Ephedrine-induced thermogenesis in man: no role for interscapular brown adipose tissue

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Summary

1. The warmest interscapular skin areas were located by thermography in six healthy subjects during ephedrine-induced thermogenesis.

2. In these interscapular areas, and in lumbar control areas, the skin temperature, subcutaneous temperature and adipose tissue blood flow were measured before and during ephedrine-induced thermogenesis.

3. The skin and subcutaneous temperatures increased in the interscapular area as well as in the lumbar area, by about 0.7–1.2°C. The interscapular skin temperature remained about 1°C higher than the lumbar; the subcutaneous temperatures in the two areas were identical during the experiments.

4. Although the interscapular subcutaneous adipose tissue blood flow increased about sixfold and the lumbar increased twofold, the absolute flows were higher in the lumbar area.

5. The oxygen uptake increased to a maximum of 30% above control level.

6. Plasma glucose and glycerol concentrations remained unchanged, and plasma non-esterified fatty acids, lactate and noradrenaline concentrations increased slightly but significantly.

7. Biopsies taken from the hot interscapular areas did not contain brown adipose tissue.

8. It is concluded that the high interscapular skin temperature may be due to a lower insulating fat thickness and that the increases in skin and subcutaneous temperatures during ephedrine-induced thermogenesis are caused by an increased blood flow. These observations weigh against the hypothesis that the interscapular temperature increase is due to functional, interscapular brown adipose tissue.

Key words: adipose tissue, body temperature, brown fat, catecholamines, ephedrine, glucose, glycerol, lactate, non-esterified fatty acids, oxygen consumption, regional blood flow, skin temperature, thermography, xenon.

Abbreviations: BAT, brown adipose tissue; NST, non-shivering thermogenesis.

Introduction

It has recently been shown that brown adipose tissue (BAT) is the major site of cold-induced non-shivering thermogenesis (NST) in adult rats and mice [1, 2], and that the ability of young lean rats to avoid the development of obesity during voluntary overfeeding is due to an increase in the energy expenditure (i.e. diet-induced thermogenesis) in this tissue [3]. The increase in heat production has been demonstrated to be mediated via the sympathetic nervous system by noradrenaline stimulation of β1-receptors on the brown adipocytes [4].

In contrast, genetically obese mice (ob/ob) show an impaired ability to diet-induced thermogenesis caused by a defect in BAT [2]. This has led to the hypothesis that at least some kinds of obesity, also in man, might be due to a similar deficiency [3].

The phenomena of cold-induced NST [5–7], diet-induced thermogenesis [8–10] and metabolic stimulation by sympathomimetic drugs [11–14] have been repeatedly described in man. For the two last-named thermography has demonstrated
areas with elevated skin temperatures, particularly between and above the scapulae and in the neck [3, 13, 15]. Since BAT has been demonstrated by necropsy in this region in children and a few adults [16-18], much speculation has been published on the possible role of BAT in the various types of thermogenesis and its deficiency in human obesity [3, 9, 11, 13, 19-23]. So far, however, no demonstration of BAT in a ‘hot’ region has been reported.

The aims of the present study were to locate the ‘hot’ interscapular areas during ephedrine stimulation, to record the cutaneous and the subcutaneous temperatures and the subcutaneous blood flow in these areas, and by biopsies to examine the ‘hot’ areas for the presence of BAT.

Methods

General

Experimental subjects were six lean, normal men (n = 2) and woman (n = 4), 21-41 years old. All gave informed consent to the investigation according to the declaration of Helsinki 2. The protocol was approved by the Municipal Ethical Committee of Copenhagen.

Mean weight was 55.5 kg (43-65 kg) and mean height 173 cm (165-184 cm). The mean body fat content was 18% (11-23%) of the body weight estimated as described in [24]. This estimate was obtained from duplicate measurements of the biceps, triceps, subscapular and supra-iliac skin folds with a Harpenden caliper at two experimental days.

All subjects participated in three experiments. There was an initial thermographic location of the interscapular, cutaneous areas with the highest temperatures during ephedrine stimulation, to record the cutaneous and the subcutaneous temperatures and the subcutaneous blood flow in these areas, and by biopsies to examine the ‘hot’ areas for the presence of BAT.

Temperature and blood flow experiments

After attainment of thermoregulatory and respiratory steady state, an experiment consisted of a control period of 40 min, intake of the same ephedrine dose as in the thermography experiment, and a 2-2.5 h post-ephedrine measuring period.

The subject was lying immobile on the right side of the body, wearing a thin shirt open in the back. The room temperature was kept constant at 23-24°C. Temperatures and adipose tissue blood flow were recorded continuously during the experiment. During the control period and a 40 min period starting about 60 min after ephedrine ingestion, the subject breathed through a low resistance Scuba mouthpiece. Preceded by 10 min adaptation, expiratory gas was collected in Douglas bags in three successive 10 min periods during the control period (C1, C2, C3) and in three similar periods during the ephedrine stimulation (S1, S2, S3). In the middle of the two last gas-collecting periods blood was sampled from an antecubital vein via a Venflon catheter (C2, C3, S2, S3). The catheter was kept open during the experiment by flushing with isotonic sodium chloride solution (154 mmol/l: saline) after each sampling.

Biopsies

The system of co-ordinates and the hot area was drawn on the back of the subject. After subcutaneous local analgesia with 2% lidocaine and
0.1% noradrenaline a cylindrical biopsy was taken under sterile conditions from the central part of the 'hot' interscapular area with a 3 mm Stiefel biopsy punch. The tissue cylinder contained all layers down to the muscular fascia. The biopsy was fixed in 4% formalin buffer, wax embedded and slices were cut perpendicular to the skin. Sections were stained in duplicate with haematoxylin and eosin and with Sirius Red (F 1125, Sigma) and Celestine Blue (C 7143, Sigma). Furthermore, biopsies were taken by the same biopsy punch from the interscapular area in 22 medicolegal autopsies (age range 19–42 years) and treated as described above. By microscopic examination adipose tissue cells were classified as white or brown as described in [18].

Measurement of adipose tissue blood flow

The co-ordinates were redrawn and the hottest area was marked. Adipose tissue blood flow was measured in the subcutaneous adipose tissue in this interscapular area and in a left lumbar subcutaneous control site. The Xenon-washout technique initially described by Larsen et al. [25] was used. Compensation for movements was obtained by the modification described by Bulow & Madsen [26]. $^{133}$Xe (100–200 μCi; 3.7–7.4 MBq), dissolved in 0.1 ml of sterile saline (XAS.21P, Amersham, U.K.) was slowly injected with a 0.4 mm φ needle into the middle of the subcutaneous adipose tissue in both regions. Measurement of the $^{133}$Xe activity in the depots was started 30 min after the injections. The activities were measured with two 2 inch NaI scintillation detectors placed perpendicular to the skin at a distance of 15–30 cm. Each detector was connected to a two-channel pulse-height analyser, with the channels set around 81 keV ($^{133}$Xe) and 122 keV ($^{57}$Co) respectively.

To prevent ‘cross-talk’ between the two depots lead collimators were used on both detectors and a lead plate was placed between the lumbar and interscapular depots. The activity measured was kept under resolving time limits of the system. The elimination rate constants were determined as described in [26].

Autopsy studies of interscapular subcutaneous adipose tissue from five young, lean bodies showed a composition of 11.2% water, 73.4% fat and 15.4% protein; thus the tissue/blood partition coefficient (λ) for $^{133}$Xe was calculated to 7.6 ml/g (SEM = 0.4) [27, 28]. This λ value was used for calculation of the interscapular adipose tissue blood flow. A λ value of 10.0 was used for the calculations of adipose tissue blood flow in the lumbar depot [25]. At the end of the experiment each tracer depot was infiltrated with 20 μg of histamine in 1 ml of saline, in order to accelerate the washout of the residual tracer [29]. Thus the radiation doses for an experiment were less than: kidneys and erythropoietic tissues, 1.20 mrem (12 μSv); gonads, 0.12 mrem (1.2 μSv); whole body, 13.2 mrem (132 μSv).

Temperature measurements

The cutaneous and subcutaneous temperatures in the interscapular and lumbar xenon-depots, as well as the rectal temperature, were continuously measured, as described in [29]. The presence of a subcutaneous thermoprobe is without effect on the locally determined adipose tissue blood flow [29].

Analyses

The subjects breathed through a low resistance Scuba one-way mouthpiece. Expiratory gas was continuously analysed for oxygen on a Godart Rapox oxygenometer and for carbon dioxide on a Beckman LB-1 Medical gas analyser. Expiratory gas was collected in Douglas bags and analysed for $\text{O}_2$ and $\text{CO}_2$ using gas-electrodes connected to an acid–base analyser (Radiometer, Copenhagen). Respiratory steady state was assumed, when the end-expiratory $\text{CO}_2$ fraction was constant.

Blood was drawn without stasis from a superficial arm vein into ice-cold syringes. The blood was immediately centrifuged at 4°C. Glucose was determined in plasma with the glucose oxidase method [30], glycerol and lactate as described in [31] and [32]. Non-esterified fatty acids were immediately extracted and later determined as described in [33]. Adrenaline and noradrenaline were determined as described in [34].

Statistics

To test differences between experimental periods and to test differences between experimental types in the same experimental period a two-way analysis of variance for randomized blocks was performed. To compare two means a modified t-test was applied [35]: $P<0.05$ was considered significant. All results are quoted as means ± SEM.

Results

Blood pressure and pulse rate

The blood pressure increased from the mean control value of 113±3/73±4 to a peak of
FIG. 1. Blood pressures before and after intake of ephedrine. Mean values of diastolic (●) and systolic (○) blood pressures are plotted with SEM values shown as vertical bars (n = 6).

150 ± 8/91 ± 6 mmHg after 50 min, and then declined steadily during the rest of the experiment to 132 ± 6/80 ± 4 mmHg at 150 min (Fig. 1). The pulse rate decreased from an initial value of 73 ± 4 min⁻¹ to 65 ± 4 min⁻¹ after 50 min, and then slowly returned to control level at the end of the experiment. The changes in blood pressure and pulse rate showed the same profile in the first and second experiments.

Thermography

In all subjects the interscapular heat emission reached a plateau that remained stationary from about 75 to about 90 min after the intake of ephedrine.

Temperatures

The interscapular skin temperature increased significantly from an absolute control mean value of 33.4 ± 0.3°C to 34.1 ± 0.2°C 120 min after the ingestion of ephedrine. The corresponding lumbar skin temperature increased significantly from 32.2 ± 0.5°C to 33.4 ± 0.7°C. The lumbar skin temperature was significantly lower than interscapular skin temperature.

The interscapular subcutaneous temperature increased from the basic value of 34.4 ± 0.4°C to 35.1 ± 0.4°C; the subcutaneous lumbar temperature increased almost identically, from 34.3 ± 0.5°C to 35.1 ± 0.6°C. In Figs. 2 and 3 the mean cutaneous and subcutaneous temperature changes are presented. The means were obtained from changes calculated separately from each experiment. No significant difference can be demonstrated between the four curves. There is a pronounced person to person variation in the time course in interscapular as well as in lumbar temperatures. In three subjects the cutaneous and

FIG. 2. Changes in interscapular and lumbar skin temperatures during ephedrine stimulation. Mean values of changes in interscapular (●) and lumbar (○) skin temperatures, with SEM, are shown (n = 6). For details of the calculations see the text.

FIG. 3. Changes in interscapular and lumbar subcutaneous temperatures during ephedrine stimulation. Mean values of changes in interscapular (●) and lumbar (○) subcutaneous temperatures, with SEM, are shown (n = 6). For details of the calculations see the text.
subcutaneous temperatures in both locations showed a steep increase, followed by a return to the control level at the end of the experiment. In the other three the increases were less abrupt and the temperatures were still increasing when the experiments were ended. The mean rectal temperature increased significantly from 36.9 ± 0.2°C to 37.1 ± 0.2°C. In six control experiments no change was seen.

**Adipose tissue blood flow**

Table 1 shows interscapular and lumbar subcutaneous adipose tissue blood flows. The flows were significantly lower in the interscapular than in the lumbar tissue, both in the control and in the post-ephedrine period. During ephedrine stimulation the blood flow increased in both depots in all subjects.

In three subjects the increases in adipose tissue blood flow were followed by an early return to control levels. In these subjects the temperatures showed a concomitant and similar course. In the other subjects both the blood flows and temperatures were still increasing at the end of the experiment.

**Oxygen uptake**

The oxygen uptakes are expressed per unit of surface area [36] and shown in Table 2. The mean surface area was 1.66 ± 0.06 m². Three almost identical basal determinations of the oxygen uptake indicated a constant resting metabolic state. In the post-ephedrine period there was a steady increase in oxygen uptake. The averages in the three post-ephedrine periods were 20, 25 and 30% above the mean control values, the effect being highly significant. The oxygen uptake increased in all subjects; in one a steep increase, to 98% above the control value, began when the temperatures and flow had returned to control values.

**R (ventilatory exchange coefficient)**

R fell insignificantly from a control average of 0.89 to 0.84 during ephedrine stimulation (Table 2).

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**TABLE 1. Interscapular and lumbar subcutaneous adipose tissue blood flows before and after ephedrine intake**

<table>
<thead>
<tr>
<th>Time after drug (min)</th>
<th>Adipose tissue blood flow (ml min⁻¹ 100 g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Interscapular</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Lumbar</td>
<td>4.9 ± 1.0</td>
</tr>
</tbody>
</table>

**TABLE 2. Oxygen uptake, R and plasma glucose, glycerol, non-esterified fatty acids (NEFA), lactate, noradrenaline and adrenaline before and during ephedrine stimulation**

Results are means ± SEM (n = 6). Values significantly different from control values are marked: *P < 0.05; **P < 0.01.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>During stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C₁</td>
<td>C₂</td>
</tr>
<tr>
<td>VO₃ (ml STPD min⁻¹ m⁻²)</td>
<td>149 ± 8</td>
<td>146 ± 7</td>
</tr>
<tr>
<td>R</td>
<td>0.89 ± 0.07</td>
<td>0.88 ± 0.06</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.02 ± 0.30</td>
<td>5.00 ± 0.16</td>
</tr>
<tr>
<td>Glycerol (mmol/l)</td>
<td>0.156 ± 0.012</td>
<td>0.148 ± 0.018</td>
</tr>
<tr>
<td>NEFA (mmol/l)</td>
<td>0.625 ± 0.111</td>
<td>0.614 ± 0.098</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td>0.658 ± 0.079</td>
<td>0.655 ± 0.085</td>
</tr>
<tr>
<td>Noradrenaline (ng/ml)</td>
<td>0.22 ± 0.03</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td>Adrenaline (ng/ml)</td>
<td>0.03 ± 0.01</td>
<td>0.02 ± 0.00</td>
</tr>
</tbody>
</table>

The times of the determinations/samplings are explained in the Methods section.
Determinations in plasma

Non-esterified fatty acids. There was a slight significant increase in their plasma concentrations from 0.619 to 0.802 mmol/l during ephedrine stimulation (Table 2).

Glycerol. Plasma glycerol concentrations did not change significantly from the control values (Table 2).

Glucose. Plasma glucose concentration remained constant during the measuring periods (Table 2).

Lactate. Plasma lactate concentration increased significantly from 0.657 to 0.783 mmol/l during ephedrine stimulation (Table 2).

Catecholamines. Plasma noradrenaline concentration increased significantly from a control mean of 0.22 ng/ml to 0.26 ng/ml. It increased in four subjects and remained unchanged in two. Plasma adrenaline concentration remained essentially constant (Table 2).

Histology

None of the sections prepared from the biopsies taken from the 'hot' interscapular areas or from the interscapular area in the necropsies contained any brown fat cells. The subcutaneous part of the biopsies consisted of loose connective tissue with white adipocytes.

Sex differences

No differences between the two sexes were seen in any of the factors examined, including the oxygen uptakes per surface area and skinfold thickness.

Discussion

The present study confirms the findings of Rothwell & Stock [3] of an increase in skin temperature on the back after administration of ephedrine. Also confirmed were their findings that the warmest skin areas on the back are located interscapularly and at the neck both before and during the influence of ephedrine.

Our findings, however, do not support their hypothesis that this temperature increase is caused by an increased metabolism in subcutaneous BAT, since no BAT could be demonstrated in biopsies from the 'hot' areas. This is in accordance with the results of the medicolegal autopsies, in which no BAT was demonstrated in the interscapular area, although it was abundant in the perirenal tissue in the majority of lean bodies (A. Astrup, unpublished work).

The increase in skin and subcutaneous temperature can be explained by the changes in subcutaneous blood flow. Skin blood flow was not determined, but no changes in skin colour suggested any major alteration.

In all the present experiments an increase in subcutaneous adipose tissue blood flow was demonstrated in both the interscapular and lumbar locations shortly after the administration of ephedrine, and subcutaneous and skin temperature rose in parallel with adipose tissue blood flow. The interscapular skin temperatures were about 1°C higher than the lumbar skin temperatures, although the perfusion was higher in the latter. Since the subcutaneous temperatures were similar in the two locations, the difference in skin temperatures may reflect differences in insulating capacity of the tissue components in the two locations [6, 37].

The mechanism of the increase in subcutaneous adipose tissue blood flow is not explained by the present experiments. Conceivably the increase in blood flow might be secondary to the increased local temperature.

However, we have previously demonstrated that subcutaneous adipose tissue blood flow changes by only about 9% per 1°C change in skin and subcutaneous temperature [29], which is in contrast to the 2.5–6.0-fold increase in adipose tissue blood flow found in the present experiments.

A thermoregulatory vasodilatation elicited by the increased core temperature can be ruled out, both because the increase in adipose tissue blood flow preceded the increase in core temperature, and because increases in core temperature of 1.0–1.5°C do not elicit significant increases in adipose tissue blood flow [38].

The increase in arterial mean blood pressure may be a factor contributing to the increase in the blood flow, but cannot be important since it amounts only to about 20%.

The actual increase in adipose tissue blood flow is of the same magnitude as that seen during physical exercise [38]. In exercise the increase seems to be secondary to an increased lipolysis [38]. In the present experiments, however, no change was seen in the plasma glycerol concentration and only a 30% increase in the concentration of non-esterified fatty acids. These figures do not suggest any marked increase in the rate of lipolysis.

The increased adipose tissue blood flow did not seem to depend on the increased oxygen uptake, since in three subjects skin temperatures and subcutaneous adipose tissue blood flow returned to the control level and the oxygen uptake continued to increase.
It is possible that the subcutaneous vasodilatation is the result of direct stimulation of vascular \( \beta \)-receptors [39]. Ephedrine is known to stimulate \( \alpha \) as well as \( \beta \)-receptors [40] and the outcome with regard to vasodilatation will depend on the balance between these two actions.

A small but significant increase in the plasma noradrenaline concentration after ephedrine administration was seen in the present experiments, and has not previously been reported. The sympathicomimetic actions of ephedrine are partly achieved by noradrenaline release [40]. The small increase in circulating noradrenaline may reflect a higher local concentration close to the adrenergic receptor. The tissues in which noradrenaline is known to be a strong lipolytic agent in white adipose tissue [41], this does not seem to be the site.

The absent or low lipolytic action of ephedrine was unexpected since ephedrine possesses well known \( \beta_1 \) and \( \beta_2 \)-agonistic properties [40, 42]. Ephedrine, however, also exerts a strong \( \alpha \)-adrenergic action, causing the rise in blood pressure. Thus ephedrine, like noradrenaline and adrenaline, is capable of interacting with both stimulatory \( \beta_1 \) and \( \beta_2 \)-receptors and inhibitory \( \alpha_2 \)-receptors on the white adipocytes [43, 44]. The actual effect upon lipolysis is then the result of a balance of the opposing actions. The balance for ephedrine might be in favour of inhibiting lipolysis via the \( \alpha_2 \)-receptors.

Ephedrine has previously been found to stimulate the oxygen uptake in man [12]. This effect was confirmed by the present experiments and found to be more pronounced than previously reported.

It is concluded that the thermogenic action of ephedrine is confirmed, but the ephedrine-induced changes in interscapular heat emission are not due to an increased metabolism in interscapular BAT, but rather to vascular effects of ephedrine. The present investigation does not offer any identification of the tissue location of the ephedrine-induced thermogenesis.

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References