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Adipose Tissue and Plasma-Free Fatty Acid and Glycerol Concentrations during Burn Shock in Guinea Pigs (40989)

KEITH M. ROBINSON AND HARVEY I. MILLER

Department of Physiology, Louisiana State University Medical Center, New Orleans, Louisiana 70112

Abstract. We have previously demonstrated a rapid decrease in the rate of free fatty acid (FFA) release from adipose tissue following burn shock in the guinea pig. A decreased release of FFA could be due to (a) decreased lipolysis, (b) increased reesterification, (c) impaired transport, or (d) any combination of the above. The purpose of this study was to investigate which of these mechanisms might actually be involved in the decreased release of FFA. This was done by measuring adipose tissue and plasma concentrations of FFA and glycerol. No accumulation of FFA within adipose tissue was detected suggesting that impaired FFA transport was not involved in the decreased release of FFA. The plasma glycerol concentration was increased from 0.17 ± 0.01 m*M* to 0.23 ± 0.01 m*M* ($P < 0.01$) 2 hr postburn when the plasma FFA concentration was decreased from 0.84 ± .06 m*M* to 0.56 ± 0.05 m*M* ($P < 0.01$) resulting in a highly significant change in the FFA:glycerol ratio. We conclude that alterations in lipolysis and reesterification are a major factor contributing to the decreased release of FFA from adipose tissue. Glycerol kinetic measurements need to be done to determine if the increased plasma glycerol concentration accurately reflects increased lipolysis during burn shock.

Within 1 hr following severe burn in the guinea pig burn shock model, cardiovascular function is decreased, core temperature is reduced, and the animal is hypometabolic (1). Altered carbohydrate metabolism is apparent from the changes in plasma glucose and lactate concentrations (1). The plasma-free fatty acids (FFA) which normally provide almost half of the metabolic energy for the organism, have been reported to be increased (2–4), unchanged (5, 6), or decreased within 24 hr postburn. Since the uptake rate of plasma FFA is directly regulated by the plasma FFA concentration under a variety of treatment conditions (8), including burn (7), a significant change in the plasma FFA concentration could seriously alter total energy substrate availability.

Previously we observed that plasma FFA concentration and turnover rate decreased approximately 50% within 2 hr following thermal injury in the guinea pig (7). The decrease in plasma FFA turnover rate was due to a decrease in the rate of FFA appearance (RA FFA) with no change in the ability of tissues to take up FFA (7). A decreased rate of FFA release from adipose tissue could be due to (a) decreased lipolysis, (b) increased reesterification, (c) impaired transport of FFA out of the adipose tissue, or (d) any combination of the above. The purpose of the present study was to investigate which of these mechanisms might contribute to the decrease in plasma FFA turnover rate following severe burn. This report examines adipose tissue (omental and epididymal) and plasma concentrations of FFA and glycerol 2 hr following burn in a guinea pig during burn shock, when plasma FFA kinetics are dramatically altered (7). An increase in adipose tissue FFA concentration would probably suggest that FFA transport from adipose tissue is impaired, whereas an increase or decrease in plasma glycerol concentration should reflect an increase or decrease in the rate of lipolysis, respectively, since glycerokinase activity is very low in adipose tissue preventing reutilization of the glycerol released from triglyceride breakdown (9).

Materials and methods. Conscious, unrestrained, adult male guinea pigs (300–400) were used in all experiments. The guinea pigs were anesthetized with sodium pentobarbital (36 mg/kg) and a PE-50 polyethylene catheter was placed in one carotid

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artery for blood collection. The surgical procedure has been described previously (1). The animals received no food or water after surgery. Experiments were begun the following morning, approximately 18 hr after surgery. At this time the catheter was unwrapped and threaded through a hole in the top of the cage. One hour later the preimmersion blood sample (3.0 ml) was collected, heparinized *in vitro*, and immediately chilled. The animal was then anesthetized with halothane and immersed to the midabdomen (approximately 50% of the body surface area) for 3 sec in 25 or 100° water. Histological examination has previously shown that a 3-sec 100° scald produced a full-skin-thickness anesthetic burn (1). All animals recovered from the halothane anesthesia within 5 min. At 2 hr postimmersion, the final blood sample (3.0 ml) was collected, heparinized, and chilled. Each animal was then anesthetized with sodium pentobarbital (ia) and adipose tissue samples (0.5–1.0 g) from the omental and epididymal pads were immediately removed, weighed, and homogenized in chloroform:methanol (2:1).

FFA and glycerol analyses were performed on adipose tissue samples by the following method. Tissue samples were homogenized in chloroform:methanol according to the Folch procedure (10). After addition of the 0.29% NaCl, the glycerol partitioned completely into the upper phase and lipids into the lower phase. The upper phase was evaporated to dryness, reconstituted to 1.0 ml with distilled water, and frozen until assaying for glycerol content (11). The lower phase, including all of the FFA, was evaporated to dryness with nitrogen, weighed to determine the total lipid content of the tissue sample, and redissolved in Dole's solution for FFA extraction and titration (12). By this procedure glycerol and FFA concentration from the same apidose tissue sample could be calculated per gram of adipose tissue or per gram of tissue lipid. Aliquots of blood plasma were used for plasma FFA (13) and glycerol (11) determinations. Statistical differences between burned and control animals were determined by Student's *t* test.

Results. The plasma concentrations of glycerol and FFA and the plasma FFA:glycerol ratio of control and burned guinea pigs are summarized in Table I. Control animals showed no change in plasma FFA, glycerol, or the FFA:glycerol ratio when compared to preimmersion values. The plasma from burned animals showed a statistically significant decrease in FFA concentration ($P < 0.01$) and increase in glycerol concentration ($P < 0.001$) following burn which resulted in a highly significant change in the calculated FFA:glycerol ratio ($P < 0.001$).

The adipose tissue (omental and epididymal) FFA, glycerol, and total lipid concentrations from control and burned guinea pigs are summarized in Table II. When compared to control animals, the adipose tissue FFA concentration was unchanged 2 hr postburn. Glycerol concentration was unchanged in the epididymal pad, but was increased ($P < 0.01$) in the

TABLE I. PLASMA FFA AND GLYCEROL CONCENTRATION AND FFA:GLYCEROL RATIO PREIMMERSION AND 2 hr POSTIMMERSION IN CONTROL AND BURNED GUINEA PIGS

Parameter	Group	Time	
		Preimmersion	Postimmersion
Plasma FFA	Control	0.84 ± .08 (10)[a]	0.87 ± .09 (10)
(μmole/ml)	Burn	0.84 ± .06 (14)	0.56 ± .05 (14)[b]
Plasma glycerol	Control	169 ± 15 (10)	185 ± 16 (10)
(nmole/ml)	Burn	173 ± 10 (14)	233 ± 12 (13)[b]
FAA:glycerol	Control	5.91 ± .86 (10)	4.85 ± .48 (10)
	Burn	4.94 ± .41 (13)	2.48 ± .30 (13)[c]

[a] Values are expressed as mean ± SEM (number of animals).
[b,c] Significantly different from preimmersion values at $P < 0.01$ and 0.001, respectively.

TABLE II. ADIPOSE TISSUE FFA AND GLYCEROL CONCENTRATION, FFA:GLYCEROL RATIO, AND LIPID CONCENTRATION IN CONTROL AND BURNED GUINEA PIGS 2 hr POSTIMMERSION

Parameter	Group	Tissue site	
		Omental	Epididymal
FFA	Control	4.68 ± .45 (9)[a]	4.04 ± .45 (9)
(μmole/g tissue)	Burn	5.44 ± .42 (11)	3.77 ± .37 (11)
Glycerol	Control	164 ± 19 (9)	193 ± 26 (9)
(nmole/g tissue)	Burn	245 ± 16 (11)[b]	187 ± 15 (11)
FFA:glycerol	Control	31.7 ± 3.9 (9)	20.9 ± 2.1 (9)
	Burn	23.8 ± 1.2 (12)[c]	22.7 ± 2.2 (12)
Lipid			
concentration	Control	132 ± 32 (9)	726 ± 25 (9)
(mg/g tissue)	Burn	133 ± 14 (11)	747 ± 26 (11)

[a] Values are expressed as mean ± SEM (number of animals).
[b,c] Significantly different from the same adipose tissue pad of control animals at $P < 0.05$ and 0.01, respectively.

omental pad, resulting in a decreased ($P < 0.05$) FFA:glycerol ratio in the omental pad. The total lipid concentration of the omental and epididymal adipose tissues was unchanged 2 hr postburn compared to that of control animals, therefore, tissue concentrations of FFA or glycerol show the same trends, whether expressed per gram tissue wet weight or per gram lipid. Assuming that water made up the great majority of the adipose tissue wet weight which was not lipid, it can also be concluded that no major changes in adipose tissue water concentration occurred within 2 hr postburn.

Discussion. Decreased plasma FFA concentration and turnover rate postburn have been shown to result from a decreased RA FFA (7). A decreased RA FFA could be produced by increased reesterification, decreased lipolysis, and decreased blood flow through the adipose tissue impairing FFA transport. By investigating the concentrations of FFA and glycerol in adipose tissue in relation to their plasma concentrations, we have attempted to further elucidate the etiology of the decrease in FFA mobilization following burn.

Several investigators have suggested that a severe decrease in adipose tissue perfusion may prevent an increased release of FFA into the blood during shock (5, 14–16), and Ferguson (17) observed an 80% reduction in adipose tissue blood flow during burn shock under similar conditions in the guinea pig. Therefore, it seemed reasonable that severely reduced adipose tissue blood flow could be preventing transport of FFA from adipose tissue into the plasma during the early period following thermal injury. However, our data indicate that FFA are not accumulating within the adipose tissue. In agreement with this finding is a report by Kovach *et al.* (14) that adipose tissue FFA concentration did not increase following hemorrhage which reduced adipose tissue blood flow greatly and the plasma FFA concentration did not rise as expected. Still, the possibility cannot be excluded that a very slight increase in adipose tissue FFA concentration stimulates a corresponding increase in reesterification and thus FFA accumulation is not observed.

Plasma and adipose tissue levels of glycerol were measured as indicators of the rate of triglyceride lipolysis. The increase in plasma glycerol concentration which we observed would suggest increased lipolysis. However, one must remember that even though the rate of plasma glycerol uptake is generally proportional to the serum concentration (18, 19), this condition may not hold true in the burned animal. Carpentier *et al.* demonstrated that plasma glycerol concentration and turnover rate were not correlated in one group of injured or septic patients (20) or following total hip replacement (21). The increased glycerol concentration in omental adipose tissue could be

related to increased plasma glycerol concentration. Since omental adipose tissue has a higher blood flow (22) and presumably is metabolically more active than epididymal adipose tissue it would be expected that changes within the omental pad would be more closely associated with plasma changes than would the epididymal pad.

The control of lipolysis during burn shock is potentially very complicated. Burned animals have elevated catecholamines (23), and glucagon (24) both of which increase lipolytic activity. However, guinea pig adipose tissue has been shown to be unresponsive to catecholamines (27). Also, the burned guinea pig is acidotic (1). Acidosis inhibits lipolysis directly and interferes with the lipolytic activity of catecholamines (25, 26). It appears that the lipolytic response is simultaneously being stimulated and inhibited. The increase in plasma glycerol would suggest that lipolytic stimuli dominate, however, caution must be exercised when inferring an increased turnover rate from an increased plasma concentration. If lipolysis either increased or remained at the preburn level, then, for plasma RA FFA to be decreased following burn as we observed (7), adipose tissue FFA reesterification must be accelerated. Reesterification of FFA within the adipose tissue could be stimulated by the high plasma lactate or glucose levels observed during burn shock (1) since either lactate or glucose can serve as a precursor of α-glycerophosphate.

Before concluding that the FFA turnover rate following burn is controlled simply by the balance between blood-borne factors which influence lipolysis and reesterification, one must consider the potential effects of greatly reduced adipose tissue perfusion on tissue metabolism. As discussed by Kovach *et al.* (14), severe hypoperfusion may cause cellular hypoxia leading to increased anaerobic metabolism and an increase in intracellular lactate and acidosis. Even though severely reduced adipose tissue blood flow does not appear to cause a build-up of FFA within the adipose tissue, it may alter the balance of lipolysis and reesterification resulting in changes in the turnover of FFA and glycerol. In conclusion, severe burn causes alterations in FFA and glycerol metabolism which appear to be due to changes in lipolysis and reesterification rather than impaired FFA transport. Simultaneous measurements of FFA and glycerol turnover rates are necessary to further elucidate the mechanisms underlying the observed alterations in adipose tissue metabolism during burn shock.

Summary. We have measured adipose tissue and plasma FFA and glycerol concentrations in order to investigate the mechanism by which FFA turnover is dramatically decreased during burn shock in the guinea pig. These data, in conjunction with previous work demonstrating acidosis and elevated plasma glucose, lactate, catecholamines, and glucagon, suggest that metabolic alterations are a major factor contributing to the decrease in release of FFA from adipose tissue. Whether or not the alterations in adipose tissue metabolism are related to decreased tissue perfusion remains unknown.

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