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Dissociation between adipose tissue expression and serum levels of adiponectin during and after diet-induced weight loss in obese subjects with and without the metabolic syndrome

Carl Johan Behre[a,b], Anders Gummesson[b], Margareta Jernås[b], Theodore C. Lystig[b,c], Björn Fagerberg[a,b], Björn Carlsson[b,c], Lena M.S. Carlsson[b,*]

[a]*Wallenberg Laboratory for Cardiovascular Research, Institute of Medicine, The Sahlgrenska Academy, Göteborg University, SE-413 45 Göteborg, Sweden*

[b]*Department of Metabolism and Cardiovascular Research, Institute of Medicine, The Sahlgrenska Academy, Göteborg University, SE-413 45 Göteborg, Sweden*

[c]*Department of Discovery Medicine, AstraZeneca R&D, SE-431 83 Mölndal, Sweden*

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Abstract

The study aimed to examine if dysmetabolic subjects (MetS+) have lower adiponectin gene expression and lower circulating adiponectin levels than non-dysmetabolic obese subjects (MetS−) at baseline, if adiponectin expression and adiponectin concentration rise more in the dysmetabolic group during weight loss, and if v-SNARE Vti1a (vesicle transport soluble NSF attachment protein receptor vps10p tail interacting 1a) expression increases during the weight loss, as a mechanism for increased adiponectin secretion. Twenty-one obese MetS+ and 19 obese MetS− subjects underwent a very low-energy diet for 16 weeks followed by 2 weeks of refeeding. Abdominal subcutaneous adipose tissue biopsies and blood samples were taken before, during, and after dieting for DNA microarray, reverse transcriptase–polymerase chain reaction, and biochemical analyses. Serum adiponectin was also assessed in a sex- and age-matched healthy, nonobese reference group. Weight decreased by 26.3 ± 9.8 kg in the MetS+ group and 28.2 ± 8.4 kg in the MetS− group with concomitant reductions in insulin, hemoglobin A_{1c}, and triglycerides that were more pronounced in the MetS+ group. Initially, the MetS+ subjects had lower serum adiponectin, but the differences disappeared at week 8, with a continuous increase in serum adiponectin throughout the study in both groups to a level that was higher than in the reference group. The expression of adiponectin and v-SNARE Vti1a did not differ between the groups or over time. In conclusion, obese subjects with the metabolic syndrome had lower circulating adiponectin than subjects without the syndrome. Weight loss increased serum levels of adiponectin without a parallel increase in adiponectin gene expression. The mechanisms involved in the regulation of adiponectin levels merits further investigation.

1. Introduction

Adiponectin is associated with improved insulin action [1,2] and anti-atherosclerotic mechanisms [3-5]. The paradoxical observation that obesity, that is, an increase in the adiponectin-producing tissue, is associated with decreased levels of circulating adiponectin is suggested to be caused by a suppressed production of adiponectin by inflammatory cytokines [6,7]. In vitro data give support for such mechanisms for tumor necrosis factor α and interleukin 6 [7]. Previous studies have also shown that obese subjects with the metabolic syndrome have lower serum adiponectin concentrations than similarly obese subjects without the metabolic syndrome [8]. We have previously shown that adiponectin and tumor necrosis factor α have opposite and reciprocal associations with insulin sensitivity in men [9].

As obesity in subjects with insulin resistance and low-grade inflammation is associated with hypoadiponectinemia, weight loss would be expected to cause parallel increases in adiponectin expression in adipose tissue and serum adiponectin concentrations, in parallel with improvement of metabolic factors. However, previously published studies have shown conflicting results. Although weight loss usually improves the dysmetabolic state, it has been shown that adiponectin expression or circulating adiponectin levels

* Corresponding author. Tel.: +46 31 342 2866; fax: +46 31 821524.
E-mail address: lena.carlsson@medic.gu.se (L.M.S. Carlsson).

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may remain unchanged [10-12], increase [13,14], or even decrease [15].

Adiponectin is exclusively secreted by mature adipose cells. It is posttranslationally hydroxylated and glycosylated, which is believed to be crucial for its polymerization and metabolic activities [16] and is stored in intracellular compartments.

Studies in 3T3L1 adipocytes by Bose et al [17] indicate that the secretion of adiponectin is regulated by v-SNARE Vti1a (vesicle transport soluble NSF attachment protein receptor vps10p tail interacting 1a), a protein that appears to affect the intracellular trafficking of adiponectin-containing vesicles without affecting the biogenesis of these vesicles or the intracellular content of adiponectin. Using a small interfering RNA–based gene silencing approach of Vti1a, the authors reduced the adiponectin secretion, suggesting a correlation between gene expression of v-SNARE Vti1a and adiponectin secretion.

In the present study we examined circulating adiponectin concentrations and adiponectin gene expression in adipose tissue in obese subjects with and without the metabolic syndrome before, during, and after diet-induced weight reduction. To our knowledge, there is as yet no published study based on repeated measurements of circulating adiponectin and adiponectin expression in adipose tissue in obese subjects with and without the metabolic syndrome, before, during, and after excessive weight loss in a refeeding state. A population-based reference group was also used for comparison at baseline. The first hypothesis was that dysmetabolic obese subjects have lower gene expression and lower circulating adiponectin levels than non-dysmetabolic obese subjects at baseline, and that gene expression and adiponectin concentration would rise more in the dysmetabolic group during weight loss. The second hypothesis was that the v-SNARE Vti1a expression would increase during the weight-reducing diet and most in the dysmetabolic group.

2. Subjects and methods

A total of 40 subjects (34 men and 6 women) were recruited among subjects referred to the Department of Body Composition and Metabolism and from advertisements in the local press. Inclusion criteria for all subjects in the study were body mass index of (BMI) 30 or higher and age of 25 to 60 years. The subjects were stratified into 2 groups by matching for BMI, age, and sex. One group had the metabolic syndrome according to slightly modified World Health Organization (WHO) criteria [18] (MetS+, n = 21), whereas the other group did not have the metabolic syndrome according to the slightly modified WHO criteria (MetS−, n = 19). Exclusion criteria were use of any medication with the exception of antihypertensive therapy in the MetS+ group, pregnancy, breast-feeding, type 1 diabetes mellitus, serious psychiatric disorder, established coronary heart disease, malignant arrhythmias, participation in any other ongoing weight reduction study, eating disorder, history of bariatric surgery or cancer treatment, drug abuse, insufficient compliance, other significant somatic disease, smoking, or unwillingness to participate. The metabolic syndrome was defined as diabetes, impaired glucose tolerance, or impaired fasting glucose according to the WHO [19] together with at least 1 of the following risk factors: (i) raised arterial (systolic/diastolic) pressure of 140/90 mm Hg or higher (either value) or use of blood pressure medication; (ii) raised triglycerides (TGs) (≥1.7 mmol/L) and/or low high-density lipoprotein (HDL) cholesterol (<0.9 mmol/L). All subjects fulfilled the criterion for obesity. Furthermore, all subjects in the MetS+ group also fulfilled the recently published International Diabetes Federation criteria [20]. At baseline, systolic blood pressure, TGs, fasting glucose and insulin, and hemoglobin A_{1c} (HbA_{1c}) differed with statistic significance between the groups because of the different inclusion criteria. The subjects received both written and oral information before they gave their consent to participate. The study was approved by the ethics committee at the Sahlgrenska University Hospital (Göteborg, Sweden).

2.1. Reference group

Serum adiponectin was available from 33 subjects. To compare circulating adiponectin with those obtained in the very low-energy diet (VLCD)–treated subjects, a population-based, sex- and age-matched reference group with 33 subjects was recruited from the Swedish Obese Subjects reference study [21].

2.2. Very low-energy diet treatment

Subjects were provided with 3 VLED meals daily from Cambridge Manufacturing, Northants, UK; the daily energy intake was 1883 kJ (450 kcal) daily. The subjects were treated with VLED for 16 weeks. From the end of week 16 until study termination at week 18, ordinary food was gradually introduced under guidance of a dietician. This diet was adapted for each individual according to their new weight. Abdominal subcutaneous adipose tissue biopsies and venous blood samples were obtained; and blood pressure, body weight, and waist circumference were measured before (week 0), during (weeks 8 and 16), and after (week 18) treatment.

2.3. Abdominal subcutaneous adipose tissue biopsies

Abdominal subcutaneous adipose tissue biopsies were obtained in local anesthesia with a syringe with manually applied vacuum. Biopsies were taken from all subjects, but complete series of biopsies large enough for microarray analysis were only available from 24 subjects. The biopsies were immediately frozen in liquid nitrogen and stored at −80°C until analysis.

2.4. RNA isolation

RNA isolation was performed using Qiagen Lipid Tissue kit (Qiagen, Hilden, Germany). The concentration was

measured spectrophotometrically, with an A_{260}/A_{280} ratio of 1.8 to 2.0, and the quality was verified by agarose gel electrophoresis.

2.5. Microarray analysis

Individual DNA microarray analyses were performed to assess adiponectin and leptin gene expressions in 24 subjects diagnosed with and without the metabolic syndrome (9 men and 3 women, in each of the 2 groups). There were no statistically significant differences between the arrayed and non-arrayed subjects, using the common clinical characteristics that were available. Preparation of complementary RNA and hybridization to DNA microarrays was performed according to the Affymetrix Gene Chip Expression Analysis (Affymetrix, Santa Clara, CA) manual. Briefly, RNA was reverse transcribed into complementary DNA (cDNA), biotin-labeled target complementary RNA was prepared by in vitro transcription (Enzo Diagnostics, Farmingdale, NY) followed by hybridization to DNA microarrays (Human Genome U133A arrays; Affymetrix) according to the Minimum Information About a Microarray Experiment guideline [22]. The HU133A arrays are composed of 22 283 probe sets representing 14 500 known human expressed genes. The microarrays were scanned with Hewlett Packard confocal laser scanner (Hewlett Packard, GeneArray scanner G2500A) and visualized using Affymetrix Genechip 4.0 software (Affymetrix, Santa Clara, CA).

2.6. Analysis of microarray data

To allow cross-comparisons of gene expression, the mean target signal on each microarray was globally scaled to an average intensity of 100.

Scanned output files were analyzed using Affymetrix Microarray Suite Version 5.0 software and Data Mining Tool 2.0 (Affymetrix). The overall percentage of detectable probe sets was 45% (10 028) and the scaling factor differed less than 3-fold, allowing for cross-comparison of all the 96 arrays. The quality of cDNA synthesis and in vitro transcription was assessed by comparing 3′ and 5′ expression levels of the housekeeping genes GAPDH and B-actin. Ratios of the 3′ and 5′ region transcripts were less than 3 (recommended by the manufacturer).

2.7. Real-time reverse transcriptase–polymerase chain reaction analysis of gene expression

In the real-time PCR analysis, 8 subjects (2 women and 6 men) with the metabolic syndrome and 8 subjects (1 woman and 7 men) without the metabolic syndrome were included for analyses of v-SNARE Vti1a expression. Reagents for real-time reverse transcriptase–polymerase chain reaction analysis of low-density lipoprotein (LDL) receptor–related protein 10 (LRP10) and Vti1a (Assays-onDemand, TaqMan Reverse Transcriptase reagents, and TaqMan Universal PCR Master Mix) were from Applied Biosystems (Foster City, CA) and used according to the manufacturer's protocol. Complementary DNA was synthesized from 1 μg of total RNA in a total reaction volume of 25 μL. Complementary DNA corresponding to 10 ng of RNA per reaction was used for real-time PCR. Specific products were amplified and detected with the ABI Prism 7900HT Sequence Detection System (Applied Biosystems) using default cycle parameters. A standard curve was plotted for each primer-probe set with a serial dilution of subcutaneous adipose tissue cDNA. Based on our previous report [23], human LRP10 was used as reference to normalize the expression levels between samples. All standards and samples were analyzed in triplicate.

2.8. Laboratory analyses

Serum levels of adiponectin were determined by an ELISA kit (R&D Systems Europe, Abingdon, UK). Samples were available from 33 subjects (3 women and 14 men with the metabolic syndrome, and 3 women and 13 men without the metabolic syndrome) and in 33 sex- and aged-matched controls. The analyses were performed at the Wallenberg Laboratory. Other blood chemistry analyses (high-sensitivity C-reactive protein, total cholesterol, HDL, LDL, TGs, fasting plasma glucose, HbA_{1c}, insulin, and leptin) were performed at the Department of Clinical Chemistry, Sahlgrenska University Hospital, accredited according to ISO/IEC17 025. High-sensitivity C-reactive protein was measured by an ultrasensitive method using particle-enhanced immunoturbidimetry (Roche Diagnostics, GmbH, Mannheim, Germany).

Cholesterol, HDL, and TGs were determined by an enzymatic technique using a Modular P analyzer (Roche Diagnostics). Low-density lipoprotein was calculated as described by Friedewald et al [24]. Hemoglobin A_{1c} was assessed by high-pressure liquid chromatography with a Kolon Mono-S HR 5/5 [25] (Pharmacia, Uppsala, Sweden). Insulin was assessed with an immunometric 2-step sandwich method with an ADVIA Centaur Insulin Ready Pack (Bayer, Tarrytown, NY).

2.9. Statistical analysis

R, an open-source statistical language, was used (www.r-project.org). Data are summarized with arithmetic means and standard deviations. For certain skewed variables, data are summarized with geometric means (GEMs) instead of arithmetic means. Variation of the data is alternatively summarized with the SEM where indicated. Differences in continuous variables between groups are assessed using linear regression with indicator variables for group status. When the indicator variable significantly improves the regression, then there is a significant difference between groups. Tests for group differences in GEM are equivalent to performing the aforementioned regression with a logarithmic transform of the response variable. Correlation between pairs of continuous variables was also assessed with linear regression, equivalent to testing Pearson

correlation coefficient. $P < .05$ (2-sided) was considered statistically significant.

3. Results

The characteristics of the subjects at study entry (week 0), during VLED (weeks 8 and 16), and after 2 weeks of refeeding (week 18) are shown in Table 1. There was no statistically significant difference in BMI or waist circumference between the MetS+ and MetS− groups, but by selection criteria the MetS+ group was more dyslipemic and had higher blood pressure.

In the sex- and age-matched randomly selected reference group, BMI was 24.1 ± 3.0 kg/m^2, waist circumference 86.5 ± 9.6 cm, fasting plasma insulin 8.22 pmol/L, TGs 1.60 ± 1.11 mmol/L, and HDL 1.32 ± 0.41 mmol/L. These characteristics differed with statistical significance from those in the MetS+ and MetS− groups (data not shown).

3.1. Changes in weight, insulin, TGs, and HbA_{1c}

As shown in Table 1, the weight decreased during the diet period by 28.2 ± 8.4 kg in the MetS− and 26.3 ± 9.8 kg in the MetS+ group. There was no statistically significant increase in weight during the final 2 weeks, when the subjects increased energy intake. There was no statistically significant difference in body weight between the MetS− and MetS + subjects at any time. The serum concentrations of insulin and TGs as well as HbA_{1c} decreased during the VLED period in both groups. These reductions in insulin, TGs, and HbA_{1c} were more pronounced in the MetS+ than in the MetS− group, during the first 8-week period, but not thereafter (Fig. 1). At the end of the VLED period the MetS+ and MetS− groups did not differ regarding these 3 variables.

3.2. Adiponectin and leptin

At baseline, serum adiponectin (GEM ± SEM) concentration was 8.85 ± 1.48 μg/mL in the reference group and 8.51 ± 1.15 μg/mL in the MetS− group (not significant). In the MetS + group, adiponectin was 6.03 ± 0.8 μg/mL, which was lower than in the MetS− and reference groups ($P < .05$). Adiponectin increased during the entire study period, also during the 2 weeks of refeeding (Fig. 2). The difference between the MetS+ and MetS− in serum adiponectin had disappeared at week 8. At week 18 the adiponectin concentration was 12.18 ± 2.08 μg/mL in the MetS+ and 12.89 ± 1.63 μg/mL in the MetS− subjects (not significant). The differences between the 18-week means and those of the reference group were statistically significant ($P < .05$) for both groups.

Table 1
Characteristics of the subjects with and without the metabolic syndrome before and during diet treatment

	Prediet	VLED		Refeeding
	0	Week 8	Week 16	Week 18
Obese, MetS+ (n = 21)				
Weight (kg)	114.3 ± 16.0	95.1 ± 14.4	85.6 ± 14.3	86.5 ± 14.1
BMI (kg/m^2)	36.7 ± 5.0	30.8 ± 4.3	27.8 ± 4.2	27.9 ± 3.8
Waist circumference (cm)	119.3 ± 11.7	105.7 ± 12.2	95.7 ± 11.5	96.3 ± 10.8
SBP (mm Hg) *	143 ± 17	122 ± 15	118 ± 11	127 ± 14
HbA_{1c} (%) *	5.20 ± 0.74	4.39 ± 0.37	4.24 ± 0.41	4.28 ± 0.43
Fasting plasma glucose (mmol/L) *	6.64 ± 1.39	4.63 ± 0.77	4.59 ± 0.82	5.29 ± 1.06
Fasting plasma insulin (mU/L) *	19.5 ± 7.81	5.38 ± 2.11	4.05 ± 1.57	6.49 ± 3.31
TG (mmol/L) *	2.48 ± 1.20	1.05 ± 0.23	0.88 ± 0.19	1.27 ± 0.39
LDL (mmol/L)	3.57 ± 0.81	2.26 ± 0.86	2.54 ± 0.74	2.98 ± 0.70
HDL (mmol/L)	1.27 ± 0.36	1.18 ± 0.26	1.34 ± 0.33	1.38 ± 0.25
hs-CRP (mg/mL)	4.46 ± 3.60	4.25 ± 5.49	2.47 ± 1.76	2.25 ± 2.38
Leptin (ng/L)	29.6 ± 16.0	7.41 ± 6.6	4.5 ± 5.3	6.7 ± 5.3
Obese, MetS− (n=19)				
Weight (kg)	121.1 ± 19.4	102.8 ± 16.4	92.8 ± 15.5	93.3 ± 15.1
BMI (kg/m^2)	37.0 ± 3.9	31.2 ± 3.3	28.2 ± 3.4	28.5 ± 3.2
Waist circumference (cm)	122.7 ± 11.0	110.0 ± 12.9	101.0 ± 13.1	100.4 ± 13.2
SBP (mm Hg)	129 ± 14	118 ± 11	115 ± 15	116 ± 13
HbA_{1c} (%)	4.35 ± 0.38	4.11 ± 0.48	4.07 ± 0.31	4.01 ± 0.39
Fasting plasma glucose (mmol/L)	5.09 ± 0.47	4.47 ± 0.59	4.31 ± 0.40	4.59 ± 0.42
Fasting plasma insulin (mU/L)	14.9 ± 7.91	7.50 ± 4.80	4.39 ± 2.69	7.06 ± 4.40
TG (mmol/L)	1.37 ± 0.46	1.02 ± 0.25	0.96 ± 0.26	1.09 ± 0.50
LDL (mmol/L)	3.43 ± 0.98	2.34 ± 0.69	2.74 ± 0.51	2.87 ± 0.60
HDL (mmol/L)	1.30 ± 0.35	1.18 ± 0.27	1.37 ± 0.32	1.38 ± 0.26
hs-CRP (mg/mL)	4.54 ± 6.05	3.33 ± 3.49	2.39 ± 1.34	2.09 ± 1.85
Leptin (ng/L)	41.3 ± 18.7	9.7 ± 7.3	6.1 ± 5.4	8.4 ± 7.0

Data are expressed as means ± SD. SBP indicates systolic blood pressure; hs-CRP, high-sensitivity C-reactive protein.
* $P < .05$ compared with MetS− at baseline, by selection criteria.

The change in serum adiponectin from baseline to week 8 did not correlate to corresponding changes in insulin, HbA_{1c}, or TGs, neither in any of the groups nor in the groups taken together (data not shown).

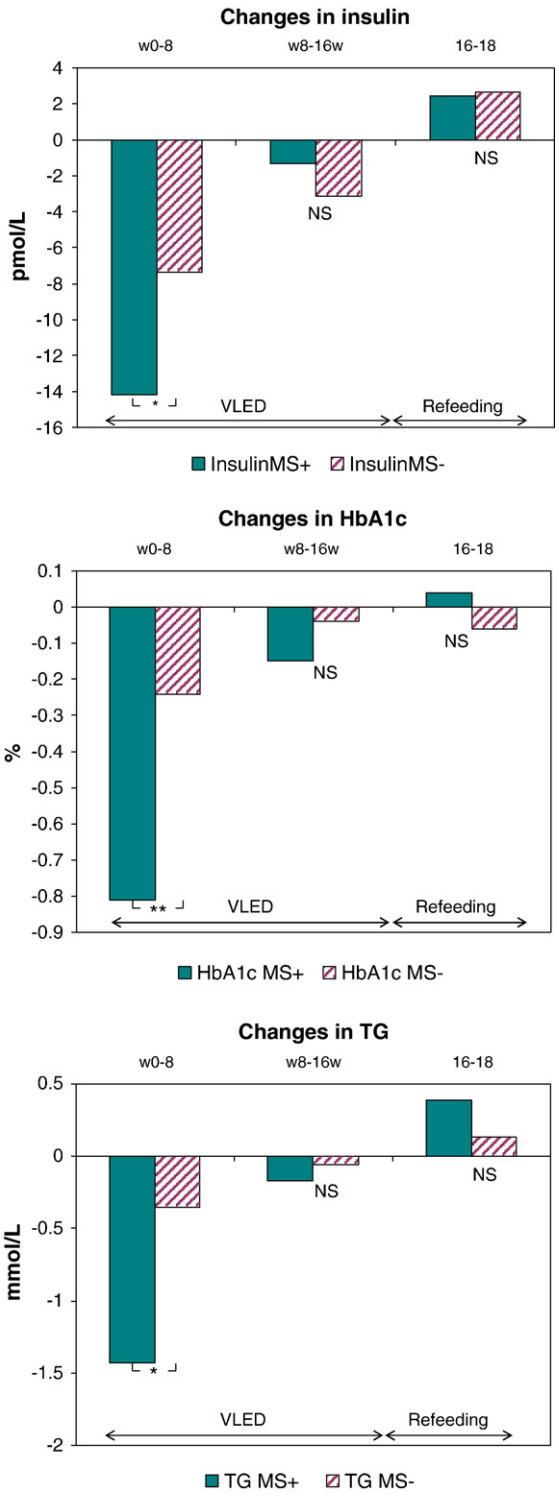

Fig. 1. Changes in serum concentrations of insulin HbA_{1c} and TGs. Differences between the MetS + and MetS− groups are shown. *$P < .05$. NS indicates no statistically significant difference between the groups.

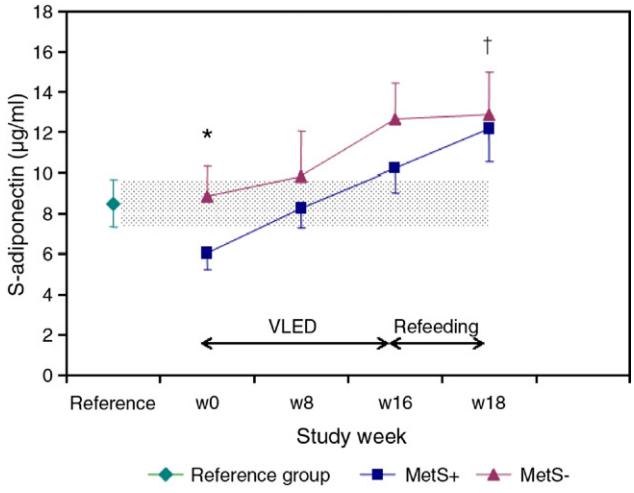

Fig. 2. Serum adiponectin concentrations in obese subjects with (MetS+, n = 16) and without (MetS−, n = 17) the metabolic syndrome, before, during, and after weight loss (GEM ± SEM). The shadow in the figure indicates GEM ± SEM for the sex- and age-matched reference group (n = 33) *$P < .05$ for MetS+ group vs the reference and MetS− groups. †$P < .05$, MetS+ and MetS− groups vs the reference group.

As expected, serum leptin concentrations decreased during weight loss ($P < .001$) from baseline to week 16 and remained unchanged during refeeding (Table 1).

3.3. Changes in gene expression: adiponectin, leptin, and v-SNARE Vti1a

There were no statistically significant changes within or between the MetS+ and MetS− groups in adipose tissue expression of adiponectin during the study (Fig. 3). The expression values for leptin at 0, 8, 16, and 18 weeks were 4280 ± 350, 3093 ± 274, 2237 ± 265, and 1670 ± 215 in the MetS+ group and 3917 ± 418, 2156 ± 282, 1991 ± 302, and 2280 ± 280 in the MetS− group. The differences in leptin expression between the groups were statistically significant only at week 8 ($P = .042$). The changes within the groups from week 0 to week 18 were statistically significant ($P = .002$ for MetS+ and $P < .001$ for MetS−).

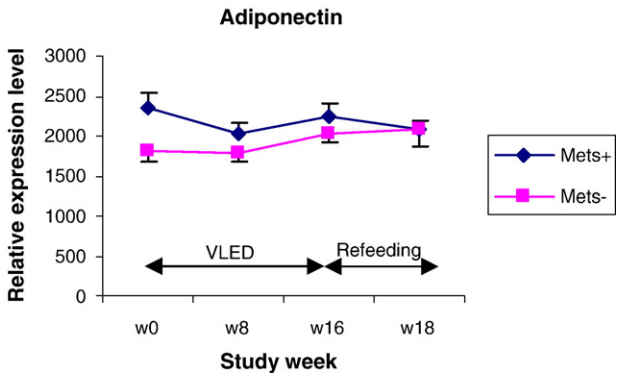

Fig. 3. Adiponectin gene expression: no statistically significant differences were seen within or between the groups during the study. Gene expression level and SEM are shown.

The gene expression of v-SNARE Vti1a did not change from week 0 to week 8 in any group, between the groups, or in the 2 groups combined. There was no statistically significant difference in the expression of v-SNARE Vti1a between the MetS+ and MetS− subjects. Mean v-SNARE Vti1a/ LRP10 messenger RNA (mRNA) ± SEM was 0.52 ± 0.069 at week 0 and 0.54 ± 0.089 at week 8 in the MetS+ group, and the corresponding values were 0.62 ± 0.083 and 0.57 ± 0.071 in the MetS− group.

4. Discussion

In this study we show that serum concentrations of adiponectin were lower in obese subjects with the metabolic syndrome compared with equally obese subjects without the metabolic syndrome. In both groups, serum adiponectin levels increased during weight loss, and interestingly, this increase continued during refeeding when weight started to increase. In addition, we report that obese subjects, with BMI of more than 30 but without the metabolic syndrome, had serum adiponectin concentrations that were similar to those in the reference group where the mean BMI was less than 25. These findings clearly indicate that factors other than obesity affect circulating adiponectin levels. Previous studies show that although weight loss invariably improves dyslipidemia or glucose intolerance, the associations with serum adiponectin are inconsistent or weak, but serum adiponectin may increase provided that the reduction in weight is considerable [26,27]. Studies showing no effect of low-energy diets on circulating adiponectin levels are typically characterized by mean weight losses of less than 10 kg [11,28]. In the present study we obtained excessive weight loss, amounting to almost 20 kg after 8 weeks and 30 kg after 16 weeks.

The excessive weight loss during the first 8 weeks of the VLED reduced circulating insulin, HbA_{1c}, and TG concentrations more in the MetS+ group than in the MetS− group. During this period, the difference in serum adiponectin between the MetS− and MetS+ groups also disappeared. The fact that the major changes in metabolic parameters and in serum adiponectin levels occurred simultaneously during the first part of the study led us to speculate that the metabolic changes could explain the changes in adiponectin levels. However, we did not find any significant association between the change in serum adiponectin and the changes in insulin, HbA_{1c}, or TGs. This dissociation between weight, insulin, and adiponectin is supported by a recent study where we showed that the variation in serum adiponectin between women with different degrees of glucose intolerance was independent of obesity, waist-to-hip ratio, and insulin resistance [29].

In an attempt to understand the molecular events responsible for alterations in circulating adiponectin levels, we measured adiponectin gene expression in adipose tissue samples. In spite of the differences in serum adiponectin levels, we found no support for the hypothesis that the MetS+ group would have lower adipose tissue expression of adiponectin or that it increased more during dieting, compared with the MetS− group. In fact, adiponectin mRNA levels did not change at all during the study. In contrast, we observed the expected parallel changes in gene expression and serum levels of leptin accompanying weight loss or refeeding. This observation also validated our study design and experimental setup.

We also measured mRNA levels of v-SNARE Vti1a, as they are suggested to correlate to the secretion of adiponectin from the cell [17]. However, we found no changes during weight loss. Hypothetically, there are other possible underlying mechanisms for the increase in circulation adiponectin during weight loss. The catabolism of adiponectin could be affected, which in turn, could be a consequence of an isoform shift, from adiponectin species with a rapid turnover to more stable forms. Studies in mice indicate that the high-molecular-weight forms have a longer half-life than hexamers [30], but the opposite has been shown in rabbits [31]. An increase of high-molecular-weight isomers in humans in response to weight loss by low-energy diet has recently been reported [13], but it is still unclear whether this isoform shift can affect adiponectin turnover. At the end of the study, the previously obese subjects had 46% higher serum adiponectin levels than the normal-weight reference group, although the former groups on average remained overweight. This intriguing rise could not be explained by changes in adiponectin or v-SNARE Vti1a gene expression.

In conclusion, our study showed that MetS+ subjects had lower circulating adiponectin than subjects without the syndrome. During weight loss this difference in adiponectin disappeared together with the difference in insulin, TGs, and HbA_{1c}. There were no indications that these changes in adiponectin concentration were explained by changes in adiponectin expression or increased adiponectin secretion mediated by v-SNARE Vti1a. The continuous increase in serum adiponectin, also during refeeding and above the level of the reference group, was unexplained.

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