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Am J Physiol Regul Integr Comp Physiol 302:R385-R390, 2012. First published 30 November 2011; doi: 10.1152/ajpregu.00245.2011

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Ilkka Heinonen,[1,2,3] Jukka Kemppainen,[1,2] Kimmo Kaskinoro,[1,4] Juha E. Peltonen,[6] Hannu T. Sipilä,[1] Pirjo Nuutila,[1,5] Juhani Knuuti,[1] Robert Boushel,[7] and Kari K. Kalliokoski[1]

[1]*Turku PET Centre, Departments of* [2]*Clinical Physiology and Nuclear Medicine,* [4]*Anesthesiology and Intensive Care, and* [5]*Medicine, and* [3]*Research Unit of Applied and Preventive Cardiovascular Medicine, Turku University Hospital, University of Turku, Turku, Finland;* [6]*Unit for Sports and Exercise Medicine, Institute of Clinical Medicine, University of Helsinki, Helsinki, Finland; and* [7]*Centre for Healthy Aging, Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark*

Submitted 6 May 2011; accepted in final form 25 November 2011

Heinonen I, Kemppainen J, Kaskinoro K, Peltonen JE, Sipilä HT, Nuutila P, Knuuti J, Boushel R, Kalliokoski KK. Effects of adenosine, exercise, and moderate acute hypoxia on energy substrate utilization of human skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* 302: R385–R390, 2012. First published November 30, 2011; doi:10.1152/ajpregu.00245.2011.—Glucose metabolism increases in hypoxia and can be influenced by endogenous adenosine, but the role of adenosine for regulating glucose metabolism at rest or during exercise in hypoxia has not been elucidated in humans. We studied the effects of exogenous adenosine on human skeletal muscle glucose uptake and other blood energy substrates [free fatty acid (FFA) and lactate] by infusing adenosine into the femoral artery in nine healthy young men. The role of endogenous adenosine was studied by intra-arterial adenosine receptor inhibition (aminophylline) during dynamic one-leg knee extension exercise in normoxia and acute hypoxia corresponding to ~3,400 m of altitude. Extraction and release of energy substrates were studied by arterial-to-venous (A-V) blood samples, and total uptake or release was determined by the product of A-V differences and muscle nutritive perfusion measured by positron emission tomography. The results showed that glucose uptake increased from a baseline value of 0.2 ± 0.2 to 2.0 ± 2.2 μmol·100 g^{-1}·min^{-1} during adenosine infusion ($P < 0.05$) at rest. Although acute hypoxia enhanced arterial FFA levels, it did not affect muscle substrate utilization at rest. During exercise, glucose uptake was higher (195%) during acute hypoxia compared with normoxia ($P = 0.058$), and aminophylline had no effect on energy substrate utilization during exercise, despite that arterial FFA levels were increased. In conclusion, exogenous adenosine at rest and acute moderate hypoxia during low-intensity knee-extension exercise increases skeletal muscle glucose uptake, but the increase in hypoxia appears not to be mediated by adenosine.

glucose uptake

SKELETAL MUSCLE IS QUANTITATIVELY the most important tissue for glucose uptake in the human body. The uptake of glucose in the quiescent skeletal muscle is importantly controlled by the anabolic hormone insulin, but during exercise the disposal of blood glucose is predominantly mediated by mechanisms other than insulin. Muscular contractions increase intracellular free calcium, which increases glucose uptake and induces the translocation of GLUT 4 transporters to the cell membrane to facilitate glucose entry into muscle cells (19, 40). During high-intensity exercise it is energetically more efficient for the muscle to utilize stored glycogen, but the reliance of muscle on blood glucose is important during low-intensity exercise and/or during prolonged exercise when glycogen stores are depleted (19, 40).

It has been proposed that an exposure to hypoxia triggers the activation of various signaling pathways that lead to an increased reliance on blood glucose both at rest, but especially, after prolonged exposure or even during acute exercise (5, 11, 35). If hypoxia per se indeed induces greater glucose uptake, one of the signaling mechanisms mediating this could be adenosine, which is formed during muscular contractions from the degradation of extracellular ATP (32) and is elevated during hypoxia (7, 8, 30). Adenosine is also known to be formed intracellularly, especially during hypoxic and/or ischemic conditions. In this regard it has been shown that adenosine via the A_1-adenosine receptors potentiates and regulates both insulin- and contraction-induced glucose uptake (14, 46), and its removal decreases glucose transport in both situations (22). Thus, it is likely that adenosine could affect glucose uptake and metabolism of other energy substrates during skeletal muscle contractions, especially in hypoxia, and we aimed to investigate this in the present study. We hypothesized that arterial infusion of adenosine would increase muscle glucose uptake and that inhibition of endogenous adenosine receptors would lower glucose uptake during exercise in hypoxia.

MATERIALS AND METHODS

Subjects. Nine healthy young men (25 ± 5 yr, 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 before 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 Intermunicipal Hospital District of Southwest Finland and National Agency for Medicines.

Study design. Skeletal muscle nutritive perfusion in the femoral region was measured using PET with [^{15}O]H_2O, as described below and in Figure 1. Muscle perfusion was measured first under normal resting conditions and then either during systemic hypoxia (14% inspired O_2 in N_2; equivalent to altitude of ~3,400 m) or local adenosine infusion in a counterbalanced order between subjects. After these measurements at rest, perfusion was measured during one-leg dynamic exercise without (always first) and with (always last) locally

Address for reprint requests and other correspondence: I. Heinonen, Turku PET Centre, PO Box 52, FIN-20521 Turku, Finland (e-mail: ilkka.heinonen@utu.fi).

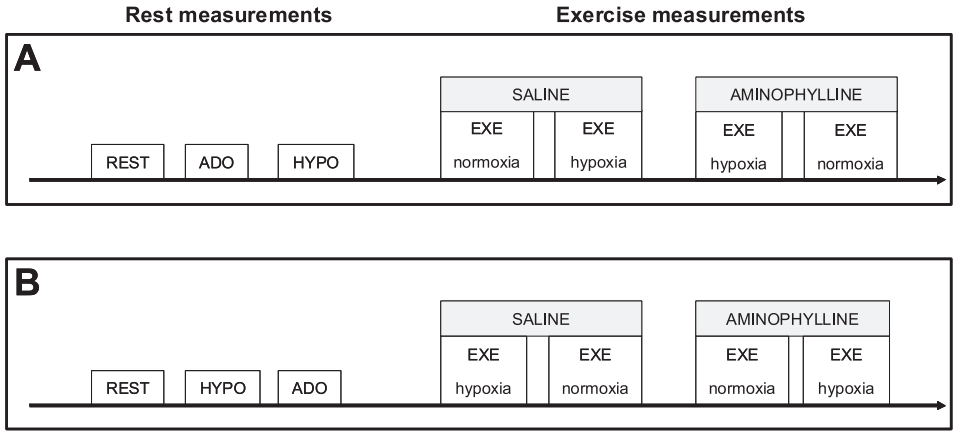

Fig. 1. Muscle perfusion was measured first under baseline conditions (REST) and then either during systemic hypoxia (HYPO) or local adenosine infusion (ADO) in a counterbalanced order between subjects (*A* and *B* lines alternating with every other subject). After these measurements at rest, perfusion was measured during one-leg dynamic exercise (EXE) without (always first) and with (always last) locally administered adenosine receptor antagonism by aminophylline. During both conditions, measurements were performed with the subject breathing either normal room air (normoxia) or hypoxic gas (hypoxia). The order of normoxia and hypoxia was counterbalanced between subjects and between adenosine receptor antagonism conditions (see lines *A* and *B*). Blood samples were drawn from the radial artery and femoral vein for blood gases and free fatty acids, glucose, and lactate in each occasion mentioned above.

administered adenosine receptor antagonism by aminophylline. During both conditions, measurements were performed with the subject breathing either normal room air or hypoxic gas. The order of normoxia and hypoxia was counterbalanced between subjects and between adenosine receptor antagonism conditions (Fig. 1). Additionally, blood samples were drawn from the radial artery and femoral vein for blood gases and free fatty acids (FFAs), glucose and lactate in each occasion mentioned above. Exercise consisted of dynamic one-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 pretesting before the actual experiments, individually appropriate workload for each subject was chosen so that they could exercise for at least ~10 min without fatigue or discomfort. The exercise load represented ~10 watts.

Other procedures before 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 drug infusion (aminophylline) 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.

Perfusion measurements and analysis. Radiowater positron-emitting tracer [^{15}O]H_2O was produced as previously described in detail (44) and the ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, TN) was used in 3D mode for image acquisition to measure muscle perfusion. 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 mg·min^{-1}·l thigh volume^{-1}) was based on the study by Rådegran and Calbet (38) at rest and Barden et al. (3) during maximal exercise. This concentration has been shown to induce maximal femoral artery blood flow as measured with Doppler ultrasound, and thus a maximal stimulus for energy substrate disposal was also expected. During exercise, scanning commenced 3 min after exercise onset to obtain a metabolic steady-state situation and continued until the end of the exercise bout, which every time lasted an additional 2.5 min. Thus exercise duration was 5.5 min. Arterial blood radioactivity was also sampled continuously with a detector during imaging for tissue perfusion quantification. Data analysis was performed using the standard models and methods (26, 41). Perfusion was analyzed from whole thigh muscles at rest, and from m. quadriceps femoris during exercise.

Adenosine receptor blockade with aminophylline. To elucidate the role of adenosine for regulating energy substrate utilization of skeletal muscle, similar procedures for adenosine receptor blockade with aminophylline were used as previously described (10, 24, 30, 34). Thus, aminophylline (2 mg·min^{-1}·l thigh volume^{-1}), was infused into the femoral artery to induce local competitive inhibition of endogenous adenosine receptor binding and to minimize any confounding systemic influence of the drug. To maximize its delivery to the site of action, the intra-arterial infusion of aminophylline was initiated simultaneously with one-leg exercise, then 3 min before scanning, and continued until the end of respective PET scan. The infusion of aminophylline was not possible to perform in two subjects for technical reasons, and the aminophylline results are therefore from seven subjects. The total volume of the experimental thigh was measured with structural MRI, as previously described (23).

Biochemical analysis. Plasma samples for energy substrates (FFAs, glucose, and lactate) and blood gases were drawn from femoral vein and radial artery in each study condition at the mid-time point of PET measurement and analyzed with standardized hospital practices. Lactate and serum FFAs were analyzed with enzymatic methods (Modular P Analyzer; Roche Diagnostics, Mannheim, Germany) and glucose by the glucose hexokinase method (Modular P analyzer, Roche Diagnostics). Blood gases were analyzed with a Radiometer ABL 835 blood gas analyzer. Uptake or release of energy substrates were determined by the Fick principle, thus arterial-to-venous (A-V) differences were multiplied with tissue perfusion, which in the case of applied radiowater directly depicts nutritive tissue perfusion that is responsible for exchange of measured energy substrates.

Statistical analysis. Statistical analyses were performed with SAS 8.2 and SAS Enterprise 4.2 programs (SAS Institute, Cary, NC). The normality of the parameters was first tested, and subsequently the statistical analyses were performed either using two-way ANOVA for repeated measures (for normally distributed parameters) or Wilcoxon signed rank test [for nonnormally distributed (negative) parameters]. In ANOVA, if significant interaction or main effect were found, pairwise differences were identified

using the Tukey-Kramer post hoc procedure. Results are expressed as means ± SD, and $P \leq 0.05$ was considered statistically significant.

RESULTS

Effects of acute hypoxia or adenosine infusion on metabolic parameters at rest. All the following results are shown in Table 1. Acute hypoxia increased arterial serum FFA concentration, but did not change the specific limb uptake of FFAs and did not have any effect on glucose or lactate parameters. Adenosine infusion increased glucose uptake 10-fold and decreased A-V difference of FFAs, but the total release of FFAs during infusion did not change significantly from resting baseline. Neither acute hypoxia nor adenosine changed muscle oxygen uptake or blood pressure, but both acute hypoxia and adenosine increased heart rate. The effect of acute hypoxia on arterial oxygen saturation differed between the subjects at rest, but this did not correlate with changes in substrate utilization (data not shown).

Effects of acute hypoxia, adenosine receptor inhibition, and combined acute hypoxia and adenosine receptor inhibition on metabolic parameters during exercise. All the following results are shown also in Table 1. Compared with resting baseline measurements, one-leg knee extension exercise in normoxia without adenosine receptor inhibition changed most of the parameters. Glucose uptake increased significantly from rest to exercise ($P = 0.03$) and added acute hypoxia tended to increase it further ($P = 0.058$). For lactate, there was a shift from a slight uptake at rest to a notable release during exercise, but acute hypoxia did not affect it further. The small FFA release at rest was shifted to a small FFA uptake during control exercise and further back to a small net release during exercise in acute hypoxia. However, because of a large individual variation, these changes were not significant. Adenosine receptor inhibition with aminophylline had no effect on utilization/release of energy substrates, although arterial FFA levels increased under inhibition. Acute hypoxia during exercise increased heart rate and decreased arterial oxygen saturation, arterial and venous oxygen content, and absolute oxygen extraction (ml/l). This latest was accompanied with increased muscle perfusion, and muscle oxygen uptake was not changed during acute hypoxia. Adenosine receptor inhibition had no effect on any of these measures, either in normoxia or in acute hypoxia. The effect of acute hypoxia on arterial oxygen saturation differed between the subjects also during exercise, but this did not correlate with changes in substrate utilization (data not shown).

Table 1. *Heart rate, blood pressure, muscle perfusion, muscle vascular resistance, muscle oxygen uptake, and arterial and venous oxygen parameters at rest and during exercise*

	Rest			Exercise			
				Without Aminophylline		With Aminophylline	
	Baseline	Hypoxia	Adenosine	Normoxia	Hypoxia	Normoxia	Hypoxia
HR, beats/min	61 ± 10	69 ± 10**	78 ± 9†	92 ± 12	102 ± 10§	97 ± 20	110 ± 13§
Mean arterial pressure, mmHg	91 ± 7	98 ± 12	95 ± 8	108 ± 6	112 ± 10	105 ± 7	107 ± 8
Systolic blood pressure, mmHg	125 ± 9	137 ± 18	133 ± 11	146 ± 7	152 ± 11	147 ± 12	149 ± 13
Diastolic blood pressure, mmHg	74 ± 6	79 ± 10	76 ± 7	90 ± 9	92 ± 12	83 ± 7	86 ± 11
Muscle perfusion, ml·100 g^{-1} ·min^{-1}	2.9 ± 1.6	3.4 ± 1.7	42.5 ± 7.5†††	36.4 ± 8.6	39.9 ± 7.1§	37.8 ± 5.0	39.0 ± 8.4§
Muscle vascular resistance, mmHg · [ml·100 g^{-1} ·min^{-1}]	39 ± 19	37 ± 22	2 ± 1†††	3.2 ± 1.0	2.8 ± 0.4	2.8 ± 0.4	2.9 ± 0.9
Arterial oxygen saturation, %	98 ± 1	91 ± 5**	98 ± 1	98 ± 1	88 ± 5§§	99 ± 0.4	89 ± 4§§
Arterial oxygen content, ml/l	199 ± 9	186 ± 13**	202 ± 7	205 ± 9	182 ± 11§§§	208 ± 24	182 ± 8§§§
Venous oxygen content, ml/l	152 ± 23	141 ± 18*	191 ± 14††	88 ± 32	78 ± 26§	96 ± 31	83 ± 35§
Oxygen extraction, ml/l	48 ± 16	45 ± 14	11 ± 14§	117 ± 33	101 ± 29§	114 ± 44	96 ± 37§
Oxygen extraction fraction, %	24 ± 9	24 ± 8	6 ± 7§	57 ± 16	56 ± 16	53 ± 18	53 ± 20
Muscle oxygen uptake, ml·100 g^{-1} ·min^{-1}	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.5	4.1 ± 1.0	4.0 ± 1.3	4.4 ± 1.9	3.9 ± 1.9
Arterial plasma glucose, mmol/l	5.94 ± 0.33	5.99 ± 0.40	6.0 ± 0.3	6.04 ± 0.57	6.06 ± 0.57	5.75 ± 0.52	5.89 ± 0.63
Plasma glucose A-V difference, mmol/l	0.07 ± 0.08	0.09 ± 0.05	0.05 ± 0.06	0.06 ± 0.10	0.16 ± 0.10	0.11 ± 0.15	0.17 ± 0.13
Glucose uptake, μmol·100 g^{-1} ·min^{-1}	0.2 ± 0.2	0.3 ± 0.3	2.0 ± 2.2*	2.2 ± 3.2	6.7 ± 4.4	4.6 ± 7.0	6.2 ± 4.8
Arterial serum FFA, mmol/l	0.446 ± 0.174	0.650 ± 0.200*	0.587 ± 0.215	0.585 ± 0.139	0.582 ± 0.141	1.103 ± 0.223[a]	1.134 ± 0.305[a]
Serum FFA A-V difference, mmol/l	−0.12 ± 0.09	−0.13 ± 0.05	−0.03 ± 0.08	0.02 ± 0.12	−0.03 ± 0.08	−0.04 ± 0.13	−0.01 ± 0.15
FFA uptake, μmol·100 g^{-1} ·min^{-1}	−0.31 ± 0.29	−0.4 ± 0.2	−1.3 ± 3.2	0.29 ± 4.4	−1.1 ± 3.2	−1.5 ± 5.0	−0.63 ± 6.2
Arterial plasma lactate, mmol/l	1.0 ± 0.3	0.9 ± 0.4	0.8 ± 0.4	1.6 ± 1.1	1.6 ± 0.8	1.2 ± 0.9	1.4 ± 0.8
Plasma lactate A-V difference, mmol/l	0.01 ± 0.24	0.05 ± 0.44	−0.1 ± 0.2	−0.4 ± 0.6	−0.3 ± 0.2	−0.2 ± 0.3	−0.5 ± 0.6
Lactate uptake, μmol·100 g^{-1} ·min^{-1}	0.2 ± 0.8	0.4 ± 1.9	−4.0 ± 7.7	−16.7 ± 25.8	−14.3 ± 12.1	−8.5 ± 11.8	−22.0 ± 31.0

Values are means ± SD. HR, heart rate; FFA, free fatty acid; A-V, arterial-to-venous. For the sake of clarity the comparisons between rest and exercise measurements are not marked in the table, but mentioned in RESULTS. Comparisons at rest: *$P < 0.05$ compared with baseline; **$P < 0.01$ compared with baseline and adenosine; †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ compared with both baseline and hypoxia. Comparisons during exercise: §$P < 0.05$, §§$P < 0.01$, §§§$P < 0.01$ compared with normoxia. [a]$P < 0.05$, compared to without aminophylline.

DISCUSSION

In the present study we found that *1*) moderate acute hypoxia led to a threefold increase in blood glucose disposal in the exercising muscle compared with normoxia, *2*) exogenous adenosine increased muscle glucose uptake at rest, and *3*) inhibition of endogenous adenosine action by aminophylline did not affect glucose uptake in human skeletal muscle during knee-extension exercise in normoxia and hypoxia.

In general, exercise increased glucose utilization from rest severalfold. This indicates that there is indeed a reliance on blood glucose even during a short exercise bout (5–10 min) and when muscle glycogen stores are well preserved. Moreover, moderate acute hypoxia corresponding to 3,400 m altitude led to an approximately threefold increase ($P = 0.058$) in blood glucose utilization during exercise compared with normoxia. This result supports findings that muscles depend more on blood glucose in hypoxic than in normoxic exercise (18) and that increased glucose oxidation may well be an early fuel efficiency adjustment mechanism during limited oxygen supply to minimize cardiovascular stress (5). Noteworthy is that in the present study, even as short a period as 5–10 min of breathing of hypoxic air resulted in this increase in glucose uptake. It is also likely that increased glucose dependency also partly explains greater metabolic economy and work efficiency during and after long-term hypoxia as observed in some (43) but not all (31) previous studies.

On the other hand, it is also known that hypoxia upregulates noninsulin-dependent glucose uptake pathways stimulated by catecholamine secretion (5), which might have been enhanced in hypoxia. This mechanism may function synergistically with exercise to account for the observed increase in glucose disposal. However, it must also be considered that increased glucose uptake in this acute hypoxic exposure was not evident in the resting state. This is in accordance with a recent hypoxia study by Chen et al. (12) of exactly the same experimental altitude, where cardiac and skeletal muscle glucose uptake was measured directly by PET and FDG-glucose analog during 1-h hypoxic exposure. They also found that while cardiac muscle showed concomitant increase in heart rate and glucose utilization in moderate hypoxia, no changes were observed in brain, liver, or skeletal muscles (12). However, we also noticed that at rest, the responses to hypoxia varied a lot between the subjects. For example, the reduction of arterial oxygen saturation, a marker of systemic hypoxia, varied from 98% to 85% at rest, and from 90% to 80% during exercise. Thus, apparently some of the subjects did not have systemic hypoxia at rest despite a largely reduced content of oxygen in the inspired air. One potential reason for this can be the short period of breathing of hypoxic gas before the measurements. It can therefore be speculated that these divergent responses in oxygen saturation could have caused also the large variation observed in metabolic responses. Therefore, we calculated correlation between changes in oxygen saturation and changes in metabolic parameters. These analyses, however, did not show any significant associations, suggesting that the variable responses to hypoxia most probably do not explain variation in responses in metabolic parameters.

Adenosine infusion at rest increased glucose uptake substantially (10-fold) in these healthy young men, which confirms the earlier finding in patients with essential hypertension (36). Since muscular vascular resistance was also decreased to minimal levels during the infusion (Table 2) a potential mechanism for the enhanced glucose uptake was a higher muscle perfusion (19). This interpretation holds that increased glucose uptake under higher flow conditions is due to insulin reaching (and acting in) previously underperfused muscle regions. Yet, glucose uptake mediated by circulating insulin under simply higher flow conditions do not account for the higher glucose uptake rates observed in resting muscle. Adenosine infusion into the forearm increases insulin-mediated glucose uptake (42), but this effect is not due to changing insulin concentration, and epinephrine does not account for changes in insulin-mediated glucose uptake during adenosine receptor antagonism (4). Alternatively, adenosine itself or its synergistic action with insulin (46) can modulate glucose uptake. It has been shown that adenosine action via the A_1-adenosine receptor potentiates and regulates both insulin- and contraction-induced glucose uptake (14, 46), and removing it decreases glucose transport in both situations (22). In heart, adenosine enhances glucose uptake only in the presence of insulin (29). It is likely that part of the effect of adenosine was mediated by nitric oxide (NO) since adenosine is well known to trigger the formation of NO in muscle (45), and NO has been shown to increase glucose uptake (25). Conversely, the formation of NO is well known to be inhibited during hypoxia (6, 9), the fact that may have also affected our hypoxic findings.

In contrast to the finding of increased glucose uptake during adenosine infusion at rest, we found that intra-arterial infusion of aminophylline to inhibit the actions of physiological endogenous adenosine during exercise did not affect glucose uptake. Most studies suggesting that endogenous adenosine may modulate muscle glucose uptake during muscle contraction have been conducted in animals (14, 22, 46, 47). One study in humans showed impaired insulin sensitivity and glucose uptake in response to the aminophylline-like methylxanthine caffeine (28). This effect was attributed to increased plasma epinephrine and FFA levels rather than peripheral adenosine receptor antagonism. Interestingly, we also found increased FFA levels during aminophylline infusion both in normoxia and hypoxia, but still this did not result in decrease in glucose uptake. Another previous study in humans investigated trained cyclists during exercise at 75% maximal oxygen uptake and showed some evidence for endogenous adenosine playing a role in controlling the uptake of blood glucose in peripheral tissues (39). In this study, theophylline infusion reduced muscle glucose uptake and enhanced glycogenolysis. This effect was independent of catecholamine release or action, which was the same in both conditions. Adenosine is thought to reduce catecholamine-mediated activation of phosphorylase by inhibiting β-receptor, adenylate cyclase coupling (15). In animals, theophylline may act synergistically with circulating catecholamines and even stimulate the release of catecholamines (2) to enhance contraction-induced glycogenolysis (47) by inhibition of phosphodiesterase and increasing cAMP. The lack of effect of adenosine inhibition on blood glucose disposal during exercise in this study is likely to be explained by the small muscle mass engaged in exercise and the relatively low exercise intensity. Previous studies have shown that adenosine partly mediates vasodilation only at higher exercise intensities, while adenosine inhibition has no effect on muscle blood flow at lower intensities (33, 34). In line with this, we did not observe any changes in muscle perfusion during exercise in the present study.

Adenosine has been shown to inhibit lipolysis in vitro (27) and in vivo (1, 37) due to activation of adenosine A_1-receptors on adipocytes causing inhibition of adenylate cyclase, and further decreasing cAMP. In this study, we did not find any significant changes in fatty acid or lactate responses to adenosine, hypoxia, and exercise, and no concrete conclusions can be drawn from these metabolic variables. It is likely that caffeine-like methylxanthine aminophylline may have blunted the well-known antilipolytic effects of adenosine in adipose tissue during exercise (37), which was seen as increased arterial FFA levels in the present study. However, this did not lead to increased reliance on FFA utilization, which is very well in line with the results of other groups, suggesting that while methylxanthines increase the mobilization of fatty acids from adipose tissue, this does not necessarily lead to increased fatty acid oxidation (20). Raguso et al. (39) found that FFA oxidation was lower with theophylline, which they attributed to carbohydrate metabolism-mediated increase in malonyl CoA that decreases mitochondrial carnitine palmitoyl transporter activity. High concentrations of theophylline (300 μmol/l) can inhibit phosphodiesterase activity (17), but a concentration (10 μmol/l) sufficient to inhibit adenosine but not alter phosphodiesterase did not inhibit lipolysis or FFA release during exercise at 75% $\dot{V}O_{2\,max}$, but glucose uptake was reduced and plasma glucose concentration was higher (39).

A noteworthy observation was that a subset of subjects did not exhibit a robust glucose uptake response to adenosine. The range of glucose uptake in response to adenosine was 0–5.4 μmol·100 g^{-1}·min^{-1}. The mean value was 2.0 ± 2.2 μmol·100 g^{-1}·min^{-1}. Four of the nine subjects showed minimal or no increase in glucose uptake, while five subjects showed very robust (~25-fold) increases in response to adenosine infusion. Battram et al. (4) observed a similar disparity in glucose uptake in a subset of subjects in response to caffeine and adrenaline, which is in line with the equivocal findings from other studies examining the effect of adenosine on glucose uptake in skeletal muscle (13, 16, 22, 46). This varied response may be due to different intracellular responses to G protein activation and cAMP. These findings are consistent with studies showing differential vasodilator responsiveness to adenosine (33, 34).

There are some additional limitations in our study that have not yet been discussed, as we, for instance, did not measure catecholamines in this study, but in studies employing the same exercise model there is no significant increase in catecholamines at a similar exercise load. Similar to our results, Graham et al. (21) found that leg glucose uptake was unchanged during one-leg knee extensor exercise when they blocked the effect of adenosine by aminophylline-like methylxanthine caffeine. Thus, adenosine may play a role in regulating glucose uptake at high-exercise intensities when adenosine is released at a sufficiently high concentration (39).

A definitive control experiment on the local effects of adenosine on glucose uptake during exercise would have been to compare the effect of adenosine inhibition with aminophylline with that of dipyridamole, which blocks adenosine transporters and activates phosphodiesterase. While dipyridamole infusion does not appear to affect glucose uptake at rest (28), this experimental approach would be of interest to examine the physiological importance of endogenous adenosine in controlling glucose uptake during exercise.

In conclusion, we showed that high-dose exogenous adenosine increases glucose uptake in human skeletal muscle at rest, but adenosine receptor inhibition does not have an effect on muscle glucose uptake during low-intensity exercise. Acute moderate hypoxia did not influence glucose uptake at rest, but increased it during exercise. However, this increase was not modified by adenosine receptor inhibition, suggesting that adenosine does not take part in glucose uptake during low-intensity exercise in moderate acute hypoxia.

Perspectives and Significance

Both exercise and hypoxia are well-known stimulators of human energy metabolism. Especially exercise, but also systemic hypoxia, can increase blood glucose disposal due to skeletal muscle utilization and particularly so when combined. However, the mechanisms of this phenomenon still remain undefined, but intra- and extracellularly formed adenosine may play a role in the process. In the present study, we therefore studied the effects of exogenous and endogenous adenosine on the energy substrate utilization of resting and exercising human skeletal muscle, with and without hypoxic stimulus. Our results indicate that exogenous, but not endogenous adenosine, increases glucose uptake in human skeletal muscle. The importance of adenosine for regulating glucose uptake seems to occur only at relatively high concentrations since endogenous adenosine inhibition does not alter glucose uptake that is elevated during voluntary low-intensity exercise in normoxia or moderate hypoxia, but high-dose adenosine infusion that has previously been shown to elicit maximal limb blood flow, increased glucose uptake. However, adenosine may play an indirect role in glucose metabolism by limiting lipolysis during exercise, and altogether, these findings advance our understanding about the regulation of tissue metabolism during exercise and acute hypoxia.

ACKNOWLEDGEMENTS

The study was conducted within the Centre of Excellence in Molecular Imaging in Cardiovascular and Metabolic Research, supported by the Academy of Finland, University of Turku, Turku University Hospital and Abo Academy.

This study could not have been performed without the contribution of the personnel of the Turku PET Centre, and the authors thank them for excellent assistance during the study. The assistance of Tero Valhberg and Tuomas Makkonen in statistical issues is also greatly acknowledged.

GRANTS

The present study was financially supported by The Ministry of Education of State of Finland (Grants 74/627/2006, 58/627/2007, and 45/627/2008), Academy of Finland (Grants 108539 and 214329, and funding from the Centre of Excellence), The Finnish Cultural Foundation and its South-Western Fund, The Finnish Sport Research Foundation, and Turku University Hospital (funding from the EVO).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

I.H., K.K., J.E.P., H.T.S., P.N., J. Knuuti, R.B., and K.K.K. conception and design of research; I.H., J. Kemppainen, K.K., H.T.S., and K.K.K. performed experiments; I.H. and K.K.K. analyzed data; I.H., J. Kemppainen, J.E.P., P.N., J. Knuuti, R.B., and K.K.K. interpreted results of experiments; I.H. and K.K.K. prepared figures; I.H., J. Kemppainen, K.K., R.B., and K.K.K. drafted manuscript; I.H., R.B., and K.K.K. edited and revised manuscript; I.H., J. Kemppainen, K.K., J.E.P., H.T.S., P.N., J. Knuuti, R.B., and K.K.K. approved final version of manuscript.

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