

Blood Levels of the Endocannabinoid Anandamide are Increased in Anorexia Nervosa and in Binge-Eating Disorder, but not in Bulimia Nervosa

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The endocannabinoid system, consisting of two cannabinoid receptors (CB₁ and CB₂) and the endogenous ligands anandamide (arachidonylethanolamide (AEA)) and 2-arachidonoylglycerol (2-AG), has been shown to control food intake in both animals and humans, modulating either rewarding or quantitative aspects of the eating behavior. Moreover, hypothalamic endocannabinoids seem to be part of neural circuitry involved in the modulating effects of leptin on energy homeostasis. Therefore, alterations of the endocannabinoid system could be involved in the pathophysiology of eating disorders, where a deranged leptin signalling has been also reported. In order to verify this hypothesis, we measured plasma levels of AEA, 2-AG, and leptin in 15 women with anorexia nervosa (AN), 12 women with bulimia nervosa (BN), 11 women with binge-eating disorder (BED), and 15 healthy women. Plasma levels of AEA resulted significantly enhanced in both anorexic and BED women, but not in bulimic patients. No significant change occurred in the plasma levels of 2-AG in all the patients' groups. Moreover, circulating AEA levels were significantly and inversely correlated with plasma leptin concentrations in both healthy controls and anorexic women. These findings show for the first time a derangement in the production of the endogenous cannabinoid AEA in drug-free symptomatic women with AN or with BED. Although the pathophysiological significance of this alteration awaits further studies to be clarified, it suggests a possible involvement of AEA in the mediation of the rewarding aspects of the aberrant eating behaviors occurring in AN and BED.

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INTRODUCTION

The physiological control of eating behavior is extremely complex, involving a balance of both central and peripheral neurotransmitters and neuropeptides that interact to stimulate or inhibit food intake. The endocannabinoid system (Di Marzo *et al*, 2004), consisting of two cannabinoid receptors (CB₁ and CB₂) and the endogenous ligands anandamide (arachidonylethanolamide (AEA)) and 2-arachidonoylglycerol (2-AG), has been shown to control feeding in both animals and humans (Cota *et al*, 2003). Indeed, both exogenous and endogenous cannabinoids stimulate food intake through several mechanisms. Con-

versely, CB₁ receptor blockade suppresses food intake, and genetically engineered mice lacking the CB₁ receptor eat less after food deprivation (Di Marzo *et al*, 2001) and are leaner and less susceptible to developing diet-induced obesity than their normal littermates (Ravinet Trillou *et al*, 2004). Finally, hypothalamic endocannabinoids have been suggested to form part of a neural circuitry regulated by leptin, the peripheral fat hormone involved in the long-term modulation of body weight (BW) and energy balance (Di Marzo *et al*, 2001). Hence, a control by the endocannabinoid system on energy homeostasis at both central and peripheral levels has been recently proposed (Cota *et al*, 2003).

Anorexia nervosa (AN) and bulimia nervosa (BN) are psychiatric disorders characterized by abnormal eating behaviors that generally result in severe food restriction with a dramatic loss of BW in AN and episodes of binge eating and vomiting without significant changes of BW in BN. Besides AN and BN, the Diagnostic and Statistical Manual of Mental Disorders-IV edition (DSM-IV) (American Psychiatric Association, 1994) includes in appendix B

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the category of binge-eating disorder (BED), which is characterized by binge eating, as in BN, but without compensatory behaviors. Since the massive ingestion of calories during bingeing is not balanced by increased energy expenditure and/or other compensatory behaviors, people with BED generally incur an overt obesity.

It is commonly accepted that eating disorders (EDs) have a complex and multifactorial etiopathogenesis, with the involvement of both psychosocial and biological factors. Central and peripheral substances known to regulate food intake and energy expenditure, including leptin, have been suggested to play a role in the pathogenesis and/or the maintenance of the altered eating behavior of these syndromes (Halmi, 2002). Based on the reported role of endocannabinoids in the regulation of feeding and energy homeostasis (Cota *et al*, 2003), of their relationship with endogenous leptin (Di Marzo *et al*, 2001; Maccarrone *et al*, 2003), and on the previously described effects of long-term food deprivation on their brain levels (Matias *et al*, 2003; Hanus *et al*, 2003), it seems plausible that these substances are involved in the pathophysiology of EDs.

In the present study, we compared the blood levels of AEA and 2-AG of women with AN, BN, or BED with those of sex-matched healthy controls, and explored the possible relationships between these endogenous cannabinoids and circulating leptin, nutritional, and psychopathological variables.

PATIENTS AND METHODS

Subjects

A total of 53 women were recruited for the study. They were 38 outpatients attending the Eating Disorder Center of our Department and 15 healthy controls. According to DSM-IV criteria, 15 patients fulfilled the diagnosis of AN restricting subtype, 12 the diagnosis of BN purging subtype, and 11 the diagnosis of BED. Diagnostic assessment was made by a trained interviewer using the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I) (First *et al*, 1995). All the bulimic patients used vomiting as the main compensatory behavior; none of them had a past history of AN; all AN women and two bulimics were amenorrheic; the remaining patients had normal regular menses.

At the time of the study, most of the patients had never taken psychotropic medications (11 anorexics, eight bulimics, 11 BED women); the remaining ones had been free from psychotropic drugs for more than 6 weeks. All of them were studied before entering specific treatment programs.

Control women were mentally healthy, as assessed by the SCID-I non-patient edition, and had no positive family history of mental disorders as assessed by the Family History Research Diagnostic Criteria (Andreasen *et al*, 1977). Healthy controls were regularly menstruating and had normal eating habits; all of them had been drug-free for more than 8 weeks.

Female controls and patients who were normally menstruating were tested in the follicular phase of their menstrual cycle (day 5–10 from menses). No subject was taking oral contraceptives or had a past history of alcohol or drug abuse.

Subjects gave written informed consent prior to study participation. The study was approved by the local ethic committee of our Department.

Experimental Procedure

Patients' psychopathological aspects were rated by means of: (a) the Eating Disorder Inventory-2 (EDI-2) (Garner, 1993) and the Bulimia Investigation Test Edinburgh (BITE) (Henderson and Freeman, 1987) that evaluated eating-related psychopathology; (b) the Montgomery Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979) that measured depressive symptoms; (c) the State-Trait Anxiety Inventory (STAI) (Spielberg, 1983) that evaluated anxiety symptoms.

In each subject, BW and height were measured, and the BMI was calculated. Moreover, all the control women, 10 anorexic patients, seven bulimic patients, and 10 BED women underwent a body composition evaluation by means of a bioelectrical impedance analyser (STA/BIA, Akern Srl, Florence, Italy). Bioelectric impedance analysis is an accurate, noninvasive, and inexpensive technique for quantitatively estimating body fat mass and body lean mass. Although in emaciated persons it may not be so accurate as the dual X-ray absorptiometry, it has been reported to possess a good reliability (Martinoli *et al*, 2003; Kyle *et al*, 2003).

After an overnight fast, each subject underwent a blood and urine sample collection between 0800 and 0900 h. Blood was collected by venipuncture in EDTA tubes and processed no later than 1 h for lipid extraction according to De Marchi *et al* (2003). Lipid extracts were analyzed for the presence of AEA and 2-AG levels by liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry as described previously (De Marchi *et al*, 2003). Intra- and inter-assay coefficients of variation were 2.5 ± 0.3 and $10.1 \pm 2.5\%$, respectively, for AEA, and 3.6 ± 0.5 and $12.1 \pm 3.4\%$, respectively, for 2-AG. Plasma leptin concentrations were determined by a sandwich enzyme-linked immunosorbent assay, using a commercial kit purchased from Alexis Biochemicals (Laufelfingen, Switzerland). The sensitivity of the method was 0.2 ng/ml; intra- and inter-assay coefficients of variation were 6.1 and 8.5%, respectively. Urine samples were screened for metabolites of the main substances of abuse (morphine, methadone, cocaine, cannabis, amphetamine derivatives, benzodiazepines, barbiturates, and ethanol) by means of EMIT. The levels of mRNAs encoding for CB₁ and CB₂ receptors were assessed by reverse transcriptase polymerase chain reaction (RT-PCR) as described previously (De Marchi *et al*, 2003).

Statistical Analysis

The BMDP statistical software package (Dixon 1985) was used for data analysis. Data distributions were examined for normality and homogeneity of variance. As there were no significant deviations from normality in both AEA and 2-AG data, parametric statistical analyses were used. Where the one-way ANOVA showed significant differences among the groups, the *post-hoc* Tuckey's test was used to assess differences in the two group comparisons. The Pearson's product \times moment correlation test was used to examine the

relationship between endocannabinoid values and circulating leptin, nutritional and psychopathological variables.

RESULTS

Clinical and demographic characteristics of the study sample are shown in Table 1. One-way analysis of variance (ANOVA) showed statistically significant inter-group differences in BW ($F_{3,49} = 44.62$, $P < 0.0001$), body mass index (BMI) ($F_{3,49} = 41.37$, $P < 0.0001$), body fat mass ($F_{3,39} = 30.59$, $P < 0.0001$), body lean mass ($F_{3,39} = 7.28$, $P < 0.0006$), and age ($F_{3,49} = 4.98$, $P < 0.005$). Statistically significant differences between healthy controls and each diagnostic group are shown in Table 1.

As far as psychopathology, one-way ANOVA showed statistically significant inter-group differences in the BITE symptoms ($F_{2,29} = 15.61$, $P < 0.0001$) and BITE total ($F_{2,29} = 8.05$, $P < 0.002$) scores, in the bulimia subitem score of the EDI-2 ($F_{2,29} = 5.92$, $P < 0.008$), and in the MADRS total score ($F_{2,29} = 43.97$, $P < 0.0001$). Differences among the groups are shown in Table 2.

One-way ANOVA showed significant inter-group differences in plasma levels of AEA ($F_{3,49} = 6.31$, $P = 0.001$), but not in plasma concentrations of 2-AG ($F_{3,49} = 0.62$; $P = 0.6$). As compared to healthy women, both AN and BED patients showed significantly enhanced plasma levels of AEA, whereas women with BN had concentrations of the two endocannabinoids similar to those of healthy women (Figure 1). When the blood of five individuals selected from each group were analyzed for the levels of mRNA encoding for either CB₁ or CB₂ receptors by semi-quantitative RT-PCR, no significant group differences were observed (data not shown).

Significant intergroup differences emerged in the plasma concentrations of leptin ($F_{3,49} = 68.09$; $P < 0.00001$), with anorexic women showing significantly decreased circulating leptin values and BED women exhibiting significantly enhanced plasma concentrations of the fat hormone as compared to healthy controls (Table 1). Bulimic patients did not show significant changes in plasma leptin levels.

Plasma AEA levels were significantly and negatively correlated to circulating leptin concentrations in both healthy controls ($r = -0.52$, $P = 0.04$) and AN women ($r = -0.53$; $P = 0.03$), but not in BN ($r = -0.30$, $P = 0.3$) and BED ($r = 0.41$, $P = 0.2$) patients (Figure 2). No

significant correlations between endogenous cannabinoids and nutritional parameters, such as BW, BMI, body fat mass, and body lean mass, were found in each diagnostic group. Moreover, no significant correlations emerged between the plasma levels of the two endocannabinoids, on the one hand, and age, duration of the illness, BITE total and subitem scores, EDI-2 subitem scores, MADRS, STAI-S and STAI-T total scores, on the other hand, in each diagnostic group. Finally, none of the subjects had positive analyses for urine metabolites of the main substances of abuse.

Table 2 Psychopathological Characteristics of the Study Sample

| | Women with anorexia nervosa | Women with bulimia nervosa | Women with binge-eating disorder |
|--------------------------------------------|-----------------------------|----------------------------|----------------------------------|
| <i>Eating disorder inventory-2</i> | | | |
| Drive for thinness | 15.0 ± 7.6 | 13.6 ± 9.2 | 13.0 ± 5.6 |
| Bulimia | 3.4 ± 4.3 | 9.6 ± 6.2* | 10.3 ± 5.5* |
| Body dissatisfaction | 11.7 ± 7.2 | 15.5 ± 6.9 | 19.1 ± 6.3 |
| Ineffectiveness | 9.3 ± 7.1 | 10.5 ± 7.2 | 13.1 ± 6.0 |
| Perfectionism | 5.0 ± 3.0 | 5.6 ± 4.3 | 4.5 ± 3.9 |
| Interpersonal distrust | 7.0 ± 5.1 | 4.3 ± 3.8 | 5.8 ± 4.2 |
| Enterceptive awareness | 11.0 ± 5.1 | 12.0 ± 6.8 | 14.4 ± 6.3 |
| Maturity fear | 9.0 ± 6.6 | 7.6 ± 5.0 | 5.4 ± 5.5 |
| Ascetism | 6.5 ± 3.3 | 7.5 ± 3.2 | 9.3 ± 4.1 |
| Impulse regulation | 10.1 ± 6.8 | 7.8 ± 3.9 | 11.2 ± 8.6 |
| Social insecurity | 7.8 ± 4.8 | 8.2 ± 4.7 | 7.6 ± 3.8 |
| <i>Bulimia Investigation Test Edinburg</i> | | | |
| Symptoms | 11.6 ± 7.0 | 23.2 ± 3.5* | 22.0 ± 4.5* |
| Severity | 6.3 ± 8.2 | 8.5 ± 3.0 | 9.4 ± 5.2 |
| Total score | 17.9 ± 12.6 | 31.7 ± 7.9* | 31.4 ± 7.2* |
| MADRS total score | 32.9 ± 7.4 | 11.2 ± 3.2* | 13.7 ± 5.9* |
| STAI—State anxiety score | 46.3 ± 11.4 | 44.7 ± 14.7 | 48.4 ± 16.0 |
| STAI—Trait anxiety score | 52.4 ± 10.5 | 47.7 ± 6.7 | 52.0 ± 9.7 |

Data are expressed as mean ± SD.

* $P < 0.001$ vs women with anorexia nervosa (Tuckey's test).

Table 1 Demographic and Nutritional Characteristics and Leptin Concentrations of the Study Sample

| | Healthy women | Women with anorexia nervosa | Women with bulimia nervosa | Women with binge-eating disorder |
|--------------------------------------|---------------|-----------------------------|----------------------------|----------------------------------|
| Age (years) | 22.9 ± 3.8 | 22.2 ± 5.7 | 26.0 ± 4.2 | 28.9 ± 5.4 ^b |
| Body weight (kg) | 61.4 ± 6.5 | 42.6 ± 6.6 ^a | 56.3 ± 7.6 | 83.5 ± 14.4 ^a |
| Body Mass Index (kg/m ²) | 22.2 ± 2.3 | 15.9 ± 1.6 ^a | 21.1 ± 2.9 | 31.2 ± 6.2 ^a |
| Body fat mass (kg) | 15.5 ± 6.7 | 6.8 ± 4.8 ^c | 12.2 ± 5.1 | 36.4 ± 11.3 ^a |
| Body lean mass (kg) | 45.0 ± 6.5 | 36.5 ± 5.1 ^d | 43.0 ± 4.9 | 47.8 ± 6.1 |
| Plasma leptin (ng/ml) | 13.4 ± 4.8 | 3.9 ± 1.9 ^a | 10.9 ± 5.5 | 41.9 ± 12.8 ^a |

Data are expressed as mean ± SD.

^a $P < 0.001$, ^b $P < 0.01$, ^c $P < 0.02$, ^d $P < 0.005$ vs healthy women (Tuckey's test).

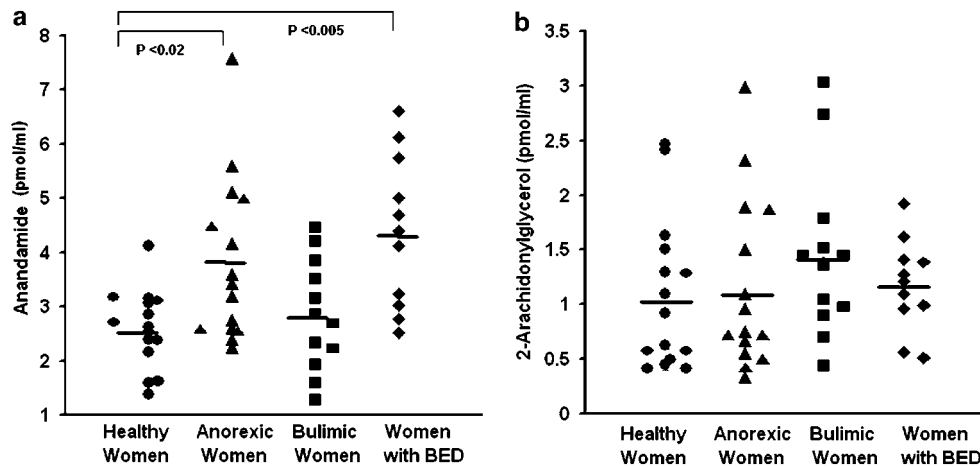


Figure 1 Plasma levels of AEA (anandamide) (panel a) and 2-AG (panel b) in healthy women and in women with AN, BN, or BED. Horizontal bars indicate mean values.

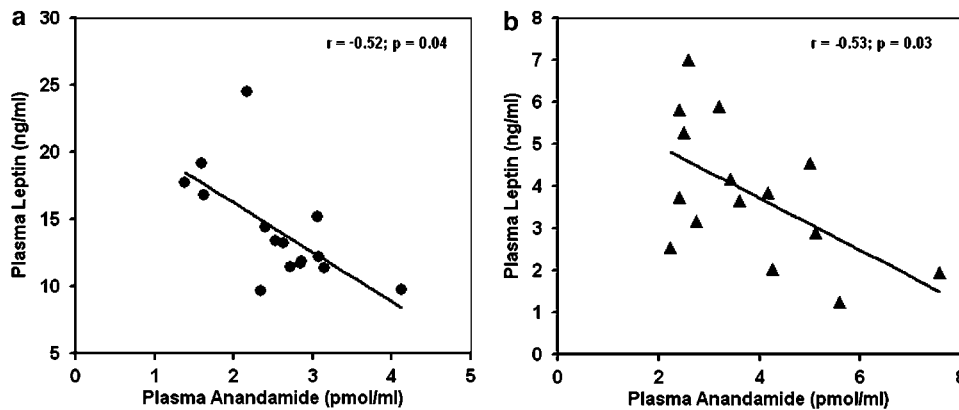


Figure 2 Correlations between plasma levels of arachidonylethanolamide (anandamide) and circulating leptin in healthy controls (panel a) and in women with anorexia nervosa (panel b).

DISCUSSION

To the best of our knowledge, this is the first study measuring circulating levels of endocannabinoids across the spectrum of EDs. We detected increased plasma concentrations of AEA, with no significant changes in plasma levels of 2-AG, in both underweight women with restricting AN and overweight/obese patients with BED. In BN individuals, instead, the circulating levels of either AEA or 2-AG did not significantly differ from control women. Moreover, as previously reported by both our group and other authors (Monteleone *et al*, 2002a,b; Adami *et al*, 2002), circulating leptin levels were drastically reduced in AN patients and significantly increased in BED individuals, but did not significantly change in BN women.

The most likely hypothesis accounting for similar changes of circulating AEA in individuals who are at the opposite of the ED spectrum is provided by the observed changes in leptin signalling. In fact, leptin has been shown to inhibit endogenous AEA levels in both rodent brain and uterus and human blood (Maccarrone *et al*, 2003; Di Marzo *et al*, 2004; Maccarrone *et al*, 2005), and since its plasma concentration was drastically reduced in underweight subjects with AN,

it is likely that increased levels of plasma AEA in these patients were secondary to their leptin deficiency. In patients with BED, where an increase in leptin production occurred because of their enhanced fat stores, one would expect decreased rather than enhanced plasma levels of AEA. In most cases, however, our BED patients were truly obese and, hence, likely characterized by reduced sensitivity to abnormally high circulating leptin levels (Proietto and Thorburn, 2003). Therefore, it is possible that impaired leptin levels or signalling, which was not observed in our BN patients, may explain why higher levels of AEA were found in subjects with BED and AN, but not in those with BN. In support of this idea, we detected a significant negative correlation between plasma AEA levels and circulating leptin in both healthy controls and anorexic patients. Alternatively, it is possible that changes in the micro- and macronutrient composition of the diet affected circulating AEA in our AN and/or BED patients, since it has been shown in animals that the diet can significantly and directly influence the levels of AEA, but not 2-AG (Berger *et al*, 2001; Matias *et al*, 2003). Finally, we cannot exclude that AEA elevation in our patients was a nonspecific phenomenon reflecting a compensatory adaptation to the disease state, since similar increases have been reported also in other

psychiatric conditions such as schizophrenia (De Marchi et al, 2003; Giuffrida et al, 2004).

The pathophysiological significance of the enhanced levels of AEA in both restricting AN and BED is not easy to explain. At present, we can propose only some hypotheses. Our findings show that changes in AEA levels do not seem to be related to psychopathological variables such as anxiety, depression or eating-related symptoms or duration of the illness, since no statistically significant correlations emerged between these variables and the plasma concentrations of the endocannabinoid. Since AEA has a stimulatory action on food intake (Williams and Kirkham, 1999; Hao et al, 2000; Jamshidi and Taylor, 2001), a possibility might be that the enhanced levels of the endocannabinoid in restricting anorexics may represent an adaptive response aiming at counteracting their restrictive behavior by increasing the drive to eat. However, this attempt has apparently no success in these patients, likely because psychological factors overwhelm biological mechanisms regulating eating behavior. In patients with BED, instead, the endocannabinoid-induced potentiation of the drive to eat may be one of the causes of their binge-eating behavior.

Finally, it is known that endocannabinoids are an essential part of the brain mechanisms controlling reward; therefore, it is possible to speculate that the enhancement of AEA levels in both AN and BED is involved in the mediation of rewarding aspects of their aberrant eating behavior. Restricting anorexics starve themselves, avoid particular foods, and adopt highly rigid eating patterns, which result in a sense of power over eating that is extremely rewarding. Therefore, the elevation of the endocannabinoid tone in restricting AN may mediate, at least in part, the patients' addiction to self-starvation, enabling them to bear with the chronic hunger associated with prolonged food restriction. In women with BED, instead, the increased levels of plasma AEA may reinforce the hedonic properties of hypercaloric nutrition, thus favoring addiction to food intake and perpetuating binge-eating behavior. The lack of enhancement of plasma AEA in people with BN who, like BED subjects, massively binge, may be explained by the presence of vomiting and other compensatory behaviors, which allow both the elimination of most of the food ingested during binge episodes and the increase in energy expenditure.

It must be pointed out that we could only measure the circulating levels of endocannabinoids. Therefore, it remains to be established whether: (i) changes in peripheral AEA levels reflect similar modifications in the brain; or (ii) peripheral changes may affect central functions, since this compound, due to its lipophilicity, is believed to act as an autocrine/paracrine mediator (Di Marzo et al, 2004). Moreover, the present results do not allow to establish the state- or trait-related nature of reported changes; the assessment of circulating endocannabinoids in AN patients after weight gain and in BED individuals after stopping bingeing and successful weight loss would help to clarify this issue.

Notwithstanding these limitations, our findings show for the first time that circulating AEA levels are increased in the blood of drug-free symptomatic women with AN or with BED. Although the pathophysiological significance of these alterations awaits further studies to be clarified, it is

intriguing that similar modifications in the endocannabinoid tone occur in disorders that are at the opposite of the ED spectrum. These results support the idea that a pharmacological manipulation of the endocannabinoid tone might be beneficial in both patients with AN and BED. In this regard, it is worth mentioning that a previous attempt to potentiate the endocannabinoid transmission by delta 9-tetrahydrocannabinol administration in AN patients showed no therapeutic benefit (Gross et al, 1983); this failure may be tentatively understood in the light of the present findings. Conversely, phase III clinical trials indicate that the CB₁ antagonist rimonabant[®] is able to reduce BW in obese individuals, and likewise it is possible to predict future similar studies on the use of this compound also to correct other EDs.

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