

The present study attempts to determine the possible influence of hemorrhage on the ability of adipocytes to respond to the lipid-mobilizing effects of norepinephrine. Adipose tissue is innervated by sympathetic nerve fibers (4), and norepinephrine is the neurotransmitter (5). Norepinephrine was therefore selected as the lipolytic agent, since it functions physiologically in the control of lipolysis (6).

Epididymal, subcutaneous, perirenal, mesenteric, and omental adipose tissue sites were chosen for study because the vascular and nervous responses to hemorrhage may not be uniform at all fat depots. In addition, it has previously been suggested that adipose tissue samples from different sites in the same animal show quantitative (7, 8) and sometimes qualitative (9) differences in responding to norepinephrine.

The present study represents an effort to investigate the effect of hemorrhagic hypotension on norepinephrine-stimulated lipolysis in vitro. In addition, the possible influences of area of origin of the adipose tissue and in vivo heparin administration were also studied.

MATERIALS AND METHODS

Male Wistar rats within a weight range of 300–350 g were used after an 18-hr fast. The animals were allowed water and Purina laboratory chow ad lib. for at least 1 wk prior to the experiment. Sodium pentobarbital (65 mg/kg) was administered by intraperitoneal injection, tracheostomy was performed, and both femoral arteries, or a single femoral artery and a single common carotid artery, were cannulated for continuous blood pressure recording and bleed-out into a reservoir when required. All "hemorrhaged" animals and one group of control animals received 200 U.S.P. units of heparin (Organon) intraarterially in a volume of 0.5 ml. An additional group of control animals received the same volume of saline.

Adipose tissue samples from epididymal (E), subcutaneous (SC), perirenal (PR), mesenteric (M), and omental (O) sites were obtained from both groups of control animals 30 min after heparin or saline administration.

A group of animals designated as "hemorrhaged" were treated as follows. Hypotension was induced gradually over a 10–15-min period by femoral arterial bleed-out into a reservoir. The mean blood pressure was lowered from a control of 125 mm Hg to 40 ± 5 mm Hg and maintained

Abbreviations: FFA, free fatty acids; E, epididymal; SC, subcutaneous; PR, perirenal; M, mesenteric; O, omental.

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at the low level for a 2.5-hr period. Adipose tissue samples were then removed from the above-mentioned sites.

The populations of animals for each group were selected in a random sequence so that both control and hemorrhaged animals were studied over the same time period, thus obviating any possible seasonal variations.

Isolated fat cells were prepared according to the method of Rodbell (10), employing a 1-hr enzymatic digestion of the tissue by 1–2 mg/ml collagenase (Worthington). The medium for tissue digestion and metabolic studies was a Krebs-Ringer bicarbonate buffer (11), pH 7.4, containing half the recommended concentration of calcium and 4% bovine serum albumin. Final concentrations of glucose and norepinephrine (Winthrop) were 2.75 μmoles/ml and 1.18 nmoles/ml, respectively. The fat cells were incubated for 2 hr at 37°C in an atmosphere of 95% O_2–5% CO_2. Total triglyceride per tube was in the range of 50–200 μmoles.

Glycerol released into the medium was assayed by the enzymatic fluorometric micromethod of Laurell and Tibbling (12), FFA release was measured by the titrimetric method of Dole and Meinertz (13), and the triglyceride content of the fat cells was determined on the basis of total fatty acid content after saponification. Protein was measured by the method of Lowry et al. (14).

Fat cell size distribution was estimated by measuring the diameters of 100 washed cells, using a light microscope equipped with a calibrated ocular. From the mean diameter and standard deviation, the mean volume and the mean triglyceride content per cell were calculated (15). The number of fat cells per incubation flask was determined by dividing the total triglyceride content of the flask by the mean triglyceride content per cell.

The results were analyzed statistically by the Student t test.

RESULTS

The apparent lipolytic activity, expressed as glycerol or FFA released per millimole of triglyceride, varied with the site of origin of the fat cells. As shown in Fig. 1, both M and O fat cells had glycerol outputs three to four times greater than fat cells of E, SC, or PR origin. The activities of the latter three sites were similar to each other.

Mean fat cell size also varied with tissue site (Table 1). E, SC, and PR fat cells had similar mean cell diameters, whereas M and O fat cells were considerably smaller, yet similar in size to each other. The differences in cell size among the adipose tissue sites are even more pronounced when expressed on the basis of mean cellular volume.

The protein content per millimole of triglyceride was similar among the E, SC, and PR sites; however, substantially higher values were obtained with M and O fat cells (Table 1). If fat cell enlargement is accomplished primarily by deposition of triglyceride with a relatively small change in cytoplasmic mass (16), the data are consistent with the variations in cell size observed among the sites. That is, smaller fat cells (M and O) have a higher protein content per millimole of triglyceride mass than larger fat cells (M vs. E, $P < 0.02$; M vs. SC, $P < 0.02$; O vs. E, $P < 0.01$; O vs. SC, $P < 0.01$; O vs. PR, $P < 0.02$). The protein content per cell is less easy to interpret. Both E ($P < 0.02$) and PR ($P < 0.01$) fat cells had a higher protein content per cell than did M cells, and there was more protein in PR cells ($P < 0.02$) than in SC cells on a per cell basis. Since a total protein measurement includes both enzymatic and structural components, variations in both components may occur among the tissue sites, with either or both influencing the final expression of hormone sensitivity. The protein content reported here for the epididymal site agrees with that reported elsewhere (17).

When cell populations from each site were compared in the same animal, the mean intraanimal differences were consistent and statistically significant. In the nonheparinized control group of six rats, lipolysis per cell was higher in M and O fat cells than in E or SC ($P < 0.025$ for M vs. E; $P < 0.05$ for M vs. SC; $P < 0.001$ for O vs. E; and $P < 0.05$ for O vs. SC). In the heparinized control group of nine rats, M fat cells showed greater lipolysis per cell than did E cells ($P < 0.025$). In addition, PR fat cells

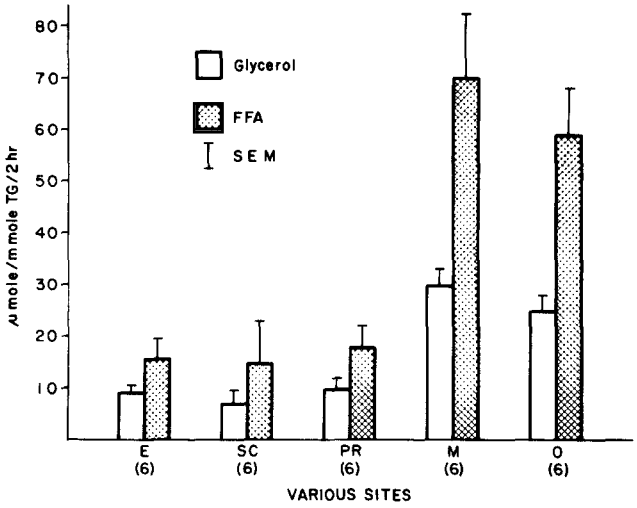

Fig. 1. Norepinephrine-stimulated glycerol and FFA release by rat adipocytes from various sites. All samples were taken from nonheparinized control animals. Numbers in parentheses refer to the number of experiments. The dosage of norepinephrine was 1.18 nmoles/ml. The number of fat cells per incubation flask for the whole group ranged from 0.5 to 1.0 × 10^6. The masses of fat from different sites varied. Usually, the mesenteric and omental sites yielded less tissue than the others. However, due to the smaller size and especially the much smaller volume of these cells, the number of cells per flask was comparable.

TABLE 1. *Mean cell size and protein content of rat adipocytes from various sites*

	E	SC	PR	M	O
Mean cell diameter, μm	62.25[a] ±1.31 (23)	58.86 ±1.30 (23)	65.82 ±1.63 (23)	44.15 ±1.51 (23)	48.07 ±1.51 (21)
Mean cell volume, pl[b]	148.84 ±8.77 (23)	130.01 ±7.86 (23)	184.37 ±12.44 (23)	54.81 ±5.31 (23)	73.76 ±6.85 (21)
Protein, mg/mmole TG[c]	3.70 ±0.29 (28)	3.70 ±0.34 (28)	3.98 ±0.43 (25)	5.37 ±0.58 (15)	6.63 ±0.96 (7)
Protein, mg/10^6 cells	0.571 ±0.058 (22)	0.452 ±0.048 (22)	0.634 ±0.058 (21)	0.341 ±0.067 (10)	0.485 ±0.125 (3)

[a] Mean ± SEM; number of rats in parentheses.
[b] Picoliters.
[c] Triglyceride.

exhibited greater lipolysis per cell than did E cells ($P < 0.05$). It seems that the high lipolysis per millimole of triglyceride by M and O fat cells observed above can be accounted for only in part by their smaller size, since their activity is still relatively higher on a per cell basis.

Influence of heparin

Our hemorrhagic shock model requires the use of an anticoagulant, and heparin was administered for this purpose. Heparin is known to stimulate intravascular lipolysis by activating lipoprotein lipase (18), an enzyme whose substrate is plasma triglyceride. Although evidence exists that heparin does not affect lipolysis by canine subcutaneous adipose tissue perfused in situ (19), it was of interest to see if in vivo heparin administration influences the in vitro lipolytic activity of rat fat cells. Table 2 shows that heparin administered under the conditions of these experiments did not influence in vitro fat cell lipolysis at any one of the five sites studied when compared with the same site in nonheparinized controls. However, it is conceivable that some subtle changes may have occurred at the level of intraanimal lipolytic differences among the various sites.

Influence of hemorrhage

Hemorrhagic shock, as described in the methods section, resulted in a 50–60% decrease in the in vitro lipolytic response, as indicated by the rate of glycerol released per cell, of fat cells from four of the five sites studied (Table 2). E and SC fat cells were most severely affected by hemorrhage; lipolytic activity at these sites was diminished to 38% of the control level. Lipolysis by PR and M fat cells was reduced to 52% of control after hemorrhage. Fat cells from the omental area seem resistant to the posthemorrhagic hypolipolysis observed at the other adipose tissue sites, since lipolysis by O fat cells was unchanged. In hemorrhaged animals, lipolysis per cell was significantly less by E and SC fat cells when compared with cells from the other three sites. Hemorrhage caused statistically significant changes in FFA released per cell at the E and SC sites, about a 30% decrease in the mean value (not significant) at the PR site, and virtually no change at the M and O sites.

DISCUSSION

Studies with rat epididymal adipocytes (20, 21) indicate that in a given tissue site, norepinephrine-stimulated lipolysis per millimole of triglyceride varies inversely with cell size. Norepinephrine-stimulated lipolysis per cell was found to be independent of cell size by Hartman et al. (20). Their data suggest that as the cells increase in size, a fixed number of adrenergic receptors are interspersed over a greater surface area. However, Zinder and Shapiro (21) observed a positive correlation between lipolysis per cell and increasing cell size.

Our results indicate that mean cell size can differ in different adipose depots and that when lipolysis is to be compared among adipocytes from differing depot areas, the comparison can best be made on the basis of cell number and not on the basis of tissue weight or total triglyceride content because the latter expressions ignore the element of cell size variability. Even after corrections for cell size variability, there were still quantitative differences among the sites, as evidenced by the higher lipolytic activities per cell obtained with M and O fat cells. Factors such as blood supply, body temperature, innervation, and enzyme content (22) are potential variables influencing adipocyte metabolic activity. In addition, there may be differences among the sites in the number, type, and affinity of the receptor sites for norepinephrine as well as differences in the efficiency of coupling of hormone stimulation to the eventual metabolic response.

TABLE 2. Effect of hypovolemia on norepinephrine[a]-stimulated glycerol and FFA release by rat adipocytes

	E	SC	PR	M	O
			μmoles/10⁶ cells/2 hr		
Control					
Glycerol	1.35[b] ±0.22 (9)	1.50 ±0.31 (9)	2.02 ±0.27 (9)	2.33 ±0.44 (9)	1.55 ±0.36 (7)
FFA	2.09 ±0.43 (9)	3.00 ±0.64 (9)	3.29 ±0.51 (9)	3.98 ±0.80 (9)	3.23 ±0.70 (7)
Hypovolemic[c]					
Glycerol	0.53 ±0.06 $P < 0.01$[d] (8)	0.55 ±0.12 $P < 0.02$ (8)	1.05 ± 0.18 $P < 0.01$ (8)	1.21 ±0.10 $P < 0.02$ (8)	1.39 ±0.26 (8)
FFA	0.80 ±0.34 $P < 0.05$ (8)	1.25 ±0.34 $P < 0.05$ (8)	2.32 ±0.46 (8)	3.30 ±0.24 (8)	3.72 ±0.87 (8)
Nonheparinized normovolemic					
Glycerol	1.55 ±0.49 (6)	1.00 ±0.27 (6)	2.36 ±0.61 (6)	2.12 ±0.44 (6)	2.17 ±0.46 (6)
FFA	2.78 ±1.04 (6)	2.07 ±0.89 (6)	4.36 ±1.02 (6)	4.83 ±0.96 (6)	5.09 ±1.05 (6)

This table represents means of samples compared between animals. The rats in the control and hypovolemic groups received 200 U.S.P. units of heparin.

[a] 1.18 nmoles/ml.

[b] Mean ± SEM; number of rats in parentheses.

[c] Hemorrhage resulting in 40 mm Hg mean arterial blood pressure for 2.5 hr.

[d] P values refer to the differences between stressed and nonstressed controls.

One of the main objectives of this study has been to measure the norepinephrine-stimulated lipolytic response, since blood catecholamine levels are elevated in hemorrhagic shock. The isolated fat cell preparation assures easy accessibility for hormones and thus provides us with a distinct advantage in this regard. Although basal rates of lipolysis were also measured, these were low compared with norepinephrine-stimulated lipolysis, making it difficult to interpret differences between control and posthemorrhagic samples.

Many of the sequelae of hemorrhagic shock could account for the hypolipolytic response observed by adipocytes obtained from hemorrhaged animals. Acidosis, a consistent finding in hemorrhagic shock (23), inhibits norepinephrine-stimulated lipolysis by adipose tissue in vivo (24) and by adipocytes in vitro (25). During hemorrhagic shock there is a pronounced elevation of the blood lactate concentration (26). Lactate has been shown to inhibit lipase activation in vitro (27) as well as FFA release in vivo by enhancing the reesterification rate (28). The fat cells were not submitted to the insults of acid and lactate in the course of our in vitro studies; however, acidosis and/or lactacidemia while the fat cells are still in situ may in some way desensitize the cells prior to removal from the animal.

Although adipose tissue blood flow was not determined in the present experiments, during hypotension induced by bleeding the adipose tissue blood flow falls to less than 10% of the control level in the dog (29). Hypoperfusion could result in a diminished delivery of nutrients such as glucose and oxygen to the adipose tissue and/or an impaired removal of waste products. Kovach et al. (29) demonstrated a 72% reduction in the oxygen uptake by canine subcutaneous adipose tissue after hemorrhage. In a different experimental shock model, in endotoxic shock in dogs, we measured regional blood flow in the subcutaneous and in the omental adipose tissue and found the flow restricted to the same extent at both sites (30).

Since lipolysis is an energy-dependent process sensitive to ATP availability (31), hypoxia may be a factor involved in the lipolytic response after shock. In addition, an elevation of adipose tissue FFA levels has been demonstrated after trauma in the rat (32). Intracellular FFA accumulation inhibits lipolysis by limiting ATP availability (33), and this too may partially explain the observed posthemorrhagic hypolipolytic response.

Hemorrhagic hypotension results in a diminished lipolytic response. Some consequence(s) of hypotension must affect the cells in vivo in such a manner that their sensitivity is altered and remains altered in the course of in vitro study. This alteration may be at the level of receptor site interaction or it may represent an impairment in subsequent adenyl cyclase activation, cyclic AMP accumulation, and/or hormone-sensitive lipase activation. It is of interest

that the effects of hypotension were not manifest at the omental site, suggesting that this area may in some way be resistant and therefore able to maintain its normal physiological response.

During the period of collagenase digestion and subsequent incubation, the cells were well nourished and oxygenated in a medium whose composition was different from the extracellular environment of these cells in vivo after 2.5 hr of hemorrhagic shock. It happens frequently that optimal conditions for study of a particular enzyme involve assay media whose electrolyte composition and osmolarity are markedly different from those of the normal extracellular environment of intact cells. The same holds true for studying respiratory control in mitochondria isolated from shocked organs. The results show the hormone-stimulated lipolytic response of fat cells in vitro when these cells were obtained from nonstressed control animals or after 2.5 hr of hemorrhagic shock. The validity of extrapolating values obtained with isolated fat cells to whole tissue is unknown. Therefore, the results do not permit a precise evaluation of metabolic function in vivo.

In summary, the data suggest an impairment in lipid mobilization during hemorrhagic hypotension and therefore imply that energy-rich substrates in the form of FFA cannot be released at the normal physiological rate. In addition, adipose tissue site of origin is an important parameter in studying lipolysis in both physiologically normal and stressed animals.

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