

Deranged endocannabinoid responses to hedonic eating in underweight and recently weight-restored patients with anorexia nervosa^{1–3}

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ABSTRACT

Background: A dysregulation of reward mechanisms was suggested in the pathophysiology of anorexia nervosa (AN), but the role of the endogenous mediators of reward has been poorly investigated. Endocannabinoids, including anandamide and 2-arachidonoylglycerol, and the endocannabinoid-related compounds oleoylethanolamide and palmitoylethanolamide modulate food-related and unrelated reward. Hedonic eating, which is the consumption of food just for pleasure and not homeostatic need, is a suitable paradigm to explore food-related reward.

Objective: We investigated responses of endocannabinoids and endocannabinoid-related compounds to hedonic eating in AN.

Design: Peripheral concentrations of anandamide, 2-arachidonoylglycerol, oleoylethanolamide, and palmitoylethanolamide were measured in 7 underweight and 7 weight-restored AN patients after eating favorite and nonfavorite foods in the condition of no homeostatic needs, and these measurements were compared with those of previously studied healthy control subjects.

Results: 1) In healthy controls, plasma 2-arachidonoylglycerol concentrations decreased after both types of meals but were significantly higher in hedonic eating; in underweight AN patients, 2-arachidonoylglycerol concentrations did not show specific time patterns after eating either favorite or nonfavorite foods, whereas in weight-restored patients, 2-arachidonoylglycerol concentrations showed similar increases with both types of meals. 2) Anandamide plasma concentrations exhibited no differences in their response patterns to hedonic eating in the groups. 3) Compared with 2-arachidonoylglycerol, palmitoylethanolamide concentrations exhibited an opposite response pattern to hedonic eating in healthy controls; this pattern was partially preserved in underweight AN patients but not in weight-restored ones. 4) Like palmitoylethanolamide, oleoylethanolamide plasma concentrations tended to be higher in non-hedonic eating than in hedonic eating in healthy controls; moreover, no difference between healthy subjects and AN patients was observed for food-intake-induced changes in oleoylethanolamide concentrations.

Conclusion: These data confirm that endocannabinoids and endocannabinoid-related compounds are involved in food-related reward and suggest a dysregulation of their physiology in AN. This trial was registered at ISRCTN.org as ISRCTN64683774. *Am J Clin Nutr* 2015;101:262–9.

Keywords anhedonia, anorexia nervosa, endocannabinoids, hedonic eating, reward

INTRODUCTION

Anhedonia, which is the reduced ability to experience reward, is a key symptom in the clinical presentation of anorexia nervosa

(AN)⁴ (1). Accordingly, in AN patients, functional brain-imaging studies that specifically assessed the processing of food-related stimuli, such as watching images of highly rewarding foods, showed, although not consistently, the hypoactivation of brain areas of the mesolimbic reward system (2–7). In addition, the hyperactivation of reward-related brain regions was documented in AN individuals on the processing of starvation-linked stimuli such as reading positive valued “thin” words (8) or watching female body images with underweight features (9). These findings are consistent with the hypothesis that an altered evaluation of reward stimuli might be involved in the pathophysiology of AN (2, 7, 10).

Eating behavior encompasses 2 different components, one that mediates the ingestion of food to maintain energy homeostasis (homeostatic eating) and the other that mediates the rewarding component of food ingestion (hedonic eating) (11). Although homeostatic eating also has a hedonic component, hedonic eating refers to the consumption of food exclusively for pleasure and not to maintain energy homeostasis (11). Thus, hedonic eating may represent a useful paradigm to specifically assess the physiologic mechanisms involved in food-related reward in humans.

The endocannabinoid system, which comprises the 2 G-protein-coupled receptors cannabinoid-1 and cannabinoid-2 receptors and their endogenous ligands arachidonylethanolamide (anandamide) and 2-arachidonoylglycerol (12), is involved in the control

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⁴Abbreviations used: AN, anorexia nervosa; PPAR α , peroxisome proliferator-activated receptor- α ; TRPV1, transient receptor potential vanilloid type-1; VAS, visual analog scale.

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of all aspects of food intake. In particular, hypothalamic and mesolimbic endocannabinoids are produced after food deprivation to activate cannabinoid-1 receptors locally and enhance appetite by stimulating neurochemical pathways underlying both homeostatic and rewarding aspects of food intake (13). We previously showed that, seemingly in a paradoxical manner, plasma endocannabinoid concentrations were elevated in both AN women (14) and obese individuals (15). Although animal and human studies strongly suggested that peripheral endocannabinoid overproduction contributes to hyperphagia, dyslipidemia, or glucose intolerance (16), the hypothesis that elevated peripheral endocannabinoid concentrations in AN contribute to dysregulated reward processes, hypothesized for this disorder, is still a matter of speculation (14, 17).

In a previous study (18), we observed that, in healthy subjects, hedonic eating was characterized by increases in peripheral concentrations of ghrelin and 2-arachidonoylglycerol, suggesting the involvement of these mediators in the modulation of food-related reward. Therefore, to investigate the physiologic modulation of food-related reward in AN, we explored peripheral endocannabinoid responses to hedonic eating in patients with active or weight-restored AN and compared them to responses of previously studied healthy control subjects. We also measured concentrations of the 2 anandamide-related mediators oleoylethanolamide and palmitoylethanolamide that act as ligands for the peroxisome proliferator-activated receptor- α (PPAR α) (19) and transient receptor potential vanilloid type-1 (TRPV1) channels (20), which are 2 receptors whose activation, in opposition to that of cannabinoid-1, may reduce food-intake and reward (16, 21, 22).

SUBJECTS AND METHODS

Patients consecutively admitted to the eating disorder inpatient unit of Villa Garda Hospital were screened for the study. Patients were required to meet Diagnostic and Statistical Manual for Mental Disorders-IV edition criteria for AN either present or past. Exclusion criteria were age <18 y, use of hormones or drugs, history of psychosis, diabetes, psychoactive substance use or head trauma, and presence of severe physical disorders or comorbid psychiatric disorders. Diagnoses were made by using the Structured Clinical Interview for Diagnostic and Statistical Manual for Mental Disorders-IV edition Axis I Disorders-Patient edition (23). A total of 30 patients were screened; 3 patients refused to participate into the study, 5 patients were excluded because they were aged <18 y, and 8 patients were excluded because of a current comorbid psychiatric disorder. The final sample included 14 participants of whom there were 7 patients (one man and 6 women) aged 18–35 y (25th, 50th, and 75th percentiles: 19.5, 22, and 27 y, respectively) with active AN (5 patients with a restricting subtype and 2 with a binge-eating/purging subtype) and 7 weight-restored patients (2 men and 5 women) aged 18–27 y (25th, 50th, and 75th percentiles: 18, 19, and 23.5 y, respectively) with a lifetime diagnosis of AN (5 patients with a restricting subtype and 2 patients with a binge-eating/purging subtype). Underweight patients were tested ≤ 2 wk from their entry into the inpatient unit before starting any specific weight-restoring program; weight-restored patients were tested 2–14 wk (25th, 50th, and 75th percentiles: 2, 4, and 8 wk, respectively) after having reached a BMI (in kg/m²) of 18.5. At the time of the study, the BMI of active AN patients ranged from

14.7 to 17.5 (25th, 50th, and 75th percentiles: 15.35, 15.8, and 16.15, respectively); the BMI of weight-restored AN patients ranged from 18.7 to 21.8 (25th, 50th, and 75th percentiles: 18.9, 19, and 19.25, respectively).

Two healthy men and 5 healthy women, aged 21–28 y (25th, 50th, and 75th percentiles: 22.5, 25, and 26 y, respectively) with BMI ranging from 19.0 to 24.5 (25th, 50th, and 75th percentiles: 19, 20, and 23, respectively), who participated in a previous study with identical methodologic procedures (18) acted as healthy controls. Controls had normal eating behaviors, were drug-free, and had normal physical examinations, normal values of routine blood and urine tests, and a normal electrocardiogram.

All subjects signed a written informed consent form. The study was approved by the Ethics Committee of the Second University of Naples, and all procedures were performed in accordance with the Helsinki Declaration of 1975 as revised in 1983. This trial was registered at ISRCTN.org as ISRCTN64683774.

Before the first experimental session, each participant was asked to indicate his and her most favorite food by answering the following question: “Which is your most favorite food that you would eat also when satiated, just for pleasure?” At the first test session, after a 12-h fast, at 0900, participants were asked to rate their hunger and satiety on visual analog scales (VASs) that used a 10-cm line with labels at the extremities indicating the most-negative and most-positive ratings; afterward, subjects received a breakfast of 300 kcal with 77% carbohydrates, 10% proteins, and 13% fat. Immediately after breakfast, participants again rated their hunger and satiety by means of VASs. After 1 h, subjects were told that they would receive their previously chosen favorite food, and an intravenous catheter was inserted into an antecubital vein to collect a first blood sample ($T = 0$); the catheter was connected to a saline solution, which was slowly infused to keep it patent. Immediately afterward, participants were exposed to the chosen favorite food for 5 min; during this time, they could smell and see the food but could not eat it. At the end of the exposure, participants were asked to rate their hunger, satiety, urge to eat the food, pleasantness to experience a mouthful of the food, and amount of the food they would eat by using VASs. Then, subjects were free to eat the favorite food ad libitum within 10 min; this time period was chosen to standardize the time of food ingestion and times of blood sample collection in participants in the 2 experimental sessions. Additional blood samples were drawn immediately after the exposure to the favorite food ($T = 5$) and 15 ($T = 30$) and 120 min ($T = 135$) after eating it. At the end of the session, the amount of food eaten by each participant was calculated by weighting the residual food and subtracting it from the initial amount of food provided, and calories eaten were calculated. At the second test session, participants underwent the same experimental procedures of the first experimental session except that they were exposed to a nonfavorite food and had to eat an amount of it with the same nutrient composition and an equal quantity of calories as the favorite food they ate in the previous session within 10 min.

Favorite foods were served in dishes from which the subject was free to eat ad libitum without being required to finish all of the food. On the basis of participants' answers to the question about their most-favorite food that they would eat also when satiated, just for pleasure, bread, milk, and butter were identified as nonfavorite foods and combined ad hoc to provide the same



nutrients and calorie amounts of the hedonic foods. Calorie and nutrient contents of favorite and nonfavorite foods were calculated by using the WINFOOD program (release 1.5, 1999; Medimatica) except for subjects who ate packaged foods with labels. To calculate calorie and nutrient contents of Italian cakes, we obtained recipes from the confectioner who prepared them.

Blood was collected in tubes containing EDTA as an anticoagulant. Plasma was separated by centrifugation and stored at -20°C . Plasma concentrations of anandamide, 2-arachidonoylglycerol, oleoylethanolamide, and palmitoylethanolamide were determined by using isotopic dilution liquid chromatography–mass spectrometry as described previously (24).

The BMDP statistical software package (release 7.0, 1992; WJ Dixon, BMDP Statistical Software) was used for data analysis. Because plasma concentrations of endocannabinoids in healthy controls were assayed in a previous experiment, their absolute values could not be directly compared with those assayed in the current study. Therefore, to make endocannabinoid response patterns of previously assayed healthy controls comparable with those of AN patients, for each subject and each compound, single values were normalized by dividing them by the correspondent $T=0$ value in the nonhedonic eating. Differences in biochemical responses to 2 isomeretic meals in the 3 groups and in each group were analyzed by using a mixed-model ANOVA with repeated measures followed by the post hoc Tukey's test. A 2-factor ANOVA with repeated measures was used to analyze differences in VAS scores. A level of significance of $P < 0.05$ was used for all data analyses.

RESULTS

VAS scores

In both hedonic and nonhedonic eating sessions, no significant differences emerged between groups in scores of hunger, satiety, urge to eat, pleasantness to experience a mouthful of presented food, and amount of food each participant would eat. In all groups, hunger and satiety scores before hedonic eating did not differ from those before nonhedonic eating, whereas scores of the urge to eat, pleasantness to experience a mouthful of presented food, and amount of food each participant would eat were significantly

higher before eating the favorite food than nonfavorite food (Figure 1).

Calories and nutrients

The calorie amount and nutrient composition of favorite and nonfavorite foods are shown in Table 1. No significant differences emerged in mean values of calories and nutrients of favorite and nonfavorite foods in each group and between groups.

Plasma 2-arachidonoylglycerol

A mixed-model 3-factor ANOVA with repeated measures disclosed significant group \times meal ($F_{[2,18]} = 6.09, P = 0.009$) and meal \times time ($F_{[3,18]} = 3.10, P = 0.03$) interactions, indicating that plasma 2-arachidonoylglycerol responses to favorite and nonfavorite meals significantly differed between groups. Indeed, when 2-arachidonoylglycerol plasma concentrations were analyzed in each group separately, significant effects for meal ($F_{[1,12]} = 5.47, P = 0.03$) and time ($F_{[3,12]} = 6.63, P = 0.001$) emerged in healthy controls, no significant effect was evident in underweight AN patients, and a significant effect only for time ($F_{[3,12]} = 3.28, P = 0.03$) was shown in weight-restored AN patients (mixed-model 2-factor ANOVA with repeated measures). In healthy subjects, plasma 2-arachidonoylglycerol concentrations progressively decreased in both test sessions; moreover, before ($T = 0$) and after ($T = 5$) the exposure to the hedonic food and 120 min after eating it ($T = 135$), 2-arachidonoylglycerol concentrations were significantly higher than correspondent time point values of nonhedonic eating (Figure 2). In contrast, in underweight AN patients, plasma concentrations of 2-arachidonoylglycerol did not show specific time patterns in either test session, whereas in weight-restored AN patients, 2-arachidonoylglycerol concentrations progressively increased in both test sessions without any significant difference ($P = 0.13$) between hedonic eating and nonhedonic eating (Figure 2).

Plasma anandamide

A mixed-model 3-factor ANOVA with repeated measures disclosed significant effects only for time ($F_{[3,18]} = 17.99, P <$

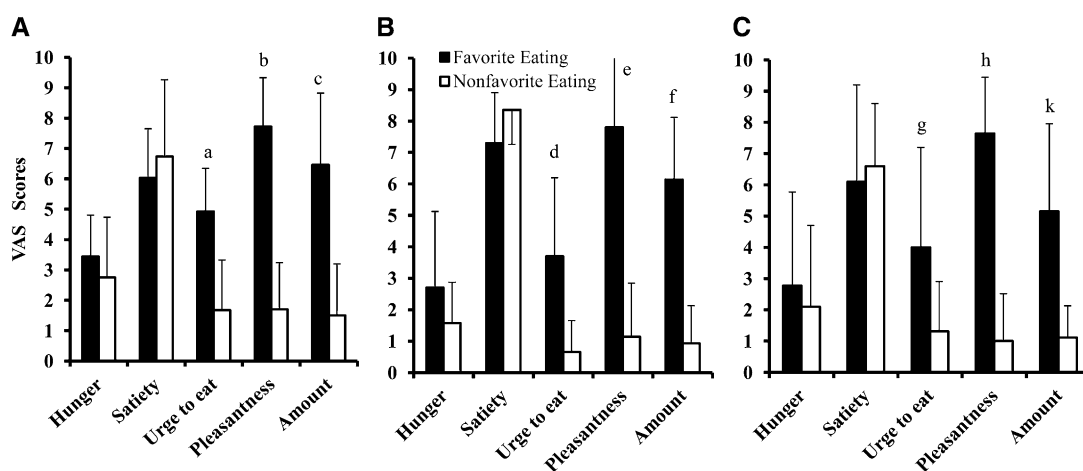


FIGURE 1 Mean (\pm SD) VAS scores in healthy subjects ($n = 7$; A), underweight patients with AN ($n = 7$; B), and weight-restored patients with AN ($n = 7$; C) before eating favorite (black bars) and nonfavorite (white bars) meals. ^{a-h,k}Compared with the nonfavorite meal: ^a $F_{[1,6]} = 10.98, P = 0.01$; ^b $F_{[1,6]} = 38.37, P = 0.0008$; ^c $F_{[1,6]} = 19.65, P = 0.004$; ^d $F_{[1,6]} = 8.64, P = 0.02$; ^e $F_{[1,6]} = 53.80, P = 0.0003$; ^f $F_{[1,6]} = 80.82, P = 0.0001$; ^g $F_{[1,6]} = 7.14, P = 0.03$; ^h $F_{[1,6]} = 50.78, P = 0.0004$; ^k $F_{[1,6]} = 20.48, P = 0.004$. AN, anorexia nervosa; VAS, visual analog scale.

TABLE 1
Calorie and nutrient contents of favorite and nonfavorite foods eaten by each subject group¹

	Favorite food				Nonfavorite food			
	Kilocalories	Carbohydrates, g	Proteins, g	Lipids, g	Kilocalories	Carbohydrates, g	Proteins, g	Lipids, g
Healthy controls (<i>n</i> = 7)	424.96 ± 76.79	56.64 ± 12.45	9.64 ± 6.36	17.76 ± 9.34	432.23 ± 80.25	61.65 ± 14.22	7.97 ± 2.68	17.08 ± 9.38
Underweight AN ² patients (<i>n</i> = 7)	409.97 ± 96.15	44.72 ± 16.82	6.92 ± 2.87	22.60 ± 8.02	444.49 ± 102.90	47.66 ± 14.88	9.05 ± 4.63	24.17 ± 9.50
Weight-restored AN patients (<i>n</i> = 7)	483.39 ± 235.19	42.24 ± 17.83	8.19 ± 6.40	31.29 ± 22.35	540.10 ± 241.15	49.52 ± 16.78	10.47 ± 8.04	33.39 ± 18.95

¹All values are means ± SDs. No significant difference emerged within each group and between groups for all variables (2-factor ANOVA with repeated measures).

²AN, anorexia nervosa.

0.00001), indicating that plasma concentrations of anandamide significantly changed over time in both test sessions without any significant difference in the 3 groups. Indeed, in healthy subjects, underweight patients with AN, and weight-restored AN patients, plasma concentrations of anandamide progressively decreased after eating either the favorite or nonfavorite meal (Figure 3).

Plasma oleoylethanolamide

A mixed-model 3-factor ANOVA with repeated measures disclosed a significant effect for meal ($F_{[1,18]} = 6.30, P = 0.02$) and time ($F_{[3,18]} = 8.35, P = 0.0001$), indicating that the plasma oleoylethanolamide response to a favorite meal differed from that to a nonfavorite meal with no significant differences between groups. Indeed, in all 3 groups, oleoylethanolamide plasma concentrations in the hedonic eating were lower than in nonhedonic eating and progressively decreased after eating both hedonic and nonhedonic meals (Figure 4).

Plasma palmitoylethanolamide

A mixed-model 3-factor ANOVA with repeated measures disclosed significant effects for meal ($F_{[1,18]} = 4.70, P = 0.04$)

and time ($F_{[3,18]} = 18.34, P < 0.00001$) and significant group × meal ($F_{[2,18]} = 7.96, P = 0.003$) and meal × time ($F_{[3,18]} = 3.12, P = 0.03$) interactions. These data indicated that plasma palmitoylethanolamide responses to favorite and nonfavorite meals were significantly different between groups. Indeed, when palmitoylethanolamide plasma concentrations were analyzed in each group separately, significant effects for meal ($F_{[1,12]} = 6.02, P = 0.03$) and time ($F_{[3,12]} = 19.70, P < 0.00001$) emerged in healthy controls, a significant meal × time interaction ($F_{[3,12]} = 3.22, P = 0.03$) was detected in underweight AN patients, and a significant effect for time ($F_{[3,36]} = 6.54, P = 0.001$) was evident in weight-restored AN patients (mixed-model 2-factor ANOVA with repeated measures). In healthy subjects, plasma palmitoylethanolamide concentrations progressively decreased in both test sessions; moreover, after the exposure to the favorite food ($T = 5$) and 15 min after eating it ($T = 25$) palmitoylethanolamide concentrations were significantly lower than correspondent time-point values of nonhedonic eating (Figure 5). In contrast, in underweight AN patients, plasma concentrations of palmitoylethanolamide showed a trend to increase ($P = 0.09$) after the exposure to the nonfavorite meal and progressively decreased after eating it, whereas plasma concentrations of palmitoylethanolamide showed only a progressive decrease in hedonic eating (Figure 5). In weight-restored AN patients,

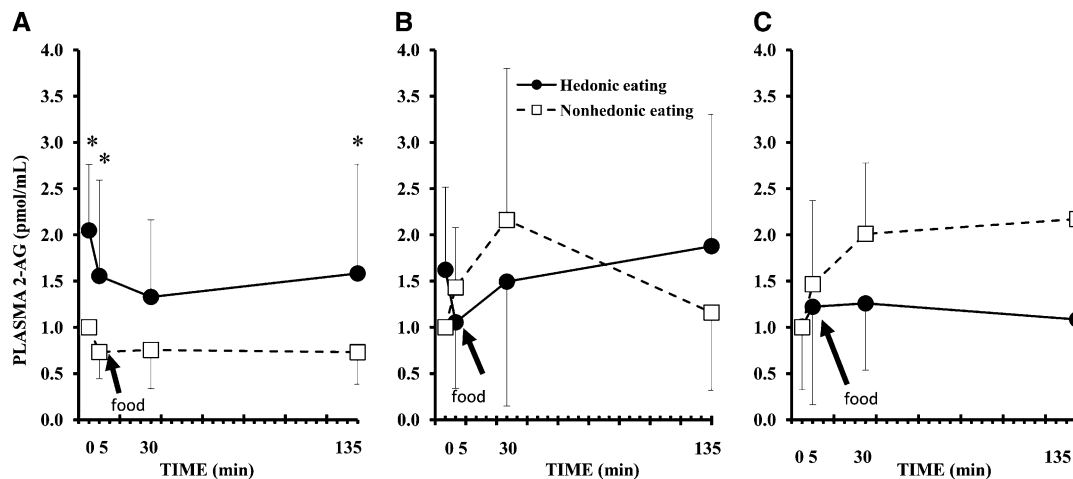


FIGURE 2 Mean (±SD) plasma concentrations of 2-AG after hedonic and nonhedonic eating in healthy subjects (*n* = 7; A), underweight patients with AN (*n* = 7; B), and weight-restored patients with AN (*n* = 7; C). Arrows indicate when subjects started to eat the test meal. *Compared with nonhedonic eating, $P < 0.001$ (post hoc Tukey's test). AN, anorexia nervosa; 2-AG, 2-arachidonoyl glycerol.

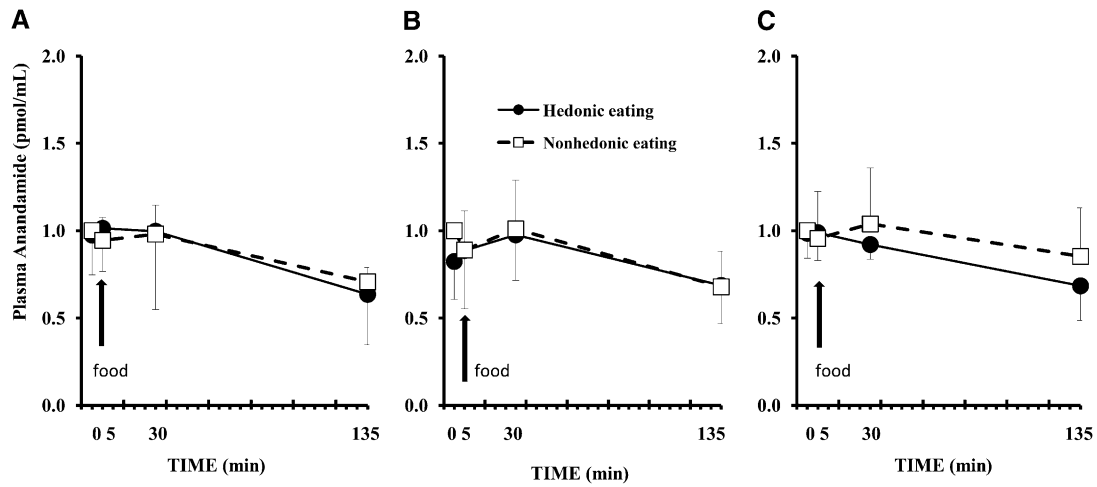


FIGURE 3 Mean (\pm SD) plasma concentrations of arachidonylethanolamide (anandamide) after hedonic and nonhedonic eating in healthy subjects ($n = 7$; A), underweight patients with AN ($n = 7$; B), and weight-restored patients with AN ($n = 7$; C). Arrows indicate when subjects started to eat the test meal. AN, anorexia nervosa.

palmitoylethanolamide concentrations showed a progressive decrease in both test sessions without any significant difference between hedonic eating and nonhedonic eating (Figure 5).

DISCUSSION

To the best of our knowledge, no previous study has been carried to investigate the endocannabinoid and endocannabinoid-related mediator responses to hedonic eating in AN. When we compared underweight and weight-restored AN patients to normal-weight healthy controls, we showed that 1) responses of plasma concentrations of 2-arachidonoylglycerol to hedonic eating were deranged in both groups of AN patients; 2) anandamide plasma concentrations exhibited no differences in their response patterns to hedonic eating in the groups; 3) plasma concentrations of palmitoylethanolamide exhibited an opposite response pattern to hedonic eating compared with that for 2-arachidonoylglycerol in healthy controls; this pattern was partially preserved in underweight AN patients but not weight-restored ones; and 4) like

palmitoylethanolamide, oleoylethanolamide plasma concentrations tended to be higher in nonhedonic eating than hedonic eating in healthy controls; however, unlike palmitoylethanolamide, no difference between healthy and AN participants was observed for food-intake-induced changes of oleoylethanolamide concentrations. The observed differences were not attributable to changes in subjective responses to hedonic food because, as ascertained by the VAS scores, both patients and controls were in a status of satiety before the exposure to favorite and nonfavorite foods, and no significant differences emerged between groups in scores of the urge to eat, pleasantness to experience a mouthful of presented food, and amount of food each participant would eat. Similarly, these results could be not explained by differences in calorie and nutrient contents of favorite and nonfavorite foods because no significant differences emerged in mean values of these variables both in each group and between groups. Finally, the lack of significant differences in BMI values between weight-restored AN patients and healthy controls suggested that deranged endocannabinoid responses persisted in recovered AN

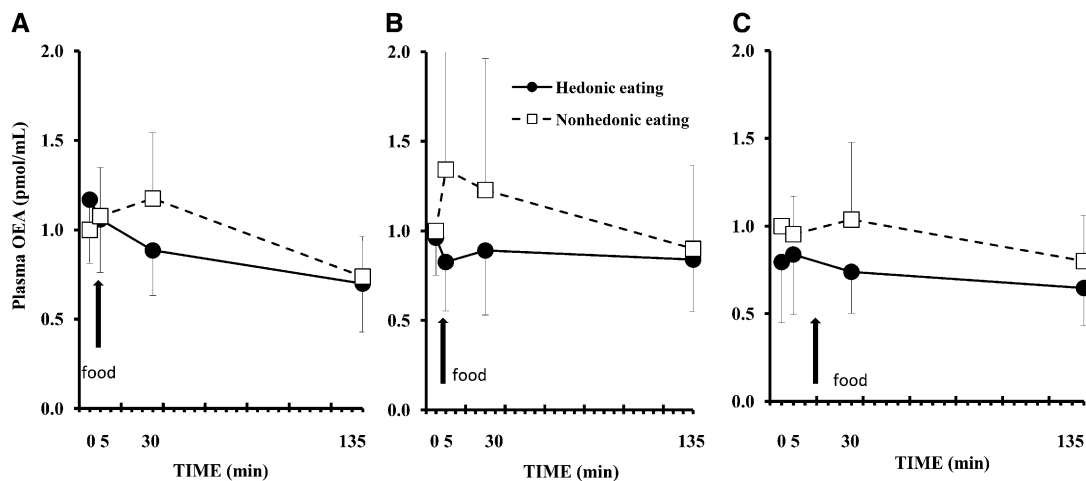


FIGURE 4 Mean (\pm SD) plasma concentrations of OEA after hedonic and nonhedonic eating in healthy subjects ($n = 7$; A), underweight patients with AN ($n = 7$; B), and weight-restored patients with AN ($n = 7$; C). Arrows indicate when subjects started to eat the test meal. AN, anorexia nervosa; OEA, oleoylethanolamide.

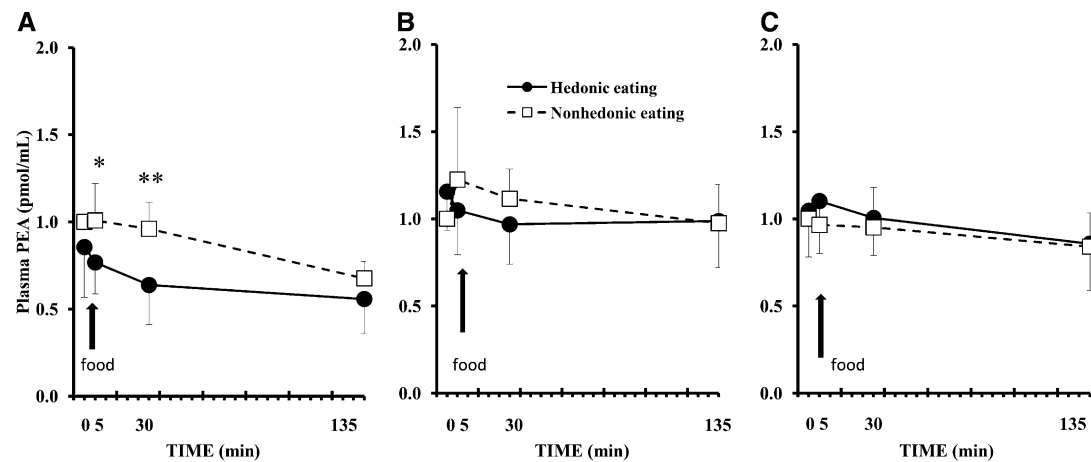


FIGURE 5 Mean (\pm SD) plasma concentrations of PEA after hedonic and nonhedonic eating in healthy subjects ($n = 7$; A), underweight patients with AN ($n = 7$; B), and weight-restored patients with AN ($n = 7$; C). Arrows indicate when subjects started to eat the test meal. ***Compared with nonhedonic eating (post hoc Tukey's test): * $P < 0.02$, ** $P < 0.001$. AN, anorexia nervosa; PEA, palmitoylethanolamide.

patients independently from body-weight restoration. Therefore, these findings support the hypothesis of the occurrence of a dysregulation of endocannabinoid and endocannabinoid-related signaling associated with hedonic eating in both underweight and weight-restored AN individuals despite the preservation of their subjective responses to the consumption of favorite and non-favorite foods and normalization of body weight.

In our healthy controls given the favorite meal, plasma concentrations of 2-arachidonoylglycerol were significantly higher than corresponding concentrations measured with the nonfavorite meal already before exposure to the meal. After eating the meal, concentrations decreased over time but remained higher than after eating the nonfavorite meal. We previously suggested (18) that this observation may have depended on our experimental design because, to adequately balance the favorite meal with the nonfavorite one, participants were obliged to eat first the favorite food, and they were aware of this. Therefore, we previously proposed that the premeal increase of plasma 2-arachidonoylglycerol in hedonic eating might have been associated with the anticipation of the pleasure of ingesting a food with highly rewarding gustatory properties, whereas the persistence of higher concentrations of 2-arachidonoylglycerol after ingesting the favorite food might have been associated with the pleasure experienced during such a meal. In the current study, in underweight AN patients, plasma 2-arachidonoylglycerol concentrations either before or after eating the pleasurable food did not significantly differ from those before or after eating the nonfavorite meal, and with both meals, they did not significantly change after food ingestion. Moreover, in weight-restored AN patients, the response pattern of plasma 2-arachidonoylglycerol to hedonic eating and nonhedonic eating was opposite to that observed in normal subjects, with values increasing with both eating conditions, and plasma concentrations after nonhedonic eating were higher than those after hedonic eating, although this latter difference was NS.

At variance with plasma 2-arachidonoylglycerol, in healthy subjects, plasma concentrations of palmitoylethanolamide progressively decreased in both test meal sessions; moreover, after the exposure to the favorite food ($T = 5$) and 15 min after eating it ($T = 30$), they were significantly lower than corresponding

concentrations measured after nonhedonic eating. In contrast, underweight AN patients, plasma palmitoylethanolamide concentrations significantly increased after the exposure to the nonfavorite food but not after the exposure to the favorite meal, and then, they progressively decreased after the ingestion of both types of food. In weight-restored AN patients, palmitoylethanolamide concentrations showed a progressive decrease with both test-meal sessions without any significant difference between hedonic eating and nonhedonic eating.

Finally, no significant main difference emerged in response patterns of plasma anandamide and oleoylethanolamide in the 3 groups, although, like palmitoylethanolamide, unlike anandamide, and opposite to 2-arachidonoylglycerol, in healthy volunteers, oleoylethanolamide concentrations were lower after the ingestion of favorite compared with nonfavorite foods.

Taken together, these findings support the idea that responses of endocannabinoid and endocannabinoid-related mediators to hedonic eating is deeply deranged both in underweight and weight-restored phases of AN. Experimental data supported the idea that endocannabinoids are involved in the mediation of food-related reward. Indeed, animal studies showed that the blockade of the cannabinoid-1 receptor by rimonabant was able to inhibit the release of dopamine in the nucleus accumbens induced by hedonic food (25) and decrease the consumption of hedonic food in non-food-deprived animals (26), with both of these effects being less strong when the animals were exposed to normal food. These data suggest that foods with high-salience and -incentive properties might stimulate an endocannabinoid tone to induce dopamine release in limbic areas involved in reward mechanisms (27). Furthermore, recent evidence also showed that endocannabinoid concentrations in the small intestine increased after the consumption of fatty foods, which, for human beings, are usually more hedonic than are nonfatty foods (28). Thus, in healthy volunteers, the selective increase of plasma 2-arachidonoylglycerol concentrations before and, especially, after exposure to a favorite food may reflect corresponding changes both in brain-reward-related areas and the gut; the altered response in AN patients may underlie the lack of motivation toward food consumption or even an increased reward from the lack of consumption of any type of food. However, note that our patients were highly motivated to participate in

this study, and this motivation may have influenced the response of 2-arachidonoylglycerol concentrations to food ingestion. Also, the observed higher plasma concentrations of palmitoylethanolamide and oleoylethanolamide (an effect significant only for the former compound) with nonhedonic eating compared with hedonic eating in healthy volunteers can be interpreted as a response aimed at enhancing the intake of favorite food because both compounds, either via PPAR α or TRPV1, were suggested to produce food-intake inhibitory or reward counteracting actions (16, 21, 22). In this sense, note again how such a potential hedonic food-intake stimulatory effect was not observed in AN patients. Finally, the lack of any response whatsoever of anandamide plasma concentrations in both healthy and AN subjects can be interpreted by the fact that this endocannabinoid, like oleoylethanolamide and palmitoylethanolamide and unlike 2-arachidonoylglycerol, has also been reported to potently activate TRPV1 (29) and, less potently, PPAR α (30). In summary, in underweight AN patients, the lack of 2-arachidonoylglycerol activation after hedonic eating and increase of concentrations of palmitoylethanolamide, a PPAR α , and TRPV1 ligand with potential anorectic and reward-counteracting actions after the exposure to the nonfavorite food but not after the favorite meal could have denoted a deranged modulation of food-related reward occurring in the acute phase of AN. Likewise, in weight-restored AN patients, the occurrence of 2-arachidonoylglycerol and palmitoylethanolamide responses different from those that occurred in healthy controls (i.e., a stronger increase of 2-arachidonoylglycerol after the exposure or ingestion of the nonfavorite meal and the lack of significant differences of palmitoylethanolamide responses to favorite and nonfavorite meals) suggested the persistence of a deranged biochemical modulation of food-related reward. However, our weight-restored patients had reached normal BMI from 2 to 14 wk only, and this may not represent a stable weight restoration. Therefore, studies in AN patients who meet more-stringent criteria of full recovery (such as maintaining a body weight >90% of the average body weight, having regular menstrual cycles, and not bingeing, purging, or restricting food intake ≥ 1 y) are necessary to define whether the deranged endocannabinoid modulation of food-related reward is part of the biological background of AN. To this regard, a functional brain imaging study (7) showed that underweight AN patients exhibited hypoactivation of brain areas involved in food-related reward after the exposure to images of favorite foods in conditions of both hunger and satiety. This hypoactivation was present also in long-term weight-restored AN patients, although only in the hunger state, which suggested that hypoactivation might be a primary or acquired biological trait of AN. It could be speculated that a deranged endocannabinoid physiology, as shown by our preliminary findings, might mediate such a brain dysfunction. If this suggestion is confirmed in future studies, it might open new potential therapeutic perspectives for AN. Alternatively, apart from underlying alterations in reward regulatory mechanisms, the differences observed in AN subjects might have also reflected their altered metabolic status because underweight AN patients normally exhibit a clear catabolic status, whereas weight-recovered AN patients are predominantly anabolic, and normal-weight healthy subjects show, on average, a neutral metabolic status. In fact, the endocannabinoid system is involved in the regulation not only of reward but also of peripheral metabolism at the level of the adipose tissue, liver, pancreas and skeletal muscle (16). This possibility seems to be supported by our

finding of differences between recovered and symptomatic AN subjects in responses to favorite foods of 2-arachidonoylglycerol and, particularly, palmitoylethanolamide plasma concentrations.

The strength of this study was that, to assess peripheral biochemical responses to hedonic eating in AN, we were able to motivate a subgroup of AN patients, who are usually very reluctant and afraid to eat, to consume a standard breakfast of 300 kcal and eat both a favorite food *ad libitum* and, in a separate session, an equivalent amount of a nonfavorite food. However, the current study also had some limitations. First, our data refer to circulating concentrations of endocannabinoids and related mediators; thus, although concentrations of these compounds are also modulated after food intake in peripheral organs, at least in rodents (31), it remains to be determined if the changes in peripheral endocannabinoid responses to hedonic eating we observed in our AN patients reflect similar changes in brain areas directly involved in reward mechanisms and if peripheral changes in endocannabinoid tone affect central functions because of the high lipophilicity of these compounds. Therefore, the relevance of our results to the physiology of brain mechanisms implicated in the modulation of food-related reward in AN remains to be determined. A second limitation was represented by the low number of subjects in each group. This number was attributable to the high difficulty in obtaining a full compliance of both underweight and weight-restored AN patients to the current study paradigm, which encompassed the exposure and ingestion of food with a high calorie content, independently from its rewarding value. Finally, our patient samples included both restricting and binge-purging individuals. A recently published study (32) showed that binge-purging AN patients have a hedonic appetite higher than that of restricting anorexics. Therefore, differences in the modulation of reward-related processes between the 2 subtypes of AN patients may have affected our findings. However, we also performed statistical analyses without binge-purging patients to have purely anhedonic-restrictive patient groups, and results did not change (data not shown). Larger patient samples would help to clarify this issue.

In conclusion, the current results show, for the first time to our knowledge, a dysregulation of peripheral endocannabinoid signaling in both underweight and recently weight-restored patients with AN who undergo hedonic eating, thereby suggesting an alteration of the biochemical mechanism implicated in food-related reward. The full assessment of the relevance of these findings to the pathophysiology of AN awaits additional studies.

The authors' responsibilities were as follows—AMM, VDM, PM, and MM: designed the study and wrote the protocol and manuscript; AMM, RDG, PS, MEG, and SC: conducted clinical tests; TA and FP: performed laboratory assays; and all authors: contributed to and approved the final version of the manuscript. None of the authors declared biomedical financial interests or potential conflicts of interest.

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