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ORIGINAL ARTICLE

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Bone Blood Flow and Metabolism in Humans: Effect of Muscular Exercise and Other Physiological Perturbations

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ABSTRACT

Human bone blood flow and metabolism during physical exercise remains poorly characterized. In the present study we measured femoral bone blood flow and glucose uptake in young healthy subjects by positron emission tomography in three separate protocols. In 6 women, blood flow was measured in femoral bone at rest and during one-leg intermittent isometric exercise with increasing exercise intensities. In 9 men, blood flow in the femur was determined at rest and during dynamic one-leg exercise and two other physiological perturbations: moderate systemic hypoxia (14 O_2) at rest and during exercise, and during intrafemoral infusion of high-dose adenosine. Bone glucose uptake was measured at rest and during dynamic one-leg exercise in 5 men. The results indicate that isometric exercise increased femoral bone blood flow from rest (1.8 ± 0.6 mL/100 g/min) to low intensity exercise (4.1 ± 1.5 mL/100 g/min, $p = 0.01$), but blood flow did not increase further with increasing intensity. Resting femoral bone blood flow in men was similar to that of women and dynamic one-leg exercise increased it to 4.2 ± 1.2 mL/100 g/min, $p < 0.001$. Breathing of hypoxic air did not change femoral bone blood flow at rest or during exercise, but intra-arterial infusion of adenosine during resting conditions increased bone blood flow to 5.7 ± 2.4 mL/100 g/min, to the level of moderate-intensity dynamic exercise. Dynamic one-leg exercise increased femoral bone glucose uptake 4.7-fold compared to resting contralateral leg. In conclusion, resting femoral bone blood flow increases by physical exercise, but appears to level off with increasing exercise intensities. Moreover, although moderate systemic hypoxia does not change bone blood flow at rest or during exercise, intra-arterially administered adenosine during resting conditions is capable of markedly enhancing bone blood flow in humans. Finally, bone glucose uptake also increases substantially in response to exercise. © 2013 American Society for Bone and Mineral Research.

KEY WORDS: BONE; BLOOD FLOW; METABOLISM; EXERCISE; HUMANS

Introduction

Among its other functions, bone provides mechanical support for stature and locomotion.[1] Physical activity, especially in a form of exercise training, is well-known for its benefits in strengthening bone.[1–4] The beneficial changes in mineral content and structure are likely made possible by increased blood flow that supplies bone with nutrients and oxygen in accordance with its metabolic needs.[4–8] However, the functional characterization of blood flow and metabolism in bone has been hampered by limitations in research techniques, and that is why the understanding of blood flow and nutrient delivery and uptake in bone in physiologically relevant situations such as during exercise remains incomplete in humans.[7] One method to address tissue metabolism is positron emission tomography (PET), which is a noninvasive imaging method to study

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physiological processes in humans in vivo. Because the method is, in principle, suitable for investigation of any tissue in the body, it has increasingly been used to measure bone blood flow and metabolism in clinical settings.[9–11]

In our previous human study applying PET methodology, we found that functional elevation in blood flow occurs in bone in response to acute heat stress,[12] but to the best of our knowledge, it is still unknown whether human bone blood flow increases along with increased workloads, or whether moderate systemic hypoxia can alter blood flow in human bone as it does in animals.[13] Moreover, the capacity of human bone blood flow remains undefined, but can be elucidated by locally administered vasodilator drugs that have also been applied in animal studies.[13] Furthermore, the uptake of glucose in response to exercise, an indication of general metabolic level, remains undefined in human bone. Along these lines, in the present study we determined femoral bone blood flow in young healthy women and men at rest and during both isometric and dynamic exercise. In addition, in men, hypoxic blood flow response was also studied at rest and when superimposed on exercise, and the functional circulatory capacity of bone blood flow was determined by local femoral arterial infusion of high-dose adenosine, which is known to induce maximal thigh blood flow in humans.[14] To this end, glucose uptake of the femoral bone was also directly determined in response to dynamic exercise. Based on previous animal studies,[13,15–17] it was hypothesized that bone blood flow increases in transition from rest to exercise, but not further with increasing workloads. Furthermore, we also expected that hypoxia decreases bone blood flow at rest[13] and adenosine infusion increases bone blood flow markedly.[13] Finally, we also hypothesized that bone glucose uptake would be increased in the exercising leg compared to the resting contralateral leg.

Subjects and Methods

This study consisted of three substudies from which muscle results have already been published.[18–20] The methods are partially described here, separately for each of the projects.

Part 1

Subjects and study design

Six healthy young women participated into this part (age 24.0 ± 2.6 years, body mass index [BMI] 21.0 ± 1.1 kg/m^2). The purpose, nature, and potential risks were explained to them before they gave their written informed consent to participate. The possibility of pregnancy was excluded by a pregnancy test before participation. They were not taking any medication other than possibly oral contraceptives and they were studied in the early follicular phase of their menstrual cycle. They fasted overnight and avoided caffeine-containing beverages such as coffee, tea, and cola drinks for at least 48 hours before the experiments. Exhaustive exercise was also prohibited for 24 hours prior to the study. The study was performed according to the Declaration of Helsinki and was approved by the Ethical Committee of the Hospital District of South-Western Finland.

The experiment day started with a magnetic resonance imaging (MRI) study to obtain anatomical references for tissues in the femoral region. Thereafter, an antecubital vein was cannulated for tracer administration and a radial artery in the contralateral arm was cannulated for blood sampling needed in blood flow calculations. Then the subjects were positioned in the PET scanner with the femoral region in the gantry and the left leg was fastened to a dynamometer (Diter Petkin; Oy Diter-Elektroniikka Ab, Turku, Finland) at a knee angle of 40 degrees. After a transmission scan, basal blood flow was measured while the subject was lying at rest, followed by exercise measurements at three workloads (50, 100, and 150 N). The exercise model consisted of 1-second isometric contractions of the knee extensors followed by 2-second relaxation interval and for each load exercise was continued for 10 minutes with 5-minute breaks in between. At all three intensities, blood flow was measured starting after 5 minutes of exercise. Instructions about maintaining the exercise intensity, and contraction and relaxation periods were provided for the subjects by light-emitting diodes (LEDs) and were also cued by specific sounds from the dynamometer. Heart rate was continuously monitored during the PET measurements and blood pressure was measured repeatedly with an automatic apparatus (Omron, M5-1; Omron Healthcare Europe B.V., Hoofddorf, The Netherlands).

Parametric blood flow images over the whole thigh area, including bone, were calculated as previously described.[12,18,19] Regions of interest were placed over the bone as previously applied,[12,21] including both cortical bone and the bone marrow cavity (Fig. 1), on seven transaxial planes. Tissue density value of 1 g/mL was used to convert flow values per milliliter of tissue to grams of tissue because bone marrow largely accounts for the

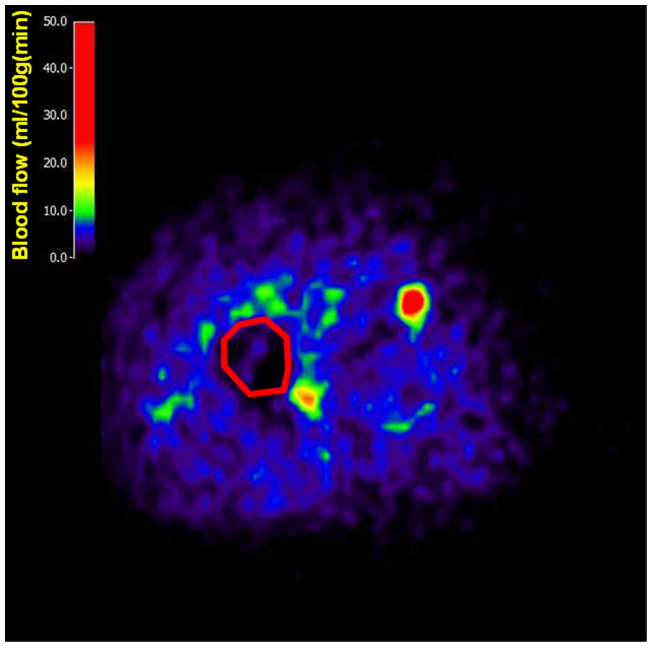

Fig. 1. A representative cross-sectional blood flow image from the middle thigh region, where the region of interest (ROI) has been drawn in femoral bone.

site of blood flow in our analyses. Vascular conductance was calculated by dividing blood flow with mean arterial blood pressure.

Part 2

Subjects and study design

Nine healthy young men (age 25 ± 5 years, height 184 ± 6 cm, weight 76 ± 9 kg) participated in this substudy. The purpose, nature, and potential risks of the study were explained for them before they gave their written informed consent to participate. As in part 1, the subjects were requested to abstain from caffeine-containing beverages for at least 24 hours before the experiments and to avoid strenuous exercise within 48 hours prior to the study. The subjects were not taking any regular medication. The study was performed at least 4 hours 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.

As in part 1, the experiment day started with an MRI study to obtain anatomical references for tissues in the femoral region. Then an antecubital vein was cannulated for tracer administration and a radial artery in the contralateral arm was cannulated for blood sampling. Additionally, cannulas were placed under local anesthesia into the femoral artery and vein for local drug infusion (adenosine) and blood sampling, respectively. Then the subject was positioned into the PET scanner with the femoral region in the gantry and the left leg was fastened to a dynamometer. Femoral bone blood flow was measured first under baseline resting conditions and then either during systemic hypoxia (14% inspired O_2 in N_2; equivalent to altitude of ~3400 m) or local adenosine infusion (order of hypoxia and adenosine was randomized between subjects), after which measurements were done during dynamic one-leg exercise, while they were breathing either normal sea-level room air or hypoxic gas. 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 to 80 degrees. During pretesting before the actual experiments, an individually appropriate workload for each subject was chosen so that they could exercise for at least ~10 minutes without fatigue or discomfort. Exercise was estimated to represent ~10 W external one-leg knee extension workload but also involved a substantial isometric component when the load had to be raised or lowered.

During systemic hypoxia measurements, breathing of hypoxic gas was started 5 minutes before imaging. Femoral arterial infusion of adenosine was initiated one minute before the blood flow measurement and continued until the end of the scan (6 minutes in total). The adenosine concentration (1 mg/min/L [thigh volume measured in liters]) was based on the study by Rådegran and Calbet[14] at rest and Barden and colleagues.[22] This concentration has been shown to induce maximal femoral artery blood flow as measured with Doppler ultrasound. During exercise measurements, scanning was started 3 minutes after the onset of exercise and continued until the end of the exercise bout (5.5 minutes of exercise in total).

Bone blood flow was analyzed similarly as in part 1.

Part 3

Subjects and study design

Five healthy men participated in this study (age 25 ± 5 years, height 182 ± 7 cm, weight 77 ± 8 kg, and BMI 23 ± 3). Written informed consent was obtained after the purpose, nature, and potential risks were explained to the subjects. The study protocol was reviewed and approved by the Ethical Committee of Copenhagen and Frederiksberg communities (11–140/03) and the study was performed in accordance with the Declaration of Helsinki.

After two intravenous catheters were put into the antecubital veins in both arms, the subjects were moved to the dynamic knee extensor ergometer[20] and one-leg exercise with the intensity of 25 W was started. Ten minutes after the beginning of the exercise 396 ± 27 MBq of [18F]-FDG in 5 mL of saline was infused. After the injection of the tracer the subject continued kicking for another 25 minutes. Immediately after the exercise subjects were moved into the PET scanner (GE Advance; General Electric Medical Systems, Milwaukee, WI, USA) and both legs distal to the mid-thigh were scanned in three 4-minute time frames from four different areas of the legs. After the emission scans, transmission scans for attenuation correction were performed for the four different areas of the leg using germanium-68 pin sources. The data sets were reconstructed using the filtered back-projection method with a Hanning filter. All data sets were corrected for dead-time and random coincidences. The axial and in-plane resolution of the reconstructed images was approximately 5 mm full-width at half-maximum. Similar regions of interest (ROIs) were drawn in femoral bone as described above in regard to blood flow, and standardized uptake value (SUV) of glucose for the ROIs was calculated as SUV = tissue radioactivity concentration $\times$ injected dose^{-1} $\times$ body weight^{-1}.

Statistical analysis

Statistical analyses were performed using SAS/STAT statistical software (version 9.2; SAS Institute Inc., Cary, NC, USA). The effects of exercise and its intensities and adenosine and hypoxia on blood flow was tested using one-way ANOVA for repeated measurements (exercise intensity as a factor). If a significant main effect(s) was found, pairwise differences were identified using the Tukey-Kramer post hoc procedure. The significance level was set at $p < 0.05$. Results are reported as means $\pm$ SD.

Results

The general cardiovascular responses to the studied physiological perturbations and exercise in blood flow investigations are shown in Table 1 and Table 2, respectively. The blood flow results indicate that in women, isometric exercise increased femoral bone blood flow from rest to low-intensity exercise ($p = 0.01$), but it did not increase further with increasing intensity ($p = 0.86$ for differences between exercise intensities) and only trended to be higher compared to rest (Fig. 2*A*). However, because perfusion pressure, as judged by mean arterial pressure, remained similar between these all four experimental conditions, vascular

Table 1. Heart Rate, Blood Pressure, and Related Calculations During Intermittent Isometric Exercise in Young Healthy Women

	REST	EXE1	EXE2	EXE3
HR (bpm)	68 ± 10	75 ± 12	81 ± 12	90 ± 15*
MAP (mmHg)	94 ± 10	94 ± 6	95 ± 7	97 ± 3
SAP (mmHg)	121 ± 11	121 ± 8	123 ± 12	126 ± 12
DAP (mmHg)	80 ± 12	81 ± 5	81 ± 6	82 ± 7

REST = rest period; EXE = exercise period; HR = heart rate, MAP = mean arterial pressure, SAP = systolic blood pressure, DAP = diastolic blood pressure.

*$p < 0.05$ compared to REST.

conductance was always higher during all intermittent isometric exercise intensities compared to rest (Fig. 2*B*) ($p < 0.05$ in all comparisons).

In men, resting femoral bone blood flow was similar to women ($p = 0.26$) and dynamic one-leg exercise increased it by almost fourfold, $p < 0.001$ (Fig. 3*A*). Similarly as in women, bone vascular conductance was also elevated from rest to exercise (Fig. 3*B*), although in men the mean arterial blood pressure was also enhanced in response to applied dynamic exercise (Table 2, $p < 0.001$). Intra-arterial infusion of adenosine increased bone blood flow and vascular conductance (Fig. 4), respectively, $p < 0.001$, to the level of moderate intensity dynamic exercise ($p = 0.09$), but vascular conductance was 1.6 times higher than during exercise ($p = 0.04$). Breathing of hypoxic air did not change femoral bone blood flow or conductance at rest (Fig. 4) or during exercise (Fig. 3), although vascular conductance trended higher during hypoxic compared to normoxic exercise ($p = 0.08$) (Fig. 3*B*).

Finally, glucose uptake of the exercising leg was 4.7-fold higher compared to resting contralateral leg during one-leg exercise (Fig. 5).

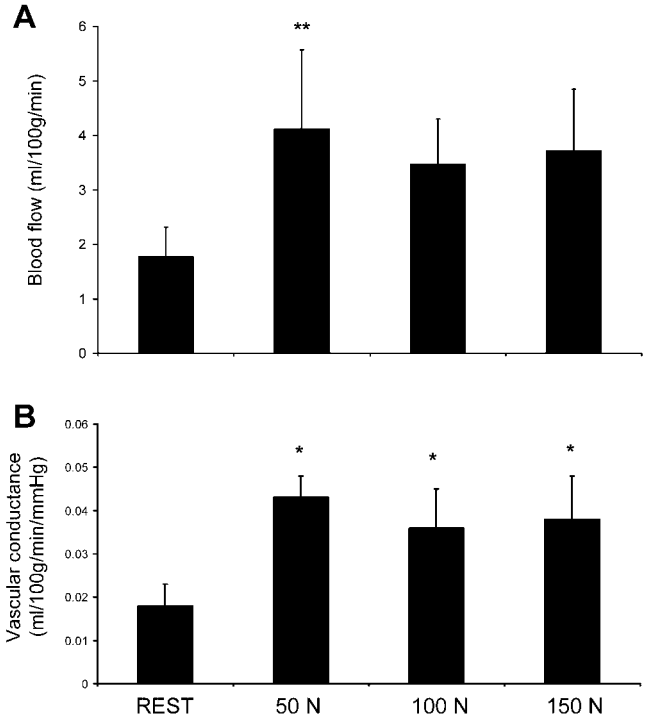

Fig. 2. The effect of intermittent isometric exercise with increasing exercise workloads on femoral bone (*A*) blood flow and (*B*) vascular conductance in young healthy women. **$p < 0.01$ and *$p < 0.05$ compared to rest. N = Newton.

Discussion

We performed this study to elucidate bone blood flow and metabolism in humans because both circulatory aspects and metabolic demands of the bone blood flow in physiologically demanding conditions, such as during physical exercise, remains poorly characterized in humans. Our major novel findings are that bone blood flow increases with physical exercise, but that the increase appears to level off with increasing intermittent isometric exercise intensities. In addition, although moderate systemic hypoxia does not change bone blood flow at rest or during exercise, intra-arterially administered pharmacological vasodilator adenosine is capable of enhancing bone blood flow in humans, to levels similar to those found during dynamic exercise. Furthermore, glucose uptake of the bone was noticed to be enhanced by almost fivefold in response to moderate intensity dynamic exercise, suggesting that metabolic requirements of bone in the moving leg are substantially enhanced.

Table 2. Heart Rate, Blood Pressure, and Arterial Oxygen Parameters at Resting Baseline, Under the Systemic Hypoxia and Local Adenosine Infusion, and During Exercise In Healthy Young Men

	Rest			Exercise	
Physiological measures	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§§§

bpm = beats per minute.

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

Comparisons during exercise: §$p < 0.05$, §§$p < 0.01$, §§§$p < 0.01$ compared to normoxia.

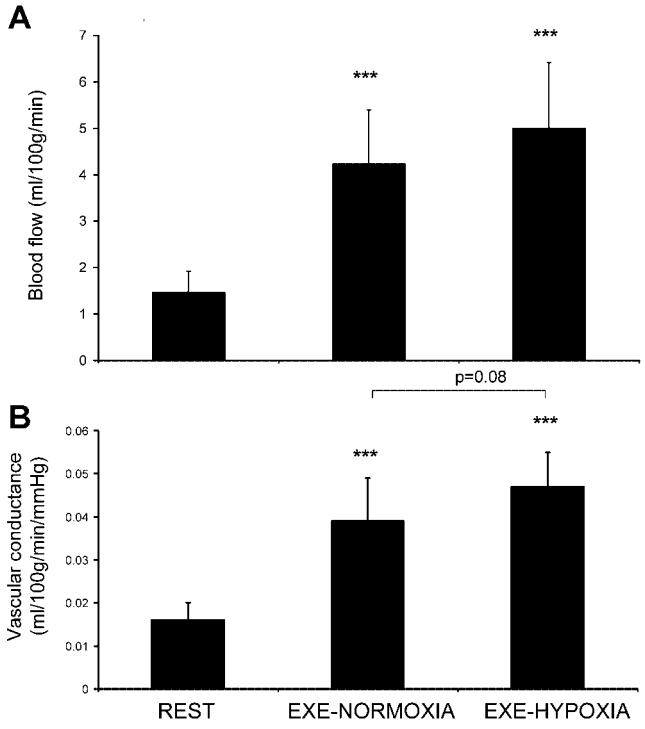

Fig. 3. The effect of dynamic one-leg knee extensor exercise without and with systemic hypoxia on femoral bone blood flow (*A*) and vascular conductance (*B*) in young healthy men. $^{***}p < 0.001$ compared to rest. EXE = exercise.

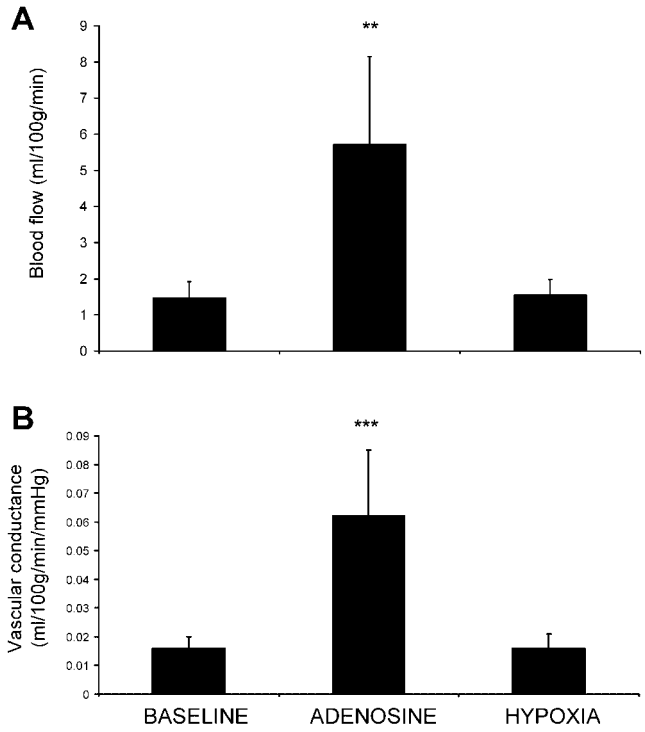

Fig. 4. The effects of intra-arterially infused adenosine and moderate systemic hypoxia on femoral bone blood flow (*A*) and vascular conductance (*B*) in young healthy men. $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to rest and hypoxia.

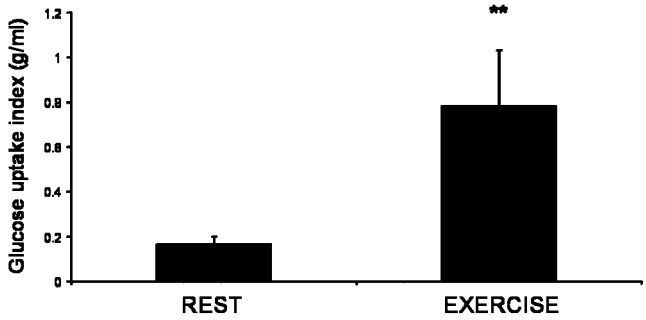

Fig. 5. The effect of dynamic one-leg knee extensor exercise on femoral bone glucose uptake in young healthy men. $^{**}p < 0.01$ compared to rest.

We document in the present study that resting bone blood flow is similar in women and men. Bone blood flow increases in response to both intermittent isometric and dynamic exercise, but appears to level off when intermittent isometric exercise intensity increases. In this respect, bone behaves differently from muscle, in which blood flow is well known to increase with increasing intensities until maximal workload is attained, although in terms of isometric exercise some limitations may be present, especially during the highest intensities and/or workloads. However, we have previously shown that in this isometric exercise model, with intermittent work and resting periods performed at fairly low exercise intensities, muscle blood flow increases proportionally with increasing exercise intensities.[18] Indirectly, these findings suggest that the metabolism of the bone increases from rest to exercise, as confirmed by glucose uptake determinations. These blood flow results are in good agreement with earlier animal studies, which suggest that when exercise intensity is low, bone blood flow may increase, but when intensity increases, vascular resistance in bone also increases and blood flow decreases.[5,13,15–17,23] It is likely that the blood flow levels off with increasing exercise intensity because muscle contractions and relaxations create pressure fluctuations in the bone marrow, and during muscle contractions the exciting veins can be occluded, especially when contraction intensity increases, resulting in increased pressure and thus reduced flow in bone marrow.[4,8] It is also highly likely that adrenergic nervous constraints play a role in this constrictor response,[13] but higher exercise intensities and modalities that involve larger muscle mass are likely needed to elicit this constrictor response.[13,15–17] Other putative mechanisms may also be involved, but they remain to be studied in humans. Interestingly, however, the increase in vascular resistance, and thus decrease in conductance, during whole-body treadmill exercise in awake animals is more prevalent in bones other than the femur.[13] Also, in the femur, the increase in vascular resistance occurs largely in bone regions other than the marrow cavity, where blood flow remains unchanged in response to exercise with increasing intensities.[13] Although resolution limitations do not allow us to differentiate cortical blood flow from blood flow in marrow, in our analysis bone marrow likely accounts for a major part of blood flow in the femur (Fig. 1). Therefore, in this regard, increased vascular conductance from rest to exercise in both intermittent isometric

and dynamic exercise in the present study is the finding that can be expected, and suggests that active vasodilation occurs in bone marrow in response to exercise, but the mediators of this phenomenon remains to be identified in humans.

In contrast to an animal study that showed reduced bone blood flow in response to systemic arterial hypoxia,[13] we did not observe in the present study either elevated or reduced blood flow in bone while subjects were breathing moderately hypoxic gas. As it appears that bone blood flow is under the control of sympathetic nervous system,[13] it is likely that in our study hypoxia did not create sufficiently high stimulus to sympathetic nervous system blood flow to be reduced, at rest or during exercise. Moreover, the finding also suggests that constrictive stimulation of arterial chemoreceptors predominates over a local hypoxic vasodilation, in bone in humans. However, the powerful vasodilator, adenosine, which was locally infused into the femoral artery, increased bone blood flow substantially. In fact, the blood flow in bone during adenosine was much higher than it has been previously observed by direct intra-arterial infusion of sodium nitroprusside,[21] and comparable to that of during dynamic exercise. Interestingly, vascular conductance during the infusion was even higher than during exercise, indicating that human bone blood flow and vascular conductance can also be increased to physiologically relevant levels with vasodilator drugs. Also in this respect our results are well in line with the previous results in anesthetized dogs, in which adenosine significantly reduced bone vascular resistance from resting baseline.[13] Therefore, in general, it appears that many of the previous findings from animal studies directly applies to humans as well, but had remained uncharacterized until the present study.

Clinical implications

Even if the present study is basic in nature, there are a number of points that might also be clinically meaningful. First, our results provide mechanistic insight that likely largely account for many of the beneficial effects of physical activity in strengthening bone.[1–4] Thus, the beneficial changes in mineral content and structure are likely made possible by increased blood flow that we document here, which supplies bone with nutrients and oxygen in accordance with its metabolic needs,[4–8] which are also increased in response to exercise according to the present results. In comparison to other connective and structural tissues of the body, it appears that the metabolism of bone is substantially larger than the metabolism of tendons in a similar exercise model that was also applied here.[24] Second, the fact that bone blood flow can be substantially increased by pharmacological means may provide some underlying mechanistic explanations of the findings, which showed positive effects of nitrates, potential vasodilators, on bone mineral density.[25,26]

Limitations

As a result of the imaging limitations, the exercise models applied in the present study were fairly localized small muscle mass exercises that also induce little vibration stimulus for bone, which is known to affect bone metabolism substantially, and it may be that the results would have been slightly different if certain whole-body exercise such as running could have been used as the exercise model. Additionally, because of the resolution limitations, differentiating cortical bone from marrow cavity in femur in the present study was not reliable; but as can also be seen from the illustration in Fig. 1, showing the imaged thigh region, bone marrow likely accounts for a major part of blood flow and glucose uptake in the femoral bone in the present study, which is also generally known based on animals studies using microspheres.[13,27] Finally, although the femoral bone represents one of the most important load-bearing and muscle-moving bones in a human body, the characteristics of blood flow and metabolism of bones other than the femoral bone warrant further investigation in humans.

In conclusion, resting femoral bone blood flow is similar in healthy young women and men and increases by physical exercise, but levels off with increasing intermittent isometric exercise intensities. Moreover, although moderate systemic hypoxia does not change bone blood flow at rest or during exercise, an intra-arterially administered pharmacological powerful vasodilator, adenosine, is capable of markedly enhancing bone blood flow and vascular conductance in humans. Finally, enhanced metabolic requirements of bone in the moving leg were documented in the present study; glucose uptake of the bone was found to be elevated by almost fivefold in response to moderate-intensity dynamic exercise. Altogether, these physiological results in healthy young subjects in vivo are likely to help in better understanding the beneficial effects of physical activity in strengthening bone.[1–4]

Disclosures

All authors state that they have no conflicts of interest.

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Authors' roles: Conceived and designed the experiments: IH, Juhani Knuuti, HL, RB, MK, KKK. Performed the experiments: IH, Jukka Kemppainen, KK, HL, RB, KKK. Analyzed the data: IH, KKK. Contributed reagents/materials/analysis tools: IH; Juhani Knuuti, RB, MK, KKK. Wrote the manuscript: IH. Edited and approved the final draft of the manuscript: All authors.

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