Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 59 (2010) 734 – 741
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Cannabinoid CB1 receptor expression in relation to visceral adipose depots, endocannabinoid levels, microvascular damage, and the presence of the *Cnr1* A3813G variant in humans

Marica Bordicchia[a], Ilaria Battistoni[a], Lucia Mancinelli[a], Elena Giannini[a], Giada Refi[a], Daniele Minardi[b], Giovanni Muzzonigro[b], Roberta Mazzucchelli[c], Rodolfo Montironi[c], Fabiana Piscitelli[d], Stefania Petrosino[d], Paolo Dessì-Fulgheri[a], Alessandro Rappelli[a], Vincenzo Di Marzo[d], Riccardo Sarzani[a,*]

[a]*Department of Internal Medicine, University of Ancona-"Politecnica delle Marche," University Hospital of Ancona, 60131 Ancona, Italy*
[b]*Department of Urology, University of Ancona-"Politecnica delle Marche," University Hospital of Ancona, 60131 Ancona, Italy*
[c]*Institute of Pathological Anatomy, University of Ancona-"Politecnica delle Marche," University Hospital of Ancona, 60131 Ancona, Italy*
[d]*Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, 80078 Pozzuoli, Italy*

Received 9 July 2009; accepted 18 September 2009

Abstract

Dysregulation of the endocannabinoid system in the visceral adipose tissue (VAT) is associated with metabolic and cardiovascular complications of obesity. We studied perirenal VAT CB1 receptor expression in relation to anthropometry, VAT area and endocannabinoid levels, kidney microvascular damage (MVDa), and the presence of the CB1 gene A3813G variant, the frequency of which was also evaluated in a large population of obese-hypertensive (OH) patients with or without the metabolic syndrome (MetS). Perirenal VAT and kidney samples were obtained from 30 patients undergoing renal surgery. Total and perirenal VAT areas were determined by computed tomography. CB1 messenger RNA expression and endocannabinoid levels in perirenal VAT were determined by quantitative reverse transcriptase polymerase chain reaction and liquid chromatography–mass spectrometry, respectively. The MVDa was evaluated in healthy portions of kidney cortex. The A3813G alleles were identified by genotyping in these patients and in 280 nondiabetic OH patients (age ≤65 years). *Metabolic syndrome* was defined according to the Adult Treatment Panel III criteria. Perirenal VAT CB1 expression was 40% lower in patients with the A3813G polymorphism, and correlated positively with perirenal and total VAT area and with perirenal VAT levels of the endocannabinoid anandamide. A 2-fold higher CB1 expression was associated with MVDa. The OH patients with the A3813G allele had lower prevalence of MetS in both unadjusted and adjusted models. Genetics influence perirenal VAT CB1 expression and the prevalence of MetS in OH. Increased VAT is associated with increased perirenal VAT endocannabinoid tone, which in turn correlates with increased MVDa. Endocannabinoid overactivity might be involved in human visceral obesity and its renal complications.

1. Introduction

Visceral obesity is strongly associated with increased risk for several chronic diseases such as the metabolic syndrome (MetS), diabetes, and hypertension [1,2]. Indeed, increased visceral adiposity is regarded as the link between overweight and cardiometabolic complications. Obesity is also causally linked, directly and indirectly, to the development of chronic renal disease that results from microvascular damage (MVDa) [3,4]. The understanding of the obesity-associated mechanisms that lead to complications is crucial. In this setting, the endocannabinoid system (ECS) plays an important role in the control of food intake, lipid and glucose metabolism through both central and peripheral effects, lipogenesis, and fat accumulation [5]. The 2 most widely studied endocannabinoids (ECs), anandamide (AEA)

* Corresponding authors. Riccardo Sarzani is to be contacted at Department of Internal Medicine, University of Ancona-Politecnica delle Marche, 60131 Ancona, Italy. Tel.: +39 071 889499; fax: +39 071 889232. Vincenzo Di Marzo, Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, 80078 Pozzuoli, Italy. Tel.: +39 081 8675093; +39 081 8675191; fax: +9 081 8041770.
E-mail addresses: vdimarzo@icmib.na.cnr.it (V. Di Marzo), r.sarzani@univpm.it (R. Sarzani).

0026-0495/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.09.018

and 2-arachidonoylglycerol (2-AG), act indeed also in the periphery through the cannabinoid CB1 receptor to regulate energy balance and body fat composition. Dysregulated ECS activity is believed to contribute to the pathogenesis of human obesity and its associated cardiometabolic risk [6-10]. Accordingly, long-term treatment with a CB1 receptor antagonist (rimonabant) reduced food intake and improved several metabolic and cardiovascular risk factors in obese subjects [11-14]. Furthermore, the Rimonabant in Obesity–Lipids trial conducted on high-risk dyslipidemic patients showed that CB1 receptor antagonism could induce substantial and not entirely weight loss–dependent increases in high-density lipoprotein (HDL) cholesterol and in the levels of adiponectin, a "protective" adipokine for the cardiovascular system [11,15].

The relevance of the overactive adipose ECS and of adipose tissue CB1 receptor to the pathogenesis of visceral obesity and associated metabolic disorders in humans, particularly those concerned with kidney dysfunction and failure, is still not fully understood, also because multiple genes and gene-environment interactions play a crucial role in this context. Several human CB1 polymorphisms have been previously described in European American, African American, and Japanese subjects in relation to addiction vulnerability [16]. However, the effect of *Cnr1* variants on obesity and related clinical parameters appears somewhat contradictory depending on the population, sex, and body mass index (BMI) of the cohorts analyzed, as well as on other factors [17-19]. Russo et al [20] showed that, in a male population with a wide range of BMI, the presence of the A3813G variant in the CB1 receptor gene (*Cnr1*) was associated with an increase of subscapular skinfold thickness, an index of general subcutaneous adiposity, and also with a slight increase of waist circumference that is usually considered an index of visceral fat. The 3813A/G (rs12720071) single nucleotide polymorphism (SNP) is localized in exon 4, which lies in sequences encoding the 3' untranslated region. This polymorphism is one of several tag SNPs of *Cnr1* for white populations (HapMap-CEU) in the Seattle SNPs database (http://gvs.gs.washington.edu/GVS/) and was thus selected as a maximally informative site for *Cnr1*, according to the tag SNPs approach in the evaluation of gene candidacy for common diseases [21]. However, it is not known if and how this or other genetic variants of the *Cnr1* gene affect CB1 expression and function, and hence ECS activity, in the adipose tissue.

The primary aim of the present study was to analyze EC levels and CB1 expression in human perirenal visceral adipose tissue (VAT), obtained from unselected patients with a wide range of BMI, from normal weight (BMI <25 kg/m^2) to obese (BMI ≥30 kg/m^2), in relation to total amount of perirenal and abdominal adipose tissue and to plasma adiponectin concentrations.

Moreover, because one of the end points of dysmetabolism is micro- and macrovascular damage, we also wanted to evaluate the association between perirenal VAT CB1 expression and the degree of MVDa in the kidney. Furthermore, to study the influence of genetics on CB1 gene expression level, we investigated whether the *Cnr1* A3813G variant was associated with different levels of CB1 messenger RNA (mRNA) in perirenal VAT. Finally, the same genetic variant was also analyzed in relation to clinical and metabolic parameters in a larger population of obese-hypertensive patients (OH), with a likely overactive ECS [6-9] and higher risk of developing a MetS.

2. Materials and methods

2.1. Patients and human tissue samples

Tissue samples were obtained during elective surgery (radical nephrectomy) from 30 patients admitted to the United Hospital of Ancona for localized clear cell renal carcinoma (T1/T2, N0, M0), a population similar to the one already described [22]. Briefly, patients were 24 men and 6 women, with average age of 64.4 ± 13.3 years, BMI = 26.8 ± 3.4 kg/m^2 (lowest BMI value, 21.4 kg/m^2; highest BMI value, 38.1 kg/m^2), waist circumference = 99.3 ± 11.6 cm, systolic blood pressure [SBP] = 139.4 ± 20.3 mm Hg, and diastolic blood pressure [DBP] = 83.6 ± 11.5 mm Hg. All women were in menopause, which contributed to lessen metabolic differences and to increase sample's homogeneity. Moreover, patients did not have *severe renal damage* (defined as creatinine clearance <60 mL/min). Samples of adipose tissue and renal tissue were taken from a healthy portion of the removed kidney and perirenal depot, at least 3 cm far from the carcinoma. After removal, samples were snap-frozen in liquid nitrogen and stored at −80°C until RNA extraction. The local Ethics Committee approved the study protocol, and all patients gave written informed consent for the collection of tissue samples and clinical data. For these patients, we also analyzed MVDa in kidney cortex samples, CB1 mRNA, and circulating adiponectin (n = 30). The amounts of ECs from perirenal VAT, and computed tomographic (CT) images to evaluate the total amount of perirenal and abdominal adipose tissue, were available for 12 patients.

2.1.1. Acquisition of CT images

Axial CT images of the abdominal region were obtained as a part of the planned kidney surgery and were available for 12 patients. The CT images corresponding to the L4-5 vertebral disk spaces were analyzed to measure whole VAT (in square millimeters) area and at the level of each renal artery to determinate perirenal adipose area (in square millimeters). Digital images were analyzed with Centricity Medora RIS (Radiology Information System) software 3.10/4.24 (GE Healthcare Medical System, Salt Lake City, UT). *Total abdominal adipose tissue* was defined as the sum of adipose tissue inside a line tracing of the skin. The VAT area was evaluated by drawing a line around the interior of the peritoneal cavity and summing all adipose tissues within this area.

Perirenal adipose tissue area was evaluated by drawing a line around the retroperitoneal VAT surrounding the kidneys at the level of the vascular peduncle. The difference between total adipose tissue and VAT was considered as subcutaneous adipose tissue (SAT) area. A single reader, blinded to the results of the study, performed all image analysis.

2.1.2. Gene expression

Total RNA was extracted from tissue samples after homogenization in guanidine thiocyanate buffer and by CsCl gradient modified as previously reported [23]. Gene expression analysis was performed as previously reported [22]. Briefly, 1.5 μg RNA was reverse-transcribed with high-capacity complementary DNA reverse transcription kits with RNase inhibitor (Applied Biosystems, Warrington, Cheshire, United Kingdom). Real-time gene expression of human CB1 receptor was performed in triplicate by using TaqMan Gene Expression Assay (Hs00275634_m1; Applied Biosystem, Weiterstadt, Germany) using an ABI 7300 for real-time polymerase chain reaction (Applied Biosystems, Darmstadt, Germany) with the standard curve method. 18S ribosomal RNA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used to normalize the differences in starting total RNA and different efficiency of complementary DNA synthesis among samples. There was no difference in the expression results when normalized to GAPDH or 18S ribosomal RNA and GAPDH expression levels were stable and independent of obesity in the whole population.

2.1.3. Extraction and quantification of ECs from adipose tissue

Tissue samples were homogenized in chloroform/methanol/TRIS-HCl 50 mmol/L, pH 7.4 (2:1:1, vol/vol), containing 10 pmol of [2H]8-AEA and 50 pmol of [2H] 5-2-AG as internal deuterated standards (purchased from Cayman Chemicals, Ann Arbor, MI). The extract was purified by means of silica gel minicolumns, and the eluted fraction containing AEA and 2-AG was analyzed by means of liquid chromatography–atmospheric pressure–mass spectrometry conducted as described previously [24]. Analyses were carried out in the selected ion-monitoring mode using *m*/*z* values of 356 and 348 (molecular ions +1 for deuterated and undeuterated AEA) and 384.35 and 379.35 (molecular ions +1 for deuterated and undeuterated 2- AG). The AEA and 2-AG levels were calculated by isotope dilution and are expressed as picomoles per gram of wet tissue weight.

2.1.4. Microvascular damage in human kidney

The evaluation was carried out in paraffin-embedded and hematoxylin and eosin–stained serial sections. The presence of the following changes in the wall of the interlobular and afferent arterioles was evaluated in 10 consecutive low-power fields (at 10×) in the renal cortex 4 cm away from the tumor in areas devoid of inflammation: subendothelial hyalinosis, intimal hyperplasia, smooth muscle hyperplasia, and wall fibrosis. Based on these features, the patients were categorized as initial MVDa (without MVDa or with subendothelial hyalinosis, intimal hyperplasia, or smooth muscle hyperplasia) or advanced MVDa (wall fibrosis and arteriolar sclerosis). The percentage of obsolescent glomeruli was also calculated.

2.1.5. Determination of adiponectin concentrations

Plasma adiponectin levels were analyzed in relation to EC levels and CB1 expression in human perirenal VAT. Indeed, adiponectin is exclusively secreted from adipose tissue; and its high–molecular-weight (HMW) multimeric form better predicts metabolic parameters than total adiponectin [15].

Serum levels of total and HMW adiponectin multimers were determined by human adiponectin enzyme-linked immunosorbent assay kit (Linco Research, St Charles, MO) on whole serum kept at 80°C before use. The intraassay and interassay coefficients of variation were 4.5% and 7.2%, respectively.

2.1.6. Genotyping

Genomic DNA was extracted from peripheral blood cells using the NucleoSpin Blood Kit (MACHEREY 180 NAGEL, Duren, Germany) following the kit-suggested procedure.

Genotyping for 3813A/G (rs 12720071) SNP in the exon 4 of the CB1 gene was carried out by polymerase chain reaction (using 5′-GAGTTGAAAGGCAAAAGCTAGGTTT-3′ [forward] and 5′ GGGACACAGAAGACAGTCACAA-TAT-3′ [reverse], respectively). The amplicons were subjected to *Hinf*I restriction enzyme digestions, and the genotype was determined by 2.5% agarose gel electrophoretic analysis.

2.2. Obese-hypertensive patients

We recruited 280 consecutive nondiabetic (exclusion by oral glucose load when required) OH patients (aged ≤65 years) from our "Hypertension Excellence Centre" (as certified by the European Society of Hypertension) that represented a larger sample of a population already described [25]. *Obesity* was defined as BMI of at least 30 kg/m^2. *Hypertension* was defined as SBP of at least 140 mm Hg, DBP of at least 90 mm Hg, or current antihypertensive therapy. Secondary forms of hypertension were excluded. *Metabolic syndrome* was defined in accordance with the Adult Treatment Panel III criteria (any 3 or more of the following criteria: waist circumference ≥102 cm in men and ≥88 cm in women, triglycerides ≥150 mg/dL, BP 140/90 mm Hg, HDL cholesterol ≤40 mg/dL in men and ≤50 mg/dL in women, and fasting glucose ≥100 mg/dL or diabetes).

In the OH population, blood was drawn after an overnight fast to measure glucose, total and HDL cholesterol, and triglyceride plasma levels. Clinical dosages were carried out using commercial kits available in our certified University Hospital Central Laboratory. The study protocol was reviewed and approved by the local Ethics Committee, and written informed consent was obtained from all subjects. Genotyping analysis of these OH patients was done as described above.

2.3. Statistical analysis

Hardy-Weinberg equilibrium was tested with χ^2 test. Difference between genotypes in CB1 gene expression levels according to CB1 variant or presence/absence of MVDa in perirenal adipose tissue of 30 patients undergoing nephrectomy was tested using the Student *t* test. The resulting gene expression data were calculated using the 2-delta delta Ct method. Correlations among fat area, EC levels, and *Cnr1* expression were assessed using the Pearson correlation test. Analysis of variance adjusting for age, BMI, number of antihypertensive drugs, and statins was used to analyze clinical values across CB1 genotypes in a subset of 256 OH patients, a subset where all these data were available. The unadjusted odds ratio (OR) for MetS in subjects carrying 3813G variant was calculated. Logistic regression analysis was then used to confirm the independent effect of this variant on MetS. Variables entered in the logistic regression model were age, sex, BMI, and hypertensive drugs. Statistical analysis was performed with SPSS 13.0 (SPSS, Chicago, IL). A level of $P < .05$ was considered as significant.

3. Results

There were no significant differences between whole population (n = 30) and subset patients (available for EC quantification and CT image analyses, n = 12) regarding clinical data and adiponectin plasma levels (Table 1).

Perirenal adipose tissue area was correlated with total VAT area ($r = 0.697$, $P = .025$) as evaluated from the analysis of the available abdominal CT scan images (n = 12), suggesting that perirenal adipose tissue expands similarly to total VAT, thus contributing to visceral obesity (Fig. 1A).

A significant positive correlation was found between normalized CB1 mRNA levels in perirenal VAT and the perirenal VAT area, and also between normalized CB1 mRNA levels in perirenal VAT and total VAT area (Fig. 1B, C; $r = 0.666$, $P = .018$ and $r = 0.728$, $P = .005$, respectively; n = 12).

Despite the small number of samples analyzed (n = 12), AEA levels in the perirenal VAT correlated with CB1 expression in these same tissues (Fig. 2; $r = 0.728$, $P = .005$). No correlation was found between 2-AG levels in perirenal

Table 1
Clinical profile of patients undergoing nephrectomy (n = 30)

	n = 30	n = 12	*P* value
Age (y)	64.8 (2.0)	66.3 (2.1)	.511
BMI (kg/m^2)	27.2 (0.3)	27.9 (1.3)	.441
Waist circumference (cm)	100.5 (1.9)	101.7 (4.5)	.676
Total cholesterol (mg/dL)	190.1 (9.1)	177.8 (22.7)	.119
HDL cholesterol (mg/dL)	38.8 (2.0)	36.1 (3.6)	.475
LDL cholesterol (mg/dL)	122.9 (8.0)	106.9 (17.5)	.160
Glycemia (mg/dL)	84.2 (4.3)	77.4 (7.9)	.266
Triglycerides (mg/dL)	131.4 (1.8)	155.9 (23.5)	.113
SBP (mm Hg)	140.4 (21.4)	139.2 (2.9)	.764
DBP (mm Hg)	82.7 (11.4)	84.8 (3.5)	.426
Total adiponectin (μg/mL)	19.0 (2.8)	18.3 (3.7)	.0884
Adiponectin HMW (μg/mL)	5.8 (1.8)	6.1 (5.5)	.899

Data are mean and standard deviation.

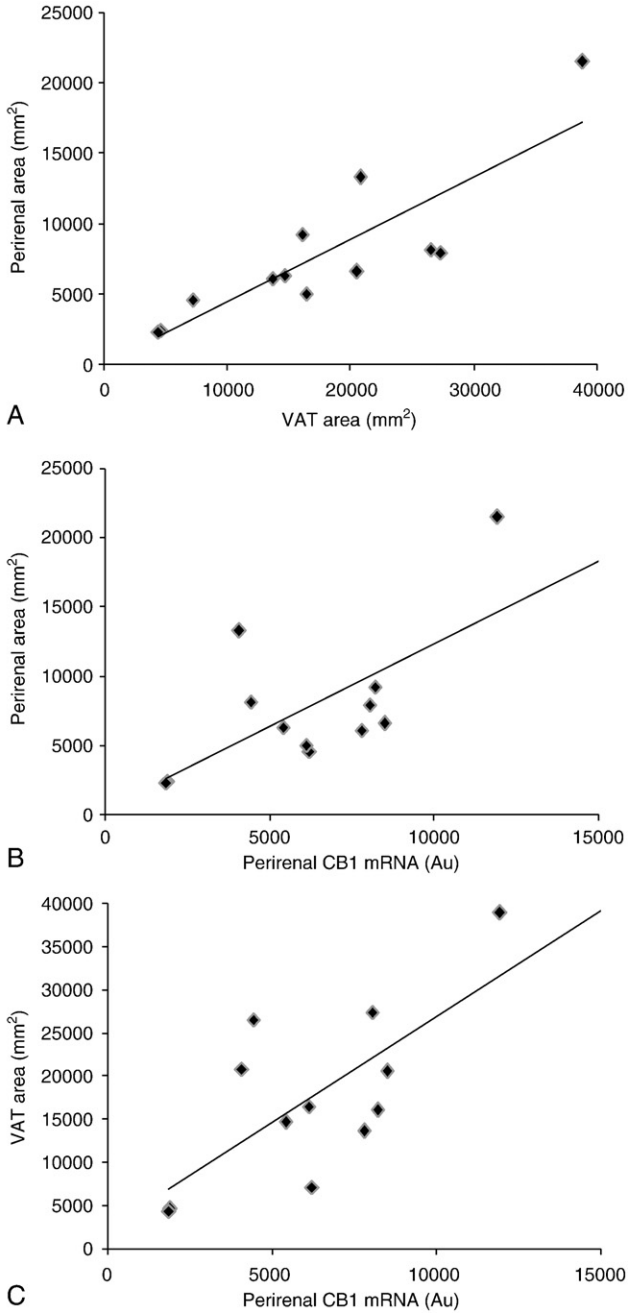

Fig. 1. A, Significant correlation between perirenal adipose tissue and VAT area (in square millimeters) measured by CT scan. B, Significant correlation between CB1 mRNA levels in the perirenal adipose tissue and perirenal adipose tissue area (in square millimeters) measured by CT scan. C, Correlation between CB1 mRNA levels in perirenal adipose tissue and total fat depots area (in square millimeters) measured by CT image analysis. $P < .05$ was considered as significant correlation (n = 12).

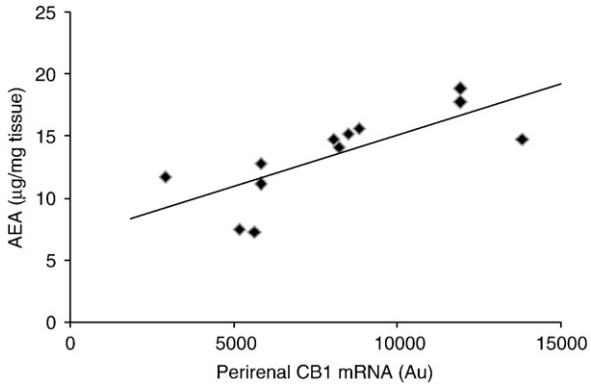

Fig. 2. Significant positive correlation between CB1 mRNA levels and AEA levels in perirenal adipose tissue. $P < .05$ was considered as significant correlation (n = 12).

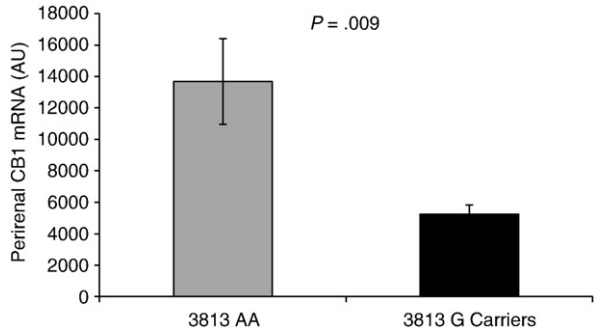

Fig. 4. Comparison between CB1 mRNA levels in perirenal VAT in carriers (n = 19) and noncarriers (n = 11) of the 3813G variant. Data are presented as means ± SEM, and $P < .05$ was considered significant.

VAT and perirenal or total VAT area, or with perirenal VAT CB1 mRNA expression.

Higher levels of VAT CB1 mRNA were found in patients with more advanced MVDa, characterized by the presence of arteriolar sclerosis, than in patients without MVDa or with initial MVDa (Fig. 3; $P = .046$, n = 30).

Total plasma adiponectin levels were associated with increased HDL cholesterol concentration ($r = 0.408$, $P = .047$, data not shown), whereas HMW adiponectin was significantly but negatively correlated with glomerular sclerosis percentage ($r = -0.39$, $P = .045$, data not shown). No relationship was found between adiponectin levels and CB1 gene variant (data not shown).

To assess the effect of the 3813A/G CB1 mutation on CB1 gene expression, we analyzed CB1 mRNA levels in perirenal VAT. A significantly lower (about 40%) expression was found in patients with the 3813G allele in comparison with 3813A carriers (Fig. 4; $P = .009$, n = 30).

Finally, to study the effect of this CB1 variant on clinical and anthropometric parameters, we analyzed a relatively large group of OH patients at high risk of developing MetS. Table 2 shows the clinical characteristics of OH patients. The 3813 A/G polymorphism distribution was in Hardy-Weinberg equilibrium ($P = .527$). Because of the low number of homozygous for the CB1 variant (G3813G, n = 3), subjects were grouped into carriers (3813G, n = 66) and noncarriers (3813A, n = 214). After characterization of OH patients by the absence or presence of MetS (as shown in Table 3), the 3813G CB1 variant was found to be an independent predictive factor for lack of MetS in both unadjusted (OR, 0.385; 95% confidence interval [CI], 0.2-0.7; $P = .003$) and adjusted models (OR, 0.398; 95% CI, 0.2-0.9; $P = .032$; n = 256), thus suggesting a possible protective role of this

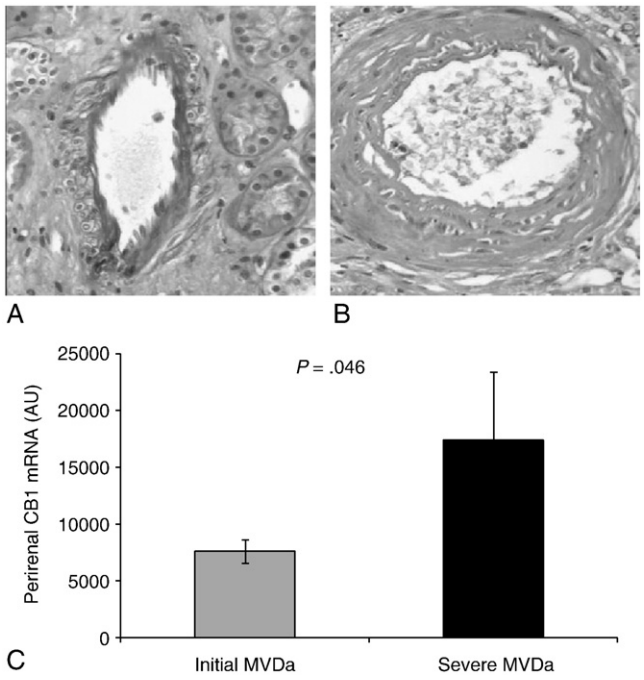

Fig. 3. A, Section of kidney cortex without MVDa evaluated by hematoxylin and eosin staining. B, Section of kidney cortex with severe hyperplasia and fibrosis evaluated by hematoxylin and eosin staining. C, CB1 mRNA levels in perirenal VAT in patients with different severity of arteriolar MVDa. Patients with more advanced MVDa (characterized by the presence of arteriolar sclerosis, n = 11) had significantly higher CB1 mRNA levels in VAT. Data are presented as means ± SEM, and $P < .05$ was considered significant.

Table 2
Clinical characteristics of OH patients

OH patients (n = 280)	
Age (y)	51.2 (0.6)
BMI (kg/m^2)	34.0 (0.3)
Waist circumference (cm)	108.8 (0.9)
Total cholesterol (mg/dL)	208.6 (2.6)
HDL cholesterol (mg/dL)	43.9 (0.7)
Triglycerides (mg/dL)	164.5 (6.4)
LDL cholesterol (mg/dL)	132.0 (2.2)
Glycemia (mg/dL)	100.9 (1.8)
SBP (mm Hg)	146.3 (1.2)
DBP (mm Hg)	92.0 (0.8)
MBP (mm Hg)	109.5 (0.9)

Data are mean and standard error. MBP indicates mean blood pressure.

Table 3
Risk factors for MetS in a subset of 256 OH patients genotyped for the CB1 A3813G SNP

	No MetS	MetS	Unadjusted OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
CB13813A homozygous	33 (16.9)	162 (83.1.)	0.39 (0.20-0.74)	.004	0.375 (0.19-0.74)	.005
3813G carriers	21 (20.4)	40 (65.6)				
Age					1.026 (1.00-1.06)	.137
Sex (male)					0.639 (0.34-1.22)	.173
BMI					1.153 (1.06-1.26)	.001

The results are from Fisher exact test and logistic regression analysis. Nagelkerke R^2 of the logistic regression model = 0.2.

variant against MetS development despite the high risk of the population studied. No differences were found between carriers and noncarriers regarding other clinical and anthropometric parameters.

4. Discussion

Our findings indicate that increased total visceral adiposity in individuals within a wide range of BMI and blood pressure is related to increased CB1 gene expression in perirenal VAT, which in turn is associated with increased VAT AEA, but not 2-AG, levels. Considering that increased total visceral adiposity was also associated with increased amounts of perirenal VAT, these findings indicate that higher amounts of VAT are very likely to be accompanied by up-regulation of the ECS in perirenal VAT.

We also found that this up-regulation of CB1 receptor expression was associated with renal MVDa, thus possibly suggesting that an overactive ECS in perirenal VAT might contribute to this typical consequence of hypertension and visceral adiposity. These findings are in agreement with previous data showing a possible causative role of the up-regulated ECS in renal failure in animal models of obesity. In fact, in the kidneys of mice with high-fat diet–induced obesity, higher levels of both AEA and 2-AG were detected [26], whereas antagonism of CB1 receptors with rimonabant in obese Zucker rats with renal complications caused a significant amelioration of renal hypertrophy and glomerular and tubulointerstitial lesions [27]. Indeed, it is well documented that chronic kidney damage is more frequent and more severe in obesity [3,4]; and increased adiposity seems to be causally linked, directly or indirectly, to the development of vascular damage, thus contributing to chronic renal disease [28]. Dysregulation of the levels of either ECs or CB1 receptor mRNA in the blood and VAT or omental fat, respectively, of obese patients has been reported in several studies. An increase of circulating 2-AG and AEA concentrations, or of AEA only, in obese postmenopausal women or in women with binge-eating disorder, respectively, was reported [6,29]. In untreated asymptomatic obese men, plasma 2-AG, but not AEA, levels were related with intraabdominal adiposity and several cardiometabolic risk factors such as high triglycerides, low HDL cholesterol, and indices of low insulin sensitivity [8]. Similar findings were also observed in obese women [7]. More importantly, correlations were found also between decreases in plasma 2-AG, but not AEA, levels and reductions in these cardiometabolic risk factors, both obtained after lifestyle-induced reduction of intraabdominal obesity [9].

In the only study in which EC levels were analyzed in a VAT depot, 2-AG, but not AEA, levels were found to be higher in obese vs normal-weight individuals [30]. In nonperirenal VAT samples from obese patients, CB1 mRNA levels were negatively correlated with VAT and circulating 2-AG [7]. Here, we did not observe any association between perirenal VAT AEA levels and most anthropometric and metabolic parameters, whereas perirenal VAT CB1 mRNA levels were positively correlated with VAT amounts and AEA levels. These discrepancies with other studies are likely to be explained by the different tissue (perirenal VAT vs blood or omental fat) and the smaller number of patients studied. Moreover, we have studied both male and female patients within a wide range of BMI, going from normal weight to obese, but with average BMI much lower than that of previously studied cohorts. The overactivity of ECS has also been related to decreased adiponectin production [30], and increased circulating 2-AG levels in male obese patients are associated with decreased circulating adiponectin [8]. Visceral adipose tissue might be linked to vascular damage through adiponectin, which is reduced in obesity and increases, concomitantly with reduction of intraabdominal fat, after CB1 antagonism in both rodent and human obese individuals [9,31,32]. The CB1 antagonists are also able to reverse several cardiometabolic risk factors that are worsened by adiponectin deficiency, such as insulin resistance, reduced HDL cholesterol, and elevation of small, dense, and oxidation-prone low-density lipoprotein (LDL) particles [9], which are key factors of vascular damage in large and small arteries. Here we found that lower circulating HMW adiponectin was associated with increased glomerulosclerosis, thus supporting the key role of adiponectin as a protective hormone for micro- and macroarterial vessels. However, we could find no association between perirenal VAT EC or CB1 mRNA levels and circulating HMW adiponectin levels.

The second major aim of the present study was to investigate the role of genetics as a contributor to ECS derangement in obesity. Indeed, we report here for the first time that reduced perirenal VAT CB1 expression levels are associated with the presence of the 3813G alleles, thus suggesting a role for genetics in determining ECS activity. It

is important to note that the 3813G variant is localized in the 3' untranslated region of *Cnr1* and is known to contain regulatory sequences for mRNA stability and for the control of the translation process [33,34]. Moreover, in our OH patients, the 3813G variant was associated with lower prevalence of the MetS, thus behaving as a protective factor against this cluster of pathologic conditions. Although statistical associations do not demonstrate cause-effect relationships, these data overall support the hypothesis that genetic variations at the CB1 locus, by potentially affecting CB1 mRNA levels, as shown above for the perirenal VAT, might reduce the risk of developing the MetS in OH patients. These data are seemingly in contrast with the findings by Russo et al [20], who showed an association of the 3813G variant in *Cnr1* with increased subscapular skinfold thickness, an index of general subcutaneous adiposity, and with high waist circumference, which is usually considered related to visceral fat. However, when visceral adiposity is on average very low, as in the case of the cohort analyzed in this previous study, waist circumference might become instead a marker of abdominal SAT. Therefore, the data by Russo et al [20] may not be at variance with the present findings, as they might be interpreted to suggest the association of 3813G with high SAT vs VAT amounts, which in turn might decrease the risk of developing MetS in obese populations.

Some limitations of the present study should be taken into account. Firstly, kidney and adipose tissues were collected from patients with intracapsular renal carcinoma, which, although localized, might have some systemic effect. Nonetheless, tissues were taken from a healthy kidney portion quite far from the cancerous lesion, this approach having been used also by others [35]. Secondly, although perirenal adipose tissue is localized in the retroperitoneal space and drained into the systemic venous system, different studies underline the similarities (in terms of anatomy, glucose uptake, lipid metabolism/fatty acid composition, adiponectin production, and clinical metabolic significance) between perirenal and intraabdominal adipose tissues [35-38]. Thus, the perirenal adipose tissue could be considered as an indicator of visceral obesity and cardiovascular risk factors in the MetS and is a depot that is believed to be particularly active in metabolism [37].

Finally, the small number of patients and tissue samples may represent another limitation of this study, although we shown that well-established correlations such as between adiponectin levels and HDL were maintained in the cohort used in this study, thus suggesting that the latter constitutes a representative sample of the general population, likely suitable to investigate the correlations between adiponectin and EC levels with clinical data.

In summary, our data suggest that increased visceral adiposity is associated with higher amounts of perirenal VAT and with overactivation of the ECS due to increased AEA levels and CB1 expression in this latter tissue. Such ECS overactivity might be indirectly related to MVDa and glomerular sclerosis, perhaps also through lower adiponectin levels. Moreover, our results on the A3813G variant of *Cnr1* indicate that genetics might impact on CB1 expression level, which appears to be a key component of the resulting ECS overactivation and, subsequently, on the risk of developing the MetS in at-risk populations such as the OH. Overall, our results support the concept that a dysregulated ECS plays an important role in augmenting cardiometabolic risk [10].

References

[1] Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, et al. American Heart Association; Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association scientific statement on obesity and heart disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation 2006;113:898-918.

[2] Sarzani R, Salvi F, Dessì-Fulgheri P, Rappelli A. Renin-angiotensin system, natriuretic peptides, obesity, metabolic syndrome and hypertension: an integrated view in humans. J Hypertens 2008;26: 831-43.

[3] de Jong PE, Verhave JC, Pinto-Sietsma SJ, Hillege HL. Obesity and target organ damage: the kidney. Int J Obes Relat Metab Disord 2002;26:21-4.

[4] Wiggins KJ, Johnson DW. The influence of obesity on the development and survival outcomes of chronic kidney disease. Adv Chronic Kidney Dis 2005;12:49-55.

[5] Cota D, Marsicano G, Tschop M, Grubler Y, Flachskamm C, Schubert M, et al. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. J Clin Invest 2003;112:423-31.

[6] Engeli S, Bohnke J, Feldpausch M, Gorzelniak K, Janke J, Batkai S, et al. Activation of the peripheral endocannabinoid system in human obesity. Diabetes 2005;54:2838-43.

[7] Bluher M, Engeli S, Kloting N, Berndt J, Fasshauer M, Batkai S, et al. Dysregulation of the peripheral and adipose tissue endocannabinoid system inhuman abdominal obesity. Diabetes 2006;55:3053-60.

[8] Cote' M, Matias I, Lemieux I, Petrosino S, Almeras N, Despres JP, et al. Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men. Int J Obes 2007;31:692-9.

[9] Di Marzo V, Côté M, Matias I, Lemieux I, Arsenault BJ, Cartier A, et al. Changes in plasma endocannabinoid levels in viscerally obese men following a 1 year lifestyle modification programme and waist circumference reduction: associations with 365 changes in metabolic risk factors. Diabetologia 2009;52:213-7.

[10] Di Marzo V. The endocannabinoid system in obesity and type 2 diabetes. Diabetologia 2008;51:1356-67.

[11] Despres P, Golay A, Sjostrom L. Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. N Engl J Med 2005;353:2121-34.

[12] Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, Rössner S, RIO-Europe Study Group. Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. Lancet 2005;365:1389-97.

[13] Pi-Sunyer FX, Aronne LJ, Heshmati HM, Devin J, Rosenstock J, RIO-North America Study Group. Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial. JAMA 2006;295:761-75.

[14] Scheen AJ, Finer N, Hollander P, Jensen MD, Van Gaal LF, RIO-Diabetes Study Group. Efficacy and tolerability of rimonabant in

overweight or obese patients with type 2 diabetes: a randomized controlled study. Lancet 2006;368:1660-72.
[15] Hara K, Horikoshi M, Yamauchi T, Yago H, Miyazaki O, Ebinuma H, et al. Measurement of the high–molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. Diabetes Care 2006;29:1357-62.
[16] Zhang PW, Ishiguro H, Ohtsuki T, Hess J, Carillo F, Walther D, et al. Human cannabinoid receptor 1: 5' exons, candidate regulatory regions, polymorphisms, haplotypes and association with polysubstance abuse. Molecular Psychiatry 2004;9:916-31.
[17] Müller TD, Reichwald K, Wermter AK, Brönner G, Nguyen TT, Friedel S, et al. No evidence for an involvement of variants in the cannabinoid receptor gene (*CNR1*) in obesity in German children and adolescents. Mol Genet Metab 2007;90:429-34.
[18] Aberle J, Flitsch J, Beck NA, Mann O, Busch P, Peitsmeier P, et al. Genetic variation may influence obesity only under conditions of diet: analysis of three candidate genes. Mol Genet Metab 2008;95:188-91.
[19] Peeters A, Beckers S, Mertens I, Van Hul W, Van Gaal L. The G1422A variant of the cannabinoid receptor gene (*CNR1*) is associated with abdominal adiposity in obese men. Endocrine 2007;31:138-41.
[20] Russo P, Strazzullo P, Cappuccio FP, Tregouet DA, Lauria F, Loguercio M, et al. Genetic variations at the endocannabinoid type 1 receptor gene (*CNR1*) are associated with obesity phenotypes in men. J Clin Endocrinol Metab 2007;92:2382-6.
[21] Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet 2004;74:106-20.
[22] Sarzani R, Bordicchia M, Marcucci P, Bedetta S, Santini S, Giovagnoli A, et al. Altered pattern of cannabinoid CB1 receptor expression in adipose tissue of dysmetabolic and overweight patients. Metabolism 2009;58:361-7.
[23] Sarzani R, Fallo F, Dessi-Fulgheri P, Pistorello M, Lanari A, Paci VM, et al. Local renin-angiotensin system in human adrenals and aldosteronomas. Hypertension 1992;19:702-7.
[24] Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, et al. The endogenous cannabinoid system controls extinction of aversive memories. Nature 2002;418:530-4.
[25] Sarzani R, Salvi F, Bordicchia M, Pietrucci F, Caraceni D, Lancioni L, et al. TCF7L2 alleles and metabolic syndrome in non-diabetic obese hypertensive patients. J Hum Hypertens 2008;22:373-5.
[26] Matias I, Petrosino S, Racioppi A, Capasso R, Izzo AA, Di Marzo V. Dysregulation of peripheral endocannabinoid levels in hyperglycemia and obesity: effect of high fat diets. Mol Cell Endocrinol 2008;16 (286):S66-78.
[27] Janiak P, Poirier B, Bidouard JP, Cadrouvele C, Pierre F, Gouraud L, et al. Blockade of cannabinoid CB1 receptors improves renal function, metabolic profile, and increased survival of obese Zucker rats. Kidney Int 2007;72:1345-57.
[28] Ejerblad E, Fored CM, Lindblad P, Fryzek J, McLaughlin JK, Nyrén O. Obesity and risk for chronic renal failure. J Am Soc Nephrol 2006;17:1501-2.
[29] Monteleone P, Matias I, Martiadis V, De Petrocellis L, Maj M, Di Marzo V. Blood levels of the endocannabinoid anandamide are increased in anorexia nervosa and in binge-eating disorder, but not in bulimia nervosa. Neuropsychopharmacology 2005;30:1216-21.
[30] Matias I, Gonthier MP, Orlando P, Martiadis V, De Petrocellis L, Cervino C, et al. Regulation, function, and dysregulation of endocannabinoids in models of adipose and beta-pancreatic cells and in obesity and hyperglycemia. J Clin Endocrinol Metab 2006;91: 3171-80.
[31] Herling AW, Kilp S, Juretschke HP, Neumann-Haefelin C, Gerl M, Kramer W. Reversal of visceral adiposity in candy-diet fed female Wistar rats by the CB1 receptor antagonist rimonabant. Int J Obes 2008;32:1363-72.
[32] Després JP, Ross R, Boka G, Alméras N, Lemieux I. ADAGIO-Lipids Investigators. Effect of rimonabant on the high-triglyceride/ low-HDL-cholesterol dyslipidemia, intraabdominal adiposity, and liver fat: the ADAGIO-Lipids trial. Arterioscler Thromb Vasc Biol 2009;29: 416-23.
[33] Shire D, Carillon C, Kaghad M, Calandra B, Rinaldi-Carmona M, Le Fur G, et al. An amino-terminal variant of the central cannabinoid receptor resulting from alternative splicing. J Biol Chem 1995;270: 3726-31.
[34] Dodson RE, Shapiro DJ. Regulation of pathways of mRNA destabilization and stabilization. Prog Nucleic Acid Res Mol Biol 2002;72:129-64.
[35] Lai KN, Leung JC, Lai KB, To WY, Yeung VT, Lai FM. Gene expression of the renin angiotensin system in human kidney. J Hypertens 1998;16:91-102.
[36] Busetto L, Tregnaghi A, Bussolotto M, Sergi G, Benincà P, Ceccon A, et al. Visceral fat loss evaluated by total body magnetic resonance imaging in obese women operated with laparascopic adjustable silicone gastric banding. Int J Obes Relat Metab Disord 2000;24: 60-9.
[37] Shen W, Wang Z, Punyanita M, Lei J, Sinav A, Kral JG, Heymsfield SB, et al. Adipose tissue quantification by imaging methods: a proposed classification. Obes Res 2003;11:5-16.
[38] Björntorp P. The regulation of adipose tissue distribution in humans 1996. Int J Obes Relat Metab Disord 1996;20:291-302.