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VASCULAR AND METABOLIC RESPONSES TO ADRENERGIC STIMULATION IN ISOLATED CANINE SUBCUTANEOUS ADIPOSE TISSUE AT NORMAL AND REDUCED TEMPERATURE

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SUMMARY

1. The circulatory and metabolic effects of temperature reduction were studied in autoperfused canine subcutaneous adipose tissue *in situ*.

2. Cooling the adipose tissue sufficiently to reduce venous effluent temperature by 5–6 °C decreased blood flow from an average of 6·4–4·1 ml. min^{-1}. 100 g^{-1}.

3. Vasoconstrictor responses to sympathetic nerve stimulation (4 Hz) and injected noradrenaline (5 n-mole) were potentiated by cooling while vasodilator components of the vascular responses, such as autoregulatory escape and post-stimulatory hyperaemia, were virtually abolished by this treatment.

4. Oxygen uptake was reduced by cooling without signs of tissue hypoxia. This reduced oxygen demand may partly cause the decrease in adipose tissue blood flow.

5. Cooling inhibited glycerol mobilization from the adipose tissue during sympathetic nerve stimulation. Post-stimulatory lipolysis was, however, not inhibited. *In vitro* studies with 'perifused' rat fat cells suggest that this may be due to impaired inactivation of the lipolytic process, rather than to changes in transmitter removal, following stimulation at low temperature.

6. Cooling inhibited the mobilization of fatty acids more than that of glycerol, suggesting increased re-esterification of fatty acids within the tissue at low temperature.

7. It is concluded that cooling increases the sensitivity to vasoconstrictor stimuli and that inhibition of metabolic vasodilator mechanisms play a role for this effect. The simultaneous inhibition of activating and inactivating mechanisms could explain the unchanged vascular and lipolytic responses to brief stimuli. Some possible implications of the present findings for the physiology of adipose tissue during cooling are discussed.

INTRODUCTION

Adrenergic responses in canine subcutaneous adipose tissue *in situ* have been studied in some detail. Sympathetic nerve stimulation or the intra-arterial (I.A.) administration of noradrenaline induces vasoconstriction mediated by α-adrenoceptors (Ngai, Rosell & Wallenberg, 1966; Fredholm, Öberg & Rosell, 1970). After α-adrenoceptor blockade these stimuli induce vasodilatation mediated by β-adrenoceptors (Ngai *et al.* 1966; Belfrage & Rosell, 1976). Furthermore, nerve

stimulation and intravenously administered catecholamines enhance lipolysis, oxygen consumption and glycogenolysis secondary to stimulation of β-adrenoceptors (Fredholm, 1970).

There is some evidence that metabolism in the canine subcutaneous adipose tissue influences the circulation. Thus, inhibition of adipose tissue metabolism by β-adrenoceptor blockade increases maximal vasoconstriction due to noradrenaline, decreases autoregulatory escape and decreases poststimulatory hyperaemia (Belfrage, 1978). However, β-adrenoceptor blocking agents inhibit also vascular β-adrenoceptors and the interpretation of these experiments is complicated. Recently it was found that acidosis, which inhibits lipolysis but not vasodilatation caused by β-adrenoceptor activation, also potentiated noradrenaline-induced vasoconstriction in the adipose tissue (Hjemdahl & Fredholm, 1976). These results were interpreted as evidence for a role of a 'metabolic' β-adrenoceptor in the regulation of adipose tissue circulation. In order to further investigate the influence of metabolism on adipose tissue vascular responses we have utilized the well known effect of hypothermia to lower the metabolic rate (Bigelow, Lindsay, Harrison, Gordon & Greenwood, 1950).

The temperature of subcutaneous tissue is decreased when the ambient temperature is lowered (Brück & Henzel, 1953). Therefore the study of the effects of local cooling of the canine subcutaneous adipose tissue may be of physiological relevance. Subcutaneous adipose tissue may play a dual role when the ambient temperature is lowered. First, it may act as an insulator of the core of the body, a function which presupposes a low blood flow to prevent heat dissipation. Secondly, it stores energy in the form of fatty acids, the mobilization of which requires an adequate blood flow to the tissue. In the present study we therefore studied the influence of local cooling on subcutaneous adipose tissue circulation, metabolic rate and on lipid mobilization. A preliminary account of some of the present findings has been reported elsewhere (Sollevi, Hjemdahl & Fredholm, 1975).

METHODS

In vivo techniques

The experiments were performed on sixteen female mongrel dogs, weighing 10–26 kg. The dogs were anaesthetized with sodium pentobarbitone (30 mg/kg I.V.) with supplements as required. Tracheotomy was performed and the dogs were mechanically ventilated with a Braun Melsungen model 74052 respirator. Subcutaneous adipose tissue in the inguinal region was subsequently isolated from skin and other surrounding tissues as described by Rosell (1966). This provided an adipose tissue preparation connected to the animal by one artery, one vein and one nerve containing adrenergic fibres. The weight of the preparation was between 30 and 190 g (average 52 g).

The adipose tissue was enclosed in a saline-perfused chamber the temperature of which was maintained at either 37 °C or 26–27 °C as measured by a thermistor (Ellab Instruments, Copenhagen). Arterial blood was diverted from the femoral artery to the adipose tissue via a drop counter and venous outflow was returned to the femoral vein via a polyethylene catheter containing a three-way stopcock, which was used for venous blood sampling. A thermistor was also inserted into the catheter for venous blood temperature monitoring. Cooling the saline-perfused plethysmograph from 37 to 27 °C reduced the venous effluent temperature from approximately 33 °C to 27–28 °C. This cooling procedure thus reduced the temperature of the effluent blood by 5–6 °C. Heparin (2500 i.u./kg) was administered before the cannulation procedure, i.e. at least 1 hr before the first experimental run. Fluid losses due to sampling and trauma were replaced with isotonic saline. Systemic blood pressure was measured with Statham P23AC transducers and

recorded together with adipose tissue blood flow on a Grass model 7B polygraph. Vascular conductance was calculated by dividing adipose tissue blood flow by the blood pressure.

The nerve supplying the adipose tissue was sectioned in all experiments. When stimulation was performed, the distal part of the sectioned nerve was placed on a bipolar silver electrode and protected from drying with Plastibase (Squibb). The nerve was stimulated at 4 Hz during periods of 5 or 30 min with impulses of supramaximal duration (1 msec) and intensity (12–14 V), which were delivered by a Grass model S4 stimulator. In four experiments 5×10^{-9} mole noradrenaline (L-norepinephrine hydrochloride, Sigma) was administered to the adipose tissue by close intra-arterial injection. In two experiments involving nerve stimulation both inguinal fat pads were isolated. One of the adipose tissue preparations was pretreated with cocaine 200–400 μg I.A. before each observation period, while the contralateral preparation served as control.

Samples of arterial blood and venous blood from the adipose tissue were collected into ice-cold plastic tubes. Aliquots of whole blood were immediately removed for the determination of lactate and pyruvate (TCC, TCB, Boehringer & Sohn, Mannheim). The samples were then centrifuged and plasma was removed for the determination of glycerol (Laurell & Tibbling, 1966), FFA (Laurell & Tibbling, 1967) and glucose (with commercially available glucose oxidase reagent, Glox, KABI, Stockholm). The venous sampling followed a standardized pattern, as illustrated in Figs. 1 and 3. Arterial samples were withdrawn at regular intervals for the determination of the hematocrit and the above-mentioned metabolites. The uptake or release of the various metabolites was subsequently calculated on the basis of arterio-venous concentration differences and blood or plasma flow values per 100 g tissue.

In experiments where the oxygen uptake of the adipose tissue was determined, blood was collected under paraffin oil. The haemoglobin content was determined by a commercially available procedure (Aculute, Ortho diagnostics). Arterial and venous pH, p_{CO_2} and p_{O_2} were determined by a Radiometer (Copenhagen) BMS 3 MK2 blood gas analyser. The oxygen content of the blood samples was subsequently obtained from a nomogram.

In vitro techniques

Isolated fat cells were prepared essentially according to Rodbell (1964). Epididymal fat pads from three to five male rats (Sprague Dawley, Anticimex strain, 180–200 g) were pooled in each experiment. After mincing, the tissue was incubated in Krebs-Ringer phosphate buffer pH 7.4, containing half the recommended calcium concentration, 5.5 mM-glucose, bovine serum albumin 30 mg/ml. (Fraction V, Sigma, St Louis) and crude bacterial collagenase 3 mg/ml. (Lot 44B231, Worthington Biochem., Freehold) for 40–60 min. After filtering through gauze and washing twice in the same medium without collagenase the cells were used either for incubation or perifusion experiments.

In the *incubation* experiments 0·5 ml. of a concentrated cell suspension were added to 2 ml. buffer in plastic vials to give a final cell concentration of 40000 cells/ml. The cells were incubated at 37 or 27 °C in shaking water-baths. The cells were preincubated for 10 min before the addition of noradrenaline. At different time intervals after the addition of noradrenaline, aliquots of cells and medium were taken for the determination of glycerol (Laurell & Tibbling, 1966) and cyclic AMP (Brown, Ekins & Albano, 1972). The treatment of samples for the cyclic AMP determination has been described elsewhere (Fredholm & Hjemdahl, 1976).

For the *perifusion* experiments 1 ml. packed fat cells were added to 2–2·5 ml. Krebs-Ringer phosphate buffer pH 7·4 in a temperature-controlled plastic chamber essentially according to Allen, Largis, Miller & Ashmore (1973). The floating fat cells in the chamber were subsequently perifused with the same medium containing albumin 10 mg/ml. at a rate of 2 ml./min. The chamber and the perifusion medium were kept at 37 or 27 °C. The perifusate was collected continuously and subsequently used for the determination of glycerol. In order to increase the sensitivity of the glycerol assay the deproteinization step was excluded. Standards and blanks were therefore prepared in the same medium and yielded expected values. Noradrenaline was infused via a side-arm to give a final concentration of 10 μM in the chamber.

Conventional statistical methods were used to calculate means ($\bar{x}$), standard deviations (S_x) and standard errors of the mean ($S_{\bar{x}}$). Hypotheses were tested by Student's t test for paired or unpaired variates or by the Wilcoxon test in case the variates were not normally distributed.

RESULTS

Vascular responses

The resting blood flow in the denervated adipose tissue averaged 6·4 ml. min^{-1}. 100 g^{-1} (range 3·0–13·0) during control conditions. When the temperature was reduced blood flow decreased to 4·1 ml. min^{-1}. 100 g^{-1} (range 2·0–7·7), which corresponded to a decrease in vascular conductance of 33·2 ± 5·5% ($n = 16, P < 0·001$).

TABLE 1. Vascular responses to noradrenaline injections (5 n-mole) in four experiments and to sympathetic nerve stimulation (5 min, 4 Hz) in six experiments. Peak vasoconstriction and peak hyperaemia are expressed as % of prestimulatory vascular conductance. Mean values ± S.E. are given

	Noradrenaline		Nerve stimulation	
	37 °C	27 °C	37 °C	27 °C
Peak vasoconstriction	25·0 ± 3·8	21·8 ± 4·3 ($P < 0·05$)	37·1 ± 3·6	28·2 ± 4·2 ($P < 0·05$)
Peak hyperaemia	167 ± 14	114 ± 20 ($P < 0·05$)	174 ± 10	118 ± 12 ($P < 0·05$)

Cooling caused a small but significant increase in the maximal vasoconstrictor response to I.A. noradrenaline injections (Table 1). The vasoconstriction was considerably prolonged and the ensuing hyperaemia was almost abolished at reduced temperature (Fig. 1, Table 1). Cooling influenced the adipose tissue vascular responses to sympathetic nerve stimulation similarly. Thus, there was an increase in maximal vasoconstriction during stimulation (Table 1) and a delay and reduction in the hyperaemia following stimulation (Figs. 2 and 3, Table 1). The autoregulatory escape normally seen during prolonged nerve stimulation was practically abolished by cooling (Fig. 3).

Lipolysis

During control conditions glycerol was spontaneously released from the denervated adipose tissue at a rate of 0·13 ± 0·07 μmole. min^{-1}. 100 g^{-1} and FFA taken up at a rate of 0·12 ± 0·12 μmole. min^{-1}. 100 g^{-1}. Cooling did not significantly alter the basal outflow of glycerol or the uptake of FFA in the adipose tissue.

Glycerol outflow from the adipose tissue was enhanced and the net FFA uptake was converted into a net release following close I.A. injection of noradrenaline, in agreement with earlier reports (e.g. Hjemdahl & Fredholm, 1976; Belfrage & Rosell, 1976). Following temperature reduction the total amount of glycerol mobilized was not significantly altered (Table 2), but the mobilization of glycerol was considerably delayed and prolonged (Fig. 1). The release of FFA was similarly delayed and prolonged (Fig. 1), but in this case there was also a significant reduction in the total amount mobilized (Table 2). The degree of reutilization of fatty acids by the adipose tissue could be calculated on the basis that glycerol is not reutilized by the tissue (cf. Fredholm, 1970) and that three moles of fatty acids are formed per mole of glycerol during triglyceride break-down. Such a calculation showed that cooling significantly increased the reutilization of FFA within the adipose tissue (Table 2), presumably by increasing the reesterification of fatty acids (cf. Fredholm, 1970).

When nerve stimulation, rather than exogenous noradrenaline, was used as the lipolytic stimulus, a similar response pattern was seen (Fig. 2). The total amount of glycerol released due to a short nerve stimulation was unaffected by cooling, while the net release of FFA was significantly reduced, again indicating an increased fatty acid reesterification (Table 2).

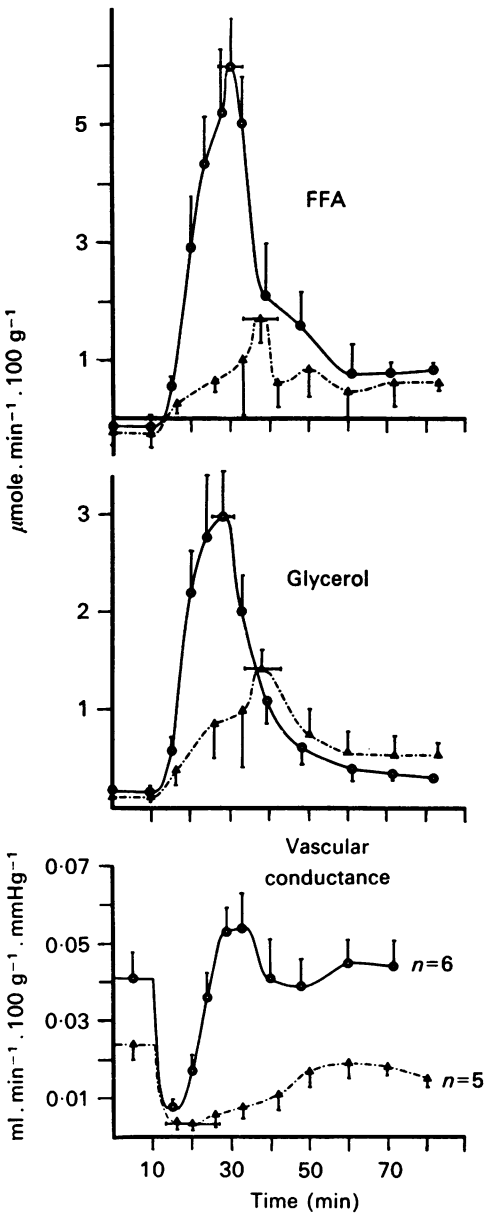

Fig. 1. Effect of I.A. noradrenaline injections (5 n-mole) on adipose tissue vascular conductance and production of glycerol and FFA. Continuous lines represent responses at 37 °C (n = 6) and interrupted lines at 27 °C (n = 5). Mean values ± S.E. The net production of glycerol and FFA in these experiments is given in Table 2.

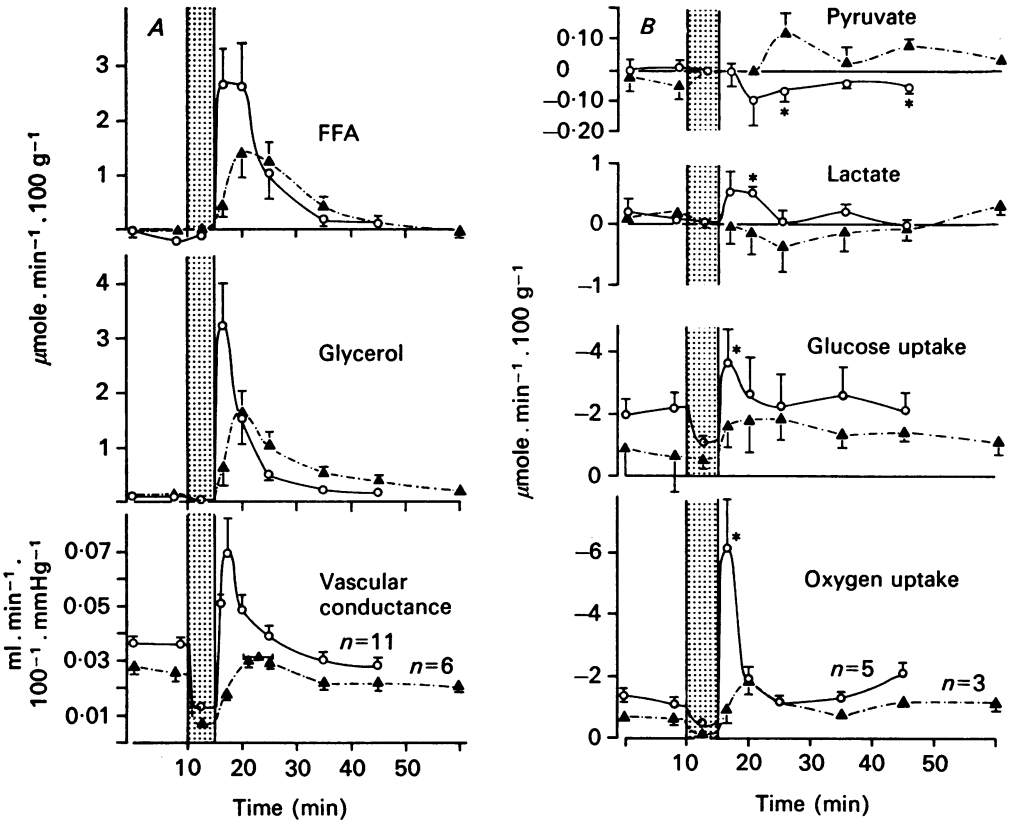

Fig 2. *A*, effects of sympathetic nerve stimulation indicated by the stippled columns (5 min, 4 Hz) on adipose tissue vascular conductance and production of glycerol and FFA. Continuous lines represent responses at 37 °C (n = 11) and interrupted lines at 27 °C (n = 6). Mean values ± S.E. are shown. The net production of glycerol and FFA is given in Table 2. *B*, effects of sympathetic nerve stimulation on adipose uptake or release of oxygen, glucose, lactate and pyruvate. Continuous lines represent responses at 37 °C (n = 5) and interrupted lines at 27 °C (n = 3). Mean values ± S.E. are shown.

TABLE 2. Total net production of glycerol and FFA (μmole.100 g^{-1}) caused by noradrenaline injections (5 n-mole I.A.) and by sympathetic nerve stimulation. The percentage of fatty acids reesterified was calculated on the basis of a production of 3 mole FFA per mole of glycerol during triglyceride breakdown. Mean values ± S.E. are given. The number of observations are shown in parentheses

	Noradrenaline		Nerve stimulation	
	37 °C	26 °C	37 °C	26 °C
Glycerol	54·7 ± 8·9 (7)	40·7 ± 12·7 (5)	20·5 ± 4·8 (11)	22·9 ± 6·0 (6)
	(n.s.)		(n.s.)	
FFA	130·4 ± 19·2 (7)	56·4 ± 11·8 (5)	31·5 ± 8·5 (11)	21·8 ± 8·0 (6)
	(P < 0·01)		(P < 0·01)	
% Re-esterification	17·2 ± 8·2 (7)	47·6 ± 6·3 (5)	45·1 ± 13·5 (11)	71·1 ± 6·2 (6)
	(P < 0·05)		(P < 0·05)	

Responses to short-lasting stimulations may be difficult to interpret since they are obtained under non-steady state conditions (Hjemdahl, 1976). Therefore, the effect of temperature on responses to prolonged sympathetic nerve stimulation was also studied. As seen in Fig. 3 the rate of glycerol outflow from the tissue increased during the first 10–15 min to approach a steady state during the later part of the nerve

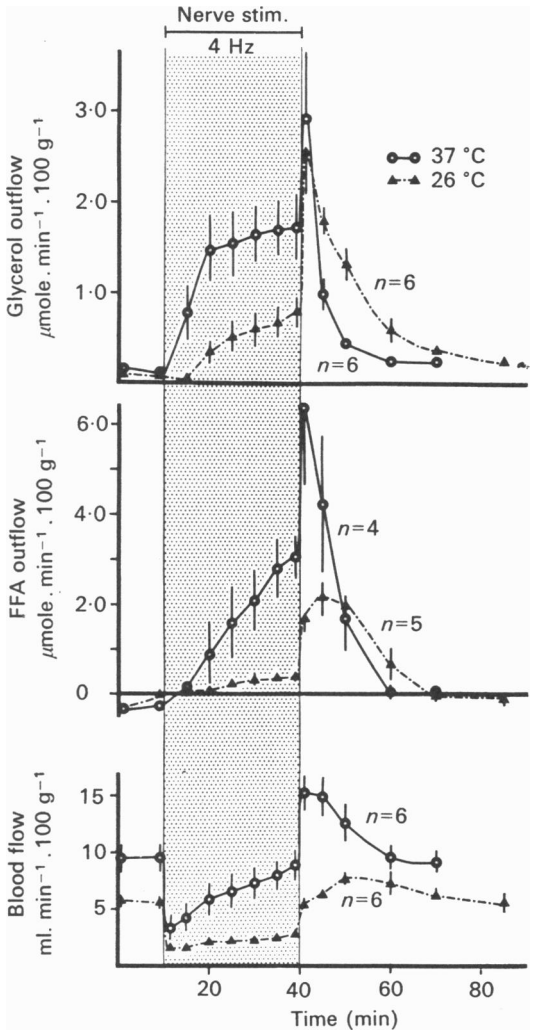

Fig. 3. The effects of prolonged sympathetic nerve stimulation (30 min, 4 Hz) on adipose tissue blood flow and outflow of glycerol and FFA. Continuous lines represent responses at 37 °C and dotted lines at 27 °C. Mean values ± S.E. are given. The production of glycerol and FFA during and after stimulation is given in Table 3. Note the absence of autoregulatory escape during nerve stimulation at low temperature.

stimulation. This 'steady state' response was markedly reduced by cooling (60 % reduction of glycerol release and 81 % reduction of FFA release (Table 3)). By contrast, the glycerol release upon cessation of nerve stimulation was significantly enhanced and the FFA release was unaltered by cooling (Fig. 3, Table 3).

The essentially unaltered glycerol release following noradrenaline injection or brief nerve stimulations and the actually enhanced post-stimulatory release after prolonged nerve stimulation could be due to impaired removal of noradrenaline in the adipose tissue during cooling. Since the most important inactivating mechanism in adipose tissue is neuronal uptake (Belfrage & Rosell, 1976; Belfrage, Fredholm & Rosell, 1977), the influence of temperature on lipolysis after pretreatment with cocaine was tested in two experiments. Cocaine, however, enhanced the lipolytic response to the same degree at normal and reduced temperature in these two experiments, giving no indication that neuronal uptake is significantly inhibited in adipose tissue by this degree of cooling.

TABLE 3. Glycerol and FFA outflow from the adipose tissue, during (i.e. between 25 and 29 min of stimulation, denoted in the Table as steady state release rate) and after cessation of the 30 min sympathetic nerve stimulations. Mean values ± S.E. from four experiments. The number of observations are shown in parentheses. For the complete outflow pattern see Fig. 3

	'Steady state' release rate (μmole.min^{-1}.100 g^{-1})		Post-stimulatory release (μmole.100 g^{-1})	
	37 °C	27 °C	37 °C	27 °C
Glycerol	1·59 ± 0·32 (6)	0·64 ± 0·16 (6)	15·8 ± 3·1	34·0 ± 3·7
	($P < 0·02$)		($P < 0·005$)	
FFA	3·18 ± 0·63 (4)	0·59 ± 0·14 (5)	55·5 ± 18·8	44·3 ± 6·0
	($P < 0·02$)		(n.s.)	

TABLE 4. The metabolism in subcutaneous adipose tissue during normal and reduced temperature. Tissue weight 60·8 ± 8·9 g. Mean values ± S.E. from seven experiments in which either nerve stimulation or noradrenaline injection was performed. The number of observations are shown in parentheses. The statistical significance was calculated by the Wilcoxon test

	37 °C	27 °C	Significance
Oxygen uptake (μmole.min^{-1}.100 g^{-1})	− 2·12 ± 0·27 (12)	− 1·52 ± 0·27 (8)	$P < 0·01$
Glucose uptake (μmole.min^{-1}.100 g^{-1})	− 1·9 ± 0·3 (12)	− 1·5 ± 0·2 (8)	$P < 0·01$
Lactate release (μmole.min^{-1}.100 g^{-1})	0·62 ± 0·23 (12)	0·33 ± 0·22 (8)	$P < 0·01$
Pyruvate uptake (μmole.min^{-1}.100 g^{-1})	− 0·020 ± 0·024 (12)	− 0·062 ± 0·023 (8)	n.s.

Effects of cooling on adipose tissue metabolism

Oxygen and glucose uptake, as well as lactate release by the adipose tissue, were significantly decreased by cooling, while the pyruvate uptake was unaltered (Table 4). The venous lactate/pyruvate ratio was similar at normal and reduced temperature (18·3 ± 3 *vs.* 16·5 ± 2). Furthermore, as shown in Fig. 4, the fate of glucose taken up by the adipose tissue was altered by cooling. The oxygen uptake and the lactate release were reduced essentially in parallel with the glucose uptake during cooling. Reesterification of fatty acids, on the other hand, was increased by cooling (Table 4 and Fig. 4) and thus contributed much more to the total utilization of glucose during cooling (75 *vs.* 39 %, $P < 0·01$). At normal temperature about 84 % of the glucose

taken up was utilized for lactate production, oxidation and reesterification, leaving 16% for other purposes such as synthesis of fatty acids. By contrast, the same three modes of glucose utilization accounted for the entire glucose and pyruvate uptake during cooling, leaving nothing for such processes as fatty acid synthesis (Fig. 4).

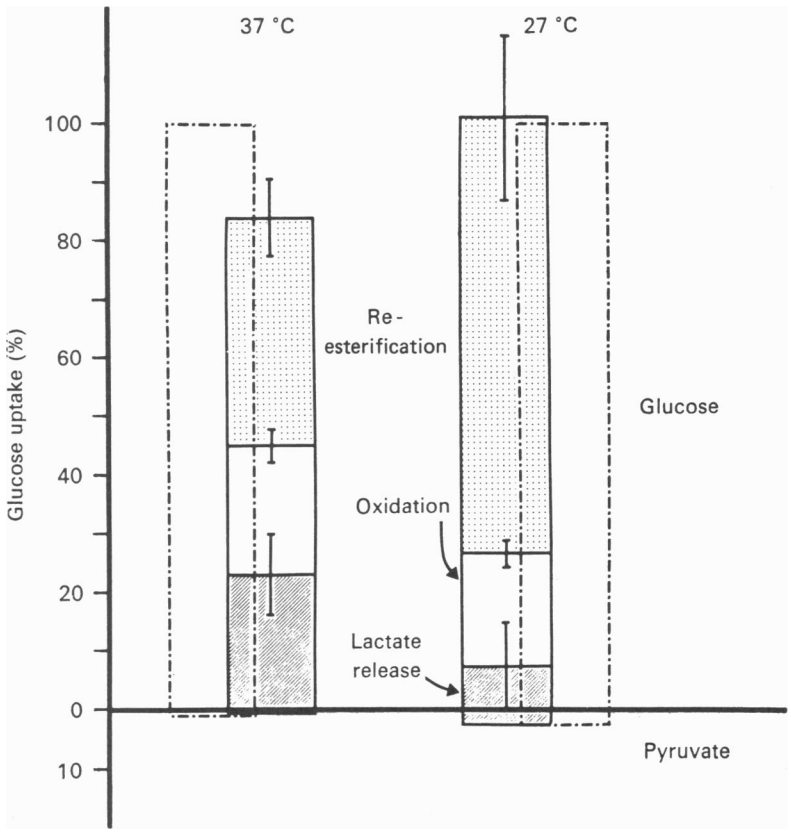

Fig. 4. The metabolism of glucose at 37 °C (n = 12) and at 27 °C (n = 8). Experiments were pooled in which either nerve stimulations or noradrenaline injections were performed. Mean values ± S.E. The total glucose uptake is shown with interrupted lines. The amount of glucose required for the observed lactate production, oxidation and reesterification (calculated as 3-times total net release of glycerol minus observed fatty acid release), is shown with continuous lines. Pyruvate has been incorporated into this Figure. On the average there was a pyruvate uptake. This has been shown beneath the x-axis. Note the increase in reesterification and in utilization of pyruvate at low temperature.

Responses of isolated fat cells

A temperature reduction from 37 to 27 °C caused a 75% inhibition of noradrenaline (3 μM) induced glycerol production in isolated rat fat cells (Fig. 5). Cyclic AMP accumulation due to noradrenaline was so depressed in the same cells that no increases over basal levels could be measured.

The kinetics of the activation and inactivation of glycerol production were studied in four experiments with 'perifused' fat cells. Glycerol production induced by 10 μM-noradrenaline was under these conditions inhibited by 54 ± 7% (Fig. 6). As shown in

Fig. 6 (insert) the glycerol production rate following a noradrenaline infusion returned towards the basal level at a slower rate when the temperature was reduced. Thus, the half-life for inactivation of lipolysis was 8·4 ± 1·2 min at 37 °C, while the half-life at 27 °C was 16·4 ± 4·0 min.

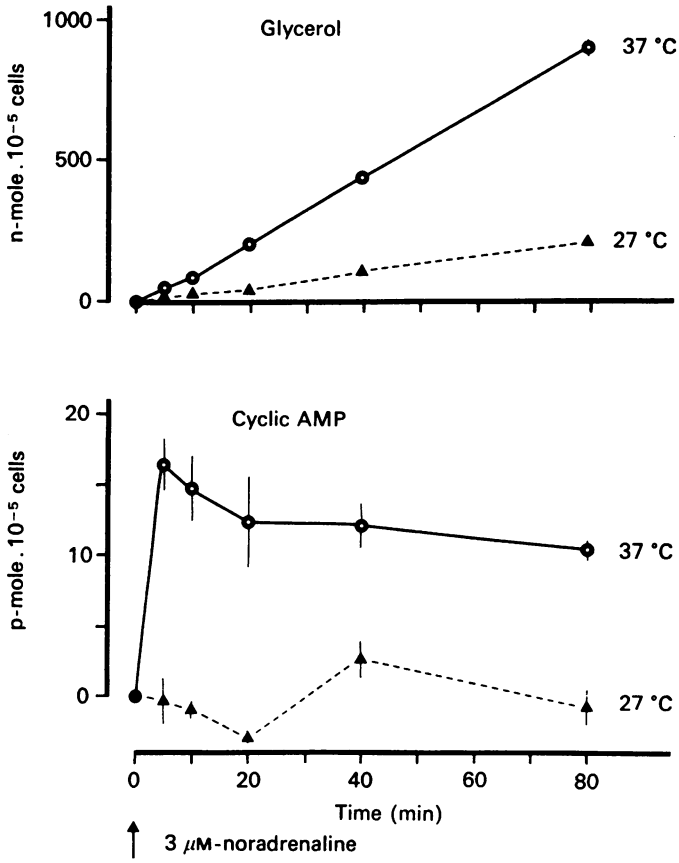

Fig. 5. Time course of glycerol and cyclic AMP production in conventionally incubated isolated rat fat cells stimulated with 3 μM-noradrenaline. Mean values ± S.E. (n = 4).

DISCUSSION

Blood flow to the denervated subcutaneous adipose tissue was markedly reduced by cooling, in agreement with earlier findings in the dog kidney (Levy, 1959) and spleen (Davies, Powis & Withrington, 1971). We recorded a fall in venous effluent temperature of 5–6 °C when the adipose tissue was cooled from 37 to 27 °C. Such a small decrease in blood temperature can not increase viscosity sufficiently (cf. Levy, 1959) to account for the decrease in vascular conductance we observed. Temperature reduction thus increases vascular tone in the adipose tissue, perhaps by removing a continuous influence of metabolic vasodilatation.

Cooling reduced the oxygen uptake by the adipose tissue. It is unlikely that this was caused by the reduction in blood flow, since there were no signs of tissue hypoxia. Instead, the metabolic demands of the tissue appear to have been reduced, as both

glucose uptake and lactate production were reduced and the venous lactate/pyruvate ratio was unchanged at low temperature. We may therefore consider the possibility that adipose tissue blood flow is controlled by the metabolic requirements of the tissue, as suggested by Hansen, Jacobsen & Madsen (1976) and consequently that cooling decreases adipose tissue blood flow by reducing the oxygen demand.

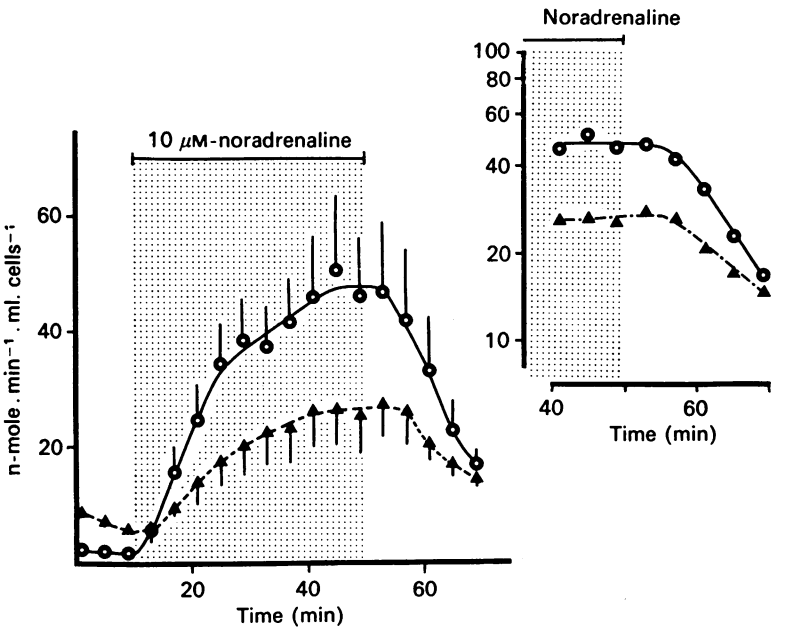

Fig. 6. Glycerol outflow from perifused fat cells stimulated with 10 μM-noradrenaline, at 37 °C (○) and 27 °C (▲). Mean values ± S.E. from four experiments. The insert shows the glycerol outflow after termination of noradrenaline infusion on a semi-logarithmic plot. Note the increase in half-life for inactivation of lipolysis following termination of the infusion at low temperature.

The vasoconstrictor response of the adipose tissue to sympathetic nerve stimulation or to injected noradrenaline was enhanced and prolonged by cooling. Similar potentiation of catecholamine effects at reduced temperature has been found in other tissues such as guinea pig atria (Trendelenburg, 1968), mouse vas deferens (Buckner, Bohuski & Ryan, 1975), canine veins (Webb-Peploe & Shepherd, 1968; Janssens & Vanhoutte, 1977) and rabbit iris dilator muscle (Matheny & Ahlquist, 1976). This increased sensitivity to catecholamines at low temperature reported in several tissues could be due to impaired inactivation of the catecholamines. The two main mechanisms of transmitter inactivation in the adipose tissue, as in most other tissues, are uptake and *O*-methylation (Belfrage & Rosell, 1976; Belfrage *et al.* 1977). There are, however, indications that temperature-induced supersensitivity is independent of changes in uptake (Opperman, Ryan & Haavik, 1971; Matheny & Ahlquist, 1976; Janssens & Vanhoutte, 1977 and present results) or in COMT activity (Wöppel & Trendelenburg, 1973; Matheny & Ahlquist, 1976).

Noradrenaline-induced vasoconstriction in the adipose tissue is modulated by simultaneously acting β-adrenergic vasodilating mechanisms, which appear to be

related to vascular β-receptors as well as to the metabolism of the tissue (Hjemdahl & Fredholm, 1976). It is possible that cooling potentiated vasoconstriction by removal of these counteracting vasodilating mechanisms. The present findings of a pronounced reduction of both the autoregulatory escape and the poststimulatory hyperaemia during cooling supports this hypothesis. In particular, the possibility that the vasodilator component linked to metabolism is influenced by temperature must be considered. It is of interest in this connection that the well known vasodilator adenosine is released from adipose tissue by nerve stimulation (Fredholm, 1976). This release of adenosine appears to be caused by tissue hypoxia (Fredholm, 1976; Hjemdahl, Fredholm & Sollevi, 1978). Inhibition of adenosine deamination enhances autoregulatory escape in the adipose tissue (Fredholm & Sollevi, 1978). Preliminary findings (B. B. Fredholm & A. Sollevi, unpublished) suggest that cooling reduces adenosine release from the subcutaneous adipose tissue, as shown earlier for the adrenal gland (Stevens, Robinson, Van Dyke & Stitzel 1975). A reduced influence of adenosine on the vascular bed of the adipose tissue may therefore be of importance for the vascular effects of cooling.

Cooling markedly reduced lipolysis, measured as glycerol production, and cyclic AMP accumulation in isolated fat cells stimulated by noradrenaline. The outflow of glycerol from the adipose tissue *in situ* was also reduced at low temperature, provided that the response *during* nerve stimulation was studied. The post-stimulatory mobilization of glycerol and that induced by brief stimuli were not inhibited by cooling. This may be due to the fact that the lipolytic response to a stimulus is the net result of two opposing influences, activation and inactivation of the lipolytic process, as discussed previously (Hjemdahl, 1976). Experiments with 'perifused' fat cells (Allen *et al.* 1973), in which transmitter removal is rapid and determined by simple wash-out, revealed a prolongation of post-stimulatory lipolysis. Therefore, the findings of uninfluenced or even enhanced glycerol outflow from the adipose tissue *in vivo* after a stimulation at low temperature may well be explained by a retardation of the events in the fat cells leading to inactivation of lipolysis. A similar line of reasoning may be followed to explain the prolongation of vasoconstriction during cooling, since temperature reduction has been shown to retard vascular smooth muscle relaxation (e.g. Webb-Peploe & Shepherd, 1968; Johansson, 1978).

Triglyceride breakdown results in the formation of glycerol and free fatty acids. Fatty acids are reutilized by the subcutaneous adipose tissue mainly by reesterification (Steinberg & Vaughan, 1965; Fredholm, 1970). It is therefore reasonable to assume that the increased utilization of fatty acids observed during cooling is caused by an increased reesterification. The rate of esterification in adipose tissue is governed by the intracellular concentration of fatty acids and α-glycerol phosphate. The α-glycerol phosphate is mainly derived from glucose (Steinberg & Vaughan, 1965), by reduction of dihydroxyacetonephosphate. Thus, the rate of reesterification should be increased by an increased glucose uptake and/or an increase in the $NADH/NAD^+$ ratio intracellularly. These assumptions have been verified in the canine subcutaneous adipose tissue *in situ* (Fredholm, 1971). However, during cooling glucose uptake was decreased and there was no change in the lactate/pyruvate ratio, which is an indicator of the $NADH/NAD^+$ ratio. Thus, our finding of increased reutilization-reesterification of fatty acids during cooling is not explained by elevated α-glycerol phosphate

levels. Recently we found that blood flow reduction in canine subcutaneous adipose tissue increased reesterification (Belfrage, Hjemdahl & Fredholm, 1976). As discussed in that report the most reasonable explanation for this finding is impaired outflow of fatty acids with consequent increases in the cellular levels of FFA. In epididymal fat pads *in vitro*, where diffusion is not limited by blood flow, esterification of fatty acids is actually reduced by cooling (Hillyard & Entenman, 1973), suggesting that the blood flow reduction caused by cooling may be particularly important. It should be pointed out that reesterification yields heat (see Smith & Horwitz, 1969), which suggests that this might be of physiological importance. In fact, an increased rate of reesterification has been invoked as an important heat generating mechanism in brown adipose tissue (Smith & Horwitz, 1969).

In conclusion, the present results demonstrate a decreased resting blood flow during hypothermia without any signs of hypoxia. The vasoconstrictor effect of catecholamines, circulating or released from nerve endings, was enhanced by cooling. Both of these factors tend to increase the insulator function of subcutaneous tissue. The break-down of triglycerides is affected to a minor degree by local hypothermia of brief to moderate duration because of a prolongation of poststimulatory lipolysis during cooling. However, the outflow of fatty acids is markedly depressed, due to enhanced reesterification. A high rate of lipolysis and reesterification might lead to heat production in the tissue. Such localized heat production could also be of importance for the insulation of the body and in the subcutaneous adipose tissue response to cooling.

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REFERENCES

ALLEN, D. O., LARGIS, E. E., MILLER, E. A. & ASHMORE, J. (1973). Continuous monitoring of lipolytic rates in perifused isolated fat cells. *J. appl. Physiol.* **34**, 125–127.

BELFRAGE, E. (1978). Vasodilatation and modulation of vasoconstriction in canine subcutaneous adipose tissue by activation of β-adrenoceptors. *Acta physiol. scand.* **102**, 459–468.

BELFRAGE, E., FREDHOLM, B. B. & ROSELL, S. (1977). Effect of catechol-*O*-methyl-transferase (COMT) inhibition on the vascular and metabolic responses to noradrenaline, isoprenaline and sympathetic nerve stimulation in canine subcutaneous adipose tissue. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **300**, 11–17.

BELFRAGE, E., HJEMDAHL, P. & FREDHOLM, B. B. (1976). Metabolic effects of blood flow restriction in adipose tissue. *Acta physiol. scand.* **98**, Suppl. 440, p. 186.

BELFRAGE, E. & ROSELL, S. (1976). The role of neuronal uptake at α- and β-adrenoceptor sites in subcutaneous adipose tissue. *Naunyn-Schmiedebergs Arch. Pharmak.* **294**, 9–15.

BIGELOW, W. G., LINDSAY, W. K., HARRISON, R. C., GORDON, R. A. & GREENWOOD, W. F. (1950). Oxygen transport and utilization in dog at low body temperature. *Am. J. Physiol.* **160**, 125–137.

BROWN, B. L., EKINS, R. P. & ALBANO, J. D. M. (1972). Saturation assay for cyclic AMP using endogenous binding protein. *Adv. cyclic Nucl. Res.* **2**, 25–40.

BRÜCK, K. & HENSEL, H. (1953). Wärmedurchgang und innentemperatur der menschlishen extremitäten. *Pflügers Arch. ges. Physiol.* **257**, 70–86.

BUCKNER, C. K., BOHUSKI, K. & RYAN, C. F. (1975). Influence of temperature and cocaine on response of the isolated mouse vas deferens to adrenergic amines. *Archs int. Pharmacodyn. Thér.* **216**, 19–27.

DAVIES, B. N., POWIS, D. A. & WITHRINGTON, P. G. (1971). Effects of lowered temperature on the responses of the smooth muscle of the isolated, blood-perfused dog's spleen to sympathetic nerve stimulation. *J. Physiol.* **212**, 18–19*P*.

FREDHOLM, B. B. (1970). Studies on the sympathetic regulation of circulation and metabolism in isolated canine subcutaneous adipose tissue. *Acta physiol. scand.* **80**, Suppl 354.

FREDHOLM, B. B. (1971). The effect of lactate in canine subcutaneous adipose tissue *in situ*. *Acta physiol. scand.* **81**, 110–123.

FREDHOLM, B. B. (1976). Release of adenosine-like material from isolated perfused dog adipose tissue following sympathetic nerve stimulation and its inhibition by adrenergic α-receptor blockade. *Acta physiol. scand.* **96**, 422–430.

FREDHOLM, B. B. & HJEMDAHL, P. (1976). Inhibition by acidosis of adenosine 3′,5′-cyclic monophosphate accumulation and lipolysis in isolated rat fat cells. *Acta physiol. scand.* **96**, 160–169.

FREDHOLM, B. B., ÖBERG, B. & ROSELL, S. (1970). Effects of vasoactive drugs on circulation in canine subcutaneous adipose tissue. *Acta physiol. scand.* **79**, 564–574.

FREDHOLM, B. B. & SOLLEVI, A. (1978). Control of lipolysis and circulation in adipose tissue by endogenous adenosine. *Acta physiol. scand.* **102**, 77–78*A*.

HANSEN, M., JACOBSEN, E. & MADSEN, J. (1976). Influence of hypoxia and hyperoxia on subcutaneous adipose tissue blood flow in man. *Scand. J. clin. lab. Invest.* **36**, 635–660.

HILLYARD, L. A. & ENTENMAN, C. (1973). Effect of low temperatures on metabolism of rat liver slices and epididymal fat pads. *Am. J. Physiol.* **224**, 148–153.

HJEMDAHL, P. (1976). Inhibition of the lipolytic response to nerve stimulation during acidosis. *Acta physiol. scand.* **98**, 80–84.

HJEMDAHL, P. & FREDHOLM, B. B. (1976). Influence of acidosis on noradrenaline-induced vasoconstriction in adipose tissue and skeletal muscle. *Acta physiol. scand.* **97**, 319–324.

HJEMDAHL, P., FREDHOLM, B. B. & SOLLEVI, A. (1978). Release of adenosine-like material from isolated 'perifused' fat cells. *Acta physiol. scand.* **102**, 81*A*.

JANSSENS, W. J. & VANHOUTTE, P. M. (1977). Effect of temperature on the disposition of neurotransmitters in the saphenous veins of the dog. *Archs int. Pharmacodyn. Thér.* **225**, 346–347.

JOHANSSON, B. (1978). Mechanics of smooth muscle relaxation. In *Mechanisms of Vasodilatation*, ed. VANHOUTTE, P. M. Karger. (In the Press.)

LAURELL, S. & TIBBLING, B. (1966). An enzymatic fluorimetric micro-method for the determination of glycerol. *Clin. chim. Acta* **13**, 317–322.

LAURELL, S. & TIBBLING, G. (1967). Colorimetric microdetermination of free fatty acids in plasma. *Clin. chim. Acta* **16**, 57–62.

LEVY, M. N. (1959). Oxygen consumption and blood flow in the hypothermic perfused kidney. *Am. J. Physiol.* **197**, 1111–1114.

MATHENY, J. L. & AHLQUIST, R. P. (1976). Role of neuronal and extra-neuronal factors in temperature mediated responsiveness of adrenoceptors. *Archs int. Pharmacodyn. Thér.* **224**, 180–189.

NGAI, S. H., ROSELL, S. & WALLENBERG, L. R. (1966). Nervous regulation of blood flow in the subcutaneous adipose tissue in dogs. *Acta physiol. scand.* **68**, 397–403.

OPPERMAN, J. A., RYAN, C. F. & HAAVIK, C. O. (1971). Temperature dependent sensitivity of isolated guinea-pig atria to sympathomimetic amines. *Life Sci., Oxford* **10**, 613–622.

RODBELL, M. (1964). Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J. biol. Chem.* **239**, 375–380.

ROSELL, S. (1966). Release of free fatty acids from subcutaneous adipose tissue in dogs following sympathetic nerve stimulation. *Acta physiol. scand.* **67**, 343–351.

SMITH, R. E. & HORWITZ, B. A. (1969). Brown fat and thermogenesis. *Physiol. Rev.* **49**, 330–425.

SOLLEVI, A., HJEMDAHL, P. & FREDHOLM, B. B. (1975). Vascular and metabolic effects of local cooling in canine subcutaneous adipose tissue. *Acta physiol. scand.* **95**, 15A.

STEINBERG, D. & VAUGHAN, M. (1965). Release of free fatty acids from adipose tissue *in vitro* in relation to triglyceride synthesis and degradation. In *Handbook of Physiology*, section V. ed. A. E. RENOLD and G. F. CAHILL, Jr., pp. 335–347. Washington D.C.: Am. Physiol. Soc.

STEVENS, P., ROBINSON, R. L., VAN DYKE, K. & STITZEL, R. (1975). The temperature dependence of [8-^{3}H]adenosine labelling of the perfused bovine and canine adrenal gland. *J. Pharm. Pharmacol.* **27**, 864–865.

TRENDELENBURG, U. (1968). The effects of cocaine on the pacemaker of isolated guinea-pig atria. *J. Pharmac. exp. Ther.* **161**, 222–231.

WEBB-PEPLOE, M. M. & SHEPHERD, J. T. (1968). Peripheral mechanism involved in responses of dog's cutaneous veins to local temperature change. *Circulation Res.* **23**, 701–708.

WÖPPEL, W. & TRENDELENBURG, U. (1973). Temperature-dependent supersensitivity of isolated atria to catecholamines. *Eur. J. Pharmac.* **23**, 302–305.