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Modulation of Adiponectin and Leptin during Refeeding of Female Anorexia Nervosa Patients

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Context: Several studies assessed adiponectin levels in anorexia nervosa (AN) patients, however, data regarding the dynamics of changes in adiponectin levels during refeeding of these patients is limited and contradicting.

Objective: Our objective was to assess adiponectin levels and the distribution of its different isoforms in AN patients before and after long-term refeeding, and to relate them to alterations in body mass index, leptin, insulin sensitivity, and additional endocrine parameters.

Design, Setting, and Participants: We conducted a longitudinal controlled study of 38 female adolescent malnourished AN inpatients, with 13 young, lean, healthy women serving as controls. Blood samples were obtained upon admission and thereafter at 1, 3, and 5 months (at target weight).

Main Outcome Measures: Changes in body mass index, leptin, adiponectin, insulin sensitivity, and adiponectin multimeric forms were measured.

Results: At admission, leptin levels of AN patients were significantly lower, whereas insulin sensitivity (assessed by homeostasis model assessment-insulin resistance), adiponectin levels, and the ratio of high molecular weight (HMW) adiponectin to total adiponectin were significantly higher compared with controls. During weight recovery, leptin levels and homeostasis model assessment-insulin resistance increased significantly, whereas adiponectin and HMW adiponectin/total adiponectin ratio decreased significantly, to levels similar to controls. An initial increase in adiponectin levels was observed after 1 month of refeeding. There was no correlation between adiponectin and either T_4 or cortisol levels.

Conclusions: Our study demonstrates hyperadiponectinemia, increased adiponectin HMW isoform, and increased insulin sensitivity in adolescent AN female patients and reversal of these findings with weight rehabilitation. We hypothesize that increased adiponectin levels may have a protective role in maintaining energy homeostasis during extreme malnourishment. **(*J Clin Endocrinol Metab* 92: 1843–1847, 2007)**

ADIPONECTIN IS AN abundant circulating hormone with insulin-sensitizing, antiatherogenic and antiinflammatory properties (1). Its levels are low in obese humans and rise after weight loss (2). Adiponectin circulates in three forms: trimers [low molecular weight (LMW)], hexamers [medium molecular weight (MMW)], and larger multimers of 12 to 18 subunits [high molecular weight (HMW)]; the latter appears to be the most active form, and the ratio of HMW adiponectin to that of total adiponectin [ratio of HMW adiponectin to total adiponetin (S_A index)] is closely correlated with insulin sensitivity (1, 3).

Anorexia nervosa (AN) is associated with multiple endocrine perturbations, including hypogonadism, hypothyroidism, hypercortisolism, GH resistance, altered glucose metabolism, insulin sensitivity (4), and hypoleptinemia (5, 6). Several studies demonstrated hyperadiponectinemia in underweight AN patients (5–10), whereas others reported that adiponectin levels were either lower (11) or not different (6) from controls. Furthermore, data regarding the dynamics of changes in adiponectin during refeeding of AN patients is limited and contradicting (7, 9, 11).

In the present study we assessed alterations in adiponectin levels in a large group of AN patients during long-term refeeding and weight restoration and related them to body mass index (BMI), leptin levels, insulin sensitivity, and additional endocrine parameters. We also describe, for the first time, the changes in distribution of adiponectin isoforms during weight gain.

Patients and Methods

Patients

A total of 38 female AN (restricting type) patients, admitted to the Pediatric Psychosomatic Department at the Sheba Medical Center, were prospectively enrolled in this study, which consisted of two substudies.

Study 1, comprising 20 consecutive AN patients, aged 16 ± 5.3 yr, with a mean BMI of 15.9 ± 1.8 kg/m^2 (group 1), was designed to evaluate adiponectin levels in AN patients before, during, and after weight rehabilitation and to determine the relation of adiponectin to weight, leptin, and hormonal changes.

Study 2, comprising another 18 AN patients, aged 15.7 ± 1.7 yr, with a mean BMI of 15.9 ± 0.9 kg/m^2 (group 2), was designed to evaluate changes in insulin sensitivity during nutritional rehabilitation.

None of the patients had evidence of bulimia nervosa, bipolar, schizo-

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Abbreviations: AN, Anorexia nervosa; BMI, body mass index; HMW, high molecular weight; HOMA, homeostasis model assessment; HOMA-IR, HOMA-insulin resistance; LMW, low molecular weight; MMW, medium molecular weight; S_A index, ratio of HMW adiponectin to total adiponectin.

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phrenic spectrum, or substance abuse disorders, or any significant medical or neurological illness. The patients were hospitalized for the entire duration of the study and were discharged after reaching their target weight and maintaining it for at least 2 wk. Blood samples were obtained from group 1 patients upon admission and thereafter at 1, 3, and 5 months after admission, and from group 2 at the time of admission and after weight rehabilitation (5.6 ± 3 months).

The study protocol was approved by the institutional ethics review committee, and a written informed consent was obtained from all patients and, in the case of minors, from their parents or legal guardians.

Controls

The control group included 13 volunteer young, lean, healthy women (age, 28.8 ± 3.2 yr; BMI, 20.2 ± 2.1 kg/m^2).

Height and weight measurements

Standing height was measured to the nearest 0.1 cm and body weight to the nearest 0.1 kg. Measurements were taken during the morning hours by a single investigator, using standardized procedures.

Biochemical assessment

Glucose was measured within 1 h of blood withdrawal by the glucose oxidase method (Olympus AU2700; Olympus, Hamburg, Germany). For other measurements, serum was recovered immediately and frozen at −30 C. Adiponectin and leptin were determined by RIA (Linco, St. Charles, MO), and insulin, 17β-estradiol, FSH, LH, TSH, free T_4, total T_3, and cortisol by a chemiluminescent immunometric method (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA).

Insulin resistance index was calculated from fasting glucose and insulin values, according to the homeostasis model assessment (HOMA) method (12).

Adiponectin multimers

Adiponectin multimers in serum samples were analyzed under nonreducing and nondenaturing conditions as described before (13). The relative proportion of each multimer to total adiponectin was obtained by dividing band densitometry of either multimer by total density in each lane.

Data analysis

Data are reported as mean ± SD. Leptin values were transformed to log scale for improving symmetry of the distributions. The repeated measures model was used for comparison of the transformed values between various time points. Within-patient factor was time after hospitalization. Comparison between S_A index at admission and after weight recovery was performed using the Student's *t* test. All tests were two-sided and *P* values < 0.05 were considered significant. Calculations were performed using SPSS 11.0 (SPSS Inc., Chicago, IL).

Results

Group 1 and group 2 were analyzed separately because different parameters were evaluated for each group. However, as detailed below, results were similar for both groups.

Weight gain

Patients' weight increased significantly ($P < 0.001$) during hospitalization: group 1, from 39.1 ± 5.3 to 48.5 ± 3.8 kg; group 2, from 40.9 ± 4.5 to 50 ± 4.9 kg. Similarly, BMI increased significantly ($P < 0.001$) during hospitalization: group 1, from 15.9 ± 1.8 to 19.5 ± 1.1 kg/m^2 (Fig. 1A); group 2, from 15.9 ± 0.9 to 19.3 ± 0.7 kg/m^2.

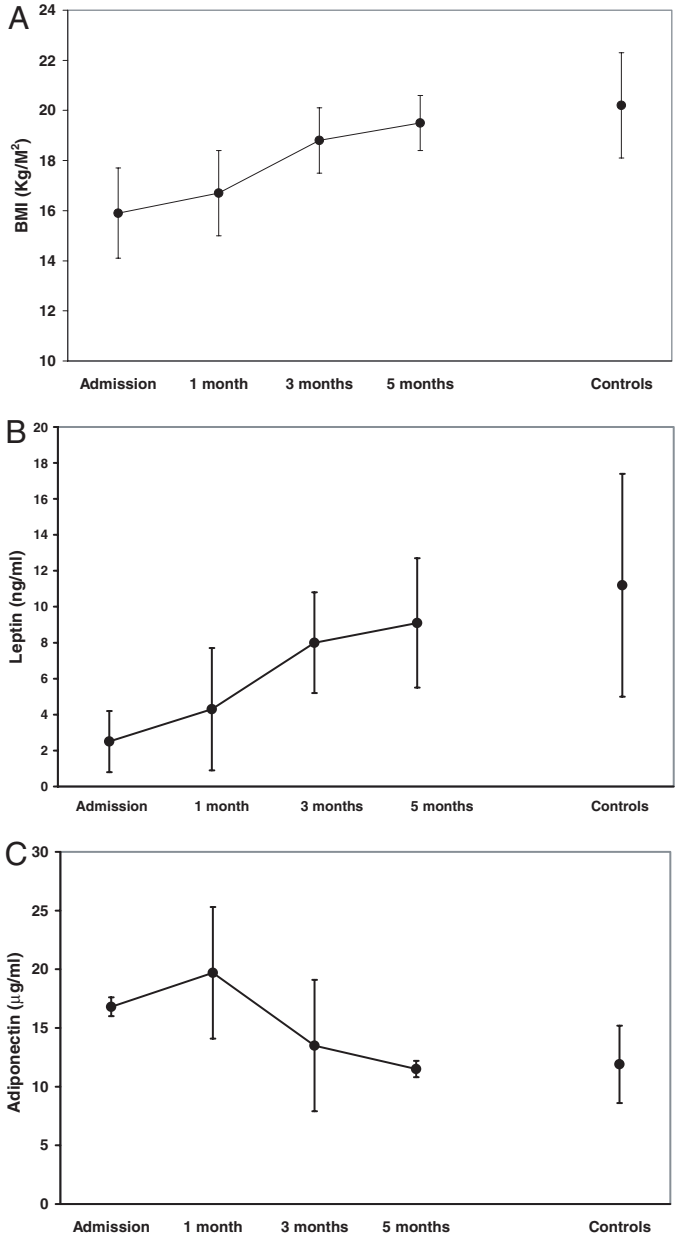

FIG. 1. A, Changes in BMI (mean ± SD) during the study period, with group 1 compared with the control group. B, Changes in leptin (mean ± SD) during the study period, with group 1 compared with the control group. C, Changes in adiponectin (mean ± SD) during the study period, with group 1 compared with the control group.

Endocrine profile

During hospitalization, cortisol levels decreased (20.7 ± 7.1 *vs.* 12.6 ± 5.1 μg/dl; $P = 0.009$), and total T_3 increased (0.97 ± 0.32 *vs.* 1.55 ± 0.45 nmol/liter; $P < 0.001$). Expected changes were seen in LH, FSH, and 17β-estradiol (data not shown).

Leptin and adiponectin

As depicted in Fig. 1B, upon admission, leptin levels in group 1 were lower compared with the control group (2.5 ± 1.7 *vs.* 11.2 ± 6.2 ng/ml; $P < 0.001$), and increased signifi-

cantly ($P < 0.001$) during the follow-up period, reaching 9.1 ± 3.6 ng/ml, which was not significantly different from the controls ($P = 0.052$). In contrast to leptin, adiponectin levels upon admission (Fig. 1C) were significantly higher compared with the control group (16.8 ± 0.8 *vs.* 11.9 ± 3.3 μg/ml; $P < 0.001$). Adiponectin levels increased significantly ($P = 0.04$) during the first month of hospitalization to 19.7 ± 5.6 μg/ml, and thereafter decreased gradually and significantly ($P < 0.001$), reaching 11.5 ± 0.7 μg /ml after 5 months of refeeding (Fig. 1C), similar to those of controls ($P = 0.228$). Likewise, in group 2, patients' adiponectin levels at admission were significantly higher than levels at discharge (16 ± 5.2 *vs.* 10.5 ± 4.1 μg /ml; $P = 0.001$). Noteworthy, upon admission, leptin to BMI ratio was significantly ($P < 0.001$) lower, whereas adiponectin to BMI ratio was significantly ($P < 0.001$) higher compared with controls. At the 5-month time point, the same trend was observed, but the differences were no longer significant. As shown in Fig. 1, adiponectin levels were inversely correlated with leptin levels and BMI during hospitalization, although these correlations failed to reach statistical significance.

There was no correlation between adiponectin and T_4 or cortisol levels, either at admission or by the end of follow-up, in accordance with previous studies (14, 15), but unlike another study (16).

Insulin sensitivity (group 2)

On admission, mean glucose and insulin were 79.8 ± 10.5 mg/dl and 6.4 ± 3.7 mU/liter, respectively. After weight recovery, mean glucose was 84 ± 6.2 mg/dl, whereas mean insulin was 8.9 ± 5.3 mU/liter. Mean HOMA-IR increased significantly ($P = 0.05$) during hospitalization from 1.28 ± 0.19 to 1.84 ± 0.25.

Adiponectin multimeric isoforms

At admission, HMW/total adiponectin ratio (S_A index) was 48.8 ± 9.7%, significantly ($P = 0.035$) higher compared with the control group (36.4 ± 11.4%). By the time of discharge, the patients' S_A index decreased significantly ($P = 0.035$) to 33.6 ± 10.3%, not significantly different than the control group. As depicted in Fig. 2, the decrease in S_A index resulted mainly from a marked decrease in the HMW multimer.

Discussion

The present study demonstrates hyperadiponectinemia in malnourished, underweight female AN patients. During refeeding, adiponectin levels displayed a biphasic pattern, with an initial rise at the first month of nutritional treatment, followed by a gradual decline with further weight gain. Adiponectin levels after weight recovery were similar to those of lean healthy female adolescents (17). Moreover, we demonstrated, for the first time, an increased proportion of adiponectin in the HMW form in the malnourished state, followed by a decrease to levels similar to control subjects after weight recovery. Insulin sensitivity, as measured by HOMA-IR, decreased significantly during weight gain. As shown in other studies, leptin levels in the underweight AN patients were low and increased significantly during refeeding (5–11).

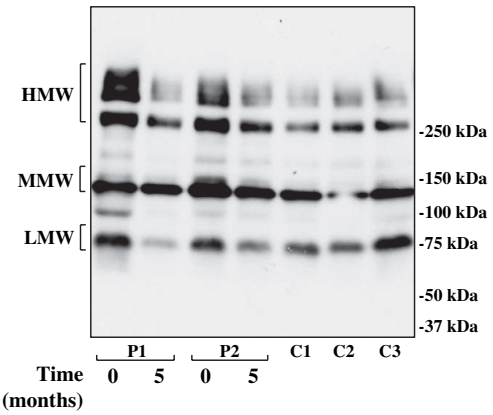

FIG. 2. Changes in the distribution of adiponectin multimeric forms during weight restoration. HMW, HMW form of adiponectin; MMW, MMW form of adiponectin; LMW, low molecular form of adiponectin. P1, P2, Representative patients. C1, C2, C3, Representative healthy controls.

Adiponectin is low in obesity, whereas weight reduction results in an increase of adiponectin levels (2). Hence, we hypothesized that adiponectin in the malnourished state of AN will be elevated and will decline during weight gain. Indeed, adiponectin in the malnourished state was significantly higher compared with the control group. This difference was further amplified when adiponectin levels were normalized for BMI. Our findings are in accordance with previous studies (5–10), however, other investigators reported that adiponectin in AN patients was lower than (11) or not different from controls (6). The increased adiponectin levels in the malnourished state may be attributed to fat mass depletion, as well as to increased vagal tone observed in AN (18), because β-adrenergic stimulation has been shown to inhibit adiponectin gene expression (19). Hyperadiponectinemia could contribute to the increased insulin sensitivity observed in AN patients in our study, as well as by others (5–11). Moreover, because adiponectin blocks B-lymphopoiesis, suppresses macrophage function, and interferes with TNF-α signaling, its elevated levels could contribute to the immune suppression observed in malnourished AN patients (20). Furthermore, it was recently suggested that adiponectin might have a role in maintaining energy homeostasis under energy shortage conditions (21).

Only a few studies investigated longitudinal changes in adiponectin levels during refeeding of AN patients, with conflicting results (7, 9, 11). Similar to our observations, Iwahashi *et al.* (9) described an initial increase, followed by a gradual decrease in adiponectin levels during the follow-up of a single patient. On the other hand, Tagami *et al.* (11) reported an increase in adiponectin levels during weight gain in 13 extremely malnourished AN patients, whereas Bosy-Westphal *et al.* (7) demonstrated a nonsignificant decrease in adiponectin levels in 11 patients after 12 wk of refeeding. The apparent disparity might be attributed to differences in both the severity of malnourishment at admission and the follow-up duration. When integrating the results of the above studies with our own data, obtained in a considerably larger

group of patients monitored for a period of 5 months (Fig. 1C), the following model can be proposed: in severely malnourished patients, refeeding results in an initial increase in adiponectin, reflecting early fat accumulation. This was observed in the current study during the first month of weight gain (Fig. 1C), by Tagami *et al.* (11) during an increase in BMI from 13.8 to 15.9 kg/m^2, and by Iwahashi *et al.* (9). Once a critical threshold of adipose mass is reached, subsequent weight gain is associated with a decrease in adiponectin, as would be expected in a normal population, and as observed by us and by Iwahashi (9) during the final months of hospitalization. We speculate that Bosy-Westpal *et al.* (7) observed only a nonsignificant decrease in adiponectin due to a shorter follow-up period.

Adiponectin expression may be more closely related to adipocyte size than adipose tissue mass or body weight (22). In addition, the early stage of adipocyte differentiation and development is characterized by an increase in adiponectin gene expression, whereas aged adipocytes display reduced expression (23). Thus, initial weight gain in AN patients might be associated with an increased proportion of young *vs.* old adipocytes, resulting in an increase in adiponectin, whereas subsequent weight gain is associated with adipocyte hypertrophy, leading to a decrease in adiponectin. Alternatively, because adiponectin levels are inversely correlated with waist-to-hip ratio (24), the changes in adiponectin levels during refeeding may also be attributed to alterations in body fat distribution, because an increase in the amount of visceral fat was observed after weight normalization in AN patients (25). Finally, the initial increase in adiponectin may reflect a lag-phase between changes in fat mass and adiponectin levels. A similar delay between weight loss and alteration in adiponectin was recently reported after bariatric surgery (22).

In the present study, we demonstrated an increased ratio of HMW to total adiponectin (S_A) in malnourished AN patients, reflecting mainly higher levels of the HMW form. After weight gain, the distribution of adiponectin multimeric forms was similar to that of controls. These findings are consistent with recent studies that reported an increase in S_A index during weight reduction in obese subjects (22). This is the first description of adiponectin multimers in AN patients before and after nutritional rehabilitation. Furthermore, only a handful of studies addressed alterations in adiponectin forms during changes in body weight, and to the best of our knowledge, changes in HMW adiponectin and S_A index during weight gain have not been previously described. The decrease in the S_A index during the study period was paralleled by a decrease in insulin sensitivity as reflected by HOMA-IR, consistent with studies showing an increase in HMW adiponectin and insulin sensitivity after weight loss (22).

One potential weakness of this study is the age difference between patients and controls. However, our patients were all postpubertal, and previous studies showed no significant age effect on adiponectin levels in postpubertal women (26). Secondly, similar to others (6, 10, 11), we demonstrated increased insulin sensitivity in the malnourished state, using HOMA-IR. However, different results were obtained when direct measures of glucose disposal were used (5).

In conclusion, our study demonstrates hyperadiponectinemia, increased HMW/total adiponectin ratio and increased insulin sensitivity in AN patients, along with reversal of these findings with weight recovery. We hypothesize that increased adiponectin levels may have a protective role in maintaining energy homeostasis during extreme malnourishment. Further studies are warranted to elucidate whether our findings are secondary to the low weight *per se* or to changes specific to AN.

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Author Disclosure Summary: D.M.-M., D.S., C.P., A.Y., A.R., M.F., R.L., E.Y., R.H., and H.K. have nothing to declare.

References

1. **Scherer PE** 2006 Adipose tissue: from lipid storage compartment to endocrine organ. Diabetes 55:1537–1545
2. **Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL, Tai TY, Chuang LM** 2001 Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. J Clin Endocrinol Metab 86:3815–3819
3. **Hara K, Horikoshi M, Yamauchi T, Yago H, Miyazaki O, Ebinuma H, Imai Y, Nagai R, Kadowaki T** 2006 Measurement of the high-molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. Diabetes Care 29:1357–1362
4. **Munoz MT, Argente J** 2002 Anorexia nervosa in female adolescents: endocrine and bone mineral density disturbances. Eur J Endocrinol 147:275–286
5. **Pannacciulli N, Vettor R, Milan G, Granzotto M, Catucci A, Federspil G, De Giacomo P, Giorgino R, De Pergola G** 2003 Anorexia nervosa is characterized by increased adiponectin plasma levels and reduced nonoxidative glucose metabolism. J Clin Endocrinol Metab 88:1748–1752
6. **Misra M, Miller KK, Almazan C, Ramaswamy K, Aggarwal A, Herzog DB, Neubauer G, Breu J, Klibanski A** 2004 Hormonal and body composition predictors of soluble leptin receptor, leptin, and free leptin index in adolescent girls with anorexia nervosa and controls and relation to insulin sensitivity. J Clin Endocrinol Metab 89:3486–3495
7. **Bosy-Westphal A, Brabant G, Haas V, Onur S, Paul T, Nutzinger D, Klein H, Hauer M, Muller MJ** 2005 Determinants of plasma adiponectin levels in patients with anorexia nervosa examined before and after weight gain. Eur J Nutr 44:355–359
8. **Delporte ML, Brichard SM, Hermans MP, Beguin C, Lambert M** 2003 Hyperadiponectinaemia in anorexia nervosa. Clin Endocrinol (Oxf) 58:22–29
9. **Iwahashi H, Funahashi T, Kurokawa N, Sayama K, Fukuda E, Okita K, Imagawa A, Yamagata K, Shimomura I, Miyagawa JI, Matsuzawa Y** 2003 Plasma adiponectin levels in women with anorexia nervosa. Horm Metab Res 35:537–540
10. **Housova J, Anderlova K, Krizova J, Haluzikova D, Kremen J, Kumstyrova T, Papezova H, Haluzik M** 2005 Serum adiponectin and resistin concentrations in patients with restrictive and binge/purge form of anorexia nervosa and bulimia nervosa. J Clin Endocrinol Metab 90:1366–1370
11. **Tagami T, Satoh N, Usui T, Yamada K, Shimatsu A, Kuzuya H** 2004 Adiponectin in anorexia nervosa and bulimia nervosa. J Clin Endocrinol Metab 89:1833–1837
12. **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC** 1985 Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
13. **Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, Hara K, Hada Y, Vasseur F, Froguel P, Kimura S, Nagai R, Kadowaki T** 2003 Impaired multimerization of human adiponectin mutants associated with diabetes: molecular structure and multimer formation of adiponectin. J Biol Chem 278:40352–40363
14. **Gavrila A, Peng CK, Chan JL, Mietus JE, Goldberger AL, Mantzoros CS** 2003 Diurnal and ultradian dynamics of serum adiponectin in healthy men: comparison with leptin, circulating soluble leptin receptor, and cortisol patterns. J Clin Endocrinol Metab 88:2838–2843
15. **Iglesias P, Alvarez Fidalgo P, Codoceo R, Diez JJ** 2003 Serum concentrations of adipocytokines in patients with hyperthyroidism and hypothyroidism before and after control of thyroid function. Clin Endocrinol (Oxf) 59:621–629

16. **Fernandez-Real JM, Lopez-Bermejo A, Casamitjana R, Ricart W** 2003 Novel interactions of adiponectin with the endocrine system and inflammatory parameters. J Clin Endocrinol Metab 88:2714–2718
17. **Andersen KK, Frystyk J, Wolthers OD, Heuck C, Flyvbjerg A** 2007 Gender differences of oligomers and total adiponectin during puberty: a cross-sectional study of 859 Danish school children. J Clin Endocrinol Metab 92:1857–1862
18. **Petretta M, Bonaduce D, Scalfi L, de Filippo E, Marciano F, Migaux ML, Themistoclakis S, Ianniciello A, Contaldo F** 1997 Heart rate variability as a measure of autonomic nervous system function in anorexia nervosa. Clin Cardiol 20:219–224
19. **Delporte ML, Funahashi T, Takahashi M, Matsuzawa Y, Brichard SM** 2002 Pre- and post-translational negative effect of β-adrenoceptor agonists on adiponectin secretion: in vitro and in vivo studies. Biochem J 367:677–685
20. **Nova E, Samartin S, Gomez S, Morande G, Marcos A** 2002 The adaptive response of the immune system to the particular malnutrition of eating disorders. Eur J Clin Nutr 56(Suppl 3):S34–S37
21. **Saito K, Arata S, Hosono T, Sano Y, Takahashi K, Choi-Miura NH, Nakano Y, Tobe T, Tomita M** 2006 Adiponectin plays an important role in efficient energy usage under energy shortage. Biochim Biophys Acta 1761:709–716
22. **Swarbrick MM, Austrheim-Smith IT, Stanhope KL, Van Loan MD, Ali MR, Wolfe BM, Havel PJ** 2006 Circulating concentrations of high-molecular-weight adiponectin are increased following Roux-en-Y gastric bypass surgery. Diabetologia 49:2552–2558
23. **Yu YH, Zhu H** 2004 Chronological changes in metabolism and functions of cultured adipocytes: a hypothesis for cell aging in mature adipocytes. Am J Physiol Endocrinol Metab 286:E402–E410
24. **Bacha F, Saad R, Gungor N, Arslanian SA** 2004 Adiponectin in youth: relationship to visceral adiposity, insulin sensitivity, and β-cell function. Diabetes Care 27:547–552
25. **Misra M, Soyka LA, Miller KK, Grinspoon S, Levitsky LL, Klibanski A** 2003 Regional body composition in adolescents with anorexia nervosa and changes with weight recovery. Am J Clin Nutr 77:1361–1367
26. **Schutte AE, Huisman HW, Schutte R, van Rooyen JM, Malan L, Malan NT** 2007 Aging influences the level and functions of fasting plasma ghrelin levels: the POWIRS study. Regul Pept 139:65–71

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