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ORIGINAL CONTRIBUTIONS

Circulating Endocannabinoids Are Reduced Following Bariatric Surgery and Associated with Improved Metabolic Homeostasis in Humans

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Abstract

Background The endocannabinoid (eCB) system plays a key role in the development of obesity and its comorbidities. Limited information exists on the changes in circulating eCBs following bariatric surgery.

Objectives This study aims to (i) assess the circulating levels of eCBs and related molecules and (ii) examine the association between their levels and numerous clinical/metabolic features pre- and post-operatively.

Methods Sixty-five morbidly obese patients (age 42.78 ± 9.27 years; BMI 42.00 ± 5.01 kg/m^2) underwent laparoscopic sleeve gastrectomy (LSG) surgery, and were followed up for 12 months. Data collected included anthropometrics and metabolic parameters. The serum levels of the eCBs, 2-arachidonoylglycerol (2-AG), anandamide (AEA); and their related molecules, arachidonic acid (AA) and oleoylethanolamine (OEA) were measured by liquid chromatography-mass spectrometry.

Results Levels of 2-AG, AEA, and AA were reduced post operatively with no differences in serum OEA levels. The delta changes in eCB levels between pre- and post-operation were correlated with the delta of different metabolic parameters. Positive correlations were found between delta AA and waist circumference (WC) ($r = 0.28$, $P < 0.05$), free fat mass ($r = 0.26$, $P < 0.05$), SteatoTest score ($r = 0.45$, $P < 0.05$), and ALT ($r = 0.32$, $P < 0.05$). Delta AEA levels positively correlated with WC ($r = 0.30$, $P < 0.05$). Delta 2-AG levels positively correlated with total cholesterol ($r = 0.27$, $P < 0.05$), triglycerides ($r = 0.55$, $P < 0.05$), and SteatoTest score ($r = 0.27$, $P < 0.05$). Delta OEA levels negatively correlated with fasting glucose levels ($r = -0.27$, $P < 0.05$).

Conclusions This study provides compelling evidence that LSG surgery induces reductions in the circulating 2-AG, AEA, and AA levels, and that these changes are associated with clinical benefits related to the surgery including reduced fat mass, hepatic steatosis, glucose, and improved lipid profile.

Keywords Endocannabinoids · Laparoscopic sleeve gastrectomy · Obesity · Metabolic parameters

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Introduction

Obesity is a global health concern and its incidence has nearly tripled in the past four decades [1]. To date, four main therapeutic options effectively combat obesity and its associated comorbidities: life style intervention, pharmacotherapy, endoluminal procedures, and bariatric surgery [2–4]. The latter is considered superior in terms of weight loss and metabolic profile improvement among morbidly obese patients [2].

The main endocannabinoids (eCBs) 2-arachidonoylglycerol (2-AG) and *N*-arachidonoylethanolamine (anandamide or AEA) are endogenous lipid ligands that activate the cannabinoid-1 and cannabinoid-2 receptors (CB_1R and CB_2R, respectively), which also recognize the psychoactive component of marijuana, Δ^9-tetrahydrocannabinol [5]. These ligands, synthesized and degraded endogenously by distinct enzymes (Fig. 1), are hypothesized to be secreted into the circulation from multiple organs and tissues, including the brain, muscle, liver, gut, stomach, adipose tissue, and circulating cells and act as effectors in homeostatic processes, such as regulation of energy balance [6]. As a whole, the eCB system plays a key role in the development of obesity and its comorbidities [7] via modulating central and peripheral signaling pathways that control appetite and energy homeostasis [5]. These effects are primarily mediated through activating the CB_1R abundantly expressed in the central nerve system [5, 8] and stimulating its activity in many peripheral tissues (reviewed in [8]). In fact, its expression in obese animals is significantly upregulated in the adipose tissue [9, 10], liver [11, 12], and kidney [13]. Moreover, elevated levels of circulating eCBs have been reported in obese patients vs. lean controls. For instance, higher levels of circulating 2-AG were found to be positively associated with waist circumference (WC), body mass index (BMI), visceral adiposity, insulin resistance, and nonalcoholic fatty liver (NAFLD) [14–18]. Therefore, circulating eCBs may predict obesity risk [19], and targeting the eCB/CB_1R system may be considered as a possible therapeutic approach against obesity.

Even though an improvement in most obesity-related health outcomes is observed after bariatric surgery [2], little and conflicting information is available regarding the association of circulating eCBs with weight loss and improved metabolic homeostasis post-operatively [20, 21]. Since increased circulating eCB levels have been linked to increased adipose fat stores, one may hypothesize that reduction in body weight and fat mass (as caused by Roux-en-Y gastric bypass (RYGB) or laparoscopic sleeve gastrectomy (LSG) surgery) would lead to their reduced levels. Therefore, we aimed to (i) examine the levels of circulating eCBs 1 year following LSG and (ii) decipher the association between levels of circulating eCB and changes in anthropometric and metabolic features 1 year post-operatively.

Materials and Methods

Subjects This prospective cohort study was a part of a randomized clinical trial (RCT) of a 6-month treatment period with probiotic vs. placebo and a 6-month follow-up of 100 NAFLD patients who underwent LSG surgery from February 2014 to January 2015 at the Tel-Aviv Assuta Medical Center [22]. Inclusion and exclusion criteria of the RCT were previously published [22]. Fatty liver was diagnosed by abdominal US using standardized criteria [23], with the same equipment (Preirius scanner Hitachi Medical Corporation, Tokyo, Japan) and by the same experienced radiologist.

Data of the combined treatment groups are presented in this study, since no difference between the treated groups was observed for the measurements discussed here. Medical history on demographic details and comorbidities were obtained from the patients' medical records. Baseline (M0) and follow-up evaluations at 12 months (M12) were performed at the Tel-Aviv Medical Center.

Anthropometric Measurements Weight and height were measured on a digital medical scale, and WC was measured twice at the level of the umbilicus according to a uniform protocol. BMI was calculated using weight (in kilograms) divided by the height squared (in square meter). Patients were measured for body composition using multi-frequency bioelectrical impedance analysis (BIA) (Inbody 220®, Biospace). Patients were evaluated after an overnight fast of 12 h and according to the manufacturer's specifications.

Biochemical Tests All blood tests were drawn following a 12-h fast and included lipids profile, liver enzymes, glucose, hemoglobin A1C (HbA1C), insulin, and C-reactive protein (CRP). Homeostasis model assessment (HOMA) was used as a surrogate marker of insulin resistance (IR) [24].

Frozen serum samples from all participants at M0 and M12 were stored at − 80 °C until analyses were conducted for eCBs (see a separate subsection), FibroTest, ActiTest, and SteatoTest (BioPredictive, Paris, France), which have been validated extensively [25]. The FibroTest included serum α2-macroglobulin, apolipoprotein-A1, haptoglobin, total bilirubin, and γ-glutamyl transpeptidase. The ActiTest included the same components plus alanine aminotransferase (ALT). The SteatoTest included the same six components of the FibroTest and ActiTest plus BMI, serum cholesterol, triglycerides, and fasting glucose. A nonalcoholic steatohepatitis (NASH) test was developed for the quantitative diagnosis of NASH using the components of the SteatoTest without glucose and BMI [26]. The pre-analytical and analytical procedures were those recommended by BioPredictive.

Endocannabinoid Measurements The extraction, purification, and quantification of AEA, 2-AG, arachidonic acid (AA), and

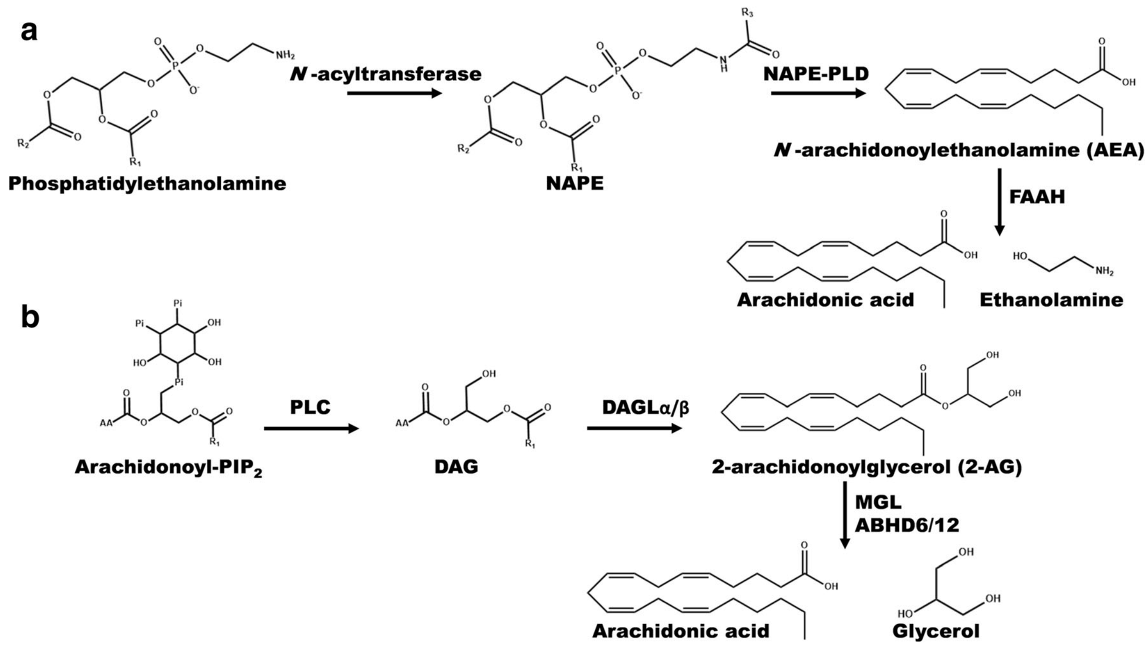

Fig. 1 Endocannabinoids and their synthesis and degradation pathways. Primary synthesis and degradative pathways for *N*-arachidonoylethanolamine (anandamide or AEA) (**a**) and 2-arachidonoylglycerol (2-AG) (**b**). Endocannabinoids (eCBs) are synthesized and degraded endogenously in almost every tissue of the human body. AEA seems to be produced from *N*-arachidonoyl phosphatidyl ethanol (NAPE), while 2-AG is produced from 2-arachidonoyl-containing phospholipids, predominantly arachidonoyl-containing phosphatidyl inositol bis-phosphate (PIP_2). As per their degradation, AEA is mainly degraded by the enzyme fatty acid amino hydrolase (FAAH) into arachidonic acid (AA) and ethanolamine, whereas 2-AG is primarily metabolized by three hydrolytic enzymes, monoacylglycerol lipase (MGL), and alpha/beta domain hydrolases 6 and 12 (ABHD6 and 12) into AA and glycerol

oleoylethanolamine (OEA) in serum were performed by stable isotope dilution liquid chromatography/tandem mass spectrometry (LC-MS/MS) as described earlier [18]. The levels of each compound were analyzed by multiple reaction monitoring. The molecular ion and fragment for each compound were measured as follows: m/z 348.3 → 62.1 (quantifier) and 91.1 (qualifier) for AEA, m/z 379.3 → 91.1 (quantifier) and 287.3 (qualifier) for 2-AG, m/z 305.2 → 91.1 (quantifier) and 77.1 (qualifier) for AA, m/z 326.2 → 62.1 (quantifier) and 55.1 (qualifier) for OEA, and m/z 352.3 → 66.1 (quantifier) and 91.1 (qualifier) for [$^2H_4$]AEA. The serum levels of AEA, 2-AG, OEA, and AA were measured in duplicates against standard curves.

Statistical Analysis Statistical analyses were performed using SPSS software version 24.0. Continuous variables are presented as means ± SD and dichotomous/categorical variables as proportions. To test the differences in continuous variables between two groups, the independent-sample *t* test was performed. For comparison of dichotomous or categorical variables, the Pearson chi-square test was performed. To compare continuous variables between two time points, the paired-samples *t* test was performed, and to compare paired dichotomous variables between two time points, the McNemar test was performed. The Pearson correlation coefficient was used to assess correlations between continuous variables. $P < 0.05$ was considered statistically significant for all analyses.

Results

Characteristics of the LSG Cohort Study at Baseline One hundred patients were recruited for the study, and 77% attended the M12 visit. A total of 65 patients (55.4% women) who completed the yearly follow-up included in the analysis. Characteristics of the LSG cohort study at M0 and changes at M12 are presented in Table 1. As expected, most measurements significantly improved 1 year following LSG. Interestingly, FibroTest showed an opposite trend, although clinically insignificant.

Changes in Circulating eCBs Levels at 12 Months Post-Surgery Participants had significantly lower levels of 2-AG (80.04 ± 35.01 vs. 109.13 ± 49.03, $P < 0.001$), AEA (682.34 ± 208.12 vs. 801.85 ± 255.54, $P = 0.001$), and AA (5424.01 ± 1459.97 vs. 5931.76 ± 1326.98, $P = 0.006$) at M12 vs. M0, respectively (Fig. 2). No differences in serum OEA levels were found (18.46 ± 4.41 vs. 18.90 ± 5.17, $P = 0.523$). Gender differences in the change in each circulating eCBs at M12 are presented in Fig. 3, indicating that 2-AG was reduced in both genders, AA reduction was restricted to men, and AEA reduction reached statistical significance only in women.

Correlations Between eCBs and Metabolic Parameters at Baseline Next, we examined the correlations between the

Table 1 Characteristics of the LSG cohort study at baseline and changes at 12 months post-surgery

Parameter[a]	Baseline	12 months post-surgery	*P* value
Age (year)	42.78 ± 9.27	–	–
Sex (%female)	55.40	–	–
Type 2 diabetes (%)	9.20	–	–
Use of type-2 diabetes medications (%)	6.20	0.00	**
Hypertension (%)	26.20	–	–
Use of anti-hypertension medications (%)	21.50	12.30	0.070
Dyslipidemia (%)	60.00	–	–
Use of lipid lowering medications (%)	10.80	3.10	0.063
Current smoker (%)	4.60	–	–
Weight (kg)	121.52 ± 19.03	83.80 ± 13.30	*< 0.001**
WC (cm)	125.03 ± 12.13	95.05 ± 10.10	*< 0.001**
BMI (kg/m^2)	42.00 ± 5.01	28.94 ± 3.34	*< 0.001**
Fat mass (kg)	56.10 ± 11.14	26.74 ± 7.62	*< 0.001**
Fat (%)	46.57 ± 6.59	32.36 ± 8.32	*< 0.001**
Fat-free mass (kg)	64.94 ± 14.03	56.39 ± 12.32	*< 0.001**
TC (mg/dL)	187.98 ± 30.12	174.66 ± 25.53	*< 0.001**
LDL-C (mg/dL)	110.56 ± 26.11	101.25 ± 23.26	*0.001**
HDL-C (mg/dL)	47.23 ± 15.15	55.46 ± 14.47	*< 0.001**
Triglycerides (mg/dL)	158.34 ± 89.43	92.62 ± 34.64	*< 0.001**
Glucose (mg/dL)	88.65 ± 11.96	77.17 ± 5.70	*< 0.001**
HbA1C (%)	5.77 ± 0.54	5.49 ± 0.33	*< 0.001**
HOMA[b]	5.77 ± 3.66	1.49 ± 0.83	*< 0.001**
SteatoTest score	0.55 ± 0.16	0.25 ± 0.11	*< 0.001**
FibroTest score	0.18 ± 0.14	0.21 ± 0.15	*0.001**
NASHTest score	0.41 ± 0.15	0.32 ± 0.14	*< 0.001**
ALT (U/L)	36.89 ± 18.53	17.69 ± 7.58	*< 0.001**
AST (U/L)	27.38 ± 9.62	20.51 ± 8.17	*< 0.001**
GGT (U/L)	35.03 ± 19.63	19.72 ± 9.29	*< 0.001**
CRP (mg/L)	12.48 ± 11.07	3.33 ± 4.42	*< 0.001**
2-AG (pmol/mL)	109.13 ± 49.03	80.04 ± 35.01	*< 0.001**
AEA (fmol/mL)	801.85 ± 255.54	682.34 ± 208.12	*0.001**
AA (pmol/mL)	5931.76 ± 1326.98	5424.01 ± 1459.97	*0.006**
OEA (pmol/mL)	18.90 ± 5.17	18.46 ± 4.41	0.523

LSG laparoscopic sleeve gastrectomy, *WC* waist circumference, *BMI* body mass index, *TC* total cholesterol, *LDL-C* low density lipoprotein cholesterol, *HDL-C* high density lipoprotein cholesterol, *HbA1C* hemoglobin A1c, *HOMA* homeostasis model assessment, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GGT* gamma-glutamyltransferase, *CRP* C-reactive protein, *2-AG* 2-arachidonoylglycerol, *AEA* *N*-arachidonoylethanolamine or anandamide, *AA* arachidonic acid, *OEA* oleoylethanolamine

[a] Values are expressed as mean ± SD unless otherwise stated

[b] HOMA was used as a surrogate marker of insulin resistance

*$P < 0.05$

**No significance of association could be computed

metabolic parameters measured in the cohort and eCBs pre-operatively. The results are shown in Table 2.

Correlations Between Changes in Circulating eCB Levels Pre- and Post-operatively and Metabolic Parameters Since reduced levels of 2-AG, AEA, and AA were reported 1 year post-operatively, we sought to assess the correlations of the delta in eCB levels between M0 and M12 and the delta in different anthropometric and metabolic parameters. The results are shown in Table 3.

Discussion

The present study, based on a relatively large cohort, investigated the changes in the circulating levels of eCBs in morbidly

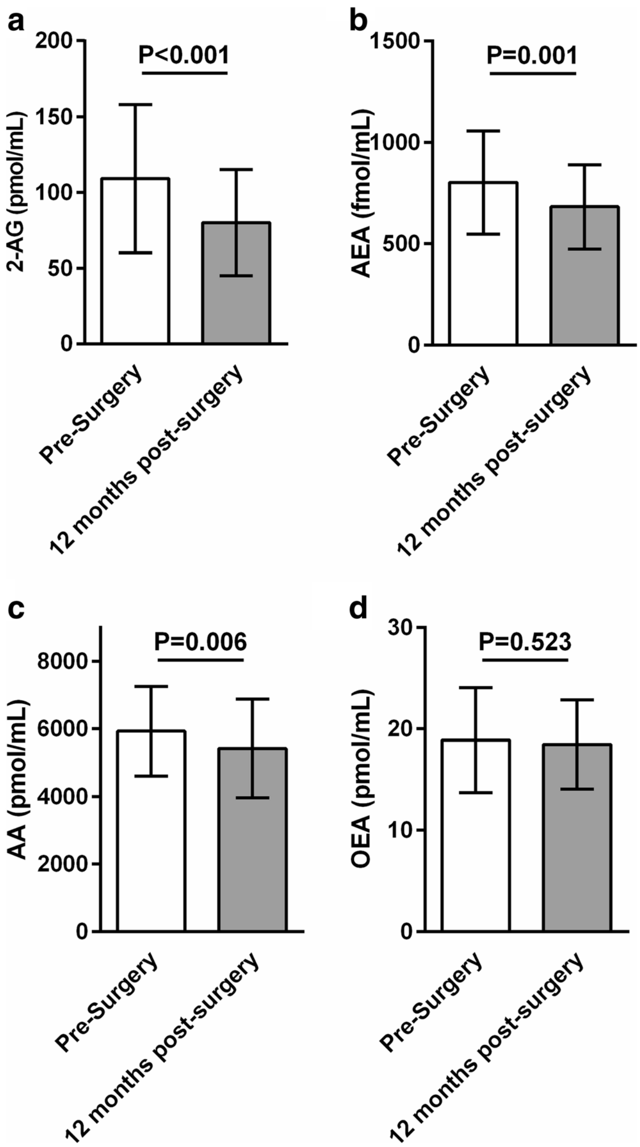

Fig. 2 Endocannabinoids levels at baseline and at 12 months post-surgery. Reduced circulating levels of 2-AG (**a**), AEA (**b**), and AA (**c**), with no changes in OEA levels (**d**) 1-year post-laparoscopic sleeve gastrectomy (LSG) surgery. Data represent the mean ± SD in 65 patients

obese patients at 1 year following bariatric surgery. The results presented here indicate that LSG surgery induces reductions in circulating 2-AG, AEA, and AA levels post-operatively, which correlate with clinical benefits related to surgery including reduced fat mass, hepatic steatosis, glucose, and improved lipid profile.

A vast amount of data regarding the role of circulating eCBs in the development of obesity exist, mostly demonstrating that 2-AG and/or AEA levels are significantly elevated in obese compared with lean subjects, and are correlated with body weight and fat gain in humans [14, 16–18, 27, 28]. However, very few studies have explored their contributing role to weight loss and improved metabolic parameters, explicitly following bariatric surgery, yielding conflicting results. In agreement with our study, Guijarro and colleagues found reduced skeletal AEA and hepatic 2-AG and AEA levels 28 days following RYGB surgery in obese rats [20]. Similarly, Quercioli and colleagues reported significant reductions in plasma 2-AG and AEA levels 22 months following RYGB performed in 18 morbidly obese patients. In fact, reduced AEA levels were found to be associated with weight loss and were closely associated with the normalization of coronary endothelial function, suggesting that AEA could serve as a potential biomarker for cardiovascular risk during obesity [29]. Our study demonstrated some gender differences, which were also demonstrated by Mallipedhi and colleagues showing significant reductions 6 months after a LSG surgery in circulating AEA and palmitoylethanolamine (PEA) levels only among women [21]. In contrast, although Montecucco and colleagues also reported elevated plasma AEA levels in pre-RYGB patients compared with lean controls, no changes in AEA, 2-AG, OEA, and PEA were found in the 11 morbidly obese patients 1 year post-operatively [30]. Interestingly, significant elevations in adipose tissue levels of all eCBs, specifically OEA, were reported in post-RYGB patients and were suggested to play an anti-inflammatory role following the procedure [30].

Our current study, which is the first to report a data set in a relatively large cohort of 65 individuals that underwent LSG surgery, is in partial agreement with the above mentioned studies. First, reduced circulating 2-AG and AEA levels as well as their breakdown molecule, AA, were found 12 months following the procedure. Second, whereas reduced AA and 2-AG levels were found in men, significant reductions in 2-AG and AEA were found in women. Third, serum OEA levels were not affected by the surgery or weight reduction. Most likely, the difference between our study and the studies published so far depends on numerous factors, including the timing of eCB assessment with respect to when (and if) the patient loses body weight, sample size, and the gender of the studied subjects. Nevertheless, the Di Marzo group [31] showed that a 1-year lifestyle modification program resulted in a significant decrease in body weight in 49 viscerally obese men, and found significant reductions in circulating AEA (−7.1%) and particularly in 2-AG (−62.3%) levels. Taken together, we can conclude that peripheral eCBs differentially decrease following surgery in men and women and among the different subclasses of eCBs. Understanding these patterns and their causal effects may contribute to the understanding of their mechanism of action in obesity.

Several lines of investigation indicate that eCBs are involved in the pathogenesis of various metabolic diseases. This notion was proposed based on examining the association between eCB levels and multiple clinical/metabolic markers. We found here distinct associations between eCBs and markers of weight and fat mass, lipid and glucose homeostasis, inflammation, and fibrosis. Preoperatively, we found that circulating AEA has significant positive correlations with WC, BMI, fat mass (Kg and %), and CRP levels. These findings are novel and were not previously reported [16, 21]. However, AEA's correlations with markers of insulin resistance were reported before, and Matias and colleagues found that fasting salivary AEA levels were significantly correlated with BMI, WC, and fasting insulin levels [21, 27].

As for the other major eCB, 2-AG, significant positive correlations with serum triglycerides and liver fibrosis were found here. These findings are in agreement with our previous report demonstrating a significant correlation between 2-AG and triglycerides as well as markers for liver injury associated with obesity [18], and are consistent with others reporting a significant association of 2-AG with several key cardiometabolic risk factors [14, 16, 28, 31]. Interestingly, AA was also positively correlated with WC, free-fat mass, and hepatic fibrosis; these findings are in accordance with our recent report [18] and in contrast to the findings by Bluher and colleagues [14]. Whereas OEA levels were positively correlated with the percentage of fat, this endogenous ligand was the only one that presented negative correlations with free-fat mass and parameters for glucose and insulin homeostasis. Likewise, previous studies have also found that OEA was positively correlated with weight, WC, and BMI [17, 21, 27]. In contrast, positive correlations between OEA and fasting insulin levels and HOMA-IR were also reported [21]. A possible explanation for the difference between the two studies could be the markedly higher body weight, WC, and the BMI of the patients in the Mallipedhi study [21].

Whereas the current state of our knowledge of the role of the eCB system in modulating human physiology and pathophysiology has been enhanced by measuring circulating levels of eCBs, many of these studies have examined only a single time point [6]. In the current study, the determination of eCBs at two time points for each individual (pre- and post-operatively) provided us an important information as to the dynamic association of eCBs and several metabolic parameters. Interestingly, a different pattern of correlations between the delta of changes in eCBs following the LSG surgery and the delta of clinical/metabolic parameters were reported here for the first time. The most important ones are those related to delta 2-AG levels, which are positively correlated with delta of circulating triglyceride and total cholesterol levels as well as the SteatoTest score. Delta AA levels were also found to be positively associated with the SteatoTest score and the serum ALT levels. Taken together, these findings may indicate their importance in regulating lipid homeostasis and the development of NAFLD, but this needs to be confirmed in studies with a temporal sequence.

The present study is a 1-year prospective follow-up and covered a wide range of anthropometric, metabolic, and hepatic parameters collected in a uniform manner. Nevertheless, there are several limitations. It is commonly known that maximal weight loss is observed at 1–1.5 year post-bariatric

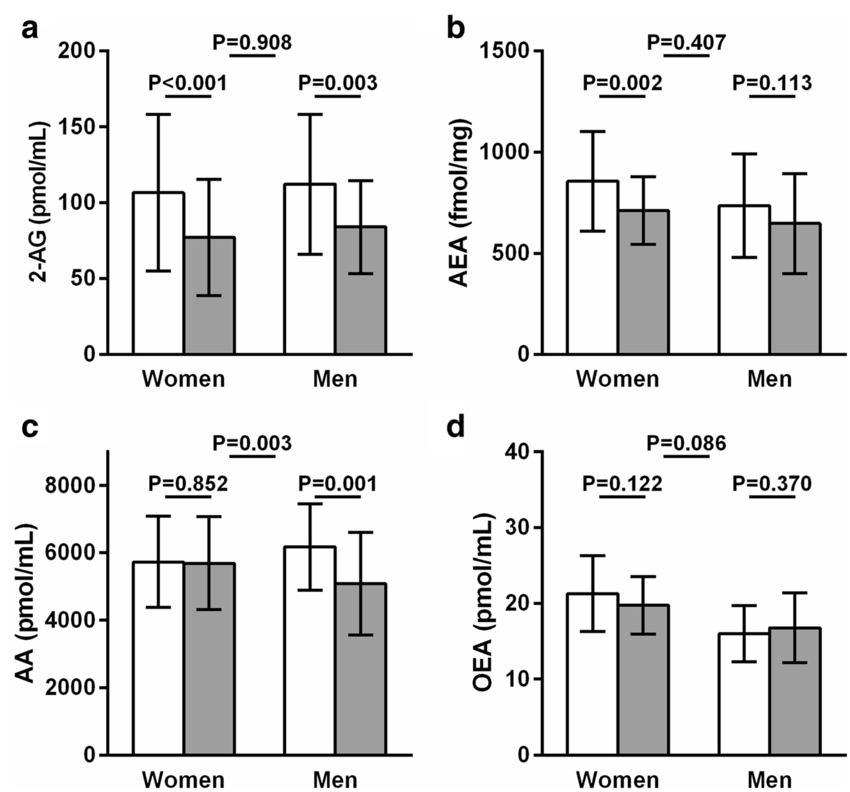

Fig. 3 The distribution of 2-AG (**a**), AEA (**b**), AA (**c**), and OEA (**d**) among the patients before (white) and after (gray) 12 months of laparoscopic sleeve gastrectomy (LSG) surgery, stratified by gender. Data represent the mean ± SD in 36 women and 29 men

Table 2 Correlations between circulating eCBs and metabolic parameters at baseline (*r*, Pearson correlation)

Parameter	AA	AEA	2-AG	OEA
WC (cm)	*0.287**	*0.246**	0.147	−0.036
BMI (kg/m^2)	0.158	*0.377**	0.119	0.131
Fat mass (kg)	0.043	*0.422**	− 0.035	0.219
Body fat (%)	− 0.197	*0.378**	− 0.166	*0.451**
Fat-free mass (kg)	*0.274**	− 0.092	0.162	*− 0.364**
TC (mg/dL)	− 0.074	− 0.149	0.100	0.054
LDL-C (mg/dL)	− 0.088	− 0.202	− 0.109	0.069
HDL-C (mg/dL)	− 0.148	0.135	− 0.018	0.148
Triglycerides (mg/dL)	0.176	− 0.046	*0.440**	− 0.040
Glucose (mg/dL)	0.202	− 0.166	0.140	*− 0.254**
HbA1C (%)	0.134	− 0.218	0.032	*− 0.362**
HOMA[a]	0.168	− 0.074	0.115	*− 0.333**
SteatoTest score	0.141	− 0.109	0.227	− 0.084
FibroTest score	− 0.018	− 0.195	−0.121	− 0.184
NASHTest score	0.204	− 0.085	0.129	− 0.122
ALT (U/L)	0.164	− 0.089	0.074	− 0.221
AST (U/L)	0.148	− 0.026	0.091	− 0.107
GGT (U/L)	− 0.003	− 0.178	0.091	− 0.162
CRP (mg/L)	0.194	*0.274**	0.037	0.131

WC waist circumference, *BMI* body mass index, *TC* total cholesterol, *LDL-C* low density lipoprotein cholesterol, *HDL-C* high density lipoprotein cholesterol, *HbA1C* hemoglobin A1c, *HOMA* homeostasis model assessment, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GGT* gamma glutamyltransferase, *CRP* C-reactive protein, *2-AG* 2-arachidonoylglycerol, *AEA N*-arachidonoylethanolamine or anandamide, *AA* arachidonic acid, *OEA* oleoylethanolamine

**P* < 0.05

[a] HOMA was used as a surrogate marker of insulin resistance

Table 3 Correlations between delta of changes** in eCBs and metabolic parameters (*r*, Pearson correlation)

Parameter	AA	AEA	2-AG	OEA
WC (cm)	*0.275**	*0.298**	− 0.061	0.181
BMI (kg/m^2)	0.051	0.224	− 0.065	0.077
Fat mass (kg)	0.126	0.217	− 0.076	0.021
Body fat (%)	0.074	0.165	− 0.066	− 0.007
Fat-free mass (kg)	*0.257**	0.158	0.008	0.101
TC (mg/dL)	− 0.034	− 0.010	*0.272**	− 0.031
LDL-C (mg/dL)	− 0.043	− 0.030	− 0.041	− 0.091
HDL-C (mg/dL)	− 0.192	0.051	0.024	0.086
Triglycerides (mg/dL)	0.100	− 0.044	*0.545**	0.023
Glucose (mg/dL)	− 0.003	− 0.215	0.090	*− 0.272**
HbA1C (%)	0.061	− 0.134	− 0.031	− 0.225
HOMA[a]	0.085	− 0.099	− 0.125	− 0.208
SteatoTest score	*0.452**	0.165	*0.266**	0.174
FibroTest score	− 0.126	0.130	− 0.128	0.201
NashTest score	0.177	0.100	0.193	0.230
ALT (U/L)	*0.320**	0.059	− 0.020	0.107
AST (U/L)	0.220	0.091	0.077	0.194
GGT (U/L)	0.166	0.019	0.048	0.014
CRP (mg/L)	0.052	0.062	− 0.041	0.020

WC waist circumference, *BMI* body mass index, *TC* total cholesterol, *LDL-C* low density lipoprotein cholesterol, *HDL-C* high density lipoprotein cholesterol, *HbA1C* hemoglobin A1c, *HOMA* homeostasis model assessment, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GGT* gamma glutamyltransferase, *CRP* C-reactive protein, *2-AG* 2-arachidonoylglycerol, *AEA N*-arachidonoylethanolamine or anandamide, *AA* arachidonic acid, *OEA* oleoylethanolamine

**P* < 0.05

**Delta of changes between M0 and M12

[a] HOMA was used as a surrogate marker of insulin resistance

surgery, and weight regain may occur at 2 years post-operatively [32]. Therefore, the first limitation is that the follow-up period in the current study ended 1 year after the surgery. Second, a control lean group would have added tremendously to the current findings. Third, our study did not establish a causal relationship between circulating eCB levels and any of the metabolic/clinical parameters tested. Therefore, further experiments are needed to unmask the underlying mechanisms regarding the importance and relevance of eCBs in obesity and its resolution following bariatric surgery. Another possible limitation was that our sample collection was limited to circulating eCBs only, and no adipose or liver tissues were analyzed. Therefore, we cannot comment on whether the surgery directly affected the activity of the enzymes responsible for eCB synthesis or degradation, which in turn, may lead to the changes in their circulating levels. Considering the potential activities of eCBs in these tissues during obesity and following weight loss, further studies are needed to investigate the specific role of tissue-related changes in eCBs and their related enzymes post-operatively.

Conclusions

LSG surgery induces reductions in circulating 2-AG, AEA, and AA in humans, and these changes are correlated with clinical benefits related to the surgery. These findings suggest a potential role for eCBs in post-bariatric surgery metabolic changes, and merit further research into their differential role in men and women, their causal role, and their potential therapeutic use. These findings also support the notion that therapeutic strategies aiming to decrease eCB "tone" (either by reducing eCBs or blocking the CB_1R in periphery) [8] in obese individuals may provide a clinically relevant tool to combat the obesity epidemic worldwide.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in this study were approved by the institutional research committees in both participating hospitals and in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was preregistered in the NIH registration website (TRIAL no. NCT01922830).

Statement of Informed Consent Informed consent was obtained from all individual participants included in the study.

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