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Elevated endocannabinoid plasma levels are associated with coronary circulatory dysfunction in obesity

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Aims Aim of this study was to evaluate a possible association between endocannabinoid (EC) plasma levels, such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and coronary circulatory function in obesity.

Methods and results Myocardial blood flow (MBF) responses to cold pressor test (CPT) and during pharmacological vasodilation with dipyridamole were measured with ^{13}N-ammonia PET/CT. Study participants ($n = 77$) were divided into three groups based on their body mass index (BMI, kg/m^2): control group $20 \leq$ BMI < 25 ($n = 21$); overweight group, $25 \leq$ BMI < 30 ($n = 26$); and obese group, BMI ≥ 30 ($n = 30$). Anandamide plasma levels, but not 2-AG plasma levels, were significantly elevated in obesity as compared with controls, respectively [0.68 (0.53, 0.78) vs. 0.56 (0.47, 0.66) ng/mL, $P = 0.020$, and 2.2 (1.21, 4.59) vs. 2.0 (0.80, 5.90) ng/mL, $P = 0.806$)]. The endothelium-related change in MBF during CPT from rest (ΔMBF) progressively declined in overweight and obese when compared with control group [0.21 (0.10, 0.27) and 0.09 (-0.01, 0.15) vs. 0.26 (0.23, 0.39) mL/g/min; $P = 0.010$ and $P = 0.0001$, respectively). Compared with controls, hyperaemic MBFs were significantly lower in overweight and obese individuals [2.39 (1.97, 2.62) vs. 1.98 (1.69, 2.26) and 2.10 (1.76, 2.36); $P = 0.007$ and $P = 0.042$, respectively)]. In obese individuals, AEA and 2-AG plasma levels were inversely correlated with ΔMBF to CPT ($r = -0.37$, $P = 0.046$ and $r = -0.48$, $P = 0.008$) and hyperaemic MBFs ($r = -0.38$, $P = 0.052$ and $r = -0.45$, $P = 0.017$), respectively.

Conclusions Increased EC plasma levels of AEA and 2-AG are associated with coronary circulatory dysfunction in obese individuals. This observation might suggest increases in EC plasma levels as a novel endogenous cardiovascular risk factor in obesity, but needing further investigations.

Keywords blood flow • endocannabinoids • circulation • coronary disease • endothelium • obesity

Introduction

Over the last decades there has been a steady increase in the prevalence of obesity in industrialized nations as high as ~30%.[1,2] Since obesity has been recognized as a risk factor of cardiovascular morbidity and mortality, the increasing prevalence of obesity emerges as a considerable public health problem.[2] The mechanisms by which obesity may initiate and accelerate coronary vascular disease remain largely unknown. Previous studies have shown an association between obesity and endothelial

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dysfunction as a functional precursor of the coronary artery disease (CAD) process.[3–5] In particular, the previously reported independent predictive value of obesity for coronary circulatory dysfunction[3,4] suggests direct mediators released from the adipose tissue, so-called adipocytokines, such as leptin, adiponectin, and/or local mediators such as the endocannabinoids (ECs) to be involved in the regulation of coronary vasomotor tone and thus in the initiation and development of CAD.[6–8] Endocannabinoids (EC), such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG), are endogenous bioactive lipid mediators deriving from arachidonic acid, which are physiologically synthesized and released upon demand from the brain, peripheral organs, and adipose tissue and exert their biological effects via interaction with specific G-protein-coupled cannabinoid receptors type 1 (CB1) and type 2 (CB2).[6] In the experimental setting, increases in adipose-derived ECs have been suggested to exert proatherosclerotic effects by signalling via CB1 and/or non-CB receptors in the vascular wall with resultant increases in oxidative stress, vascular smooth muscle cell proliferation, and recruitment of monocytes and neutrophils into the arterial wall.[6,9] This may contrast with recent findings in ApoE$^{-/-}$ mice, in whom the stimulation of CB2 receptor appeared to mediate anti-inflammatory and anti-atherosclerotic effects.[6,10] More recently, elevated plasma levels of the EC 2-AG, but not AEA, were found to be associated with atherosclerotic disease in a hypercholesterolaemic mouse model.[9] In light of this, it is intriguing to hypothesize that an abnormal function of the coronary circulation as a result of increases in EC plasma levels may provide a mechanistic link between obesity and the initiation and progression of cardiovascular disease.

To this end, we aimed to evaluate in normal, overweight, and obese individuals, without traditional cardiovascular risk factors, a possible association between EC plasma levels, such as AEA and 2-AG, and coronary circulatory function.

Methods

Study population and design

The study population comprised 77 individuals (41 men and 36 women; mean age 43.3 ± 11.2 years) with no arterial hypertension (blood pressure <140/90 mmHg), smoking, and diabetes mellitus (fasting plasma glucose obtained on more than two occasions ≤126 mg/dL) (*Table 1*). Study participants underwent an initial screening visit that comprised a physical examination, electrocardiogram (ECG), blood pressure measurements, and routine blood chemistry in a fasting state. Any study applicants with any cardiac or vasoactive medication, a history of variant angina, a family history of premature CAD, or clinically manifest cardiovascular or any other disease were excluded from the study. Physical examination revealed normal findings in all study participants and they had normal resting ECGs. Study participants then underwent ^{13}N-ammonia PET/CT (64-slice Biograph HiRez TruePoint PET-CT scanner, Siemens, Erlangen, Germany) measurements of myocardial blood flow (MBF) at rest and during vasomotor stress in the morning in a fasting state to evaluate coronary circulatory function. A prerequisite for the study inclusion was a normal stress-rest perfusion imaging on ^{13}N-ammonia PET/CT, which widely excluded the presence of flow-limiting CAD lesions.[3] Recruited study participants were subsequently grouped according to their body mass index (BMI, kg/m^2): control group, 20≤ BMI <25 (CON; $n = 21$); overweight group, 25≤ BMI <30 (OW; $n = 26$); obese group, ≥30 (OB; $n = 30$).

Blood chemistry included plasma glucose, haemoglobin A_{1c}, insulin, total cholesterol, HDL and LDL cholesterol, triglycerides levels, and high-sensitive C-reactive protein. Assessment of insulin resistance using the Homeostasis Model Assessment (HOMA) was determined as described previously.[3] In each study participant, EC plasma levels were determined from peripheral arm vein blood samples. Endocannabinoids, AEA, and 2-AG were extracted from 100 μL of human plasma by liquid–liquid extraction and separated by liquid chromatography (Ultimate 3000 RS, Dionex, CA, USA). Analyses were performed on a 5500 QTrap® triple quadrupole/linear ion trap (QqQLIT) mass spectrometer equipped with a TurboIon-SprayTM interface (AB Sciex, Concord, ON, Canada).[11] The study was approved by the University Hospitals of Geneva Institutional Review Board (No: 07-183), and each participant signed the approved informed consent form.

Assessment of myocardial flow with PET/CT

Following the topogram used to define the axial field-of-view and a low-dose CT scan (120 kV, 30 mA) for attenuation correction, PET emission data were acquired during shallow breathing following intravenous injection of ^{13}N-ammonia. At first, PET scanning in fully 3D mode at rest started immediately following injection of ~500–550 MBq of ^{13}N-ammonia for a total duration of 18 min in list-mode format and 21 dynamic frames of the ungated first 6 min data (12 × 10, 3 × 20, 6 × 30 s) were reconstructed using iterative normalized attenuation-weighted ordered subsets expectation maximization (NAW-OSEM). The CT-based attenuation correction map was used to reconstruct the PET emission data. The default parameters used were ordered OSEM with eight iterations and four to six subsets followed by a post-processing Gaussian filter. Time-activity curves from the first 12-dynamic frames (12 for 10 s each), in concert with a two-compartment tracer kinetic model,[12] were used to calculate MBF in mL/g/min of the left ventricle with the use of the PMOD software package (version 2.8 PMOD Technologies Ltd, Zurich, Switzerland).[13] The tracer kinetic model applied, corrected for physical decay of ^{13}N-ammonia, partial- volume-related underestimations of true myocardial tissue concentrations (by assuming a uniform myocardial wall thickness of 1 cm). Further, correction for scatter and random counts, and spillover of radioactivity between the left ventricular blood pool and myocardium was performed according to Hutchins *et al.*[14] Following, 12 min ECG-gated PET emission data were acquired with 10 frames per cardiac cycle. The gated PET emission data were used for the analysis of the relative ^{13}N-ammonia uptake of the left-ventricle and, thus, myocardial perfusion was evaluated visually on reoriented short- and long-axis myocardial slices and semiquantitatively on the corresponding polar map from the last static 18 min transaxial PET image. Second, sympathetic stimulation with cold pressor test (CPT) was performed with the study participant immersing one hand in a slush of ice water. At 60 s, ~500–550 MBq ^{13}N-ammonia was administered and serial PET images were acquired while immersion of the hand in ice water continued for at least another 60 s. The initially acquired low-dose CT scan was applied for attenuation correction of the PET emission data. The CPT-induced and predominantly endothelium-related flow alteration from rest was defined as the difference between rest and CPT-related MBF (ΔMBF). This endothelium-related flow parameter was chosen as it had been described to be widely independent of age, gender, haemodynamic conditions, and resting MBF.[15] Third, hyperaemic MBF was induced with the intravenous standard dose of 140 μg/kg/min with dipyridamole over 4 min. After an additional 3 min at the peak effect of

Table 1 Characteristics of study population ($n = 77$)

	CON ($n = 21$)	OW ($n = 26$)	*P*-value	OB ($n = 30$)	*P*-value
BMI, kg/m^2	21.1 (20.5, 22.6)	26.93 (25.91–28.22)	0.0001	41.3 (31.75–45.45)	0.0001
Waist–hip ratio	0.84 (0.80, 0.88)	0.89 (0.84–0.93)	0.023	0.96 (0.91–0.98)	0.0001
Age, years	40.0 (33.5, 46.5)	44.0 (33.0, 51.5)	0.304	44.0 (35.5, 56.5)	0.162
Gender, F/M	10/11	12/14	0.146	14/16	0.354
AEA, ng/mL	0.56 (0.47, 0.66)	0.52 (0.41, 0.67)	0.630	0.68 (0.53, 0.78)	0.020
2-AG, ng/mL	2.0 (0.80, 5.90)	1.4 (0.72, 2.08)	0.181	2.2 (1.21, 4.59)	0.806
Lipid status					
Cholesterol levels, mg/dL	191.1 (165.8, −228.2)	202.8 (170.6, 224.2)	0.563	191.1 (158.0–212.6)	0.871
LDL level, mg/dL	121.7 (105.5, 145.1)	128.5 (113.0, 146.1)	0.531	118.6 (102.3, 141.4)	0.934
HDL level, mg/dL	54.6 (39.4, 64.0)	45.0 (37.7, 54.2)	0.033	42.1 (37.2, 47.8)	0.006
Triglyceride level, mg/dL	54.3 (50.3, 89.9)	93.0 (60.1, 117.0)	0.016	117.5 (77.9–126.4)	0.0001
Glucose level, mg/dL	91.9 (81.9, 99.9)	97.3 (87.8, 102.7)	0.090	100.9 (92.3, 106.0)	0.007
Insulin, mUI/L	3.1 (1.9, 5.2)	5.4 (3.4, 10.9)	0.016	9.4 (7.3, 19.4)	0.0001
HOMA	0.72 (0.44, 1.33)	1.44 (0.71, 2.61)	0.023	2.25 (1.79, 4.29)	0.0001
C-reactive protein levels, mg/L	0.9 (0.9, 3.0)	1.0 (0.9, 2.8)	0.616	5.0 (1.4, 7.5)	0.0001
HbA_{1c}, %	5.2 (4.9, 5.4)	5.2 (5.0, 5.3)	0.745	5.3 (5.1, 5.8)	0.289

Values are median (Q1, Q3). CON, controls; OW, overweight; OB, obesity; AEA, anandamide; 2-AG, 2-arachidonoylglycerol; BMI, body mass index; HbA_{1c}, haemoglobin A_{1c}; HDL, high-density lipoprotein; HOMA, Homeostasis Model Assessment; LDL, low-density lipoprotein.
P-values vs. CON (Mann–Whitney *U* test for independent samples).

pharmacological vasodilation of the arteriolar vessels, ~500–550 MBq ^{13}N-ammonia was injected intravenously again and acquisition of PET emission data was started. The stress PET scan was followed by a low-dose CT scan for attenuation correction of the PET stress images during pharmacological vasodilation. This was deemed necessary to account for possible changes in cardiac and pulmonary volumes owing to dipyridamole-induced hyperaemia. Finally, the physical half-life (9.8 min) of ^{13}N-ammonia necessitated a time interval of ~45 min between repeat assessments of MBF with PET.[7]

A heart rate, blood pressure, and a 12-lead ECG were recorded continuously during each MBF measurement. From an average of heart rate and systolic blood pressure (SBP) during the first 2 min of each image acquisition, the rate–pressure product (RPP) was derived as an index of cardiac work. To account for possible interindividual variations in coronary driving pressure, an index of global coronary vascular resistance (CVR) was determined as the ratio of mean arterial blood pressure (mmHg) to MBF (mL/g/min). In addition, MBF was normalized to the RPP, and thus myocardial work (averaged during the first 2 min of image acquisition; MBF divided by RPP multiplied by 10 000). Finally, the short- and long-term reproducibility of MBF responses to CPT and pharmacological vasodilation have been reported previously.[7,16]

Statistical analysis

Since continuous variables are not always normally distributed, they are presented as median and inter-quartile range (25th to 75th percentile: Q1, Q3). For comparison of differences, Mann–Whitney *U* test for independent samples was used (Statistical Analysis Software Institute, Cary, NC, USA). A comparison of CPT-induced change in MBF and dipyridamole MBFs between the different groups was performed by one-way analysis of variance (ANOVA), followed by Scheffe's multiple comparison test. Pearson's correlation coefficient (r), assuming a linear regression, and the standard error of the estimate (SEE) was calculated to investigate the associations between CPT- and dipyridamole-induced changes in MBFs and laboratory parameters. Multivariate analysis was performed by means of a linear step-wise regression model adjusting for the following *a priori* selected predictors of the CPT-induced ΔMBF and hyperaemic MBF during dipyridamole stimulation: age, BMI, gender, AEA, 2-AG, SBP, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, glucose, HOMA, and C-reactive protein. All test procedures were two-tailed, and $P \leq 0.05$ was considered statistically significant. Based on a standard deviation of 0.18 and 0.17 of ΔMBF to CPT and hyperaemic MBF,[17] respectively, and a minimum clinically relevant difference in ΔMBF of 0.20 mL/g/min and in hyperaemic MBF of 0.22 mL/g/min, $\alpha = 0.05$, and a power $(1 - \beta)$ of 0.8, the number of patients necessary for the cross-sectional baseline analysis was calculated to be 25 and 23, respectively.

Results

Patient characteristics and metabolic profile

The clinical characteristics and laboratory measurements among the groups studied are shown in *Table 1*. The increase in BMI among study participants was paralleled by the waist–hip ratio (WHR). The BMI significantly correlated with the WHR ($r = 0.62$, SEE $= 12.32$, $P < 0.0001$). Endocannabinoid plasma levels did not differ significantly between CON and OW. Conversely, AEA plasma levels were significantly higher in OB than in CON and OW, while this was not observed for 2-AG (*Table 1*).

Correlations between metabolic parameters

With respect to the entire study population, Pearson's regression analysis denoted significant correlations between BMI and insulin resistance (HOMA) ($r = 0.58$, SEE $=0.45$; $P = 0.0001$), BMI and AEA ($r = 0.46$, SEE $=5.12$; $P = 0.0001$), and a non-significantly for the relation between BMI and 2-AG ($r = 0.20$, SEE $=0.22$; $P = 0.088$). In addition, HOMA correlated with AEA ($r = 0.36$, SEE $=1.59$; $P = 0.006$), and 2-AG ($r = 0.30$, SEE $=0.063$; $P = 0.028$), respectively. These observations may denote a close interrelation between these metabolic parameters. As regards the HDL cholesterol, it also showed significant and inverse correlations with BMI ($r = -0.34$, SEE $=0.079$ $P = 0.004$), and HOMA ($r = -0.29$, SEE $=0.76$; $P = 0.035$), but not with AEA ($r = 0.08$, SEE $=8.37$; $P = 0.510$) or 2-AG ($r = -0.14$, SEE $=0.32$; $P = 0.239$), respectively. Furthermore, the regression analysis demonstrated significant correlations between BMI and C-reactive protein plasma levels ($r = 0.57$, SEE $=0.19$; $P = 0.0001$), HOMA, and C-reactive protein ($r = 0.32$, SEE $=0.26$; $P = 0.017$), AEA and C-reactive protein ($r = 0.25$, SEE $=2.87$; $P = 0.034$), but none between 2-AG and C-reactive protein ($r = 0.12$, SEE $=0.115$; $P = 0.312$). These findings may suggest some association between metabolic and inflammatory cardiovascular disease mechanisms.

Haemodynamic parameters

At baseline, the heart rate and SBP were comparable between CON and OW, while they were higher in OB (*Table 2*). As a consequence, the resting RPP was significantly higher in OB than in OW ($P = 0.005$) and CON, while it did not differ between OW and CON ($P = 0.716$). Sympathetic stimulation with CPT induced a significant increase in the heart rate and SBP in each group, so that the RPP was significantly higher during CPT than at baseline. The increase in the RPP (ΔRPP) as a result of the CPT-induced sympathetic stimulation was not significantly different between CON, OW, and OB (*Table 2*), indicating comparable increases in myocardial workload among the groups studied. As regards the pharmacologically induced hyperaemia with dipyridamole, there was a significant increase in the heart rate in the three study groups, while blood pressures mildly decreased. The change in RPP (ΔRPP) during dipyridamole-stimulation remained similar among these groups (*Table 2*).

Myocardial blood flow

At baseline, MBF was comparable between CON and OW but significantly higher in OB (*Table 3*). When adjusted for RPP, the normalized myocardial blood flow (NMBF) at rest was comparable among groups (*Table 3*). Conversely, the MBF and NMBF during CPT progressively decreased from CON to OW and OB. As regards the endothelium-related change of MBF during CPT from rest (ΔMBF to CPT), it was significantly less in OW and OB than in CON, respectively (*Table 3*, *Figure 1A*). In addition, ΔMBF to CPT was significantly more diminished in OB than in OW (*Table 3*, *Figure 1A*). The group comparison between the ΔMBF to CPT in CON was significant when compared with OW and OB ($P = 0.0001$ by ANOVA). As changes of CVR mirrored those of MBF for each study group (*Table 3*), differences in haemodynamic responses can be widely ruled out as possible cause for the observed alteration in MBF response to CPT.

As regards the MBFs during dipyridamole-induced pharmacological vasodilation, they were significantly lower in OW and

Table 2 Haemodynamic findings PET/CT exam

	Group				
	CON	**OW**	***P*-value**	**OB**	***P*-value**
Haemodynamics at rest					
Heart rate (b.p.m.)	62.0 (58.0, 69.5)	62.5 (54.8, 69.3)	1.000	67.0 (63.0, 75.0)	0.015
Systolic blood pressure (mmHg)	115 (109.5, 126.0)	119.0 (112.8, 126.5)	0.341	124.0 (111.5, 134.0)	0.047
RPP	6831.0 (6420.5, 7815.0)	7239.5 (6474.8, 7841.5)	0.716	8580.0 (7155.0, 9619.5)	0.003
CPT					
Heart rate (b.p.m.)	74.0 (62.5, 85.0)	70.5 (60.0, 78.0)	0.239	75.0 (66.0, 82.0)	0.637
Systolic blood pressure (mmHg)	135 (127.0, 144.0)	135.5 (126.8, 148.5)	0.615	151.0 (139.5, 158.5)	0.002
RPP	9240.0 (8649.0, 11002.0)	9637.5 (8290.0, 10572.0)	0.781	11376.0 (9379.5, 12528.0)	0.031
ΔRPP (CPT-rest)	2469.0 (1834.0, 3036.0)	1861.5 (999.5, 3032.8)	0.185	2648.0 (1013.0, 3839.0)	0.914
Pharmacological vasodilation					
Heart rate (b.p.m.)	85.0 (73.0, 92.5)	85.0 (79.3, 91.8)	0.983	87.0 (82.0, 101.0)	0.137
Systolic blood pressure (mmHg)	114 (106.0, 118.5)	118 (111.0, 122.5)	0.139	120.0 (115.0, 133.0)	0.002
RPP	9118.0 (8258.5, 10451.0)	9895.5 (8977.2, 11001.25)	0.280	11310.0 (10353.0, 12272.0)	0.0001
ΔRPP (pharmacological-rest)	2044.0 (1133.5, 3023.5)	2221.0 (1532.5, 3827.1)	0.460	2533.0 (1725.0, 3984.0)	0.201

Values are median (Q1, Q3). CON, controls; OW, overweight; OB, obesity; RPP, rate-pressure product; CPT, cold pressor test.
P-values vs. CON (Mann–Whitney *U* test for independent samples).

Table 3 Myocardial blood flow findings during PET/CT exam

MBF (mL/min/g)	Group				
	CON	OW	*P*-value	OB	*P*-value
MBF at rest	0.69 (0.63, 0.79); 0.71 ± 0.10	0.70 (0.61, 0.76); 0.70 ± 0.11	0.756	0.81 (0.64, 0.95); 0.81 ± 0.18	0.040
NMBF at rest	0.92 (0.85, 1.13); 0.99 ± 0.17	0.95 (0.83, 1.09); 0.98 ± 0.18	0.608	0.95 (0.84, 1.15); 0.97 ± 0.21	0.776
MBF during CPT	1.0 (0.86, 1.21); 1.04 ± 0.21	0.88 (0.80, 1.04); 0.91 ± 0.19	0.040	0.91 (0.74, 0.96); 0.88 ± 0.18	0.008
△MBF to CPT from rest	0.26 (0.23, 0.39); 0.34 ± 0.17	0.21 (0.10, 0.27); 0.21 ± 0.17	0.010	0.09 (−0.01, 0.15); 0.07 ± 0.12	0.0001
NMBF during CPT	1.05 (0.92, 1.15); 1.06 ± 0.18	0.93 (0.85, 1.09); 0.96 ± 0.18	0.087	0.763 (0.67, 0.93); 0.81 ± 0.17	0.0001
MBF during pharmacological vasodilation	2.39 (1.97, 2.62); 2.37 ± 0.49	1.98 (1.69, 2.26); 1.96 ± 0.43	0.007	2.10 (1.76, 2.36); 2.05 ± 0.40	0.042
MFR	3.4 (2.95, 3.85); 3.38 ± 0.67	2.92 (2.36, 3.28); 2.88 ± 0.75	0.012	2.70 (2.27, 3.15); 2.63 ± 0.76	0.001
CVR (mmHg/mL/min/g)					
At rest	119.0 (103.4, 133.6)	130.9 (116.8, 142.0)	0.091	111.9 (95.2, 136.0)	0.356
During CPT	96.1 (73.4, 110.9)	114.6 (97.3, 134.4)	0.003	128.2 (111.9, 140.7)	0.0001
△ change to CPT from rest	−25.3 (−36.9, −15.7)	−11.6 (−31.3, −0.6)	0.023	10.3 (−2.8, 18.3)	0.0001
Pharmacological vasodilation	33.6 (29.0, 37.9)	43.3 (36.2, 49.6)	0.001	42.7 (36.9, 49.5)	0.002

Values are median (Q1, Q3) and mean ± SD. CON, controls; OW, overweight; OB, obesity; MBF, myocardial blood flow; NMBF, normalized MBF; MFR, myocardial flow reserve; CVR, coronary vascular resistance.
P-values vs. CON (Mann–Whitney *U* test for independent samples).

OB compared with CON, respectively, while they remained comparable between OW and OB (*Table 3*, *Figure 1B*). Similarly, the MFR progressively declined from CON to OW and OB, while it did not differ between OW and OB ($P = 0.270$) (*Table 3*). The group comparison of dipyridamole-stimulated MBFs and MFR in CON was significantly different from those in the OW and OB ($P = 0.010$ by ANOVA). Further, the CVR during dipyridamole stimulation was significantly higher in OW and OB than in CON, confirming the reduced vasodilatory capacity in individuals with increased body weight (*Table 3*).

Changes in myocardial blood flow related to endocannabinoid plasma levels

With regard to the entire study population, Pearson regression analysis demonstrated significant and inverse correlations between AEA and 2-AG plasma levels and endothelium-related ΔMBF to CPT, respectively ($r = -0.37$, SEE =0.18; $P = 0.001$ and $r = -0.22$, SEE =0.18; $P = 0.052$), as well as for dipyridamole-induced hyperaemic MBFs, respectively ($r = -0.23$, SEE =0.45; $P = 0.052$ and $r = -0.30$, SEE =0.44; $P = 0.010$) (*Table 4*). In OW, AEA, and 2-AG plasma levels were not correlated with ΔMBF to CPT ($r = -0.19$, SEE =0.17; $P = 0.337$ and $r = -0.17$, SEE =0.17; $P = 0.401$) and hyperaemic MBFs ($r = -0.11$, SEE =0.44; $P = 0.604$ and $r = -0.28$, SEE =0.43; $P = 0.174$), respectively. Conversely, in OB there were significant associations between AEA and 2-AG plasma levels and endothelium-related ΔMBF to CPT, respectively (*Figure 2*), and for dipyridamole-induced hyperaemic MBFs, respectively (*Figure 3*). These correlations were also maintained when corresponding CVR values of the CPT- and

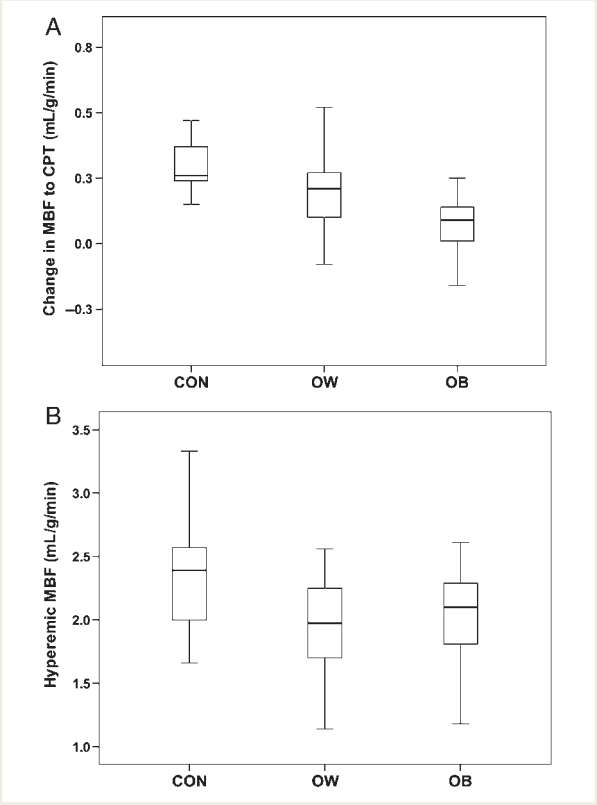

Figure 1 (*A*) Change in endothelium-related myocardial blood flow from rest in response to cold pressor testing, and (*B*) hyperaemic MBFs during pharmacological vasodilation with dipyridamole in controls (CON), overweight (OW), and obesity (OB).

Table 4 Entire study group

	ΔMBF to CPT				Hyperaemic MBF			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	Coefficient β (95% CI)	*P*-value	Coefficient β (95% CI)	*P*-value	Coefficient β (95% CI)	*P*-value	Coefficient β (95% CI)	*P*-value
Age, years	−0.23 (−0.008 to 0.0001)	0.043	—	—	−0.27 (−0.021 to −0.002)	0.017	—	—
BMI, kg/m^2	−0.54 (−0.016 to −0.008)	0.0001	−0.56 (−0.017 to −0.007)	0.0001	−0.16 (−0.020 to 0.004)	0.177	—	—
Gender, M	−0.33 (−0.22 to −0.044)	0.003	−0.36 (−0.23 to −0.050)	0.002	−0.23 (−0.43 to 0.0001)	0.216	—	—
AEA, ng/mL	−0.36 (−0.618 to −0.159)	0.001	—	—	−0.227 (−1.183 to 0.006)	0.052	—	—
2-AG, ng/mL	−0.22 (−0.019 to 0.0001)	0.052	—	—	−0.297 (−0.058 to −0.002)	0.010	−0.297 (−0.058 to −0.002)	0.034
SBP, mmHg	−0.27 (−0.008 to 0.0001)	0.016	—	—	−0.17 (−0.016 to 0.003)	0.157	—	—
Total cholesterol, mg/dL	−0.024 (−0.002 to 0.001)	0.841	—	—	0.047 (−0.003 to 0.004)	0.697	—	—
LDL cholesterol, mg/dL	−0.11 (−0.002 to 0.001)	0.336	—	—	−0.027 (−0.004 to 0.003)	0.824	—	—
HDL cholesterol, mg/dL	0.38 (0.002 to 0.009)	0.001	—	—	0.27 (0.001 to 0.018)	0.023	—	—
Triglycerides, mg/dL	−0.28 (−0.002 to 0.0001)	0.016	—	—	−0.070 (−0.002 to 0.001)	0.557	—	–
Glucose, mg/dL	−0.22 (−0.005 to 0.0001)	0.054	—	—	−0.18 (−0.010 to 0.001)	0.121	—	—
HOMA	−0.22 (−0.042 to 0.004)	0.108	—	—	−0.18 (−0.096 to 0.020)	0.196	—	—
C-reactive protein, mg/L	−0.23 (−0.019 to 0.0001)	0.049	—	—	0.048 (−0.020 to 0.030)	0.688	—	—

MBF, myocardial blood flow; CPT, cold pressor testing; BMI, body mass index; AEA, anandamide; 2-AG, 2-arachidonoylglycerol; SBP, systolic blood pressure; LDL cholesterol, low-density lipoprotein cholesterol; HDL cholesterol, high-density lipoprotein cholesterol; HOMA, homeostasis model assessment for insulin resistance. *P*-values by analysis of variance.

dipyridamole-induced flow response were evaluated, respectively (ΔCVR to CPT and AEA: $r = 0.41$, SEE =17.7; $P = 0.028$ and with 2-AG: $r = 0.38$, SEE =0.60; $P = 0.039$; and between hyperaemic CVR and AEA: $r = 0.47$, SEE =11.6; $P = 0.013$, and with 2-AG: $r = 0.63$, SEE =0.34; $P = 0.0001$).

Determinants of change in myocardial blood flow to cold pressor test

Considering the entire study group, on univariate analysis age, BMI, gender, AEA and 2-AG plasma levels, SBP, HDL cholesterol, triglycerides, glucose, and C-reactive protein levels were significantly associated with endothelium-related ΔMBF to CPT (*Table 4*). As denoted in *Table 4*, by multivariate analysis only BMI and gender correlated in an independent fashion with ΔMBF. The univariate analysis in OW (*Table 5*) identified only gender to be significantly associated with ΔMBF. Conversely, in OB (*Table 5*), gender, SBP, AEA, and 2-AG plasma levels were significantly associated with ΔMBF.

Determinants of hyperaemic myocardial blood flows

As regards the dipyridamole-stimulated hyperaemic MBFs in the entire study group, on univariate analysis they correlated with age, AEA and 2-AG plasma levels, and HDL cholesterol (*Table 4*). By multivariate analysis, only 2-AG plasma levels remained independently associated with hyperaemic MBFs. On the univariate analysis in OW (*Table 5*) no association with hyperaemic MBFs was identified. Conversely, as denoted in *Table 5*, age, gender, and AEA and 2-AG plasma levels were significantly associated with hyperaemic MBFs in OB.

Discussion

The present study is unique in that it identifies increases in EC plasma levels to be significantly associated with an impairment of coronary circulatory function in OB. Increases in EC plasma levels, such as of AEA and 2-AG, were associated with an abnormal functioning of the coronary endothelium and arteriolar vascular smooth muscle cells in obese individuals. As an impairment of

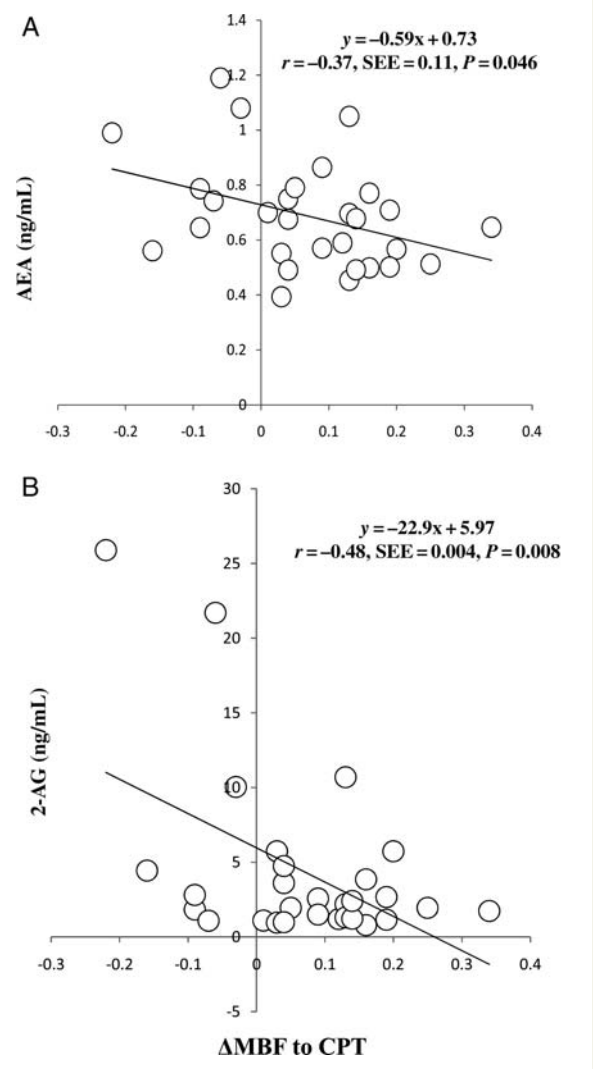

Figure 2 Correlation between (*A*) anandamide, (*B*) 2-arachidonoylglycerol plasma levels and change of endothelium-related myocardial blood flow during cold pressor testing in obesity, respectively.

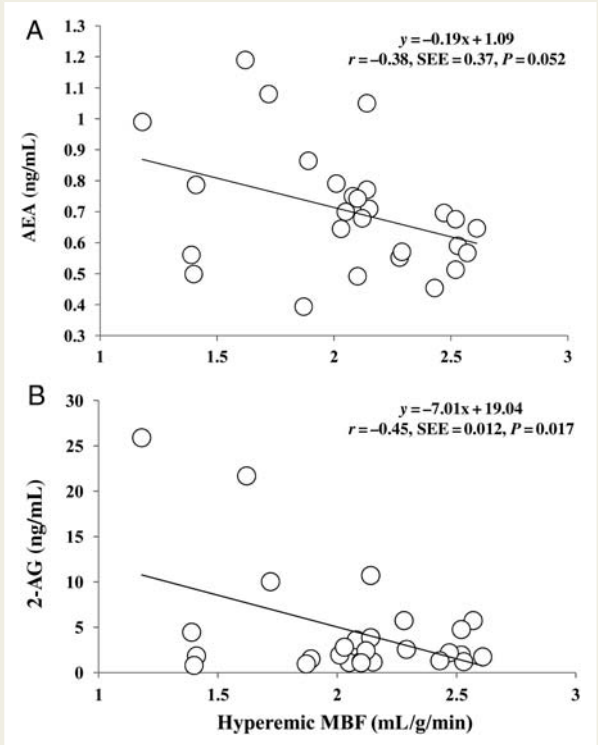

Figure 3 Correlation between (*A*) anandamide, (*B*) 2-arachidonoylglycerol plasma levels and hyperaemic myocardial blood flows during pharmacological vasodilation with dipyridamole in obesity, respectively.

coronary circulatory function has been widely appreciated as a functional precursor of the CAD process and future cardiovascular events,[7,13] the observed direct and close associations between increases in EC plasma levels and coronary circulatory dysfunction may suggest increases in EC plasma levels such as of AEA and 2-AG as a potential novel risk factor for the initiation and development of CAD in OB, which, however, warrants further investigations.

Metabolic profiles and interrelations

As observed in the current and in a recent investigation,[18] the median value of AEA plasma levels were significantly increased in OB when compared with CON, while this was not observed for 2-AG. In the OW again, AEA and 2-AG plasma levels did not differ significantly from those in CON. Differences in the characteristics of the present cohort of participants, and in particular the prevalence of women vs. men, might also explain why the plasma levels of AEA but not those of 2-AG were found here to be significantly increased in OB. In fact, previous studies have demonstrated that elevated plasma levels of 2-AG are most often found in men with abdominal OB, whereas elevated plasma levels of AEA are observed in post-menopausal women.[19,20] These divergent characteristics of AEA and 2-AG concentrations in individuals with increasing body weight remain to be further elucidated but they may be related to different (patho-)physiological roles of these compounds. For example, AEA also stimulates non-CB receptors such as transient receptor potential vanilloid type-1 channels, and, with increasing concentrations, PPAR-γ.[6] Conversely, 2-AG has been described to act more selectively on CB receptors. It is also conceivable that AEA concentrations are increased in OB, in part at least, due to a malfunctioning of AEA degradation owing to a missense polymorphism in fatty acid amide hydrolase. Interestingly, EC plasma levels have been shown to be diminished post-prandially or subsequent to oral glucose load and euglycaemic hyperinsulinaemic clamp in lean,[18] but not in insulin-resistant OB,[6,18] suggesting an uncoupling of insulin-induced negative feedback regulation of ECs derived from the peripheral and adipose tissues in obese individuals with an insulin-resistant state. The current study adds further information in that EC plasma levels also appear to be increased in OB even in a fasting state and, as it has been also reported more recently,[21] in obese type 2 diabetes mellitus patients.

Table 5 Univariate analysis in overweight and obesity group

	OW				OB			
	ΔMBF to CPT		Hyperaemic MBF		ΔMBF to CPT		Hyperaemic MBF	
	Coefficient β (95% CI)	*P*-value	Coefficient β (95% CI)	*P*-value	Coefficient β (95% CI)	*P*-value	Coefficient β (95% CI)	*P*-value
Age, years	−0.18 (−0.008 to 0.003)	0.378	−0.13 (−0.019 to 0.010)	0.525	−0.30 (−0.007 to 0.001)	0.106	−0.49 (−0.029 to 0.005)	0.009
BMI, kg/m^2	−0.035 (−0.060 to 0.051)	0.865	0.078 (−0.11 to 0.16)	0.704	−0.20 (−0.010 to 0.003)	0.291	−0.066 (−0.027 to 0.019)	0.742
Gender, M	−0.45 (−0.31 to −0.030)	0.010	0.23 (−0.17 to 0.61)	0.138	−0.28 (−0.17 to −0.03)	0.057	−0.52 (−0.811 to −0.153)	0.006
AEA, ng/mL	−0.19 (−0.65 to 0.23)	0.337	−0.10 (−1.42 to 0.84)	0.604	−0.37 (−0.46 to −0.005)	0.046	−0.38 (−1.52 to 0.003)	0.051
2-AG, ng/mL	−0.17 (−0.034 to 0.014)	0.401	−0.27 (−0.10 to 0.019)	0.174	−0.48 (−0.017 to −0.003)	0.008	−0.45 (−0.055 to −0.006)	0.017
SBP, mmHg	−0.24 (−0.010 to 0.003)	0.231	0.17 (−0.009 to 0.024)	0.388	−0.28 (−0.007 to 0.001)	0.130	−0.23 (−0.021 to 0.006)	0.246
Total cholesterol, mg/dL	−0.032 (−0.003 to 0.002)	0.875	0.09 (−0.005 to 0.008)	0.643	−0.16 (−0.002 to 0.001)	0.433	0.013 (−0.005 to 0.005)	0.949
LDL cholesterol, mg/dL	−0.16 (−0.004 to 0.002)	0.436	0.055 (−0.006 to 0.008)	0.797	−0.22 (−0.003 to 0.001)	0.286	−0.020 (−0.006 to 0.005)	0.926
HDL cholesterol, mg/dL	0.35 (0.0001 to 0.01)	0.076	0.17 (−0.008 to 0.020)	0.387	0.25 (−0.002 to 0.009)	0.226	0.20 (−0.009 to 0.026)	0.324
Triglycerides, mg/dL	−0.23 (−0.001 to 0.0001)	0.251	−0.028 (−0.003 to 0.002)	0.891	−0.13 (−0.001 to 0.001)	0.530	−0.118 (−0.004 to 0.002)	0.573
Glucose, mg/dL	−0.35 (−0.013 to 0.001)	0.077	−0.068 (−0.022 to 0.016)	0.742	0.036 (−0.002 to 0.002)	0.857	−0.22 (−0.010 to 0.003)	0.265
HOMA	−0.021 (−0.048 to 0.043)	0.925	−0.29 (−0.15 to 0.029)	0.174	0.13 (−0.022 to 0.023)	0.955	0.061 (−0.068 to 0.086)	0.811
C-reactive protein, mg/L	0.16 (−0.016 to 0.037)	0.435	−0.23 (−0.10 to 0.028)	0.240	−0.015 (−0.009 to 0.008)	0.940	0.26 (−0.010 to 0.045)	0.204

OW, overweight, OB, Obesity; MBF, myocardial blood flow; CPT, cold pressor testing; BMI, body mass index; AEA, anandamide; 2-AG, 2-arachidonoylglycerol; SBP, systolic blood pressure; LDL cholesterol, low-density lipoprotein cholesterol; HDL cholesterol, high-density lipoprotein cholesterol; HOMA, homeostasis model assessment for insulin resistance. *P*-values by analysis of variance.

Endocannabinoids and coronary circulatory function

As regards PET/CT determined MBF measurements, resting MBFs in OB were observed to be significantly higher than in CON and OW, which were related to higher resting heart rates, SBPs and resulting RPP, indicative of the myocardial workload. The observation of an increase in myocardial workload in OB associated with higher resting MBFs is in agreement with a previously reported activation of the sympathetic nervous system and renin–angiotensin–aldosterone system and an increase in resting MBF in OB.[22] This may contrast with previous findings,[3] where the resting MBF was not significantly different between CON, OW, and OB. The reason for this discordant observation remains uncertain but, most likely, is related to the presence of a more advanced stage of OB in the current study population associated with a more enhanced activation of the sympathetic nervous- and renin–angiotensin–aldosterone system.[22] In the evaluation of coronary circulatory function, however, as observed in the current and recent investigations,[3,4] there was a progressive decrease in endothelium-dependent MBF responses to CPT from normal weight CON to OW, and OB. The total vasodilator capacity was also altered in individuals with increases in body weight. Thus, alterations of coronary circulatory function were not only confined to the endothelium but they had already affected vascular smooth muscle cell function in both groups of OW and OB.

Concerning the obese study population increases in both AEA and 2-AG plasma levels were significantly and inversely correlated with the extent of CPT-induced alterations in MBF and hyperaemic MBFs, respectively. Such findings may strongly suggest that increases in EC plasma levels may mediate an abnormal function of the coronary circulation as a functional precursor of CAD in OB. Confounding effects related to obesity such as low HDL cholesterol, insulin resistance, inflammation, various adipocytokines, and/or undetermined factors, however, most likely had also altered coronary circulatory function. By multivariate analysis, 2-AG plasma levels remained independently associated with altered hyperaemic MBFs in the current study population with increasing body weight, whereas no independent association was observed for the endothelium-related MBF response to CPT. Thus, altered MBF responses to CPT related to elevated EC plasma levels appear to contribute to the adverse effects increases in body weight associated with low HDL cholesterol, insulin resistance, increases in triglycerides, and micro-inflammation. Such observations may also suggest differential binding of both ECs to different parts of the coronary arterial wall. Thus, someone could speculate that 2-AG may have a higher affinity and efficacy for CB1- and CB2-receptors of the arteriolar vascular smooth muscle cells than for the coronary endothelium,[6,23] which, however, needs to be elucidated by experimental investigations. With regard to possible effects of elevated ECs plasma levels in OB on coronary circulatory function, the current observations provide first direct *in vivo* evidence that increases in AEA and 2-AG may confer adverse effects on both the coronary endothelium and vascular smooth muscle function of the arteriolar vessel. Support for these *in vivo* observations comes also from a more recent experimental investigation in apolipoprotein E-deficient ($ApoE^{-/-}$) mice.[24] Inhibition of CB1 receptor by rimonabant in mice, which were previously shown to exhibit elevated 2-AG levels in the aorta and visceral adipose tissue,[9] resulted in a decrease in aortic reactive oxygen species (ROS) production and NADPH oxidase activity paralleled by an improvement in endothelium-dependent vasodilation. These results emphasize that stimulation of CB1 receptor stimulation by ECs may indeed stimulate proatherosclerotic mechanisms.[24] Endocannabinoids have also been shown to specifically stimulate CB1 and CB2 on the vascular endothelium and smooth muscle cells,[6] that, as predominantly demonstrated by *in vitro* studies, may lead to EC stimulated proatherosclerotic effects, such as increases in oxdidative stress and vascular adhesion molecules like VCAM-1 expression, monocyte adhesion, and migration into the arterial wall.[6,9,24,25] Further investigations, however, are warranted for the identification and characterization of the molecular mechanisms by which ECs affect coronary circulatory function in OB.

Conceptually, an improvement of coronary circulatory function by preventive medical intervention, for example, with the CB1 antagonist rimonabant, and/or behavioural interventions related to weight, diet, and physical activity may diminish the association between obesity and the risk for future cardiovascular events.[7,26] A recent study of coronary circulatory function is in support of this consideration. For example, an improvement of coronary endothelial function in type 2 diabetes after 1 year follow-up in response to glucose lowering treatment with metformin and/or glyburide has been shown more recently to mediate direct preventive effects on the progression of epicardial structural disease.[27] These preliminary observations may indeed give rise to the consideration that medical therapy strategies aiming to decrease EC plasma concentrations in obese individuals, perhaps using new generation CB1 antagonists with lesser central side effects,[6] may improve coronary circulatory function, thereby leading to an improvement in cardiovascular prognosis. This hypothesis, however, necessitates further clinical outcome studies. Finally, it should be noted that the findings presented were obtained from a relatively small sample size of patients with predominantly advanced obesity (median BMI of 41 kg/m^2), which may not necessarily allow definite answers but may stimulate further investigations in this emerging research field.

Conclusions

This study demonstrates that increased EC plasma levels of AEA and 2-AG are associated with abnormal coronary circulatory function in OB. As an impairment of coronary circulatory function may reflect an early abnormal functional stage of the CAD process, increases in EC plasma levels may emerge as a novel cardiovascular risk factor in OB.

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