Myocardial blood flow and adenosine A_{2A} receptor density in endurance athletes and untrained men

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Previous human studies have shown divergent results concerning the effects of exercise training on myocardial blood flow (MBF) at rest or during adenosine-induced hyperaemia in humans. We studied whether these responses are related to alterations in adenosine A_{2A} receptor ($A_{2A}R$) density in the left-ventricular (LV) myocardium, size and work output of the athlete's heart, or to fitness level. MBF at baseline and during intravenous adenosine infusion, and $A_{2A}R$ density at baseline were measured using positron emission tomography, and by a novel $A_{2A}R$ tracer in 10 healthy male endurance athletes (ET) and 10 healthy untrained (UT) men. Structural LV parameters were measured with echocardiography. LV mass index was 71% higher in ET than UT ($193 \pm 18 \, \mathrm{g \, m^{-2}}$ *versus* $114 \pm 13 \, \mathrm{g \, m^{-2}}$, respectively). MBF per gram of tissue was significantly lower in the ET than UT at baseline, but this was only partly explained by reduced LV work load since MBF corrected for LV work was higher in ET than UT, as well as total MBF. The MBF during adenosine-induced hyperaemia was reduced in ET compared to UT, and the fitter the athlete was, the lower was adenosine-induced MBF. $A_{2A}R$ density was not different between the groups and was not coupled to resting or adenosine-mediated MBF. The novel findings of the present study show that the adaptations in the heart of highly trained endurance athletes lead to relative myocardial 'overperfusion' at rest. On the other hand hyperaemic perfusion is reduced, but is not explained by $A_{2A}R$ density.

Since oxygen extraction in the myocardium is high, increases in myocardial blood flow (MBF) are of importance for matching substrate delivery to demands of the heart (Feigl, 1983). It is known that in human skeletal muscle, blood flow capacity is increased through endurance training (Snell *et al.* 1987; Martin *et al.* 1987). Endurance training also enhances coronary blood flow capacity in laboratory animals (Laughlin *et al.* 1998), but no consensus has been reached on whether endurance-trained athletes (ET) have a better MBF reserve than untrained (UT) humans. While some studies suggest that ET have a supranormal coronary blood flow reserve (Toraa *et al.* 1999; Hildick-Smith *et al.* 2000; Kjaer *et al.* 2005), some do not support the view (Heiss *et al.* 1976; Radvan *et al.* 1997; Kalliokoski *et al.* 2002).

A possible interfering factor in human studies is the size of the athlete's heart. Extreme phenotypes of the athlete's heart can structurally resemble hypertrophic cardiomyopathy, which is associated with reduced MBF reserve (Camici & Crea, 2007). However, it is not known to what extent the adaptations in the athlete's heart (Maron & Pelliccia, 2006) reflect maladaptive cardiac hypertrophy or how MBF and flow reserve are affected. Typically, endurance-trained athletes with varying left ventricle (LV) mass are reported to have similar or reduced basal MBF compared to untrained humans, despite similar or increased MBF reserve as determined by adenosine-induced vasodilatation (Heiss *et al.* 1976; Radvan *et al.* 1997; Toraa *et al.* 1999; Hildick-Smith *et al.* 2000; Kalliokoski *et al.* 2002; Kjaer *et al.* 2005; Laaksonen

et al. 2007) However, pronounced LV hypertrophy may impact total MBF and flow reserve per unit ventricular mass.

In addition to the importance of global MBF and blood flow reserve in health and disease, the regulatory aspects of MBF are also of great interest. It is well recognized that control of MBF involves a dynamic interplay of neural, mechanical and metabolic mechanisms (Feigl, 1983; Duncker & Bache, 2008). Metabolic regulation is still considered to exert a major role for matching flow to the local energy demand in the myocardium by altering vascular resistance in the coronary circulation (Camici & Crea, 2007; Duncker & Bache, 2008). The role of adenosine as a metabolic regulator of MBF in humans is apparent (Edlund & Sollevi, 1995; Namdar *et al.* 2006), although some animal studies cast a doubt in this belief, as recently reviewed (Tune *et al.* 2004). The effects of adenosine are mediated via four different adenosine receptors; A_1, A_{2A}, A_{2B} and A_3 (Shryock & Belardinelli, 1997). Adenosine is a widely used agent to study coronary perfusion and evidence from animal studies suggests that adenosine-induced vasodilatation in the coronary circulation is mediated primarily through A_{2A} receptors (A_{2A}Rs) (Belardinelli *et al.* 1998; Hein *et al.* 1999, 2001). However, no study has sought to determine whether this is also the case in humans *in vivo*. Interestingly, the density of A_{2A}Rs in the human heart has been found to be higher in endurance-trained triathletes (Mizuno *et al.* 2005). However, it is not known whether A_{2A}R density and MBF are interrelated in humans, especially during adenosine-induced hyperaemia, and whether increased LV mass in athletes has an effect on A_{2A}R density.

The purpose of the present study was to investigate the relation of MBF at rest and during adenosine-induced vasodilatation to A_{2A}R density and LV mass in endurance-trained athletes and untrained men. It was hypothesized that ET have higher MBF reserve and adenosine A_{2A}R density in the LV. Additionally, based on animal studies addressing adenosine receptor expression implicating an important role of A_{2A}R in adenosine-induced coronary vasodilatation, it was expected that A_{2A}R density and MBF would be proportional, thus subjects with higher adenosine A_{2A}R density also exhibit a larger capacity to increase MBF during hyperaemia.

Methods

Subjects

Twenty healthy men volunteered for the study (Table 1). Ten of them were highly trained endurance athletes (ET) and 10 were untrained men (UT). The subjects were apparently healthy as determined by health questionnaire and medical screening by a doctor in addition to pre-ECG

Table 1. Characteristics of the subjects

	ET	UT	P value
Age (years)	24.6 ± 3.6	25.7 ± 4.2	0.54
Height (cm)	182.3 ± 7.5	179.3 ± 6.6	0.35
Weight (kg)	78.1 ± 6.6	78.1 ± 7.0	1
BMI (kg m^{-2})	23.4 ± 0.9	24.4 ± 2.9	0.33
BSA (m^2)	1.99 ± 0.13	1.97 ± 0.09	0.60
Body fat (%)	9.7 ± 2.4	19.0 ± 3.9	<0.001
Power$_{max}$ (W kg^{-1})	4.7 ± 0.3	3.5 ± 0.4	<0.001
Power$_{max}$ (W)	367 ± 26	271 ± 35	<0.001
$\dot{V}_{O_2,max}$ (ml kg^{-1} min^{-1})	61.7 ± 5.0	46.2 ± 3.2	<0.001
$\dot{V}_{O_2,max}$ (l min^{-1})	4.7 ± 0.3	3.5 ± 0.3	<0.001

BMI, body mass index; BSA, body surface area; Power$_{max}$, highest workload in $\dot{V}_{O_2,max}$ test.

evaluation. The subjects were not under any medication and were normotensive non-smokers with no history of hypercholesterolaemia and no family history of coronary disease. The purpose, nature and potential risks were verbally explained to the subjects before they gave their written informed consent to participate. 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 ET participants in the study were cross-country skiers, who also trained and competed in other endurance sport events such as running, cycling and orienteering. The training volume of their last training season was on average 626 ± 61 h in a year. The ET had been training on a regular basis for 12.3 ± 3.9 years, 8.7 ± 1.4 times and 12.8 ± 1.5 h a week primarily in endurance exercise at various intensities. They had started sport training at 12.5 ± 2.4 years of age, competed in cross-country skiing for 15.6 ± 4.5 years, and won numerous medals especially in national junior championships, and also at the senior level. Thus, the athletes that participated in the present study were among the best Finnish athletes in cross-country skiing or orienteering. The UT men, on the other hand, had been performing physical activity only occasionally, less than three times per week, mainly walking, jogging, gym, ball games and swimming, for less than one hour at a time, in addition to other physical activities such as commuting by bike or walking.

Study design

Before the PET experiments, an echocardiography (ECHO) study was performed as described below. PET studies were performed at least 2 h after a light breakfast. The subjects were instructed to abstain from caffeinated drinks and to avoid exhausting exercise 48 h prior to the study. Before the PET experiment, an antecubital vein was cannulated for the administration of tracers. For blood

sampling, a radial artery cannula was placed under local anaesthesia in the opposite hand. Thereafter, the subject was positioned into the PET scanner in a supine position with the thoracic region in the gantry. In the PET study (as a PET study), myocardial blood flow (MBF) was measured at rest and during adenosine administration with PET and ^{15}O-labelled water $[^{15}O]H_2O$), which was produced as previously described (Sipilä *et al.* 2001). After the MBF measurements, A$_{2A}$R density was measured with a recently developed tracer, [7-methyl-^{11}C]-(E)-8-(3,4,5-trimethoxystyryl)-1,3,7-trimethylxanthine [^{11}C]TMSX) and PET (Mizuno *et al.* 2005).

PET measurements

The PET imaging was performed using either ECAT EXACT HR + PET scanner (Siemens/CTI, Knoxville, TN, USA) or GE Advance PET scanner (General Electric Medical System, Milwaukee, WI, USA). The subjects as a whole and subgroups studied were evenly distributed between these scanners but the same scanner was used for a single subject. Before the emission scans, a transmission scan for the correction of photon attenuation was performed. MBF measurements were performed first at baseline and then during adenosine infusion. At baseline, $[^{15}O]H_2O$ (1070 ± 130 MBq) was infused intravenously and a dynamic PET scanning was performed. After radioactivity decay, a similar perfusion measurement was repeated (1029 ± 120 MBq of $[^{15}O]H_2O$) during 7 min of intravenous infusion of adenosine (140 μg min^{-1} (kg body weight)$^{-1}$). Distribution volume (DV) of A$_{2A}$Rs in the cardiac left ventriculum (LV), thus the density of A$_{2A}$Rs in LV, was determined according to the established principles of Mizuno *et al.* (Mizuno *et al.* 2005) by intravenous bolus injection (430 ± 130 MBq) of [^{11}C]TMSX tracer and measured uptake of the tracer with dynamic PET imaging for 60 min in two-dimensional mode. Plasma radioactivity was measured from the arterial blood and unaltered [^{11}C]TMSX in the plasma was analysed by HPLC (Mizuno *et al.* 2005). Adenosine A$_{2A}$R DV values were obtained and are reported (and related correlations) from 16 subjects (9 athletes and 7 untrained men).

PET data analysis

Regions of interest (ROIs) encompassing the anterior, septal and lateral myocardial walls, and all three together – the whole LV – were drawn manually on six transaxial slices of the left ventricle (LV), and another ROI was drawn in the LV cavity. MBF was calculated using the previously introduced methods employing the single-compartment model (Iida *et al.* 1995). Measured MBF reserve was defined as a ratio of the perfusion during adenosine administration to the flow at rest. Total MBF in the whole

LV was determined as the product of the measured MBF and the LV mass measured with ECHO. In addition, for normalizing the effect of different LV work loads on MBF observed between the groups, individual measured baseline MBF was corrected by multiplying it with LV work obtained from the whole subject population and divided by the individual LV work. Coronary resistance was calculated by dividing the mean arterial pressure (MAP) by the respective perfusion value, and coronary vascular resistance index was defined as vascular resistance during adenosine infusion divided by resistance at baseline. DV of A$_{2A}$R was calculated with Logan's graphical analysis for irreversible tracers (Logan *et al.* 1990) using the time–activity curves (TACs) of myocardium and the metabolite, spillover and partial volume-corrected TAC of plasma.

Echocardiographic measurements and analyses

ECHO measurements and analyses for LV parameters were performed using a commercially available ultrasound scanner (Acuson 128 XP-10 ultrasound system, Siemens, USA) according to the recommendations of the American Society of Echocardiography by an experienced specialist in cardiology as previously described (Kalliokoski *et al.* 2002). Total LV work output was calculated as a product of MAP and cardiac output. Relative LV work output per unit mass was calculated by dividing total LV work output by LV mass.

Other measurements

A continuous incremental $\dot{V}_{O_2,max}$ bicycle test with electrically braked cycle ergometer and direct respiratory measurements was performed for the evaluation of fitness level. Volumes of oxygen consumed ($\dot{V}_{O_2,max}$) during exercise were obtained from minute ventilation, and maximal load at the end of the test was measured. For the UT, the fitness test was performed at Paavo Nurmi Centre, University of Turku, Turku, Finland with Ergoline 800 S ergometer (Ergoline, Bitz, Germany) and $\dot{V}_{O_2,max}$ was measured with a Medikro 202 gas analyser (Medikro Oy, Kuopio, Finland). Since the ET were recruited from Central Finland, the fitness test for them was performed with a Monark ergomedic 839E ergometer (Monark Exercise AB, Vansbro, Sweden) and $\dot{V}_{O_2,max}$ was obtained with Medgraphics Cardiorespiratory diagnostic system (Medical Graphics Corporation, St Paul, USA) at the Department of Biology of Physical Activity, University of Jyväskylä, Finland. Additionally, percentage body fat (skinfolds) was measured and a specific questionnaire concerning physical activity and health state was obtained from the subjects.

Table 2. Echocardiography parameters of the study subjects

	ET	UT	P value
EDD-l (mm)	106.6 ± 7.1	96.9 ± 4.2	<0.001
EDD (mm)	58.1 ± 2.5	53.2 ± 3.1	<0.01
SWd (mm)	12.4 ± 0.8	9.0 ± 0.9	<0.001
PWd (mm)	12.3 ± 0.7	9.1 ± 0.9	<0.001
AWd (mm)	12.3 ± 0.7	9.0 ± 0.9	<0.001
LV mass (g)	385 ± 43	225 ± 34	<0.001
LV mass index (g m^{-2})	193 ± 18	114 ± 13	<0.001
SV (ml)	110 ± 8	91 ± 10	<0.001
CO (l min^{-1})	5.8 ± 1.1	5.6 ± 0.9	0.60
EF (%)	66.0 ± 4.5	66.3 ± 3.9	0.87
FS (%)	37.1 ± 2.9	37.3 ± 3.6	0.89
E/A ratio	1.8 ± 0.2	2.2 ± 0.6	0.07

EDD-l, longitudinal end-diastolic diameter; EDD, end-diastolic diameter; SWd, septal wall thickness in diastole; PWd, posterior wall thickness in diastole; Awd, anterior wall thickness in diastole; SV, stroke volume; CO, cardiac output; EF, ejection fraction; FS, fractional shortening; E/A ratio, ratio between early and late diastolic filling rate.

Statistical analysis

Statistical analysis was performed using SAS/STAT statistical software (version 8.2, SAS Institute Inc., Cary, NC, USA). Two-way ANOVA for repeated measurements was used for the analysis of statistical differences between the values obtained at baseline, during adenosine and [^{11}C]TMSX administration, and between the groups. Student's two-tailed *t* test was used for comparisons of group differences in body composition, fitness test parameters and echocardiographic measurements. Correlation values were calculated using Pearson's correlation coefficient. *P* values < 0.05 were considered statistically significant. All data are shown as mean ± S.D.

Results

Characteristics of the subjects

The groups were closely matched in age, height, weight and body mass index (BMI) (Table 1), but differed in body fat percentage, fitness level and the LV structural parameters as determined by ECHO (Table 2). On average, both the total LV mass and the body surface area (BSA)-normalized LV mass were 71% higher in ET compared to UT subjects.

Haemodynamic parameters during imaging

Heart rate, blood pressure, and LV work values during the PET measurements are shown in Table 3. Adenosine infusion increased heart rate, but had no effect on blood pressure parameters. All haemodynamic parameters except systolic blood pressure were similar between the groups during the imaging procedures.

Myocardial blood flow (Table 3) (Figs 1–3)

Adenosine infusion increased MBF ~5-fold both in the whole LV (Fig. 3) as well as in all three regions of it (Fig. 2) as compared to the resting values. ET had lower measured MBF per gram of tissue both at rest and during adenosine infusion (Figs 1 and 3), but the MBF reserve was similar between the groups (Fig. 3). When calculated for the whole LV, total LV MBF was substantially higher in ET than UT both at baseline (217 ± 61 *versus* 155 ± 28 ml min^{-1}, respectively, *P* < 0.001) and during adenosine (1056 ± 272 *versus* 777 ± 223 ml min^{-1}, *P* < 0.001). When MBF was normalized for myocardial work, baseline MBF was significantly higher in ET than in UT (*P* < 0.01) (Fig. 1). Coronary resistance was higher in ET than UT at baseline (156 ± 32 *versus* 122 ± 15 mmHg ml min^{-1} g^{-1}, respectively, *P* < 0.04), but the difference was not statistically significant during adenosine-induced hyperaemia (34 ± 11 and 25 ± 7 mmHg ml min^{-1} g^{-1} in ET and UT, respectively, *P* = 0.17) and coronary vascular resistance index was similar in both groups (0.23 ± 0.08 in ET and 0.21 ± 0.06 in UT, *P* = 0.65).

Adenosine A$_{2A}$R density

The total volume of cardiac A$_{2A}$R in LV was significantly higher in ET compared to UT (Fig. 4*A*), but the DV of A$_{2A}$R (receptor density) did not differ between the groups (Fig. 4*B*). Spillover and partial volume-corrected DV values did not differ from the reported uncorrected values (*P* = 0.7). Only a small fraction of [^{11}C]TMSX was metabolized during the scan duration (< 20% at 60 min). There were no differences in metabolism (*P* = 0.7) nor in Area under curve (AUC) of TACs (*P* = 0.4) between the groups (data not shown).

Relations of measured parameters

In the whole subject population, LV mass and LV mass index correlated negatively with MBF at rest (*r* = −0.46, *P* = 0.04 and *r* = −0.49, *P* = 0.03, respectively) and almost significantly during adenosine infusion (*r* = −0.43, *P* = 0.057 and *r* = −0.42, *P* = 0.067, respectively), but not with MBF reserve. In addition, a negative correlation between MBF at rest and $\dot{V}_{O_2,max}$ (*r* = −0.59, *P* < 0.01) was observed. However, significance levels of these correlations were not reached when parameters were correlated separately in the two groups.

Table 3. Haemodynamic variables during the PET measurements

	Rest		ADO		[^{11}C]TMSX	
	ET	UT	ET	UT	ET	UT
HR (beats min^{-1})	47 ± 7***	59 ± 8	80 ± 9†***	103 ± 11†	47 ± 6***	61 ± 8
BPs (mmHg)	123 ± 7*	115 ± 11	127 ± 8*	117 ± 10	126 ± 12*	115 ± 11
BPd (mmHg)	64 ± 5	68 ± 7	66 ± 5	67 ± 8	70 ± 9	69 ± 8
MAP (mmHg)	84 ± 4	84 ± 8	86 ± 5	84 ± 9	89 ± 9	84 ± 8
LV work (mmHg l min^{-1} g^{-1})	1.1 ± 0.2***	2.0 ± 0.4	2.0 ± 0.2†***	3.5 ± 0.6†	1.2 ± 0.2***	2.1 ± 0.5
Total LV work (mmHg l min^{-1})	433 ± 79	448 ± 82	753 ± 89†	765 ± 93†	474 ± 103	456 ± 85
MBF (ml min^{-1} g^{-1})	0.56 ± 0.13***	0.69 ± 0.09	2.75 ± 0.75***	3.48 ± 0.94	—	—
Normalized MBF (ml min^{-1} g^{-1})	0.79 ± 0.16**	0.56 ± 0.13	—	—	—	—
Total MBF (ml min^{-1})	217 ± 61***	155 ± 28	1056 ± 272***	777 ± 223	—	—
Coronary resistance (mmHg ml min^{-1} g^{-1})	156 ± 32*	122 ± 15	34 ± 11	25 ± 7	—	—

Rest, ADO and [^{11}C]TMSX represent values at baseline, during adenosine and [^{11}C]TMSX infusions, respectively. HR, heart rate; BPs, systolic blood pressure; BPd, diastolic blood pressure; MAP, mean arterial blood pressure; LV work, left ventricular myocardial work; MBF, myocardial blood flow. Adenosine increased HR as well as relative and total LV work significantly from rest to ADO in both groups, †$P < 0.001$. Percentage increase in these parameters was similar between the groups (∼75%). However, adenosine induced no changes in BPs, BPd and MAP, but ET had higher BPs, and lower HR and LV work in every measurement, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ compared to UT.

Measured MBF at rest or during adenosine administration in the LV did not correlate significantly with A$_{2A}$R DV values in the whole study population or within the groups (P not significant in any group). However, in all subjects, DV in the whole LV tended to be negatively related to measured MBF reserve ($r = -0.46$, $P = 0.07$). In addition, there was a tendency for a negative relation of whole LV [^{11}C]TMSX DV values and LV mass in ET ($r = -0.63$, $P = 0.07$) but not in UT ($r = -0.38$, $P = 0.27$). Finally, the fitter the athlete was, the lower the MBF during adenosine-induced hyperaemia (Fig. 5).

Discussion

Human studies have shown divergent results concerning the effects of exercise training on MBF in healthy subjects and furthermore, the mechanisms of the detected changes have remained unresolved. We measured myocardial perfusion at rest and during adenosine-induced hyperaemia in absolute terms in untrained and trained subjects. Furthermore, we studied whether the training responses are related to structural or functional changes in the athlete's heart or alterations in cardiac A$_{2A}$R density. The results show that elite endurance athletes had significantly lower MBF per gram of tissue both at rest and during adenosine-induced vasodilatation as compared with untrained but relatively fit subjects. Due to clearly increased cardiac mass in the athletes the whole heart total MBF is, however, significantly increased while the flow reserve is unchanged. Furthermore, when MBF is related to the myocardial work at rest, i.e. the energy demands of heart, MBF per gram of tissue was also significantly higher in athletes than untrained men, suggesting relative overperfusion of elite athletes'

myocardium. Finally, no differences in adenosine A$_{2A}$R density was found between the groups and A$_{2A}$R density was not coupled with MBF.

Structural and functional changes in the athlete's heart are considered a favourable physiological phenomenon with no known harmful consequences except for increased incidence of atrial fibrillation at older age (Karjalainen *et al.* 1998). However, extreme forms of myocardial hypertrophy due to heredity and vigorous physical conditioning can resemble a structural heart disease such as hypertrophic cardiomyopathy, which is associated with reduced MBF reserve (Camici & Crea, 2007) and substantially increased risk of cardiac event (Maron & Pelliccia, 2006; Thompson *et al.* 2007). LV cavity dimensions and wall thicknesses of athletes in the present study were in the near
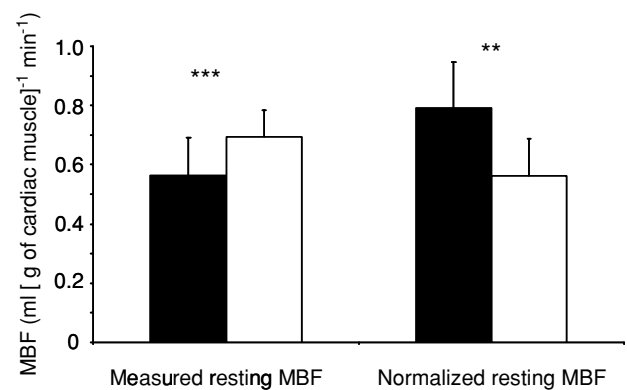

Figure 1. Measured and normalized myocardial blood flow (MBF) at rest
Filled bars, male endurance athletes (ET); open bars, healthy untrained men (UT). **$P < 0.001$ and ***$P < 0.001$. After considering the significantly lower myocardial work load of ET, ET showed significantly higher MBF per gram of cardiac tissue at rest.
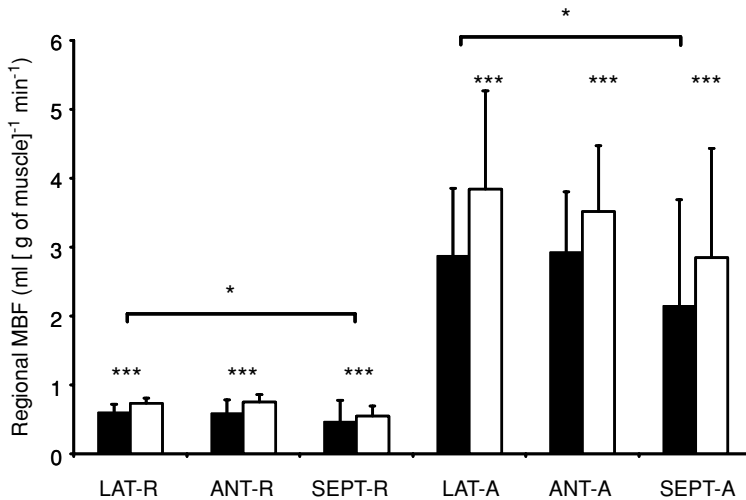
Figure 2. Regional MBF

ET (filled bars) had lower MBF in all three regions of LV compared to UT men (open bars) both at rest and during adenosine-induced hyperaemia (ADO), ***$P < 0.001$. LAT-R, MBF in lateral part of LV at rest and during ADO (LAT-A); ANT-R, MBF in anterior part of LV at rest and during ADO (ANT-A); SEPT-R, MBF in septal part of LV at rest and during ADO (SEPT-A). Among all subjects, MBF in lateral region of LV was higher compared to septum, *$P < 0.05$ both at rest and during ADO, but not compared to anterior region ($P = 0.94$). There was no statistically significant difference in MBF between anterior and septal regions ($P = 0.06$).

upper normal limits observed previously in world-class athletes (Pelliccia *et al.* 1991, 1999). LV mass normalized to BSA ranged in athletes from 159 g m^{-2} to 221 g m^{-2}, even exceeding the upper normal limits found among athletes (Pelliccia *et al.* 1991, 1999; Rodriguez Reguero *et al.* 1995; Pluim *et al.* 2000).

Myocardial blood flow at rest

To the best of our knowledge, there are no reported studies of MBF in athletes with as high LV mass as the subjects had in the present study. Untrained subjects in the present study had good fitness, as judged by their relatively low heart rate and high $\dot{V}_{O_2,max}$, highlighting our findings with respect to the two groups. Compared to the previous PET study where highly trained rowers were studied (Kjaer *et al.* 2005), our athletes had on average 51% higher LV mass index (128 *versus* 193 g m^{-2}, respectively). In line with the study by Kjaer and colleagues, uncorrected MBF was significantly lower in ET than UT at rest in the present study. In other previous human studies with PET, uncorrected MBF has been similar between the trained and untrained groups at rest

(Radvan *et al.* 1997; Toraa *et al.* 1999; Kalliokoski *et al.* 2002; Laaksonen *et al.* 2007) despite the larger LV mass in athletes. Given the known close relationship between LV work output, myocardial oxygen consumption, and myocardial blood flow, it is necessary to relate the measured blood flow values to LV work load. When this was done in the present study, MBF at rest was significantly higher in ET than UT. This leads to two alternative consequences: athletes had either (1) impaired myocardial efficiency of work with comparable myocardial oxygen extraction between the groups or (2) reduced oxygen extraction with unchanged efficiency of work.

Myocardial oxygen extraction and efficiency have been sparsely studied in athletes mainly due to methodological difficulties. Takala and colleagues found no differences in myocardial efficiency and oxygen extraction between ET and UT subjects during insulin clamp at rest (Takala *et al.* 1999). A recent report of a study performed with monozygotic twins discordant for physical activity and fitness showed that myocardial oxygen extraction tended to be lower and efficiency higher at rest in the more active and fitter group, despite relatively small difference in fitness level between the groups (Hannukainen *et al.* 2007).
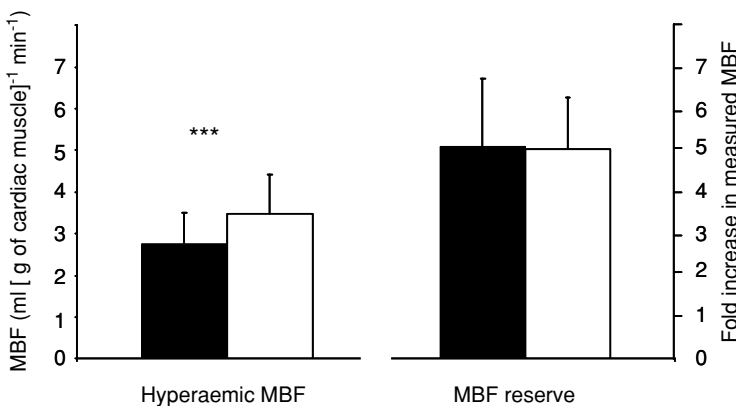

Figure 3. Adenosine-induced hyperaemic myocardial bood flow (MBF) and measured MBF reserve

Filled bars, ET; open bars, UT. Adenosine increased MBF significantly compared to resting MBF (Fig. 1) in both groups, $P < 0.001$. Endurance athletes had significantly lower hyperaemic MBF, but their MBF reserve calculated from measured MBF values was similar compared to untrained men. ***$P < 0.001$.
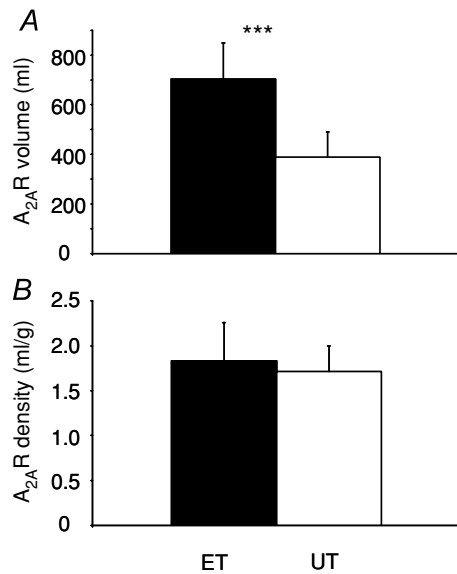
Figure 4. Total volume (*A*) and density (*B*) of myocardial adenosine A$_{2A}$R

No difference in A$_{2A}$R density was found, but ET had a higher total volume of A$_{2A}$Rs.

Additionally, in a study by Heiss and colleagues (Heiss *et al.* 1976), resting oxygen extraction also tended to be lower in endurance athletes than in untrained subjects (calculated from the individual data presented in the article). Thus, although solid evidence on the relation between flow and oxygen extraction in the present study is lacking the data from this study point to the conclusion that the athletes may have lower myocardial oxygen extraction at rest,

which may then, in turn, allow a higher increase in oxygen extraction whenever myocardial demand is elevated, such as during strenuous exercise. This conclusion is also supported by the well-established fact that fit endurance athletes have substantially higher resting cardiac vagal tone, a well-documented direct coronary vasodilator, which may indeed allow them to take advantage of their larger stroke volume 'overperfusing' more relaxed and bigger coronary arteries, and ultimately, myocardium. This issue however, definitely warrants further research.

Myocardial blood flow during hyperaemia and blood flow reserve

The finding of a similar measured MBF reserve between the groups is concordant with many (Radvan *et al.* 1997; Kalliokoski *et al.* 2002; Hannukainen *et al.* 2007), but not all (Toraa *et al.* 1999; Kjaer *et al.* 2005) previous studies. Although there is some evidence from animal studies that endurance training could lead to improved MBF capacity (Laughlin *et al.* 1998) our findings suggest that endurance-trained athletes expressing remarkable LV hypertrophy have no supranormal MBF reserve. Coronary vasculature may simply increase in parallel with increases in exercise-induced LV mass (Hudlicka, 1982), which is also supported by the known close connection between the size of epicardial coronary arteries and LV mass (Rodriguez & Robbins, 1959; Lewis & Gotsman, 1973; O'Keefe *et al.* 1987; Leung *et al.* 1991; Dodge *et al.* 1992). Yet, it has also been found already decades ago that enlargement of even the healthy coronary arteries may not necessarily
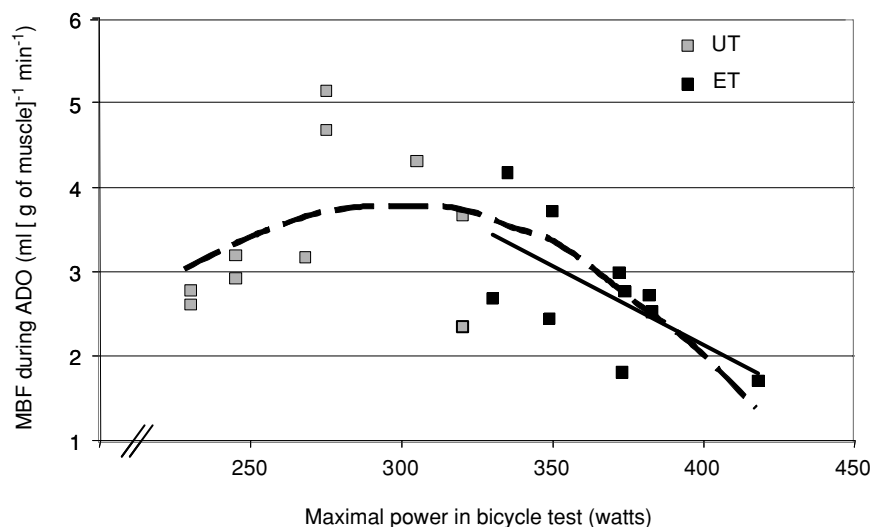

Figure 5. Relationship between maximal sustained power obtained during $\dot{V}_{O_2,max}$ bicycle test (W_{max}) and myocardial blood flow during adenosine infusion (MBF during ADO)

In UT, W_{max} was not related to MBF during ADO ($r = 0.28$, $P = 0.44$, grey squares), but in ET these parameters were inversely related (black squares and line, $r = 0.64$, $P = 0.04$). Interestingly, if the subjects are considered showing a continuum of inborn and acquired fitness, a curvilinear relation between fitness level and MBF during adenosine-induced hyperaemia in the whole subject population is observed (dashed line, $y = 0.0002x^2 + 0.0981x - 10.824$, $r^2 = 0.42$, $P = 0.002$).

keep pace with that of the cardiac mass (Roberts & Wearn, 1941). In this respect, considering the reduced hyperaemic MBF per gram of tissue in our athletes, our findings suggest that pronounced myocardial hypertrophy in healthy athletes closely resembles hypertrophic cardiomyopathy in terms of blood flow (Knaapen *et al.* 2008). Regarding the reduced hyperaemic MBF per gram of myocardium in ET, it was also found that the fitter the athlete, the lower the MBF during adenosine-induced hyperaemia. The mechanism of this is unclear but may involve differing effects of nervous regulation during adenosine stimulation (Kaufmann *et al.* 2007). The possibility also exists that due to a few seconds half-life of adenosine (Moser *et al.* 1989) caused by its rapid cellular uptake through high-affinity nucleoside transporters (Loffler *et al.* 2007) especially in endothelium and erythrocytes abundant in athletes (Heinicke *et al.* 2001), the efficiency of a standard dose of adenosine inducing hyperaemia may not be similar in athletes and untrained men. However, it is unlikely that the degradation of adenosine would be that much different between the groups. Yet, our observation of the effect of fitness level of the subjects on adenosine-induced hyperaemia (Fig. 5) suggests that fitness may at least partly explain previously observed divergent inter-individual responses to intravenously infused adenosine (Chan *et al.* 1992). Another issue that could have modulated the response to adenosine is cardiac $A_{2A}R$ density. In particular, many animal studies addressing adenosine receptor expression have implicated an important role for $A_{2A}R$ in adenosine-induced coronary vasodilatation.

Adenosine $A_{2A}R$ density

In the present study in humans myocardial $A_{2A}R$ density and MBF were not tightly coupled and receptor density was not different between the groups. Athletes are, however, found to have higher total volume of $A_{2A}Rs$ due to their significantly larger LV mass despite similar receptor density. Many animal studies suggest that coronary vasodilatation caused by adenosine is solely or at least primarily mediated via $A_{2A}Rs$ (Belardinelli *et al.* 1998; Hein *et al.* 1999, 2001). Although direct human studies attempting to elucidate the coupling of adenosine-induced vasodilatation and $A_{2A}Rs$ are lacking, it has been shown that specific $A_{2A}R$ agonists cause MBF increases comparable to adenosine with fewer side-effects (Udelson *et al.* 2004; Iskandrian *et al.* 2007), supporting the essential role of $A_{2A}Rs$ also in the vasodilatation of adenosine in humans. In the present study, however, there was a trend of inverse relationship between $A_{2A}R$ density and MBF reserve among all the subjects, which may suggest that the amount/density of $A_{2A}Rs$ may not be the most important factor in adenosine-induced

myocardial hyperaemia in humans. In addition to a likely role of other adenosine receptors modulating (Shryock & Belardinelli, 1997) or directly evoking vasorelaxation, such as A_{2B} (Talukder *et al.* 2003), the divergent interindividual effects of intravenously infused adenosine (Chan *et al.* 1992) may obscure a direct comparison between our non-invasive *in vivo* correlation approach of examing the role of $A_{2A}Rs$ as a predominant adenosine receptor responsible for adenosine-induced vasodilatation in humans. Moreover, it has to also be considered that A_2Rs are not completely cell membrane-fixed receptors but rather, are up-regulated from the cytosol when needed (Milojevic *et al.* 2006). Thus, it may appear that there are, indeed, no differences between the groups at rest, but strenuous exercise for instance may induce receptor up-regulation that could be variable. On the other hand, continuous stimulus due to extensive infusion of adenosine may also induce $A_{2A}R$ down-regulation or inactivation (stimulus desensitization), and possibly more extensively in ET, potentially also explaining their reduced hyperaemic MBF. Finally, one possible reason for poor coupling of $A_{2A}R$ density and MBF is that the extent of adenosine-induced hyperaemia is not due solely to the amount/density of $A_{2A}Rs$, but also the sensitivity and activity of different signalling cascades downstream of $A_{2A}R$ activation is also important. These factors associated with adenosine-induced vasodilatation in humans may have precluded a direct correlation between MBF and $A_{2A}R$ density in the present study.

The current method measures total cardiac $A_{2A}Rs$, but cannot separate the receptors at specific tissue compartments. The density of $A_{2A}Rs$ is high in myocardial resistance vessels and microcirculation, where the vasodilator action of adenosine also happens. This tracer probably binds mostly to the cell surface receptors of endothelial cells, but may also reach smooth and cardiac muscle cells. Since our findings were in contrast to a recent study in which a higher density of $A_{2A}Rs$ in ET triathletes was observed (Mizuno *et al.* 2005), we tested whether possible differences in arterial input curves or tracer metabolism affected the findings, but no differences were found to exist between the groups in these calculations.

In conclusion, the novel findings of the present study indicate pronounced structural changes in the heart of highly trained endurance athletes leading to myocardial 'overperfusion' at rest. This is probably associated with a lower oxygen extraction in athletes, although inefficiency caused by maladaptive cardiac hypertrophy cannot be totally excluded. Furthermore, the density of adenosine $A_{2A}Rs$ is similar in athletes as compared to sedentary people and this is in accord with a similar minimal coronary resistance during adenosine-induced hyperaemia. Finally, adenosine-induced hyperaemia and adenosine $A_{2A}R$ density are not directly coupled and linked with changes in myocardial perfusion.

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