Capacity and Hypoxic Response of Subcutaneous Adipose Tissue Blood Flow in Humans

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Background: The blood flow capacity in subcutaneous adipose tissue in humans remains largely unknown, and therefore the aim of this study was to determine the physiological range of blood flow in this tissue.

Methods and Results: The subcutaneous adipose tissue blood flow (ATBF) was measured in 9 healthy young men by positron emission tomography using radiowater tracer. Subcutaneous ATBF was determined in regions adjacent to knee extensors at rest and during dynamic knee extensor exercise, and with 2 physiological perturbations: while breathing moderate systemic hypoxic air (14% O₂) at rest and during exercise, and during intra-femoral artery infusion of high-dose adenosine infusion. ATBF was $1.3 \pm 0.6 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ at rest and increased with exercise ($8.0 \pm 3.0 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$, $P < 0.001$) and adenosine infusion ($10.5 \pm 4.9 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$, $P = 0.001$), but not when breathing moderate systemic hypoxic air ($1.5 \pm 0.4 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$). ATBF was similar during exercise and adenosine infusion, but vascular conductance was lower during adenosine infusion. Finally, ATBF during exercise in moderate systemic hypoxia was reduced ($6.3 \pm 2.2 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$) compared to normoxic exercise ($P = 0.004$).

Conclusions: The vasodilatation capacity of human subcutaneous adipose blood flow appears to be comparable to, or even higher, than that induced by moderate intensity exercise. Furthermore, the reduced blood flow response in subcutaneous adipose tissue during systemic hypoxia is likely to contribute, in part, to the redistribution of blood flow to exercising muscle in a condition of reduced oxygen availability. (*Circ J* 2014; **78**: 1501–1506)

Key Words: Adipose tissue; Blood flow; Capacity; Human; Hypoxia

Adipose tissue plays an important role in controlling metabolism in the human body,^{1–3} and the circulation to adipose tissue is an integral part of its metabolic and endocrine function.^{4,5} Despite the fact that adipose tissue has a capillary surface area less than one-third that in skeletal muscle,⁵ it has long been acknowledged that adipocytes are surrounded by an extensive network of capillaries.⁴ This circulatory feature importantly affects the adaptability of subcutaneous adipose tissue to excess caloric overload, which is, in contrast, also associated with a hypoxic state in adipose tissue.^{1,6} While chronic hypoxia in expanded adipose tissue is now well known to lead to metabolic disturbances due to the insufficient blood supply,^{1,6} the effects of acute environmentally induced hypoxia on adipose blood flow in both lean healthy humans and patients with cardiovascular, respiratory and metabolic diseases needs further clarification.^{1–3,7–9} Along these lines, in the present study, we measured the capacity and hypoxic response of adipose blood flow in 9 healthy young men by positron emis-

sion tomography. Blood flow capacity was determined by direct intra-femoral infusion of adenosine, with a dose that has previously been shown to induce maximal thigh blood flow.¹⁰ The hypoxic response was elucidated by letting subjects breathe moderate systemic hypoxic air (14% O₂) at rest and during exercise. It was hypothesised that the blood flow capacity of adipose tissue is at least comparable, or even higher, than that induced by moderate intensity exercise. We also expected a higher adipose tissue blood flow in response to hypoxia at rest, but a lower blood flow during exercise, compared to the respective normoxic conditions.

Methods

Subjects

Nine healthy young men (25 ± 5 years, 184 ± 6 cm, 76 ± 9 kg) volunteered to participate in the study. The purpose, nature, and potential risks of the study were explained to the subjects be-

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fore they gave their written informed consent to participate. The subjects were requested to abstain from caffeine-containing beverages for at least 24 h before the experiments, as well as to avoid strenuous exercise within 48 h prior to the study. The subjects were not taking any regular medication. The study was performed at least 4 h after the subjects had eaten a light breakfast. The study was performed according to the Declaration of Helsinki and was approved by the Ethics Committee of the Intermunicipal Hospital District of Southwest Finland and the National Agency for Medicines.

Study Design

Subcutaneous adipose tissue blood flow (ATBF) in the femoral region was measured using positron emission tomography (PET) with [^{15}O]- H_2O , as described in detail previously^{11,12} and briefly below, and in **Figure 1**. ATBF was measured first under normal resting conditions and then either during systemic hypoxia (14% inspired O_2 in N_2 ; equivalent to an altitude of ~3,400 m) or local adenosine infusion. The order of hypoxia and adenosine measurements was randomized. After these measurements at rest, blood flow was measured during

dynamic 1-leg exercise in a counterbalanced setting with the subject breathing either normal room air or hypoxic gas. Additionally, blood samples were drawn from the radial artery for blood gas analyses in the middle of each study occasion mentioned above. Exercise consisted of dynamic 1-leg exercise at 40 rpm with individually chosen workloads (4.3 ± 2.1 kg) with a knee angle range of motion of ~70–80 degrees. During pre-testing before the actual experiments, individually appropriate workloads for each subject were chosen so that they could exercise for at least ~10 min without fatigue or discomfort. Exercise was estimated to represent ~10 watts of an external 1-leg knee extension workload.

Other Procedures Before and After PET Measurements

Before the PET experiments, the antecubital vein was cannulated for tracer administration. For blood sampling, a radial artery cannula was placed under local anesthesia in the contralateral arm. Additionally, cannulas were placed under local anesthesia into the femoral artery and vein for local adenosine infusion and blood sampling, respectively. Subjects were then moved to the PET scanner with the femoral region in the gantry and the right leg was fastened to a custom-designed dynamometer.

Blood Flow Measurements and Analysis

Radiowater positron-emitting tracer [^{15}O]- H_2O was produced, as previously described in detail,¹³ and the ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, TN, USA) was used in 3D mode for image acquisition to measure ATBF. Photon attenuation was corrected by 5-min transmission scans performed both at the beginning of the resting and exercise PET studies. All data were corrected for dead time, decay and measured photon attenuation. During systemic hypoxia, breathing air with 14% oxygen gas began 5 min before imaging. Femoral arterial infusion of adenosine was initiated 1 min before the PET scanning and continued until the end of the scan (6 min in total). The adenosine concentration ($1 \text{ mg} \cdot \text{min}^{-1} \cdot \text{L thigh volume}^{-1}$) was based on the study by Rådegran and Calbet¹⁰ at rest and Barden et al¹⁴ during maximal exercise. This concentration has been shown to induce maximal femoral artery blood flow, as measured with ultrasound Doppler, and thus a maximal stimulus for ATBF was also expected. During exercise, scanning commenced 3 min after exercise onset to obtain a metabolic steady-state situation and was continued until the end of the exercise bout, which every time lasted an additional 2.5 min. Thus, the exercise duration was 5.5 min. Arterial blood radioactivity was also sampled continuously with a detector during imaging for blood flow quantification. Data analysis was performed using the standard models and methods.^{15,16} In addition to ATBF, muscle blood flow was also measured for comparison, as described and reported previously.^{17,18}

Other Measurements and Analysis

Structural magnetic resonance imaging (MRI) of the experimental thigh was performed to be combined with PET blood flow images for precise adipose tissue localisation (**Figure 1**), as previously described.¹⁷ Arterial oxygen was analyzed with a Radiometer ABL 835 blood gas analyzer.

Statistical Analysis

Statistical analyses were performed with SAS 9.2 version (SAS Institute, Cary, NC) using an ANOVA. If a significant main effect(s) was found, pairwise differences were identified using the Tukey-Kramer post hoc test. Relationships between variables were investigated by using Pearson correlation coefficient.

Table. Heart Rate, Blood Pressure and Arterial Oxygen Parameters at Resting Baseline, and Under the Systemic Hypoxia and Local Adenosine Infusion, and During Exercise in Healthy Young Men

Physiological variables	Rest			Exercise	
	Baseline	Hypoxia	Adenosine	Normoxia	Hypoxia
Heart rate (bpm)	61±10	69±10**	78±9†	92±12	102±10§
Mean arterial pressure (mmHg)	91±7	98±12	95±8	108±6	112±10
Systolic blood pressure (mmHg)	125±9	137±18	133±11	146±7	152±11
Diastolic blood pressure (mmHg)	74±6	79±10	76±7	90±9	92±12
Arterial oxygen saturation (%)	98±1	91±5**	98±1	98±1	88±5§§
Arterial oxygen content (ml/L)	199±9	186±13**	202±7	205±9	182±11§§§

Comparisons at rest: **P<0.01 compared to baseline and adenosine, †P<0.05 compared to both baseline and hypoxia.

Comparisons during exercise: §P<0.05, §§P<0.01, §§§P<0.001 compared to normoxia.

cient. Results are expressed as mean±SD and P≤0.05 was considered statistically significant.

Results

The general cardiovascular responses to the studied physiolog-

ical perturbations and exercise are shown in [Table](#). Heart rate increased in response to both adenosine and hypoxia at rest, but blood pressure remained unchanged from baseline. Directly determined arterial oxygen saturation and content decreased from baseline in response to hypoxia, and further reductions were observed during hypoxic exercise compared to normox-

ic exercise. Similarly, compared to at rest, the heart rate was increased but the blood pressure was similar during hypoxia compared to normoxia during exercise.

Adipose tissue blood flow was $1.3 \pm 0.6 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ at rest and was increased by adenosine infusion (Figure 2) and exercise (Figure 3), but not by breathing in moderate systemic hypoxic air (Figure 2). ATBF was similar during exercise and adenosine infusion ($P=0.23$). ATBF during exercise in moderate systemic hypoxia was reduced compared to during normoxic exercise (Figure 3, $P=0.004$).

At rest, adipose vascular conductance was elevated from rest ($0.014 \pm 0.005 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$) to adenosine infusion ($0.118 \pm 0.058 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$; $P=0.001$), but not during hypoxic air breathing ($0.016 \pm 0.005 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$). Adipose vascular conductance was also elevated from rest by exercise ($0.073 \pm 0.026 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$, $P<0.0001$), although the mean arterial blood pressure was also enhanced from rest in response to the applied dynamic exercise (Table, $P<0.001$). Vascular conductance was higher during adenosine infusion compared to exercise ($P<0.05$). Finally, vascular conductance was lower during hypoxic ($0.056 \pm 0.022 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$) compared to normoxic exercise ($P=0.005$).

Finally, adipose tissue blood flow correlated significantly with muscle blood flow at resting baseline, but not under any other conditions (Figure 4).

Discussion

The capacity and hypoxic response of blood flow in human subcutaneous adipose tissue remains incompletely understood. We show in the present study that the vasodilatation capacity of human subcutaneous adipose blood flow appears to be at least comparable to, or even higher, than that induced physiologically by moderate intensity exercise. In contrast, acute exposure to moderate systemic hypoxic air did not affect adipose tissue blood flow at rest, but during exercise, it was significantly reduced by systemic hypoxia compared to respective normoxic exercise.

White adipose tissue is known to be a highly responsive tissue to hypoxia, as exposure of adipocytes to reduced oxygen

availability in cell culture changes the expression of over 1,000 genes.⁶ In the present study, no change was observed in subcutaneous adipose tissue blood flow in response to moderate systemic hypoxia at rest. As it appears that adipose blood flow in humans is under the direct control of the sympathetic nervous system,¹⁹ it is likely that in our study, hypoxia did not create a sufficiently high stimulus for sympathetic neural vasoconstriction activation to reduce blood flow. In contrast, the finding also suggests that stimulation of vasoconstriction by arterial chemoreceptors predominates over a local hypoxic vasodilation in adipose tissue in humans. In this regard, adipose tissue possesses similar features as bone,²⁰ while increases in blood flow in response to systemic hypoxia have been reported in human skin.²¹ Further studies are warranted to investigate whether unchanged blood flow is of importance to explain the pathophysiological features of adipose tissue function under chronically low oxygen supply that is not compensated by higher blood flow.^{1,6} These conditions include, but are not limited to obesity, diabetes and cardiorespiratory disease, which are known or speculated to be associated with impaired adipose tissue function.^{1-3,7-9}

In contrast to rest, subcutaneous adipose blood flow was reduced during exercise when subjects were breathing hypoxic air. This novel finding is likely based on the constriction of adipose tissue arterioles by hypoxia-induced increased sympathetic nervous activity, which redistributes limb blood flow to exercising muscles that are more critically dependent on adequate oxygen delivery during exercise. We have previously reported that adipose tissue blood flow is reduced by the acute local infusion of norepinephrine, the principal neurotransmitter released from sympathetic nerve endings, and that the inhibition of α -adrenergic receptors by phentolamine tends to increase adipose blood flow, both at rest and during exercise.¹⁹ Romijn et al have also previously suggested that the reduction of adipose tissue blood flow could be one mechanism to explain decreased free-fatty acid release in response to high-intensity exercise,²² leading to preferential utilization of glucose and increased efficiency of ATP generation for a limited O_2 availability. Similarly, Layden et al have proposed that reduced adipose tissue blood flow might be one mechanism to explain reduced lipolysis and/or mobilization during exercise in cold

temperatures.²³ Furthermore, it has been recently reported that the inability to increase adipose tissue vascular resistance during exercise or maintain mean arterial pressure during orthostatic stress with old age is largely a result of diminished α -adrenergic responsiveness of adipose tissue arterioles.^{24,25} Hence, reduced adipose tissue blood flow is one potential acute physiological adjustment in response to short-term and/or high-intensity exercise. Accordingly, our results indicate that part of the redistribution of blood flow towards exercising muscle in hypoxia¹⁸ involves vasoconstriction of adipose tissue, while increased adipose blood flow is required in response to prolonged exercise that also associates with increased lipolysis to provide free-fatty acids for muscular work.²⁶ Mechanistically, the possibility remains that in addition to sympathetic nervous system influence, reduced oxygen content also directly contributed to reduced adipose blood flow as hypoxia, per se, is known to constrict arteries.^{27,28} It would be of interest to investigate whether adipose tissue blood flow changes in normoxic exercise below and above aerobic threshold, but unfortunately this threshold does not occur physiologically in incremental exercise to maximum in this well-established 1-leg knee extension model used in this study.

In addition to the hypoxic response, the peak blood flow capacity of human subcutaneous adipose tissue has remained largely unexplored. In this regard, our main novel finding is that the vasodilatation capacity of human subcutaneous adipose blood flow approximates the physiological level reached during moderate-intensity exercise. Vascular conductance can reach a level even higher than that induced by exercise. In terms of absolute values, the comparison of adipose tissue blood flow capacity to skeletal muscle is of interest. We have previously reported that blood flow in skeletal muscle during a similar adenosine infusion protocol, reaches a level of $40 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$.¹⁷ As the mean value of adenosine-induced adipose blood flow was $10.5 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$, it represents ~26% of that in muscle. In this light, the functional vascular capacity appears to closely follow that of structural anatomy, as adipose tissue has a capillary surface area that is slightly less than one-third than that of skeletal muscle.⁵ In terms of fold-increase from rest to adenosine, the percentage is even higher; 57%, as adipose blood flow increased 8-fold and muscle blood flow increased 14-fold. In contrast to skeletal muscle,¹⁷ adenosine-induced blood flow was not, however, positively related to subjects' whole body maximal oxygen consumption (data not shown), meaning also that adipose and muscle blood flow do not simply parallel each other under most physiological conditions, although they closely correlate at resting baseline (Figure 4). The data nevertheless suggest that the functional blood flow capacity is quite large in healthy human subjects. It, however, needs to be determined if this capacity is lost in pathological states, and if a loss of functional vascular capacity associates with an impaired ability to store fats in white adipose tissue that contributes to metabolic and cardiovascular derangements in the human body.¹ These investigations would be timely and highly relevant as obesity and diabetes, in particular, have become increasingly prevalent, and impairments in adipose tissue blood flow capacity could possibly contribute to explain other functional impairments of adipose tissue in patients.^{1-3,7-9}

In conclusion, the vasodilatory capacity of human subcutaneous adipose blood flow appears to be at least comparable to that induced by moderate intensity exercise, and is some one-third of that in skeletal muscle. Acute breathing of moderate systemic hypoxic air does not affect adipose tissue blood flow at rest, but during exercise, systemic hypoxia reduces blood flow

in subcutaneous adipose tissue and might serve as a mechanism to redistribute blood flow from inactive tissues to exercising skeletal muscle. Further mechanistic studies are warranted to investigate whether these reported responses are impaired in patients with cardiovascular and metabolic diseases, as could be hypothesized based on the known other impairments of adipose tissue function. It would also be worth determining whether exercise training, which maintains or even increases whole body fitness, could also improve the circulatory and functions of adipose tissue.²⁹

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