Blood Flow, Oxygen Consumption, and Free Fatty Acid Release in Subcutaneous Adipose Tissue during Hemorrhagic Shock in Control and Phenoxybenzamine-Treated Dogs

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ABSTRACT

Dogs were anesthetized with α-D(+) -glucocorticosterone. After bleeding to a mean arterial pressure of 55 mm Hg, blood flow in subcutaneous adipose tissue decreased from 6 ± 0.9 (mean ± SE) to 0.6 ± 0.21 ml/min/100 g (P < 0.001), and remained at that low level during bleeding to 35 mm Hg for an additional 90-minute period. In five out of nine experiments the blood flow ceased completely. Sixty minutes after reinfusion, the blood flow was significantly lower than control, and in two there was no blood flow after reinfusion. In animals previously treated with phenoxybenzamine (5 mg/kg), the decrease in blood flow was significant only at 35 mm Hg arterial pressure (2.6 ± 0.67 ml/min/100 g). After reinfusion blood flow increased to a mean above 10 ml/min/100 g, significantly higher than resting blood flow (P < 0.05). There was no significant change in arterial FFA concentration or FFA release in subcutaneous adipose tissue during bleeding and after reinfusion. In phenoxybenzamine-treated animals there was a tendency to have higher arterial FFA concentration and FFA release. The oxygen uptake fell from 0.47 ± 0.07 to 0.13 ± 0.05 ml/min/100 g (P < 0.01) during 55 mm Hg arterial pressure and remained significantly lower also during 35 mm Hg arterial pressure. Previous treatment with phenoxybenzamine prevented a fall in O_2 uptake in subcutaneous adipose tissue. The decrease in blood flow in subcutaneous adipose tissue during bleeding was more pronounced than found in other organs with same hemorrhagic shock procedure. After reinfusion, flow in subcutaneous adipose tissue could not be restored, indicating irreversible vascular damage. α-receptor activity seems to play a significant part in the development of vascular and metabolic changes in subcutaneous adipose tissue during bleeding.

ADDITIONAL KEY WORDS

bleeding shock lipid metabolism

During hemorrhagic shock the blood flow in different organs is impaired to various degrees (1, 2). However, the vascular reactions in adipose tissue have not been studied under such conditions.

There is a gross lipemia in animals subjected to bleeding (3). During tourniquet shock in rabbits, Johnson and Wadstörm (4) observed increased serum glycerol levels, and Stoner and Mathews (5) found an increased FFA concentration in the epididymal fat pad of rat during such treatment. These observations suggest that there is an increased lipolysis after trauma.

The aim of the present study was to elucidate the circulatory and some of the metabolic changes in canine subcutaneous adipose tissue during standardized hemorrhagic shock (1, 6).
Following hemorrhage there is an increased rate of release of catecholamines from the adrenals (7-10) as well as a rise in the sympathetic nervous activity (11, 12). Since these factors, to a large extent, influence blood flow and release of FFA in subcutaneous adipose tissue (13), we have also investigated the effects of denervation and α-receptor blockade in dogs subjected to acute hemorrhage.

**Material and Methods**

Twenty-five female mongrel dogs, fasted for about 18 hours, were anesthetized with α-D(+) -glucocloralose (Merck, Darmstadt), 100 mg/kg iv. Heparin (Chemical Fabrik, Gedeon Richter, Budapest), 5 mg/kg, was given about one hour before the experimental run to prevent clotting. The subcutaneous adipose tissue was isolated from surrounding tissues including skin in the right inguinal region, with the principal artery and nerve left intact as described earlier (14).

For continuous recording of blood flow the vein was cannulated with polyethylene tubing directing the flow to a drop recorder (Jaquett, Switzerland). After having passed the drop recorder, the venous blood was collected in ice-cooled centrifuge tubes for subsequent analysis of FFA and under paraffin oil for determination of oxygen content. Arterial samples were withdrawn from a cannula in one brachial artery for the same purpose. Blood was drained from the artery into a reservoir during the bleeding periods (15). Arterial blood pressure was measured in the other brachial artery. Total cardiac output and three fractions of it were measured according to the thermodilution principle (16, 17). Saline at room temperature was injected into the aorta at three different levels: through the aorta ascendens, at the level of the diaphragm, and below the renal arteries. The thermistor was inserted at the level of the bifurcation of the aorta through a branch of the femoral artery. The dilution curves were calculated with the aid of a Fischer cardiac output computer (A. G. Fischer, Göttingen). Respiratory rate was measured with a thermistor in the tracheal cannula, ECG was recorded from two fronto-occipital leads and EEG from the standard lead II. These parameters were registered continuously on an Alvar polygraph and were used to detect cerebral and cardiac hypoxic damage during the standardized shock procedure (18).

As an α-receptor blocking agent, 5 mg/kg phenoxybenzamine (Dibenzyline, Smith, Kline and French, Philadelphia, Pa.) was injected intravenously in 11 dogs on the day preceding the experiment. In four experiments, subcutaneous adipose tissue was denervated before the start of the experimental period.

FFA in arterial and venous samples was analyzed according to Dole (19) as modified by Trout et al. (20). The extraction of FFA from the ice-cooled plasma samples was performed on the experimental day and titrated within 48 hours. Oxygen saturation in blood samples collected under paraffin oil was measured by an oximeter (AB Elema-Schönander, Stockholm) and the oxygen uptake calculated from the arteriovenous difference in the oxygen content, blood flow, and the hematocrit values.

An ordinary experimental run was performed as follows: After anesthesia, operative procedures, and a resting period of at least 60 minutes, the dogs were bled into a reservoir to stabilize the mean arterial blood pressure at about 55 mm Hg for 90 minutes (B I). This was followed by further bleeding to 35 mm Hg for an additional 90 minutes (B II). The shed blood was then reinfused and the experiment was, if possible, continued for 90 minutes. As reported earlier (18), after such a procedure the dogs developed irreversible shock. Blood was sampled and cardiac output, respiratory rate, ECG, and EEG were recorded every 15 minutes.

The statistical analysis was performed according to Student's t-test.

**ADIPOSE TISSUE IN HEMORRHAGIC SHOCK**

![Graph](http://circres.ahajournals.org/figure/1)

**FIGURE 1**

Mean values (±SE) during control period, bleeding periods (B I and B II), and after reinfusion of the shed blood (R). Solid circles show experiments without phenoxybenzamine and open circles show experiments with phenoxybenzamine.
Results

**BLOOD FLOW**

The mean value of the blood flow of subcutaneous adipose tissue was $6 \pm 0.9$ (mean $\pm$ se, $N = 10$) ml/min/100 g (Fig. 1, Table 1). After the reduction of the arterial blood pressure to 55 mm Hg, the blood flow was very much restricted. The lowest mean value, $0.6 \pm 0.2$ ml/min/100 g, was obtained after 10 minutes. The difference was significant ($P < 0.001$). During the rest of B I the blood flow remained at that level. In three out of ten experiments the blood flow ceased completely. In B II the mean blood flow remained at around 10% of the control value. However, in five out of nine experiments there was no blood circulation. During B I and B II there was a pronounced increase in peripheral resistance expressed as the ratio between arterial pressure and blood flow. Since in some of the experiments the flow ceased completely, calculation of the mean numerical value of the peripheral resistance would be meaningless. After reinfusion the adipose tissue blood flow approached the control level but began to fall again after 15 minutes. Thus 60 minutes after reinfusion the average flow was around 2 ml/min/100 g. In two of seven dogs, still living at that time, it was not possible to restore blood flow. One such experiment is illustrated in Figure 2.

In the phenoxybenzamine-treated animals the resting circulation was not significantly different from that in the control animals ($5.5 \pm 0.9$ ml/min/100 g, $N = 11$) (see Fig. 1, Table 1). After bleeding to 55 mm Hg, initially the mean blood flow was reduced to about 3 ml/min/100 g and after 30 minutes the flow was $3.9 \pm 1.1$ ml/min/100 g and remained at that level, i.e., at about 70% of the resting blood flow. However, the difference between control and B I at 30 minutes was not significant ($P > 0.20$). During B II there was a further reduction to 2.5 ml/min/100 g at 60 minutes ($P < 0.02$ for the difference between control and B II). During B I and B II the mean numerical value of the peripheral resistance did not change. After reinfusion
there was a pronounced reactive hyperemia. Thus, the blood flow increased to $11 \pm 2$ ml/min/100 g at 15 minutes and remained above the control level for a period of 1 hour. The peripheral resistance after reinfusion was significantly lower than before bleeding ($P < 0.05$). A typical experiment with a phenoxybenzamine-treated animal is illustrated in Figure 3.

**ARTERIAL FFA VALUES AND NET FFA RELEASE**

The mean FFA concentration in arterial plasma was $0.68 \pm 0.13$ μmoles/ml at 15 minutes before bleeding (Fig. 1 and Table 1). During B I at 30 minutes the FFA concentration fell to about 60% of that value ($0.42 \pm 0.06$), however, this fall was not significant ($P = 20$). The FFA concentration remained at about 40 μmoles/ml during B II. After reinfusion the mean arterial FFA concentration tended to rise but still there was no significant change.

Before bleeding there was no significant net uptake or release of FFA in subcutaneous adipose tissue (Fig. 1, Table 1), which was also the case during B I or B II. In six of the animals the blood flow was too low to allow collection of blood for FFA measurements of venous plasma; consequently, the significance levels were calculated from only three experiments. After reinfusion the FFA release was not significantly different from control.

As also illustrated in Figure 1, in the phenoxybenzamine-treated animals the arterial FFA concentration was about the same as in the untreated animals during the control period. During B I and B II, arterial FFA concentration was not significantly higher than before bleeding. However, in comparison with the control experiments the difference was significant during B II ($P < 0.05$). Following reinfusion the arterial FFA concentration returned to the prebleeding value.

The phenoxybenzamine-treated animals had a tendency for a net release of FFA during B I and the first part of B II, but the
difference in comparison with the prebleeding values was not significant. After reinfusion the net release of FFA gradually increased, but there was a great variation between the individual experiments. Thus in five of the animals there was a net uptake, and in six a pronounced net release of FFA during the prebleeding period.

**OXYGEN UPTAKE**

The arteriovenous $O_2$ difference was $7.5 \pm 0.8\%$ of the volume in the control period. In B I the arteriovenous $O_2$ difference increased to $15.5 \pm 1.8$ and was around $18\%$ of the volume during B II. The increment in the arteriovenous difference in B I and B II was significant ($P < 0.01$). After reinfusion the arteriovenous difference returned to the control range.

Before bleeding, the oxygen uptake (see Fig. 1, Table 1) was $0.47 \pm 0.07$ ml/min/100 g, this initially decreased to $0.13 \pm 0.05$ in B I ($P < 0.01$), went down to $0.08 \pm 0.04$ at the end of this period, and stayed at a significantly lower level also during B II. Due to the low blood flow in the latter part of B II, blood $O_2$ content could be determined in only three cases. After reinfusion the oxygen uptake remained significantly low compared with the prebleeding value ($P < 0.05$).

In the phenoxybenzamine-treated animals, the arteriovenous oxygen difference was $8.8 \pm 0.80\%$ of the volume, which was not significantly different from the untreated group. After bleeding, the arteriovenous difference increased but not to the same extent as in the untreated animals. Thus, during B I the arteriovenous oxygen difference was significantly different from the control period only at 30 minutes ($P < 0.02$). After reinfusion it diminished to $5.0 \pm 0.86\%$ of the volume and subsequently returned to the control value.

During the control period the oxygen uptake was the same in the phenoxybenzamine-treated and the untreated animals. In the phenoxybenzamine-treated animals the oxygen uptake in B I and B II remained between 0.40 and 0.45 ml/min/100 g. Both in B I and B II there was a significant difference in $O_2$ uptake between the control and phenoxybenzamine-treated groups ($P < 0.01$). After reinfusion the $O_2$ uptake remained at the prebleeding level.

**DENERVATION**

In four experiments the adipose tissue was denervated at the start of the experiment. The blood flow, FFA release, and $O_2$ uptake in the adipose tissue changed during bleeding and after reinfusion in much the same way as in the control experiments.

**Discussion**

The present experiments show that during hemorrhage blood flow in subcutaneous adipose tissue was severely diminished due to a pronounced rise in peripheral resistance. As a consequence, tissue metabolism was impaired as indicated by the marked decrease in oxygen uptake. Furthermore, in some of the experiments the blood flow could not be restored by reinfusion (see Fig. 2). This suggests that the vascular bed was irreversibly damaged during the bleeding period.

In contrast, the diminution in blood flow was much less pronounced in the phenoxybenzamine-treated animals and was restored to normal or above normal values after reinfusion of the shed blood. The calculated peripheral resistance was not significantly altered during the oligemic state. Furthermore, oxygen uptake in the adipose tissue remained normal. These findings indicate that phenoxybenzamine protected the vascular bed of subcutaneous adipose tissue from damage during severe bleeding.

The subcutaneous adipose tissue seems to be especially sensitive to bleeding. With a similar experimental procedure Kováč (1) found that blood flow in the hypothalamus, liver, skeletal muscle, and myocardium fell to about 60% of the resting blood flow and the renal cortical blood flow fell to about 40% during bleeding to an arterial pressure of 55 mm Hg. During bleeding to 35 mm Hg arterial pressure, there was a further small decrease in blood flow, except in the myocardium. In the subcutaneous adipose tissue, however, the blood flow was reduced to about
10% during the first bleeding period and persisted at that low level during B II. Furthermore, in five out of nine dogs, the blood flow ceased completely during B II. After reinfusion, the blood flow in liver, muscle, myocardium, and the kidney recovered. Partial recovery occurred in the hypothalamus and, according to the present results, in the subcutaneous adipose tissue. These comparisons suggest that the subcutaneous adipose tissue may be one of the organs where irreversible shock is manifested.

The fact that the peripheral resistance did not increase in the phenoxybenzamine-treated animals during bleeding may have been due to at least two factors: (a) protection from vasoconstriction caused by α-receptor blockade, and (b) vasodilatation because it has been shown that provided the α receptors are blocked, sympathetic nerve activity produces vasodilatation caused by β-receptor stimulation (13).

Bleeding induces an increased activity in sympathetic nerves and a pronounced release of catecholamines from the adrenals. Both noradrenaline and adrenaline cause vasoconstriction in the subcutaneous adipose tissue (13). The finding that the blood flow in acutely denervated adipose tissue was reduced to the same extent as in the innervated tissue suggests that in this case the blood-borne catecholamines may be of importance in producing vasoconstriction during oligemic shock.

Since the adipose tissue constitutes approximately 15% of the body weight and has a resting blood flow of 6 to 9 ml/min/100 g which can diminish greatly following bleeding, as the present experiments show, the tissue may function as a blood reserve by shifting blood during short term emergency periods to other organs. On the other hand, if the vasoconstriction in adipose tissue persists for a long time, this may have local metabolic and vascular consequences.

Arterial plasma FFA and the release rate of FFA from the subcutaneous adipose tissue did not rise during bleeding, despite a presumably high sympathoadrenal activity, although it is well established that catecholamines, to a large extent, promote lipolysis (13, 21, 22). There may be several explanations for this. Already during the control period the arterial FFA concentration was high, presumably due to the chloralose anesthesia, and bleeding did not elevate it further. Recently, Halmagyi et al. (23) found that the arterial FFA concentration increased from 0.163 to 0.257 μmoles/ml during bleeding. Thus the control level was much lower than in the present experiments. They used thiopentone for anesthesia, which presumably decreases the arterial FFA level (24). Furthermore, acidosis, a constant finding in hemorrhagic shock (6, 25), inhibits the noradrenaline-induced lipolysis (26, 27). Isselkutz et al. (28) have found that infusion of Na-L(+) lactate reduced the rate of release and uptake of FFA in unanesthetized dogs.

In addition, it has been shown that infusion of buffered lactate in concentrations above 6 to 7 μmoles/ml inhibits the outflow of FFA from the same type of adipose tissue as was used in the present experiments (29). This effect of lactate seems to be due to an enhanced reesterification rate, because the outflow of glycerol was not altered (29). During hemorrhagic shock there is a pronounced elevation of the blood lactate concentration (30-32). Thus both acidosis and lactate may counteract the lipomobilizing effect of sympathetic nerve activity and catecholamines. To what extent such mechanisms may be responsible for the present findings remains to be studied. However, it is quite reasonable to assume that the pronounced reduction in blood flow must have played a significant role in counteracting the FFA outflow from adipose tissue.

In the phenoxybenzamine-treated animals the level of FFA in plasma was significantly elevated during B II. This effect may be explained by a more efficient blood flow in the adipose tissue and, furthermore, by the fact that α-receptor blocking agents potentiate the release rate of FFA induced by sympathetic nerve activity (33). Phenoxybenzamine treatment inhibits the pronounced elevation of blood lactate concentration and acidosis after standardized hemorrhagic shock (18, 25).
References


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