Assessment of Serum Apelin Levels in Girls with Anorexia Nervosa

Katarzyna Ziora, Joanna Oświęcimska, Elżbieta Świętochowska, Dariusz Ziora, Zofia Ostrowska, Małgorzata Stojewska, Ewa Klimacka-Nawrot, Antoni Dyduch, and Barbara Błońska-Fajfrowska

Department of Pediatrics in Zabrze (K.Z., J.O., M.S., A.D.), Medical University of Silesia in Katowice, 41-800 Zabrze, Poland; Department of Biochemistry in Zabrze (E.S., Z.O.), Medical University of Silesia in Katowice, 41-808 Zabrze, Poland; Department of Pneumonology and Tuberculosis in Zabrze (D.Z.), Medical University of Silesia in Katowice, 41-800 Zabrze, Poland; and Department of Basic Biomedical Science in Sosnowiec (E.K.-N., B.B.-F.), Medical University of Silesia in Katowice, 41-200 Sosnowiec, Poland

Context: Pilot studies in rats have suggested that apelin (APE) is involved in the control of appetite and food intake. APE is secreted in the organs involved in the control of hunger and satiety: the stomach, hypothalamus, and fat tissue. Anorexia nervosa (AN) is an eating disorder that represents a good biological model of chronic fat tissue atrophy in humans. To date, there are no reports of APE expression in the fat tissue and its circulating concentrations in patients with AN.

Objective: Our objective was to assess serum APE concentrations in girls with AN.

Design, Participants, and Setting: APE-36 and APE-12 serum concentrations were evaluated in 87 Polish girls with restrictive AN, in 61 healthy (H) controls, 17 girls with no otherwise specified eating disorders (NOS), and 30 girls with simple obesity (OB).

Results: Mean serum APE-36 and APE-12 concentrations in patients with AN and NOS were significantly lower than in the H and OB groups. However, no differences between AN, H, and NOS groups were observed when APE concentrations were calculated per body mass index (BMI). In participants with normal BMI, serum APE-36 ($r = 0.35$) and APE-12 ($r = 0.37$) concentrations correlated positively with BMI.

Conclusions: We conclude that compared with H controls, serum APE-36 and APE-12 concentrations decreased as a result of fat tissue depletion in patients with AN. Conversely, obese adolescents had elevated APE-36 and APE-12 due to excessive fat mass as well as increased APE production in adipose tissue. **(*J Clin Endocrinol Metab* 95: 2935–2941, 2010)**

Apelin (APE) is an endogenous ligand of the previously discovered "orphan" receptor named APJ, isolated by Tatemoto *et al.* (1) from bovine stomach extracts in 1998. Several different isoforms of APE have been identified that are thought to exist *in vivo*. The predominant form of circulating APE is believed to be APE-36; however, shorter C-terminal fragments with biological activity have been found, including APE-17, APE-16, APE-13 and its pyroglutamylated form (APE-p[Glu]-13), and APE-12 (2, 3). It has been demonstrated that the biological activity of APEs is related inversely to the peptide length; thus, APE-12 is the most potent isoform. However, APE-11 and shorter peptides are inactive (4).

APE belongs to the adipokines group because its mRNA expression has been demonstrated in mature adipocytes and vascular stroma of fat tissue in rodents and humans as well as in murine preadipocyte cell lines (3T3F442A) (5, 6). APE peptide expression has been also

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2009-1958 Received September 15, 2009. Accepted March 11, 2010.
First Published Online April 9, 2010

Abbreviations: AN, Anorexia nervosa; APE, apelin; BMI, body mass index; BMI-SDS, SD score for BMI; H, healthy; ICV, intraventricular; NOS, not otherwise specified (eating disorders); OB, simple obesity.

detected in brain cells (APE-p[Glu]-13 and APE-17), lungs, placenta, breasts in pregnant and lactating women (APE-36 and APE-p[Glu]-13), pancreas, kidneys, prostate, testes (APE-36), uterus (APE-36), gastrointestinal tract, and osteoblasts (7).

The apelinergic system distribution over such a variety of tissues has suggested that it might play relevant roles in human physiology. Indeed, APE is involved in the regulation of cardiovascular, gastrointestinal, and immune functions, as well as bone physiology, fluid homeostasis, and cardiovascular system embryonal development (8). It has been demonstrated that APE exerts long-term positive inotropic effects, and its plasma concentrations are decreased in patients with chronic heart failure (9). APE may reduce blood pressure due to endothelium-dependent nitric oxide-mediated vasodilation, but it causes vasoconstriction from its direct actions on vascular smooth muscle cells. This peptide and its receptor, APJ, are expressed in human osteoblasts, stimulating their proliferation and inhibiting apoptosis (10). APE suppresses the production of proinflammatory cytokines (11) and chemotactic activity of CHO-A10 (Chinese hamster ovary) cells (12). In the gastrointestinal system, APE *in vivo* stimulates gastric cell proliferation and cholecystokinin secretion, and it may be involved in the pathogenesis of peptic ulcer, ulcerous colitis, and Crohn's disease (13).

Immunocolocalization of APE and APJ in the organs involved in the control of food intake (stomach, hypothalamus, and fat tissue) suggests their role in eating behavior; however, the available data are still scarce and contradictory (14–17). In a few studies, higher serum APE concentrations in obese people compared with lean controls (5, 17) and positive correlations between APE and body mass index (BMI) were observed (17, 18). However, to our knowledge, there are no reports on APE expression in the fat tissue and its serum concentrations in undernutrition.

Anorexia nervosa (AN) is an eating disorder that predominantly affects girls and young women, and it has many severe complications, including amenorrhea and other disturbances of the endocrine system, low bone mass, increased peripheral vascular resistance despite the presence of hypotension, low cardiac output and bradycardia, disturbances of gastrointestinal motility, and alterations in the immune system (19–22).

Considering previously demonstrated positive correlations between APE serum concentrations and BMI, marked fat tissue depletion in AN, the fact that the spectrum of biological activity of APE-36 and APE-12 corresponds with the majority of negative consequences of prolonged starvation, and the paucity of published research on this topic, we hypothesized that serum concentrations of APE in patients with AN are significantly lower than in healthy (H) and obese subjects. The aim of the study was to evaluate the serum concentrations of the most abundant circulating isoform of APE (APE-36) and its most potent C-terminal fragment, APE-12, in girls with AN.

Subjects and Methods

Subjects

This study involved 195 girls aged 11 to 18.9 yr. In all participants, BMI [body weight (kilograms)/height (meters)^2], and SD score for BMI (BMI-SDS) were calculated according to current Polish populational normal ranges (23). The examined group consisted of 87 girls with the restrictive form of AN according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) classification (24) (mean age, 15.2 ± 0.3 yr). The control group included 61 H, regularly menstruating female volunteers with BMI-SDS between −2.0 and +2.0 who were recruited from secondary schools (mean age, 15.4 ± 0.8 yr). We also examined a group of 17 girls with not otherwise specified eating disorders (NOS) per the DSM-IV (24) (mean age, 16.4 ± 0.9 yr) and 30 girls with simple obesity (OB) defined as BMI-SDS greater than 2.0 (mean age, 14.6 ± 0.8 yr).

All examined girls were at pubertal stage of Tanner IV–V. Clinical characteristics of the investigated patients are shown in Table 1.

The average disease duration was 12.1 months (range, 3–36 months) in the AN group and 8.7 months (4–12 months) in the NOS group. The average body weight loss from the beginning of the slimming process until hospitalization was 13.6 kg (5.8–48.7 kg) in the AN group and 12.3 kg (3.7–27.7 kg) in the NOS group.

Patients in the AN and NOS groups were examined during the first 3 d of hospitalization before therapy was started. Eligibility criteria consisted of stable general medical condition and the absence of clinical signs of dehydration. The initial results of additional laboratory investigations (serum electrolytes, aspartate and alanine aminotransferases, creatinine) excluded those with hepatic and renal pathologies. Patients with any organic or psychiatric disorders, other than eating disorders that could cause cachexia, were excluded. None of the participants took any medications, including hormonal drugs, within the past 3 months, or had any infections within the last month before the study.

This study was approved by the Bioethics Committee at the Medical University of Silesia in Katowice (no. L. dz. KNW-6501-62/08), and written informed consent was obtained from all examined participants and their parents or legal guardians before their participation.

Laboratory assays

Blood samples for analyses were collected in the fasting state between 0700 and 0830 h. After centrifugation at 1000 × *g* for 15 min at 4 C, the serum samples were frozen at −70 C until assays were performed.

APE-36 and APE-12 concentrations were determined using commercial human APE-12 and APE-36 enzyme immunoassay kits (Phoenix Pharmaceuticals Inc., Burlingame, CA) following the manufacturer's instructions. Before the assay, serum samples were extracted to isolate analyzed peptides before assay. Buffer

TABLE 1. Clinical characteristics of the examined groups of girls

	AN (n = 87)	NOS (n = 17)	OB (n = 30)	H (n = 61)
Age (yr)	15.18 ± 0.32 (11.3 to 18.5)	16.35[a] ± 0.85 (11.9 to 18.9)	14.62 ± 0.84 (11.0 to 18.3)	15.36 ± 0.80 (11.7 to 17.9)
Body weight (kg)	38.46 ± 1.20 (26.7 to 51.6)	47.50[b] ± 2.90 (39.2 to 60.7)	85.87[c] ± 7.58 (57.7 to 134.0)	52.26[c] ± 3.66 (31.5 to 71.7)
Height (cm)	162.00 ± 1.49 (143.5 to 175.0)	163.57 ± 3.51 (146.5 to 178.0)	163.40 ± 4.50 (145.0 to 186.5)	162.6 ± 3.49 (138.0 to 183.0)
BMI (kg/m^2)	14.67 ± 0.33 (10.85 to 17.85)	17.86[b] ± 1.04 (14.34 to 21.85)	31.86[c] ± 2.19 (25.5 to 52.0)	19.75[c] ± 1.12 (15.29 to 23.93)
BMI-SDS	−2.65 ± 0.20 (−5.21 to −1.08)	−1.42[a] ± 0.68 (−3.23 to −1.41)	6.91[c] ± 1.23 (3.23 to 17.83)	−0.18[c] ± 0.54 (−2.11 to 1.89)

Data are expressed as mean ± 1.96 SE (range). BMI-SDS, [Current patient's BMI (kg/m^2) − BMI equal to 50th percentile (kg/m^2)]/[BMI equal to 50th percentile (kg/m^2) − BMI equal to 3rd percentile (kg/m^2)].

[a] $P < 0.05$ NOS *vs.* OB.

[b] $P < 0.05$ NOS *vs.* AN.

[c] $P = 0.000008$ OB and H *vs.* AN.

A (1% trifluoroacetic acid aqueous solution), buffer B (60% acetonitrile solution in 1% trifluoroacetic acid solution), and SEP-PAK C chromatographic columns (Waters Associates, Milford, MA) were used for extraction. The obtained extract was lyophilized and then dissolved in the assay buffer before analysis. The absorbance measurements for all samples were performed using the Quant Universal Microplate Spectrophotometer (BioTek Instruments Inc., Winooski, VT). The sensitivity was 0.09 ng/ml for APE-36 and 0.07 ng/ml for APE-12 kit; the intraassay coefficient of variance was 5% or less, and the extraassay coefficient of variance was 14% or less for both kits.

Statistical analysis

Statistical analysis was performed using Statistica 6.0 software (StatSoft Inc., Tulsa, OK). Normal data distribution was assessed using Shapiro-Wilk test, and the homogeneity of variance was assessed using Levene's test. Comparisons between the examined groups were performed using the ANOVA and *post hoc* RIR Tukey's multiple comparison test for different sample sizes or Kruskal-Wallis and median tests if data distribution was not normal. Correlations were analyzed by Pearson's linear correlation test or Spearman's test if data distribution was not normal. All results were considered statistically significant at $P < 0.05$.

Results

The mean age of girls in the AN group was similar to that of girls in the other study groups (H, NOS, and OB). The mean age of patients in the NOS group was statistically significantly ($P < 0.05$) higher than the OB group. The mean body weight and BMI in the AN group were statistically significantly lower compared with the H ($P < 0.00001$), NOS ($P < 0.05$), and OB ($P < 0.00001$) groups. BMI-SDS in patients with AN was significantly ($P < 0.00001$) lower only when compared with H and OB subjects. In the NOS group, BMI-SDS was significantly lower ($P < 0.05$) than in the OB group (Table 1).

Mean APE-36 and APE-12 serum concentrations in the AN group (96.9 ± 9.2 and 96.0 ± 8.8 pg/ml, respectively) and in the NOS group (103.6 ± 17.8 and 97.9 ± 16.3 pg/ml, respectively) were significantly lower ($P < 0.0001$) compared with values obtained in H (132.2 ± 10.8 and 130.7 ± 9.8 pg/ml) and OB (246.5 ± 50.3 and 245.3 ± 57.1 pg/ml) groups. There were no significant differences in the mean serum concentrations of APE-12 and APE-36 between the AN and NOS groups. In the OB group, the mean APE-36 and APE-12 serum concentrations were statistically significantly increased compared with the H group ($P < 0.0001$) (Fig. 1).

When APEs were calculated per BMI, the mean serum APE-36 (APE-36/BMI) and APE-12 (APE-12/BMI) concentrations observed in the AN group (6.709 ± 0.982 and 6.649 ± 1.009 pg/ml/kg/m^2, respectively) and in the NOS group (5.837 ± 1.041 and 5.499 ± 0.840 pg/ml/kg/m^2,

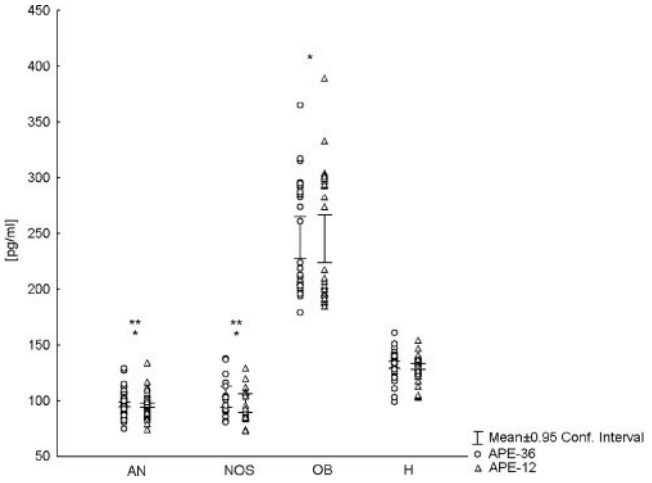

FIG. 1. Mean APE-36 and APE-126 serum concentrations (pg/ml) in examined groups. *, $P < 0.00001$ *vs.* H group; **, $P < 0.00001$ *vs.* OB group.

respectively) were significantly lower ($P < 0.001$) than in the OB group (7.917 ± 1.850 and 7.896 ± 2.139 pg/ml/kg/m^2). However, mean APE-36/BMI and APE-12/BMI values in the AN and NOS groups did not differ significantly from those in the H group (6.716 ± 0.921 and 6.643 ± 0.919 pg/ml/kg/m^2, respectively). In the OB group, mean APE-36/BMI and APE-12/BMI were significantly increased ($P < 0.001$) compared with the H group (Fig. 2).

Among participants from H and NOS groups analyzed together with normal BMI (between −2.0 and +2.0 SD), statistically significant positive correlations between BMI and serum APE-36 concentrations (r = 0.35; $P < 0.005$) (Fig. 3) as well as between BMI and serum APE-12 concentrations (r = 0.37; $P < 0.005$) (Fig. 4) were found. No significant linear correlations between BMI and APE-36 or APE-12 serum concentrations were demonstrated

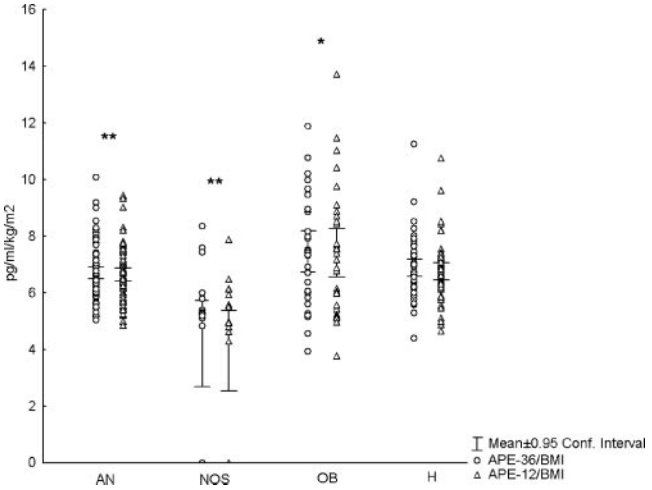

FIG. 2. Mean APE-12/BMI and APE-36/BMI (pg/ml/kg/m^2) values in examined groups. *, $P < 0.001$ *vs.* H group; **, $P < 0.001$ *vs.* OB group.

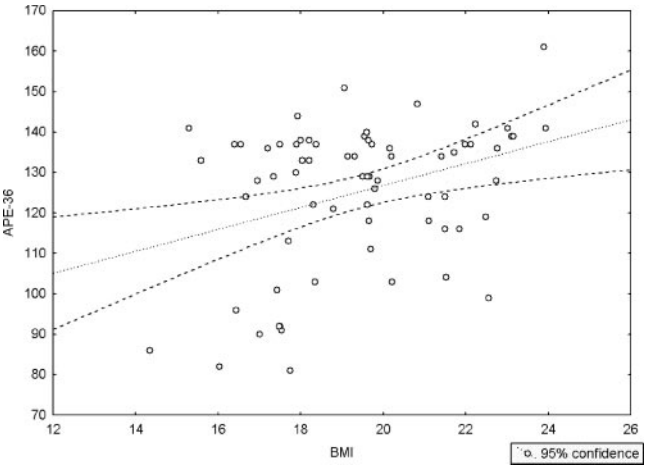

FIG. 3. Correlation between BMI (kg/m^2) and serum APE-36 (r = 0.35; $P < 0.005$) concentrations (pg/ml) in participants with normal BMI (H and NOS subjects analyzed together).

when analyzed only in the AN group (r = −0.12, P = 0.9930; and r = −0.10, P = 0.8930, respectively) or in the OB group (r = 0.26, P = 0.1664; and r = 0.21, P = 0.26, respectively).

Discussion

To our knowledge, this is the first published report on serum APE concentrations in AN. We evaluated a large homogenous group of late-pubertal (Tanner IV–V) patients with a restrictive form of AN. Additionally, we analyzed patients with so-called "not otherwise specified" eating disorders and girls with simple obesity.

AN is a chronic disease of psychosomatic nature, with peak incidence between the ages of 14 and 18 yr. Patients with AN struggle for slim silhouette of the body by deliberate limitation of the amount of food intake, excessive physical exercising, provocation of vomiting, or other

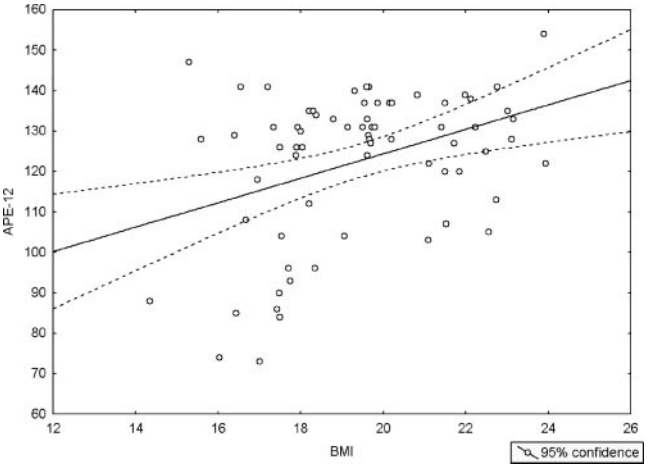

FIG. 4. Correlation between BMI (kg/m^2) and serum APE-12 (r = 0.37; $P < 0.005$) concentrations (pg/ml) in participants with normal BMI (H and NOS subjects analyzed together).

methods to lose weight. The disease leads to a significant decrease in the overall adipose tissue mass, which is manifested by low body weight or even cachexia (25). The clinical picture of NOS eating disorders is similar to that in AN; however, these patients have not met all *DSM-IV* diagnostic criteria for AN because some of them, despite intensive dieting, have normal body weight and BMI greater than 17.5 kg/m^2.

Our findings showed that in girls with AN and NOS, serum APE-12 and APE-36 concentrations were significantly lower than in H and OB subjects. After the obtained results had been calculated per BMI, we showed that the values of APE-36/BMI and APE-12/BMI in AN and NOS groups were similar to those recorded in the H group and decreased compared with the OB group. Positive linear correlations between serum APE-12 and APE-36 concentrations and BMI were also noted. Because age- and sex-adjusted BMI is a reliable parameter used to evaluate nutritional status in children and adolescents and because there is a strong, positive correlation between fat mass and BMI (26, 27), the BMI-adjusted values of APE concentrations allowed us to not only eliminate the effect of different fat mass in the examined groups but also to provide an approximate overview of their secretion in adipose tissue. Thus, we speculate that APE secretion in the adipose tissue in patients with AN or NOS is similar to that in H persons, but it is significantly increased in obesity. Indeed, in animal models of obesity, APE mRNA expression in adipocytes is elevated (5).

It is noteworthy that within each individual group, serum concentrations of APE-36 and APE-12 were similar, with the APE-36/APE-12 ratio close to 1. This observation may have some clinical implications and sets new directions of research in this field because there are some differences in the activity of APE isoforms. Namely, it has been thought that APE-36 acts as a precursor with limited biological activity, up to the moment of its proteolysis and posttranslational change to produce biologically more active peptides (8). It has been demonstrated that the APJ receptor is a coreceptor for HIV infection in CD4-expressing cells and in preincubation of APJ+ and CD4+ cells wherein APE blocks the entry of the virus into the host's cells (26). The potency of different APE peptides to protect against HIV infection is inversely correlated with the molecular weight of the peptide (27). On the other hand, shorter forms of APE seem to be more powerful in their hypotensive and positive inotropic actions (2).

In some animal studies, it has been suggested that shorter forms of APE (APE-13 and APE-12) may be involved in the regulation of food intake. Sunter *et al.* (29) demonstrated that acute intraventricular (ICV), but not iv, injection of APE-13 reduced food intake in both fed and fasted rats. However, these findings have not been confirmed by others (16). Conversely, a prolonged ICV infusion of APE-13 in mice caused increased food intake, body weight, energy expenditure, and body temperature (30). A study by O'Shea *et al.* (15) suggested that APE may control the appetite in a central circadian rhythm-dependent manner. These researchers observed that APE-12 exerted a delayed inhibitory effect on nocturnal feeding in rats. In contrast, daytime ICV administration of APE-12 to satiated rats stimulated feeding. These conflicting reports may have occurred because of differences in the species of rodent used, the form of APE used, and the variation in the doses or time points of injections. Although these animal data cannot be directly extrapolated to humans without more thorough analysis (31), it should be noted that high identities between human, bovine, and rat preproapelin with 100% identity in the C-terminal 13 amino acids, which encodes the mature APE peptide, have been established (32).

Colocalization of APE peptide in a wide range of tissues, including the gastrointestinal tract, stomach, adipose tissue, and central nervous system (arcuate and paraventricular nuclei of the hypothalamus, hypophysis, and extrahypothalamic structures) (7, 14, 33) suggested that APE should be added to the list of "adiposity signals" generated in proportion to body fat stores, which already includes leptin, insulin, and amylin (3). Interestingly, APE and leptin demonstrate several other similarities, including increases in core body temperature and locomotor activity, which may contribute to negative energy balance (30). Insulin stimulates APE expression in adipocytes, and APE in turn inhibits glucose-induced secretion (5, 10, 14, 34). The same mechanism of negative feedback, the so-called "adipoinsular axis," has been postulated for insulin and leptin (17). In the conditions of low fat mass such as AN or lipodystrophies, serum leptin levels are diminished, and in obesity they are elevated (17, 35, 36). This pattern also appears valid for APE.

Boucher *et al.* (5) compared APE expression in fat cells, evaluated its plasma levels in four different models of obesity in mice, and demonstrated a large increase in all hyperinsulinemia-associated obesities. They also confirmed their results in obese men, showing elevated plasma APE concentrations compared with lean controls. In a group of 25 adults with morbid obesity (BMI, 48 ± 1 kg/m^2), Heinonen *et al.* (17) observed significantly increased serum APE-12 concentrations (736 ± 50 pg/ml) compared with a control group consisting of 12 normal-weight subjects (174 ± 14 pg/ml). APE-12 serum concentration values in obese patients were almost three times higher than those recorded in our study. Thus, it is possible that these

may be influenced by age or the severity of obesity because all participants in the study by Heinonen *et al.* (17) were morbidly obese.

In our study, there was a strong positive correlation between blood APE-36 and APE-12 concentrations and BMI in participants from H and NOS groups analyzed together with BMI between −2.0 and +2.0 SD. However, no such relationship was found in the AN and OB groups. This might have occurred simply from low sample size, although this is likely not the case because our group included a total of 87 patients with AN. Considering the broad expression of APE in different tissues, one may suspect that low secretion of APE in fat tissue, due to its low mass in AN, may be partly compensated by its production in other organs.

Published data on correlations between circulating APE concentrations and BMI are scarce. Some researchers obtained positive correlations in patients with morbid obesity (17) and in a group of obese patients with glucose intolerance and type 2 diabetes (18). Yet there are other studies that did not observe such findings (9, 37). Because Boucher *et al.* (5) demonstrated that plasma APE levels in obesity are associated with hyperinsulinemia and hyperglycemia, it is possible that in the studies where no correlations between BMI and APE concentrations were observed, including our research, the obese patients were heterogenous with regard to insulin resistance. This may be the case particularly in adolescents and young adults because they may have not yet developed the metabolic consequences of obesity.

We identified at least four potential limitations to this study. The first was a cross-sectional design of the study. The second was the heterogenous character of the NOS group. We realized that this group comprised a mixture of patients with normal and low BMI, those who were menstruating, and those who presented with amenorrhea. However, due to the increasing knowledge and awareness of eating disorders among adolescents, their parents, and their teachers (28), we believed that this group should not be excluded from research. The third limitation was the lack of data on insulin sensitivity in our patients, which could have been used to better characterize study participants. Another limitation was that body composition was not analyzed using bioimpedance or other methods, because this would have been useful.

We conclude that compared with H controls, serum APE-36 and APE-12 concentrations decreased as a result of fat tissue depletion in patients with AN. Conversely, obese adolescents had elevated APE-36 and APE-12 due to excessive fat mass as well as increased APE production in adipose tissue.

Acknowledgments

Address all correspondence and requests for reprints to: Joanna Oświęcimska, M.D., Ph.D., Department of Pediatrics, Medical University of Silesia in Katowice, ul. 3-Maja 13/15, 41-800 Zabrze, Poland. E-mail: smina@poczta.onet.pl.

This work was supported by Grants NN-1-011/2007, NN-1-081/07, and KNW-1-022/08 from the Medical University of Silesia in Katowice, Poland.

Disclosure Summary: The authors have nothing to disclose.

References

1. **Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou MX, Kawamata Y, Fukusumi S, Hinuma S, Kitada C, Kurokawa T, Onda H, Fujino M** 1998 Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Biochem Biophys Res Commun 251:471–476
2. **Kleinz MJ, Davenport AP** 2005 Emerging roles of apelin in biology and medicine. Pharmacol Ther 107:198–211
3. **Bełtowski J** 2006 Apelin and visfatin: unique "beneficial" adipokines upregulated in obesity? Med Sci Monit 12:RA112–RA119
4. **Tatemoto K, Takayama K, Zou MX, Kumaki I, Zhang W, Kumano K, Fujimiya M** 2001 The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. Regul Pept 99:87–92
5. **Boucher J, Masri B, Daviaud D, Gesta S, Guigné C, Mazzucotelli A, Castan-Laurell I, Tack I, Knibiehler B, Carpéné C, Audigier Y, Saulnier-Blache JS, Valet P** 2005 Apelin, a newly identified adipokine up-regulated by insulin and obesity. Endocrinology 146:1764–1771
6. **Kawamata Y, Habata Y, Fukusumi S, Hosoya M, Fujii R, Hinuma S, Nishizawa N, Kitada C, Onda H, Nishimura O, Fujino M** 2001 Molecular properties of apelin: tissue distribution and receptor binding. Biochim Biophys Acta 1538:162–171
7. **Wang G, Anini Y, Wei W, Qi X, O'Carroll AM, Mochizuki T, Wang HQ, Hellmich MR, Englander EW, Greeley Jr GH** 2004 Apelin, a new enteric peptide: localization in the gastrointestinal tract, ontogeny, and stimulation of gastric cell proliferation and of cholecystokinin secretion. Endocrinology 145:1342–1348
8. **Ladeiras-Lopes R, Ferreira-Martins J, Leite-Moreira AF** 2008 The apelinergic system: the role played in human physiology and pathology and potential therapeutic applications. Arq Bras Cardiol 90:343–349
9. **Chong KS, Gardner RS, Morton JJ, Ashley EA, McDonagh TA** 2006 Plasma concentrations of the novel peptide apelin are decreased in patients with chronic heart failure. Eur J Heart Fail 8:355–360
10. **Xie H, Tang SY, Cui RR, Huang J, Ren XH, Yuan LQ, Lu Y, Yang M, Zhou HD, Wu XP, Luo XH, Liao EY** 2006 Apelin and its receptor are expressed in human osteoblasts. Regul Pept 134:118–125
11. **Habata Y, Fujii R, Hosoya M, Fukusumi S, Kawamata Y, Hinuma S, Kitada C, Nishizawa N, Murosaki S, Kurokawa T, Onda H, Tatemoto K, Fujino M** 1999 Apelin, the natural ligand of the orphan receptor APJ, is abundantly secreted in the colostrum. Biochim Biophys Acta 1452:25–35
12. **Hosoya M, Kawamata Y, Fukusumi S, Fujii R, Habata Y, Hinuma S, Kitada C, Honda S, Kurokawa T, Onda H, Nishimura O, Fujino M** 2000 Molecular and functional characteristics of APJ. Tissue distribution of mRNA and interaction with the endogenous ligand apelin. J Biol Chem 275:21061–21067
13. **Guzik TJ, Mangalat D, Korbut R** 2006 Adipocytokines—novel link between inflammation and vascular function? J Physiol Pharmacol 57:505–528
14. **Lee DK, George SR, O'Dowd BF** 2006 Unravelling the roles of the apelin system: prospective therapeutic applications in heart failure and obesity. Trends Pharmacol Sci 27:190–194

15. **O'Shea M, Hansen MJ, Tatemoto K, Morris MJ** 2003 Inhibitory effect of apelin-12 on nocturnal food intake in the rat. Nutr Neurosci 6:163–167
16. **Taheri S, Murphy K, Cohen M, Sujkovic E, Kennedy A, Dhillo W, Dakin C, Sajedi A, Ghatei M, Bloom S** 2002 The effects of centrally administered apelin-13 on food intake, water intake and pituitary hormone release in rats. Biochem Biophys Res Commun 291:1208–1212
17. **Heinonen MV, Purhonen AK, Miettinen P, Pääkkönen M, Pirinen E, Alhava E, Akerman K, Herzig KH** 2005 Apelin, orexin-A and leptin plasma levels in morbid obesity and effect of gastric banding. Regul Pept 130:7–13
18. **Li L, Yang G, Li Q, Tang Y, Yang M, Yang H, Li K** 2006 Changes and relations of circulating visfatin, apelin, and resistin levels in normal, impaired glucose tolerance, and type 2 diabetic subjects. Exp Clin Endocrinol Diabetes 114:544–548
19. **Casiero D, Frishman WH** 2006 Cardiovascular complications of eating disorders. Cardiol Rev 14:227–231
20. **Oświęcimska J, Ziora K, Adamczyk P, Roczniak W, Pikiewicz-Koch A, Stojewska M, Dyduch A** 2007 Effects of neuroendocrine changes on results of ambulatory blood pressure monitoring (ABPM) in adolescent girls with anorexia nervosa. Neuro Endocrinol Lett 28: 410–416
21. **Oświęcimska J, Ziora K, Pluskiewicz W, Geisler G, Broll-Wacœka K, Karasek D, Dyduch A** 2007 Skeletal status and laboratory investigations in adolescent girls with anorexia nervosa. Bone 41:103–110
22. **Marcos A** 2000 Eating disorders: a situation of malnutrition with peculiar changes in the immune system. Eur J Clin Nutr 54:S61–S64
23. **Palczewska I, Niedźwiecka Z** 2001 Somatic development indices in children and youth of Warsaw. Med Wieku Rozwoj 5(2 Suppl 1): 108–118
24. **American Psychiatric Association** 1994 Diagnostic and statistical manual of mental disorders (DSM-IV). Washington, DC: APA
25. **Nogal P, Lewiñski A** 2008 Anorexia nervosa. Endokrynol Pol 59: 148–155
26. **Choe H, Farzan M, Konkel M, Martin K, Sun Y, Marcon L, Cayabyab M, Berman M, Dorf ME, Gerard N, Gerard C, Sodroski J** 1998 The orphan seven-transmembrane receptor APJ supports the entry of primary T-cell-line-tropic and dualtropic human immunodeficiency virus type 1. J Virol 72:6113–6118
27. **Zou MX, Liu HY, Haraguchi Y, Soda Y, Tatemoto K, Hoshino H** 2000 Apelin peptides block the entry of human immunodeficiency virus (HIV). FEBS Lett 473:15–28
28. **Ziora K, Pilarz LB, Sztylc J, Oswiecimska J** 2009 Assessment of knowledge on anorexia nervosa in adolescents. Endokrynol Otyl Zab Przem Mat 5:12–18
29. **Sunter D, Hewson AK, Dickson SL** 2003 Intracerebroventricular injection of apelin-13 reduces food intake in the rat. Neurosci Lett 353:1–4
30. **Valle A, Hoggard N, Adams AC, Roca P, Speakman JR** 2008 Chronic central administration of apelin-13 over 10 days increases food intake, body weight, locomotor activity and body temperature in C57BL/6 mice. J Neuroendocrinol 20:79–84
31. **Arner P** 2005 Resistin: yet another adipokine tells us that men are not mice. Diabetologia 48:2203–2205
32. **Lee DK, Cheng R, Nguyen T, Fan T, Kariyawasam AP, Liu Y, Osmond DH, George SR, O'Dowd BF** 2000 Characterization of apelin, the ligand for the APJ receptor. J Neurochem 74:34–41
33. **De Mota N, Lenkei Z, Llorens-Cortès C** 2000 Cloning, pharmacological characterization and brain distribution of the rat apelin receptor. Neuroendocrinology 72:400–407
34. **Sörhede Winzell M, Magnusson C, Ahrén B** 2005 The APJ receptor is expressed in pancreatic islets and its ligand, apelin, inhibits insulin secretion in mice. Regul Pept 131:12–17
35. **Estrada V, Serrano-Ríos M, Martínez Larrad MT, Villar NG, González López A, Téllez MJ, Fernández C** 2002 Leptin and adipose tissue maldistribution in HIV-infected male patients with predominant fat loss treated with antiretroviral therapy. J Acquir Immune Defic Syndr 29:32–40
36. **Nakai Y, Hamagaki S, Kato S, Seino Y, Takagi R, Kurimoto F** 1999 Leptin in women with eating disorders. Metabolism 48:217–220
37. **Tasci I, Dogru T, Naharci I, Erdem G, Yilmaz MI, Sonmez A, Bingol N, Kilic S, Bingol S, Erikci S** 2007 Plasma apelin is lower in patients with elevated LDL-cholesterol. Exp Clin Endocrinol Diabetes 115: 428–432