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Clinical Research Article

Higher Plasma Levels of Endocannabinoids and Analogues Correlate With a Worse Cardiometabolic Profile in Young Adults

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Abstract

Context: The endocannabinoid system (ECS) is a signaling system composed of endocannabinoids (eCBs), their receptors, and the enzymes involved in their synthesis and metabolism. Alterations in the ECS are linked to the development of cardiometabolic diseases.

Objective: Here, we investigated the relationship between plasma levels of eCBs and their analogues with body composition and cardiometabolic risk factors.

Methods: The study included 133 young adults (age 22.1 ± 2.2 years, 67% women). Fasting plasma levels of eCBs and their analogues were measured using liquid chromatography-tandem mass spectrometry. Body composition, brown adipose tissue (BAT) volume, glucose uptake, and traditional cardiometabolic risk factors were measured.

Results: Plasma levels of eCBs and several eCB analogues were positively correlated with adiposity and traditional cardiometabolic risk factors (eg, serum insulin and triacylglyceride levels, all $r \geq 0.17$ and $P \leq .045$). Plasma levels of 2-arachidonoyl glycerol and N-pentadecenoylethanolamine were negatively correlated with BAT volume and glucose uptake (all $r \leq -0.17$ and $P \leq .047$). We observed that the plasma levels of eCBs and their analogues were higher in metabolically unhealthy overweight–obese participants than in metabolically healthy overweight–obese participants.

Conclusion: Our findings show that the plasma levels of eCBs and their analogues are related to higher levels of adiposity and worse cardiometabolic profile.

Key Words: body composition, cardiometabolic risk factors, anandamide, endocannabinoid system, 2-arachidonoyl glycerol, visceral adipose tissue

Abbreviations: 2-AG, 2-arachidonoylglycerol; 2-LG, 2-linoleoylglycerol; 2-OG, 2-oleoylglycerol; ^{18}F-FDG, ^{18}F-fluorodeoxyglucose; AEA, anandamide; APOA1, apolipoprotein A1; APOB, apolipoprotein B; ATP III, Adult Treatment Panel III; BAT, brown adipose tissue; BMI, body mass index; CB1R, cannabinoid receptor type 1; CB2R, cannabinoid receptor type 2; CMD, cardiometabolic disease; DGLEA, N-dihomo-gamma-linolenoylethanolamine; DHEA, N-docosahexaenoylethanolamine; eCB, endocannabinoid; ECS, endocannabinoid system; FBM, fat body mass; HDL-C, high-density lipoprotein-cholesterol; HOMA index, homeostatic model assessment for insulin resistance index; IDF, International Diabetes Federation; LC-MS/MS, Liquid chromatography-tandem mass spectrometry; LBM, lean body mass; LDL-C, low-density lipoprotein cholesterol; LEA, N-linoleoylethanolamine; LMI, lean mass index; MHOO, metabolically healthy overweight–obese; MUOO, metabolically unhealthy overweight–obese; NAE, N-acyl ethanolamine; NAPE, N-acyl phosphatidylethanolamine; N-PEA, palmitoylethanolamine; OEA, N-oleoylethanolamine; POEA, N-palmitoleoylethanolamine; PDEA,

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N-pentadecanoylethanolamine; QC, quality control; RSD, relative standard deviation; SD, standard deviation; SEA, N-stearoylethanolamine; SUV, standardized uptake value; TC, total cholesterol; TG, triglyceride; VAT, visceral adipose tissue; WAT, white adipose tissue.

Cardiometabolic diseases (CMDs) are the leading cause of mortality worldwide (1, 2). The increase in obesity and obesity-related cardiometabolic disorders, including dyslipidemia, hyperglycemia, hypertension, and abdominal fat accumulation partially drives the increments in the prevalence of CMD (3, 4). Despite the recent advances in understanding the pathological mechanisms underlying the onset and progression of CMD, additional efforts are required to improve the prognosis and diagnosis of these diseases.

Emerging research suggests that the endocannabinoid system (ECS) may be involved in the onset and progression of CMD. The ECS is composed of cannabinoid receptor type 1 (CB1R) and cannabinoid receptor type 2 (CB2R), their endogenous agonists anandamide (AEA), and 2-arachidonoylglycerol (2-AG), as well as the metabolic enzymes of these 2 endocannabinoids (eCBs) (5). In mice, the activation of CB1R plays a significant role in the development of obesity by regulating appetite and feeding behavior, influencing food intake and energy balance, and regulating the browning of adipose tissue (BAT) (5). Moreover, CB1R-mediated eCB signaling has been directly implicated in the development of insulin resistance and type 2 diabetes mellitus, as well as in cardiovascular diseases such as atherosclerosis or myocardial infarction (6, 7).

Besides the eCBs, their structural analogues could also be important in the development of CMD (8). These structural analogues include N-acyl ethanolamines (NAEs), such as N-palmitoylethanolamine (PEA), N-oleoylethanolamine (OEA), and N-linoleylethanolamine (LEA), as well as other 2-acylglycerols, such as 2-linoleoylglycerol (2-LG) and 2-oleoylglycerol (2-OG). These structural homologues do not have affinity for CB1R or CB2R, but can enhance the effects of AEA and 2-AG on their receptors by increasing their affinity or inhibiting their hydrolysis (so-called *entourage effect*) (9, 10).

In human, several previous studies showed correlations between eCBs with obesity and cardiometabolic risks; however, inconsistent results among 2-AG and AEA were observed (11-14). Moreover, these studies were focused on middle-aged or elderly populations, but the correlation of these metabolites with cardiometabolic risk factors in younger populations has never been studied. Therefore, in this study, we aimed to investigate the association of plasma levels of eCBs and their analogues with body composition parameters and cardiometabolic risk factors in a cohort of young adults.

Material and Methods

Study Design and Participants

This cross-sectional study was performed under the framework of the ACTIBATE study (ClinicalTrials.gov, ID: NCT02365129) (15, 16). The study included 136 young adult participants, 45 males and 91 females (Table 1). All participants were recruited via advertisements in electronic media and leaflets. The inclusion criteria included an age of 18 to 25 years; being engaged in less than 20 minutes of moderate or vigorous physical activity per day on <3 days/week; not smoking; having a stable body weight over the past 3 months (change <3 kg); without any CMD (eg, hypertension, diabetes); not taking any medication that might affect cardiovascular function; and no history of cancer among first-degree relatives. The study protocol and experimental design were applied in accordance with the last revised ethical guidelines of the Declaration of Helsinki. The study was approved by the Ethics Committee on Human Research of the University of Granada (no. 924) and the Servicio Andaluz de Salud (Centro de Granada, CEI-Granada); all participants gave informed consent.

Determination of Plasma Levels of Endocannabinoids and Endocannabinoid Analogues

Plasma levels of AEA, 2-AG, and their analogues (ie, 2-LG, 2-OG, N-α-linolenoylethanolamine [α-LEA], N-dihomo-gamma-linolenoylethanolamine [DGLEA], N-docosahexaenoylethanolamine [DHEA], LEA, OEA, PEA, N-pentadecanoylethanolamine [PDEA], N-palmitoleoylethanolamine [POEA], and N-stearoylethanolamine [SEA]), together with arachidonic acid (ie, a downstream metabolite of AEA and 2-AG) were assessed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) after liquid–liquid extraction. The liquid–liquid extraction–LC-MS/MS method has been described previously (17, 18). Deuterated internal standards (listed in Table S1 (19)) were used to correct for analytical errors. Quality control (QC) samples were regularly injected during the measurements and used to evaluate the data quality and correct for between-batch variations using the in-house developed mzQuality workflow (available at http://www.mzQuality.nl) (20). Relative standard deviations (RSDs) were calculated for each analyte present in the QC samples using the peak area ratios (ie, peak area of the target analyte divided by the peak area of the respective internal standard). Metabolites with RSDs ≤ 15% were included in further data analyses. Metabolites showing RSDs higher than 30% on peak area ratios in QC samples were excluded. Metabolites with 15% ≤ RSDs < 30% were interpreted with caution (Table 1). The experimental procedure is detailed elsewhere (19).

Anthropometry and Body Composition

Body weight and height were measured using a SECA model 799 electronic column scale and a stadiometer (SECA, Hamburg, Germany). The waist circumference was measured in the minimum perimeter, at the end of a normal breath expiration, with the arms relaxed on both sides of the body. The measurements were taken just above the umbilicus following a horizontal plane when the minimum perimeter could not be detected, such as in overweight or obese participants. The waist circumference was measured twice with a plastic tape measure, and the 2 measurements were averaged. Lean body mass (LBM), fat body mass (FBM), and visceral adipose tissue (VAT) were determined with a Hologic Discovery Wi dual-energy X-ray absorptiometer (Hologic, Marlborough, MA, USA). Body mass index (BMI), lean mass index (LMI), and fat mass index were calculated by dividing body weight, LBM, and FBM (in kilograms) by the square of the height (in meters), respectively. The fat mass percentage (%) was calculated as the FBM divided by total body mass and multiplied by 100.

Table 1. Characteristics of the study participants

	N	Total	N	Men	N	Women
Age (years)	136	22.1 (2.2)	45	22.3 (2.3)	91	21.9 (2.2)
Body composition						
Body mass index (kg/m^2)	136	24.9 (4.6)	45	26.8 (5.5)	91	23.9 (3.7)
Lean body mass (kg)	136	41.8 (9.7)	45	52.8 (7.2)	91	36.3 (5.0)
Lean mass index (kg/m^2)	136	14.7 (2.4)	45	17.2 (2.1)	91	13.5 (1.4)
Fat body mass (kg)	136	24.7 (8.8)	45	24.8 (11.0)	91	24.6 (7.5)
Fat mass (%)	136	35.5 (7.6)	45	29.7 (7.6)	91	38.3 (5.9)
Fat mass index (kg/m^2)	136	8.8 (3.0)	45	8.1 (3.6)	91	9.1 (2.7)
Visceral adipose tissue (g)	136	336.4 (174.1)	45	417.9 (175.9)	91	296.1 (159.2)
Waist circumference (cm)	130	81.0 (4.6)	43	89.9 (15.2)	87	76.5 (10.5)
Brown adipose tissue						
BAT volume (mL)	131	68.5 (57.4)	42	78.9 (66.0)	89	63.6 (52.6)
BAT metabolic activity	131	332.9 (328.7)	42	326.8 (327.8)	89	335.8 (331.0)
BAT SUVmean	131	3.7 (1.9)	42	3.2 (1.3)	89	4.0 (2.1)
BAT SUVpeak	131	11.1 (8.2)	42	9.9 (7.3)	89	11.6 (8.6)
BAT SUVmax	131	12.2 (9.0)	42	10.8 (8.1)	89	12.8 (9.4)
Cardiometabolic risk factors						
Metabolic syndrome ATP III	128	0.5 (0.9)	42	1.0 (1.3)	86	0.2 (0.5)
Metabolic syndrome IDF	128	0.7 (1.1)	42	1.1 (1.5)	86	0.5 (0.7)
Fatty liver index	132	20.4 (25.0)	43	36.9 (32.0)	89	12.5 (15.7)
GTP (IU/L)	131	19.0 (17.5)	43	28.4 (26.8)	88	14.4 (6.7)
GGT (IU/L)	131	19.8 (20.0)	43	29.9 (29.8)	88	14.9 (9.9)
ALP (IU/L)	132	71.3 (18.5)	43	79.3 (19.4)	89	67.5 (16.9)
C-reactive protein (mg/L)	132	2.4 (3.4)	43	2.1 (2.3)	89	2.5 (3.8)
C3 (mg/dL)	132	137.4 (23.8)	43	143.0 (26.2)	89	134.7 (22.2)
C4 (mg/dL)	132	28.7 (8.8)	43	30.3 (9.9)	89	27.9 (8.1)
Insulin glucose ratio	132	14.1 (7.0)	43	14.8 (8.8)	89	13.8 (6.0)
HOMA index	132	1.8 (1.2)	43	2.1 (1.6)	89	1.7 (1.0)
Glucose (mg/dL)	132	87.6 (6.6)	43	88.9 (7.4)	89	87.0 (6.1)
Insulin (μIU/mL)	132	8.3 (4.9)	43	9.1 (6.4)	89	8.0 (4.0)
Total cholesterol (mg/dL)	132	165.1 (32.2)	43	160.1 (30.9)	89	167.6 (32.7)
HDL-C (mg/dL)	132	52.8 (11.0)	43	46.0 (7.4)	89	56.0 (11.0)
LDL-C (mg/dL)	132	96.0 (25.3)	43	96.5 (26.2)	89	95.8 (25.0)
APOA1 (mg/dL)	113	144.7 (27.5)	37	130.0 (16.8)	76	151.9 (28.9)
APOB (mg/dL)	113	69.7 (19.9)	37	72.7 (24.4)	76	68.3 (17.3)
Triglycerides (mg/dL)	132	82.5 (44.6)	43	88.2 (47.2)	89	79.7 (43.2)
Leptin (μg/L)	129	6.2 (4.4)	42	4.4 (4.0)	87	7.1 (4.3)
Adiponectin (mg/L)	127	11.4 (7.9)	42	7.7 (5.2)	85	13.3 (8.3)
Systolic blood pressure (mmHg)	134	116.7 (11.6)	44	125.3 (10.9)	90	112.5 (9.5)
Diastolic blood pressure (mmHg)	134	70.9 (7.6)	44	72.2 (9.2)	90	70.3 (6.7)
Circulating endocannabinoids (peak area ratio)						
AEA	133	0.14 (0.06)	43	0.14 (0.05)	90	0.13 (0.06)
2-AG[a]	133	0.18 (0.13)	43	0.21 (0.21)	90	0.16 (0.07)
AA	133	64.3 (20.7)	43	61.14 (18.42)	90	65.81 (21.64)
2-LG[a]	133	0.17 (0.28)	43	0.22 (0.44)	90	0.15 (0.15)
2-OG[a]	133	0.04 (0.07)	43	0.05 (0.12)	90	0.03 (0.04)
DHEA[a]	133	0.1 (0.24)	43	0.12 (0.36)	90	0.08 (0.16)
DGLEA[a]	132	0.21 (0.08)	43	0.2 (0.06)	89	0.21 (0.09)
LEA	133	0.01 (0)	43	0.01 (0)	90	0.01 (0)
α-LEA	133	1.72 (0.27)	43	1.71 (0.22)	90	1.72 (0.29)
PEA	132	0.02 (0.01)	43	0.02 (0.01)	89	0.02 (0.01)
PDEA[a]	133	0.26 (0.2)	43	0.18 (0.12)	90	0.3 (0.22)

(*continued*)

Table 1. Continued

	N	Total	N	Men	N	Women
POEA	133	0.68 (0.2)	43	0.65 (0.17)	90	0.69 (0.21)
OEA	133	1.26 (0.22)	43	1.27 (0.21)	90	1.26 (0.23)
SEA	133	0.14 (0.06)	43	0.14 (0.05)	90	0.13 (0.06)

Data are presented as mean (SD).
Abbreviations: 2-AG, 2-arachidonoylglycerol; 2-LG, 2-linoleoylglycerol; 2-OG, 2-oleoylglycerol; α-LEA, α-linolenoylethanolamine; AA, arachidonic acid; AEA, anandamide; ALP, Alkaline phosphatase; APOA1, apolipoprotein A1; APOB, apolipoprotein; BAT, brown adipose tissue; DGLEA, N-dihomo-gamma-linolenoylethanolamine; DHEA, N-docosahexaenoylethanolamine; C3, complement component 3; C4, complement component 4; GGT, gamma-glutamyl transferase; GTP, glutamic pyruvic transaminase; HDL-C, high-density lipoprotein cholesterol; HOMA index, homeostatic model assessment for insulin resistance index; LDL-C, low-density lipoprotein cholesterol; LEA, N-linoleoylethanolamine; Metabolic syndrome ATP III, Metabolic syndrome prevalence calculated following the National Cholesterol Education Program Adult Treatment Panel III classification; Metabolic syndrome IDF, Metabolic syndrome prevalence calculated following the International Diabetes Federation (IDF) classification; OEA, N-oleoylethanolamine; PEA, N-palmitoylethanolamine; PDEA, N-pentadecanoylethanolamine; POEA, N-palmitoleoylethanolamine; SEA, N-stearoylethanolamine; SUV, standardized uptake value.
[a]Analytes to be considered with caution, as relative SDs were between 15% and 30% in quality control samples.

Activation and Determination of ^{18}F-fluorodeoxyglucose Uptake by Brown Adipose Tissue

Activation of BAT was carried out using a personalized cooling protocol for each participant on 2 independent days. This personalized cooling protocol has been extensively described elsewhere (21). Briefly, we first determined the shivering threshold of each participant; 48 to 72 hours later, the uptake of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) by BAT was determined. During the shivering threshold test, participants were exposed for 30 minutes to a warm room for acclimation purposes, before moving to a mild-cold room. Next, participants wore a water-perfused cooling vest (Polar Products Inc., Stow, OH, USA) during the second part of the test. The water temperature of this cooling vest was reduced from 16.6 °C to ∼2.2 °C per 10 minutes until participants began shivering; 48 to 72 hours later, the participants went to the hospital, where they were exposed to the same cooling protocol for 2 hours at ∼4 °C above the water temperature, for which they reported shivering. A subgroup of individuals did not report shivering and were therefore exposed to the lowest water temperature of the device. After 1 hour of cold exposure, a bolus of ∼185 MBq of ^{18}F-FDG was intravenously injected, before a positron emission tomography/computed tomography scan (Siemens Biograph 16 positron emission tomography/computed tomography, Siemens Healthcare, Erlangen, Germany) 2 hours later. The BAT volume and BAT ^{18}F-FDG activity were determined following standard guidelines (22) using the Beth Israel plugin for the FIJI program. This required the determination of the number of pixels in the region of interest with a radiodensity range of −190 to −10 Hounsfield Units, as well as the individualized, threshold ^{18}F-FDG standardized uptake values (SUVs) [1.2/(LBM/body mass)] (22). BAT volume was determined as the number of pixels in the range described above, with an SUV value above the SUV threshold. BAT metabolism activity was determined concerning the mean SUV (SUVmean, ie, the mean quantity of ^{18}F-FDG contents in 3 pixels within a volume of <1 cm^3), peak SUV (SUVpeak, ie, the mean of the 3 highest ^{18}F-FDG contents in 3 pixels within a volume of <1 cm^3), max SUV (SUVmax, ie, the maximum quantity of ^{18}F-FDG contents in 3 pixels within a volume of <1 cm^3) (23).

Blood Sample Collection and Determination of Cardiometabolic Risk Factors

Blood samples were taken at baseline after 10 hours of fasting (15). Serum glucose, total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), apolipoprotein A1 (APOA1) and apolipoprotein B (APOB), triglyceride (TG), as well as liver enzyme (ie, glutamic pyruvic transaminase, gamma-glutamyl transferase, and alkaline phosphatase) levels were assessed following standard methods using an AU5832 automated analyzer (Beckman Coulter Inc., Brea, CA, USA) with Beckman Coulter reagents (OSR6521, OSR6116, OSR60118, OSR446410, OSR447730, OSR6507, OSR6520, OSR6204, and OSR6187, respectively). Low-density lipoprotein-cholesterol (LDL-C) was estimated as [total cholesterol – HDL-C – (TG/5)], with all units in mg/dL (24). Serum insulin was measured using the Access Ultrasensitive Insulin chemiluminescent immunoassay kit (Beckman Coulter Cat# 33410, RRID:AB_2756878). The homeostatic model assessment for insulin resistance index (HOMA index) was calculated as insulin levels (μU/mL) multiplied by glucose levels (mmol/L)/22.5 (25), whereas the fatty liver index was calculated following standard guidelines (26). C-reactive protein, complement component C3, and complement component C4 concentrations were measured by immunoturbidimetric assays (Beckman Coulter Cat# OSR6159, RRID:AB_3073653; Beckman Coulter Cat# OSR6160, RRID:AB_3073654; Beckman Coulter Cat# OSR6160, RRID: AB_3073655) using an AU5832 spectrophotometer. Leptin and adiponectin concentrations were measured using the MILLIPLEX MAG Human Adipokine Magnetic Bead Panel 2 and MILLIPLEX MAP Human Adipokine Magnetic Bead Panel 1, respectively (Luminex Corporation, Austin, TX, USA). The metabolic syndrome prevalence was calculated following the National Cholesterol Education Program Adult Treatment Panel III (ATP III) (27) and International Diabetes Federation (IDF) classifications (28). An Omron M6 upper arm blood pressure monitor (Omron Healthcare Europe B.V., Hoofddorp, The Netherlands) was used to determine the systolic blood pressure and diastolic blood pressure, with subjects seated and relaxed; measurements were taken at 3 time points with the mean used in later analyses.

Classification of Metabolically Healthy Overweight–Obese and Metabolically Unhealthy Overweight–Obese Participants

Overweight or obese individuals were divided into 2 groups: metabolically healthy overweight–obese (MHOO) and metabolically unhealthy overweight–obese (MUOO) groups. MHOO participants were defined as having a BMI ≥25 kg/m^2 and not presenting any of the following criteria (29): (1) serum

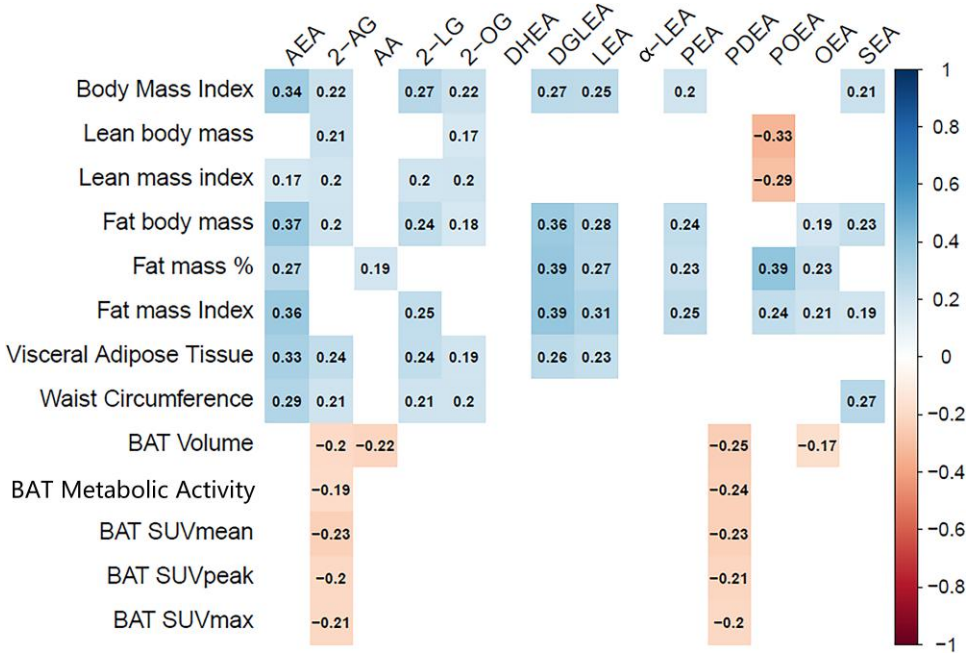

Figure 1. Pearson correlations between plasma levels of endocannabinoids and their analogues with body composition and brown adipose tissue parameters in young and sedentary adults (n = 133). Every box represents a statistically significant correlation coefficient ($P < .05$), whereas empty spaces represent no statistically significant correlations. 2-AG, 2-arachidonoylglycerol; 2-LG, 2-linoleoylglycerol; 2-OG, 2-oleoylglycerol; α-LEA, N-α-linolenoylethanolamine; AA, arachidonic acid; AEA, anandamide; BAT, brown adipose tissue; DGLEA, N-dihomo-gamma-linolenoylethanolamine; DHEA, N-docosahexaenoylethanolamine; LEA, N-linoleoylethanolamine; OEA, N-oleoylethanolamine; PEA, N-palmitoylethanolamine; PDEA, N-pentadecanoylethanolamine; POEA, N-palmitoleoylethanolamine; SEA, N-stearoylethanolamine; SUV, standardized uptake value.

TG concentration ≥150 mg/dL; (2) serum HDL-C concentration ≤40 mg/dL for men and ≤50 mg/dL for women; (3) systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg; or (4) serum glucose concentration ≥100 mg/dL. MUOO participants were defined as having a BMI ≥25 kg/m^2 and presenting at least 1 of the cardiometabolic risk factors mentioned above factors.

Statistical Analysis

Categorical and continuous variables were used to describe the clinical and demographic characteristics of the study participants. Since peak area ratios of the plasma eCBs and their analogues and blood cardiometabolic risk factors did not follow a normal distribution, they were $\log_{10}$ transformed to obtain normal distributions. Data were presented as mean ± SD unless otherwise stated. Since no sex interaction was observed (all $P > .05$), data from both sexes were pooled together for all the statistical analyses, unless otherwise stated. Pearson correlations of plasma levels of eCBs and their analogues with body composition and cardiometabolic risk factors were obtained using R (V.3.6.0). Correlation plots were built using the R package "corrplot". False discovery rate was not performed in the data analysis, as this requires the analytes to be independent, while the eCBs and their analogues are not. In that case, overcorrection may occur with false discovery rate, leading to false negatives. In separate models, forward stepwise regression analyses were conducted with FBM and VAT as dependent outcomes. The measured plasma eCBs and their analogues and leptin and adiponectin values were introduced as predictors using a "forward stepwise" procedure. This procedure introduces predictor components step by step into the model (if $P < .05$) according to the strength of their association with the dependent outcome. All forward stepwise regression analyses were performed with Statistical Package for the Social Sciences v.22.0 (IBM Corporation, Chicago, IL, USA), with a significance level set at $P < .05$. The differences in the plasma levels of eCBs between MHOO and MUOO individuals were assessed using 1-way analyses of covariance, including either sex or VAT as a confounder. Box plots were made using GraphPad Prism software v.9 (GraphPad Software, San Diego, CA, USA).

Results

Characteristics of the Study Participants and Plasma Levels of Endocannabinoids and Their Analogues

The characteristics of the study participants are shown in Table 1. The LC-MS/MS method enabled the relative quantitation of 14 eCBs and their analogues. Among those, 8 metabolites showed RSD values for peak area ratios in QC samples lower than 15%, while 6 metabolites were detected with RSDs between 15% and 30% in QC samples (Table 1). The data quality was confirmed based on the acceptance criteria typically used in metabolomics-based experiments (30, 31).

Plasma Levels of Endocannabinoids and Their Analogues Are Positively Correlated With Adiposity and Cardiometabolic Risk Factors

In the pooled data of males and females, the plasma levels of AEA, 2-AG, and most of the eCB analogues (ie, 2-LG, 2-OG, DGLEA, LEA, PEA, POEA, OEA, and SEA) were positively correlated with adiposity (ie, BMI, waist circumference, FBM, and VAT, Fig. 1). Notably, POEA showed a negative correlation with LBM ($r = -0.33$, $P < .001$) and LMI

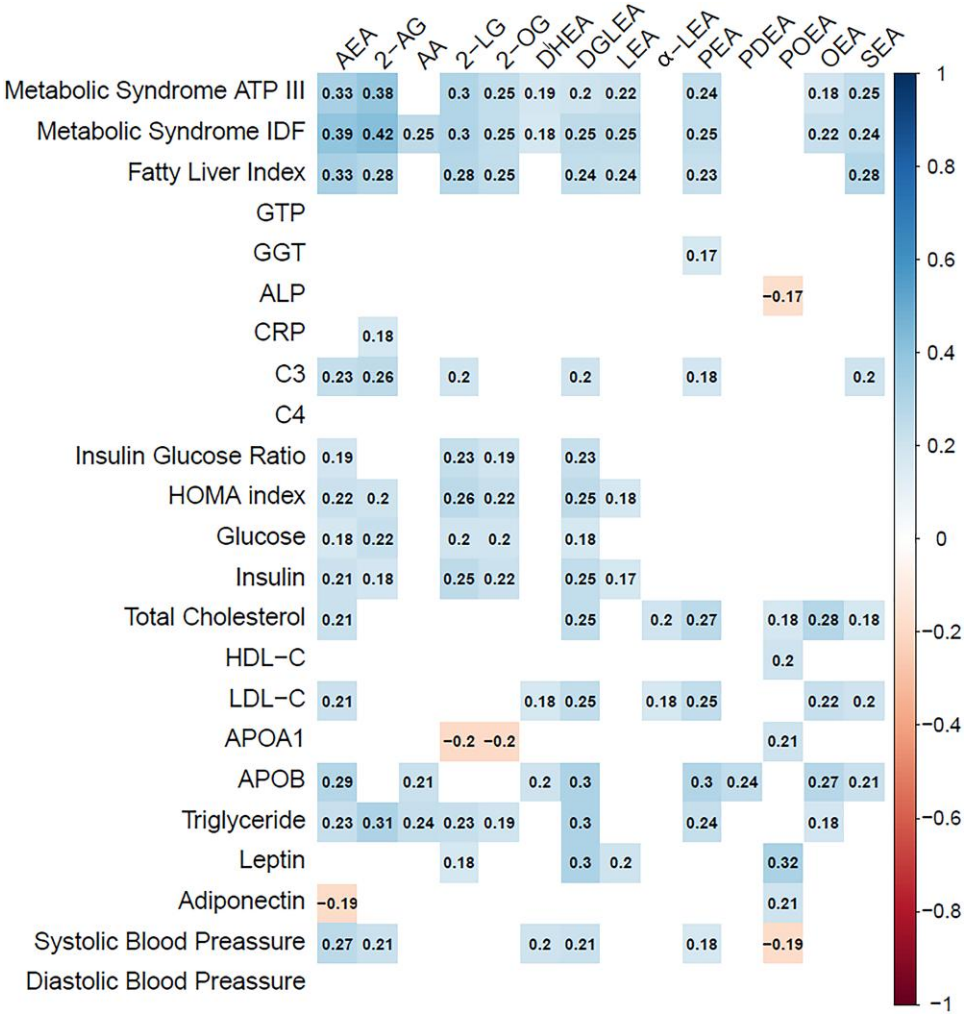

Figure 2. Pearson correlations between plasma levels of endocannabinoids and their analogues with cardiometabolic risk factors in young sedentary adults (n = 133). Every box represents a statistically significant correlation coefficient ($P < .05$), whereas empty spaces represent no statistically significant correlations. 2-AG, 2-arachidonoylglycerol; 2-LG, 2-linoleoylglycerol; 2-OG, 2-oleoylglycerol; α-LEA, α-linolenoylethanolamine; AA, arachidonic acid; AEA, anandamide; ALP, alkaline phosphatase; APOA1, apolipoprotein A1; APOB, apolipoprotein B; BAT, brown adipose tissue; C3, complement component C3; C4, complement component C4; CRP, C-reactive protein; DGLEA, N-dihomo-gamma-linolenoylethanolamine; DHEA, N-docosahexaenoylethanolamine; GTP, transaminase; GGT, gamma-glutamyl transferase; HDL-C, high-density lipoprotein cholesterol; HOMA index, homeostatic model assessment for insulin resistance index; LDL-C, low-density lipoprotein cholesterol; LEA, N-linoleoylethanolamine; Metabolic syndrome ATP III, Metabolic syndrome prevalence calculated following the National Cholesterol Education Program Adult Treatment Panel III classification; Metabolic syndrome IDF, metabolic syndrome prevalence calculated following the International Diabetes Federation (IDF) classification; OEA, N-oleoylethanolamine; PEA, N-palmitoylethanolamine; PDEA, N-pentadecanoylethanolamine; POEA, N-palmitoleoylethanolamine; SEA, N-stearoylethanolamine.

($r = -0.29$, $P < .001$). DHEA, α-LEA, and PDEA did not significantly correlate with body composition parameters (Fig. 1). 2-AG and PDEA, but not AEA, showed negative correlations with BAT parameters (ie, BAT volume and glucose uptake by BAT; all $r \leq -0.19$, $P \leq .031$, Fig. 1).

The plasma levels of AEA, 2-AG, and eCB analogues were positively correlated with cardiometabolic risk factors (Fig. 2). Both 2-AG and AEA showed positive correlations with the prevalence of metabolic syndrome assessed by ATP III ($r \geq 0.33$, $P < .001$, Fig. 2) and IDF ($r \geq 0.39$, $P < .001$, Fig. 2). Positive correlations were observed between the eCBs and glucose parameters (ie, insulin glucose ratio, glucose, insulin, HOMA index), as well as some of the lipid parameters (ie, TC, LDL-C, APOB, and TG levels) and the fatty liver index (Fig. 2). Only AEA showed a weak and negative correlation with adiponectin levels ($r = -0.19$, $P = .035$, Fig. 2). 2-AG and AEA were not correlated with leptin levels. 2-LG, 2-OG, and most of the NAEs showed positive correlations with the prevalence of metabolic syndrome assessed by ATP III ($r \geq 0.18$, $P < .038$) and IDF ($r \geq 0.18$, $P < .012$) (Fig. 2). 2-LG, 2-OG, and DGLEA were positively correlated with parameters related to glucose and insulin (insulin glucose ratio, glucose, insulin, HOMA index; $r \geq 0.17$, $P < .046$, Fig. 2). DGLEA, PEA, POEA, OEA, and SEA showed positive correlations with lipid parameters (ie, TC, LDL-C, APOB, and TG levels; $r \geq 0.18$, $P < .044$, Fig. 2). Interestingly, POEA showed positive correlations with both leptin ($r = 0.32$, $P < .001$, Fig. 2) and adiponectin levels ($r = 0.21$, $P < .02$, Fig. 2). We have performed sensitivity repeating the analyses of Fig. 2 but adjusting for BMI, and most of the significant results with glucose and lipoprotein metabolism remained significant (Table S2 (19)). However, other significant correlations, such as the relationship between eCBs and liver markers or inflammation, disappeared (Table S2 (19)).

Similar findings were observed when VAT was included instead of BMI as a confounder (data not shown).

Based on the significant correlations observed, we performed stepwise linear regression models to study whether the plasma levels of eCBs and their analogues could improve the prediction of FBM and VAT by classical markers (ie, leptin and adiponectin). These analyses showed that AEA and POEA improved the prediction of FBM by leptin and adiponectin by 14.2% and the prediction of VAT by 13.7%, respectively (Table S3 (19)).

Plasma Levels of Endocannabinoids and Several Endocannabinoid Analogues Are Higher in Metabolically Unhealthy Than in Metabolically Healthy Overweight–Obese Participants

To further understand the biological meaning of all correlations observed, we divided the cohort between individuals who were MHOO (n = 38) and individuals who were MUOO (n = 20). These analyses showed that these groups were similar in terms of BMI, LMI, and FBM ($P \geq .27$); however, MUOO participants showed higher VAT depots than MHOO participants ($P = .028$, Table S4) (19). In addition, we found that individuals who were MUOO showed higher plasma levels of AEA and 2-AG than MHOO (all ≥18% difference, $P \leq .034$, Fig. 3). Similarly, the plasma levels of NAEs (ie, DHEA, LEA, PEA, and OEA) and 2-OG were also higher in MUOO than in MHOO participants (all ≥8% difference, $P \leq .045$, Fig. 3). However, all the significant differences disappeared when VAT was included as a confounder (data not shown).

Discussion

In this study, we found that plasma levels of eCBs and their analogues were positively correlated with adiposity and cardiometabolic risk factors in young adults. Moreover, participants who were MUOO displayed significantly higher plasma levels of eCBs and their analogues than participants who were MHOO, although these differences disappeared when VAT was included as confounder. These findings suggest a significant association between plasma levels of eCBs and their analogues and body composition parameters such as VAT. While these results point to a potential link between these metabolites and cardiometabolic complications, further studies are warranted to elucidate whether these metabolites may be involved in the onset of cardiometabolic complications.

Positive correlations between plasma levels of eCBs and adiposity have been reported in previous studies, but with inconsistent results for AEA and 2-AG (11-14). For instance, 1 study observed that 2-AG, not AEA, was positively correlated with BMI and intra-abdominal adiposity in a cohort of 62 males (14). Another study showed AEA, but not 2-AG, was positively correlated with adiposity in a cohort of 60 males and 84 females (13). Moreover, most of the studies published so far were focused only on middle-aged or elderly adults. Our study was performed in a large cohort and, based on that, our findings are more robust and have higher statistical power. This allows us to detect statistically significant findings where smaller and/or underpowered studies may have failed. Additionally, we observed the same correlation patterns between both genders. Thus, we found that both AEA and 2-AG are correlated with adiposity, which might be explained by (1) the role of ECS in regulating energy metabolism or (2) the secretion of eCBs and other analogues by white adipose tissue (WAT) and VAT. In the central nervous and digestive systems, elevated eCB levels stimulate food intake and increase food-seeking behavior in mice (5, 32-34). In human WAT, CB1R activation increases fat storage by stimulating fatty acid uptake, de novo lipogenesis, and adipocyte differentiation (11, 35). Moreover, VAT synthesizes and secrets eCBs into the circulation (35, 36) (which supports the correlation we observed) and, in turn, may further stimulate food intake. On the other hand, our results show that 2-AG was negatively correlated with BAT glucose uptake, suggesting that activation of ECS might be related to a decreased BAT activity in humans. The possible explanation for this observation could be linked to a preclinical investigation that demonstrated increased BAT activity when CB1R was blocked (37). This suggests that in obese individuals, higher levels of 2-AG might have activated CB1R, resulting in reduced BAT activity and subsequently lowering their energy expenditure via BAT. Interestingly, PDEA also negatively correlated with BAT glucose uptake, whereas the other 8 NAEs with longer fatty chains did not exhibit such a connection. Hence, it would be worthwhile to explore whether BAT activity is linked to other NAEs with short fatty acid chains, which were not considered in this particular study. We also observed a positive correlation between 2-AG and 2-OG with lean body mass. In this sense, evidence suggest that circulating eCBs come from different organs and tissues, including brain, muscle, adipose tissue and circulating cells (38). Since skeletal muscle is the main determinant of the lean body mass, it could be speculated that this tissue can synthesize and secrete these eCBs. Contrarily, we only show a negative correlation between POEA and lean body mass, and not between the remaining NAEs. Although these metabolites can have different physiological functions in skeletal muscle, our results should be interpreted with caution (37). We also observed a positive correlation between plasma levels of eCBs and cardiometabolic risk factors, including parameters related to insulin resistance, dyslipidemia, and metabolic syndrome. Similar results have been reported in a clinical study where plasma levels of 2-AG, but not AEA, were positively correlated with cardiometabolic risk factors (ie, HDL-C, TG, insulin, and glucose levels) in a cohort of 62 middle-aged males (age = 42.2 ± 7.8 years, BMI = 27.4 ± 4.5 kg/m^2) (14). These results are in accordance with a preclinical experiment, where chronic exposure to corticosterone led to an increase of plasma eCBs levels and the development of metabolic syndrome (39). Another study in mice revealed that a high-fat diet-induced activation of ECS in the liver and WAT to insulin resistance (40). Interestingly, these mice studies reported that higher hepatic and plasma eCB levels, as well as a higher hepatic expression of eCB synthesis enzymes, are linked to a deteriorated liver function (38, 39). The deterioration of liver function is attenuated by CB1R deficiency or CB1R inhibition (38), suggesting that the activation of ECS mediates this deterioration. At the same time, we did not observe any correlations between plasma eCBs and liver function parameters (ie, glutamic pyruvic transaminase, gamma-glutamyl transferase, and alkaline phosphatase), which could be explained by the relatively young age of our cohort. Despite the premenopausal age of the participants in this study, no difference of all the aforementioned correlations between the sexes was observed, which is in line with previous findings in an elderly cohort (13).

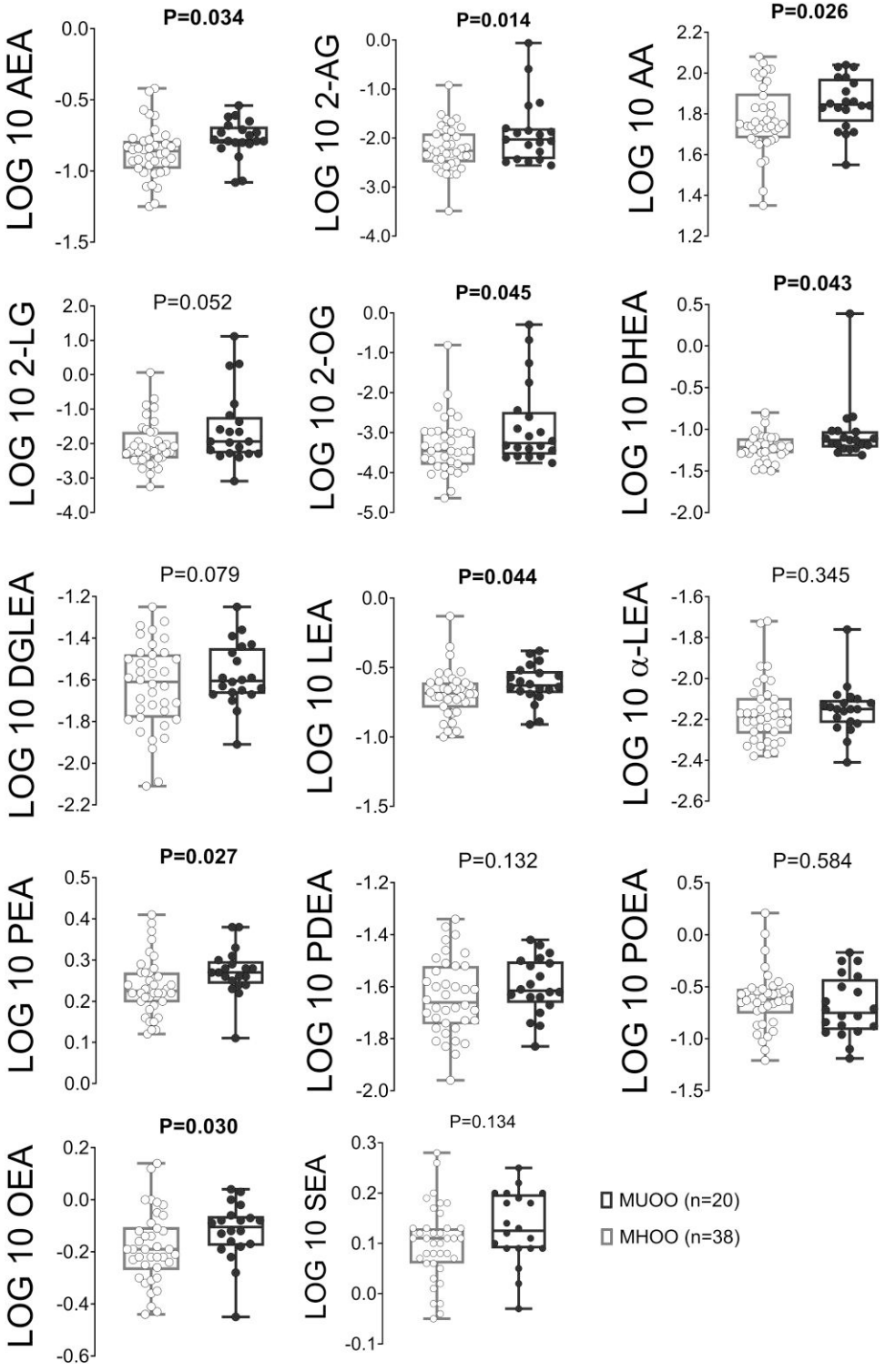

Figure 3. Differences in plasma levels of endocannabinoids and their analogues between individuals who were metabolically healthy overweight–obese (MHOO) and individuals who were metabolically unhealthy overweight–obese (MUOO). *P* values were obtained from 1-way analyses of covariance (ANCOVA) and were adjusted for sex. 2-AG, 2-arachidonoylglycerol; 2-LG, 2-linoleoylglycerol; 2-OG, 2-oleoylglycerol; α-LEA, N-α-linolenoylethanolamine; AA, arachidonic acid; AEA, anandamide; DGLEA, N-dihomo-gamma-linolenoylethanolamine; DHEA, docosahexaenoylethanolamine; HOMA, homeostatic model assessment for insulin resistance index; LEA, N-linoleoylethanolamine; OEA, N-oleoylethanolamine; PEA, N-palmitoylethanolamine; PDEA, N-pentadecanoylethanolamine; POEA, N-palmitoleoylethanolamine; SEA, N-stearoylethanolamine.

Similar to AEA and 2-AG, their analogues (ie, 2-OG, DHEA, LEA, PEA, and OEA) also showed positive correlations with adiposity parameters and cardiometabolic risk factors. NAEs are produced from N-acyl phosphatidylethanolamines (NAPEs) by NAPE-specific phospholipase D and catabolized by fatty acid amide hydrolase (8, 41).

Alterations of the metabolic enzymes involved in eCBs metabolism have been observed in adipose tissues from obese individuals (42, 43), including downregulation of fatty acid amide hydrolase that could explain the higher levels of AEA and other NAEs with increased adiposity.

This is the first study to investigate differences in eCBs between MUOO and MHOO participants. Previously, it has been reported that proinflammatory cytokines, such as interleukin 13, interleukin 33, or succinate were higher in MUOO than in MHOO participants (44-46). Interestingly, we observed that the plasma levels of eCBs and their analogues were significantly higher in MUOO participants than MHOO participants. However, these differences disappeared when VAT was included as a confounder. This result shows that VAT may be a key endocrine organ that could regulate the plasma levels of eCBs and their analogues. Thus, further studies are required to unveil whether individuals with higher eCBs and their analogues levels in the circulation have higher VAT mass, or whether VAT differently contributes to the synthesis of eCBs and their analogues compared with other adipose tissue depots (eg, subcutaneous adipose tissue). These findings also suggest that investigating novel biomarkers in these particular phenotypes is worth it since it can reveal whether their concentrations are altered in the onset of a metabolic complication.

Strengths and Limitations

Most eCB analogues, including analogues typically less studied, such as POEA, have been analyzed with our LC-MS workflow. Moreover, body composition was measured with a dual-energy X-ray absorptiometer scan, which is a validated method. Additionally, our study population size is the largest cohort reporting eCBs and their analogues in combination with BAT parameters so far. This study also has limitations. The area peak ratio but not the absolute plasma concentration of eCBs and their analogues were reported (relative quantitation). No causality can be established due to the inherent limitation of all cross-sectional studies. Since we only included young adults, we cannot extrapolate our results to older or unhealthy populations. Finally, the results related to BAT parameters should be treated with caution, as the method for quantifying BAT volume and activity has limitations, as described elsewhere (47, 48).

Conclusions

The plasma levels of eCBs and their analogues are related to higher levels of adiposity and cardiometabolic risk factors in young adults. MUOO participants showed higher plasma levels of eCBs and their analogues than their MHOO counterparts; these differences disappeared when visceral adipose tissue was included as a confounder.

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Disclosures

The authors have nothing to disclose.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Clinical Trial Information

ClinicalTrials.gov, ID: NCT02365129 (registered February 10, 2015).

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