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Inherent insulin sensitivity is a major determinant of multimeric adiponectin responsiveness to short-term weight loss in extreme obesity

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High molecular weight (HMW-A) adiponectin levels mirror alterations in glucose homeostasis better than medium (MMW-A) and low molecular weight (LMW-A) components. In 25 patients with wide-range extreme obesity (BMI 40-77 kg/m²), we aimed to explore if improvements of multimeric adiponectin following 4-wk weight loss reflect baseline OGTT-derived insulin sensitivity (ISI_{OGTT}) and disposition index (DI_{OGTT}). Compared to 40 lean controls, adiponectin oligomers were lower in extreme obesity ($p < 0.001$) and, within this group, HMW-A levels were higher in insulin-sensitive ($p < 0.05$) than -resistant patients. In obese patients, short-term weight loss did not change total adiponectin levels and insulin resistance, while the distribution pattern of adiponectin oligomers changed due to significant increment of HMW-A ($p < 0.01$) and reduction of MMW-A ($p < 0.05$). By multivariate analysis, final HMW-A levels were significantly related to baseline ISI_{OGTT} and final body weight (adjusted $R^2 = 0.41$). Our data suggest that HMW adiponectin may reflect baseline insulin sensitivity appropriately in the context of extreme obesity. Especially, we documented that HMW-A is promptly responsive to short-term weight loss prior to changes in insulin resistance, by a magnitude that is proportioned to whole body insulin sensitivity. This may suggest an insulin sensitivity-dependent control operated by HMW-A on metabolic dynamics of patients with extreme obesity.

Obesity is associated with a state of chronic low-grade inflammation, insulin resistance and metabolic disturbances, collectively defined as the metabolic syndrome, which are linked to an increase in cardiovascular morbidity and mortality¹. Key to the metabolic syndrome is the disproportionate accumulation of abdominal adipose tissue (AT), the body's largest endocrine organ, comprised of subcutaneous (SAT) and visceral AT (VAT). An extensive vascular network supplies adipocytes, thus that even modest alterations in fat accumulation can impact cellular secretions and physiologically result in variations in metabolism, energy storage, immunity and inflammation. Of great importance is the role of adipose tissue as a key regulator of lipid and glucose metabolism via several adipokines such as leptin, adiponectin, TNF- α and numerous interleukins².

Unlike the majority of adipokines, circulating adiponectin is inversely related to body mass index (BMI) and fat accumulation, and its ability to control insulin sensitivity has been extensively characterized^{3,4} in relation to states of insulin resistance, obesity, type 2 diabetes mellitus (T2DM) and coronary heart disease⁵⁻⁷. Peculiarly, as a result of the obesity epidemic, there is an increasing number of patients in the extremely obese category (defined as BMI ≥ 40 kg/m²). It is shown that the effect of extreme obesity on mortality is greater among young than older adults, greater among men than women, and greater among whites than blacks⁸. However, data on the dynamics of adiponectin is currently limited in this group of patients⁹.



Circulating adiponectin is comprised of distinct multimeric moieties including a trimeric low molecular weight (LMW), a hexameric medium molecular weight (MMW) and an oligomeric high molecular weight complex (HMW)⁴. Three different adiponectin receptors have been identified: the structurally highly related G-protein-coupled seven-transmembrane-domain receptors AdipoR1 and AdipoR2, and T-cadherin, a glycosyl-phosphatidyl-inositol-anchored extracellular protein responsive to HMW and MMW adiponectin^{4,10}. AdipoR1 is expressed in muscle, whereas AdipoR2 is liver specific and T-cadherin is present in muscle, as well as in cardiovascular and nervous systems¹⁰. Previous investigations in humans revealed that plasma HMW adiponectin levels reflect insulin sensitivity and high density lipoprotein cholesterol levels more accurately than MMW and LWM oligomers^{11,12}. In obese and non-obese subjects, profiling of the distribution of multimeric adiponectin by enzyme immunoassay resulted in a better correlation of HMW adiponectin concentrations to parameters of insulin sensitivity, circulating lipids, VAT, and waist-to-hip ratio than MMW or LMW adiponectin levels¹³. It is also acknowledged that there are gender-related differences in the circulating levels of adiponectin and its complexes, with women harboring higher levels than men⁷.

In obese individuals, previous studies on responsiveness of the composition of multimeric adiponectin following dietary intervention or bariatric surgery have yielded contradictory results, with some investigations failing to demonstrate significant changes following weight loss¹⁴, while others reported increases in HMW, MMW and LMW concentrations^{15,16}. Due to differences in the inclusion criteria, sample size and comorbidities across diverse studies, much uncertainty remains as to the metabolic determinants, or timing and the magnitude of weight loss, required to modify adiponectin moieties in the setting of extreme obesity.

The aim of this study was thus to investigate multimeric adiponectin complexes in patients with extreme obesity (BMI > 40 kg/m²) previously unknown to be diabetic, in relation to different measures of insulin sensitivity and in response to short-term weight loss.

Methods

Subjects. Participants were consecutively recruited into the study after signing an informed consent upon admission to our Institution for routine diagnostic workup and inpatient rehabilitation for extreme obesity. The study was approved by the local Ethic Committee. The study population consisted of 25 extreme obese patients (9 females/16 males; mean age, 36.4 ± 9.3 yr; mean BMI, 51.5 ± 8.4 kg/m², BMI range, 40–77 kg/m²). As healthy controls, 40 subjects (15 females, age 35.1 ± 7.3 yr, BMI 22.4 ± 2.1 kg/m²) were recruited among the Institution's employees and donated plasma for biochemical analyses. Exclusion criteria were menopause, endocrine disturbances causing obesity, type 1 diabetes mellitus (T1DM), previous diagnosis of T2DM, autoimmune or chronic inflammatory disorders, chronic obstructive pulmonary disease, history of neoplasms or degenerative diseases, previous chronic steroid treatment, kidney or cardiac disorders. No patient was undergoing pharmacological therapies at the time of the study, with body weight stable for at least three months prior to hospital admission. Obese subjects were studied at baseline upon admission and following a four-week inpatient metabolic rehabilitation consisting of individualized caloric restriction equivalent to 75% of basal resting energy expenditure measured by indirect calorimetry¹⁷, physical exercise comprising of three sessions per week of aerobic activity, supported by a nutrition and lifestyle action program consisting of three 1-hr classes per week dedicated to an educational approach on dietary behavior, nutrition knowledge and motor activity. Inpatient hypocaloric diet consisted of 30% lipids, 50% carbohydrates, and 20% proteins. Neither supplements nor anti-obesity therapies were used during the study period. The methods were carried out in accordance with the approved guidelines.

Body Measurements. Subjects underwent body measurement wearing light underwear, in a fasting condition after voiding. Weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively. BMI was expressed as weight (kilograms)/height (meters)² and obesity was defined for any BMI over 30 kg/m²; all patients included in this study were affected with extreme obesity (BMI range, 40–77 kg/m²). Waist circumference was measured midway between the lowest rib and the top of the iliac crest after gentle expiration; hip measurements were taken as the greatest circumference around the nates. Anthropometric data were expressed as the mean of two measurements. All obese subjects underwent dual-energy x-ray absorptiometry (GE-Lunar, Madison, WI) for measurement of lean and fat body mass, the morning after an overnight fasting and after voiding.

Metabolic Determinations and Laboratory. Obese patients underwent 2-h 75 g oral glucose tolerance test (OGTT) for the determination of glucose, insulin and C-peptide levels. The areas under the curves for glucose (G_{AUC}), insulin (I_{AUC}) and C-peptide (P_{AUC}) were calculated using the trapezoidal formula. Upon OGTT, ADA guidelines¹⁸ were applied for the assessment of glucose tolerance as follows: normal fasting plasma glucose (FPG) if <100 mg/dl (5.6 mmol/l); impaired FPG if 100–125 mg/dl (6.9 mmol/l); impaired glucose tolerance (IGT) if 2 h OGTT-PG 140–199 mg/dl (7.8–11.0 mmol/l); T2DM if FPG ≥ 126 mg/dl (≥7 mmol/l) two days apart or if 2-h OGTT-PG ≥ 200 mg/dl (≥11.1 mmol/l). Glycated haemoglobin (HbA_{1c}) values of 5.7 and 6.5% were considered as the threshold for normal glucose metabolism and T2DM, respectively. Insulin sensitivity was investigated by indices obtained in fasting conditions or derived from the OGTT, previously validated against the euglycemic hyperinsulinemic clamp, as follows: FPG; fasting insulin (FI); fasting C-peptide (FCP); homeostatic model of insulin resistance (HOMA-IR), calculated as insulin (μU/ml) × [glucose (mmol/l)/22.5]; whole-body insulin sensitivity index (ISI_{OGTT}), also indicated as the Matsuda index, measured during OGTT by the composite index¹⁹ as $ISI_{OGTT} = 10,000/\text{square root of } (FG \times FI) \times (\text{mean glucose} \times \text{mean insulin during OGTT})$; insulinogenic index (IGI_{OGTT}), an index of first-phase insulin secretion²⁰, calculated as $[(\text{insulin at time 30} - \text{insulin at time 0})/(\text{glucose at time 30} - \text{glucose at time 0})]$; disposition index (DI_{OGTT}), a product of the insulin sensitivity index and the absolute insulin secretion index, calculated as $ISI_{OGTT} \times IGI_{OGTT}$ ¹⁹.

Blood glucose, electrolytes and HbA_{1c} were measured by enzymatic methods (Roche Molecular Biochemicals, Mannheim, Germany). A two-site, solid-phase chemiluminescent immunometric assay or competitive immunoassay was used for insulin and C-peptide levels (Immulite 2000 Analyzer; DPC, Los Angeles, CA). Serum total adiponectin and adiponectin multimeric forms were determined using the Adiponectin (Multimeric) enzyme immunoassay (EIA) (ALPCO Diagnostics, Salem, NH), according to the manufacturer's instructions. Assay sensitivity is 0.019 ng/ml; as reported by the manufacturer, overall intra- and inter-assay coefficient of variations (CV) for total, HMW and MMW+LMW are 5.4–7.3% and 5.0–6.0%, respectively.

Statistical Analysis. Data are expressed as the mean ± SD. Data were tested for normality of distribution by the Kolmogorov-Smirnov test. Statistical analyses were carried out using unpaired and paired two-tailed Student's *t* test to compare baseline characteristics of the two groups and within the obese population at the different time-points. Linear regression analyses were performed to determine correlation coefficients between different parameters. Stepwise multivariate regression was used to evaluate the association between adiponectin oligomers and metabolic or anthropometric variables after controlling for potential associated variables. β coefficients and related significance values obtained from the models are reported. *P* < 0.05 was considered as statistically significant.

Results

Baseline characteristics of the obese patients are shown in Table 1. Their mean BMI was 51.6 ± 8.4 kg/m² and the prevalence of IGT and newly diagnosed T2DM was 52% and 26%, respectively. Two patients with diabetic FPG levels did not undergo OGTT. Compared to controls, adiponectin overall was significantly decreased in obese patients when calculated as total (2.98 ± 1.01 vs. 5.62 ± 1.28 μg/ml, *p* < 0.001) or measured as HMW (1.20 ± 0.57 vs. 2.47 ± 0.94 μg/ml, *p* < 0.001), MMW (0.70 ± 0.26 vs. 1.52 ± 0.29 μg/ml, *p* < 0.001) and LMW isoforms (1.09 ± 0.64 vs. 1.63 ± 0.14 μg/ml, *p* < 0.001). The HMW-to-total adiponectin ratio was similar between patients and controls (42.4 ± 7.2 vs. 39.5 ± 11.9%, NS). Within the obese group, adiponectin multimers were similar between nondiabetic and newly diagnosed diabetic patients (Table 2). However, when obese patients were stratified by ISI_{OGTT} according to a cutoff of 2.5, previously demonstrated to be a conservative threshold of insulin sens-

Table 1 | Baseline characteristics of the obese study participants

Parameters	Obese patients (N = 25)
Age	36.5 ± 9.4
Gender (F/M)	9/16
Weight (kg)	145.5 ± 25.5
Body mass index (kg/m ²)	51.6 ± 8.4
Waist (cm)	141.8 ± 16.5
Waist-to-hip ratio	0.98 ± 0.10
Fat mass (kg)	70.7 ± 18.8
Fat mass (%)	49.4 ± 7.3
Fat free mass (kg)	72.2 ± 16.0
Fat free mass (%)	50.6 ± 7.3



Table 2 | Baseline glucose homeostasis and multimeric adiponectin in the obese population as a whole and after stratification as non-diabetic and diabetic by OGTT. Significance is shown (unpaired T test) for nondiabetic and newly diagnosed diabetic patients. For abbreviations: FPG, fasting plasma glucose; FI, fasting insulin; FCP: fasting C-peptide; AUC, area under the OGTT curve; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model of insulin resistance; HMW, high molecular weight; MMW, medium molecular weight; LMW, low molecular weight

Parameters	Whole obese population (n = 25)	Nondiabetic patients (n = 18)	Diabetic patients (n = 7)	p
FPG (mg/dl)	99.4 ± 30.1	87.8 ± 10.1	129.3 ± 43.3	0.0007
FI (mU/l)	19.0 ± 8.6	16.1 ± 6.55	26.5 ± 9.26	0.005
FCP (µg/l)	3.80 ± 1.55	3.42 ± 1.39	5.17 ± 1.43	0.02
HbA1c (%)	6.03 ± 1.1	5.58 ± 0.25	7.17 ± 1.69	0.0006
Glucose AUC	17 831.6 ± 3 363.1	16 531.8 ± 2 497.2	22 251.0 ± 1 685.2	0.0001
Insulin AUC	10 324.9 ± 4 691.3	9 502.4 ± 4 684.1	13 121.4 ± 3 890.1	ns
C-peptide AUC	1 210.8 ± 317.4	1 144.7 ± 294.5	1 435.6 ± 316.7	0.07
HOMA-IR	4.77 ± 2.75	3.47 ± 1.45	8.12 ± 2.48	0.0001
Matsuda index (ISI _{OGTT})	2.66 ± 1.29	3.02 ± 1.26	1.42 ± 0.46	0.02
Disposition index	3.41 ± 4.21	4.11 ± 4.56	1.05 ± 0.85	ns
Insulinogenic index	1.28 ± 1.30	1.46 ± 1.43	0.70 ± 0.38	ns
Total adiponectin (µg/ml)	2.98 ± 1.28	3.34 ± 0.82	2.52 ± 1.06	ns
HMW adiponectin (µg/ml)	1.20 ± 0.57	1.43 ± 0.38	1.01 ± 0.62	ns
MMW adiponectin (µg/ml)	0.70 ± 0.26	0.64 ± 0.23	0.72 ± 0.26	ns
LMW adiponectin (µg/ml)	1.09 ± 0.64	1.30 ± 0.65	0.78 ± 0.73	ns
HMW/total adiponectin (%)	39.5 ± 11.9	39.7 ± 11.3	39.1 ± 13.1	ns

itivity²¹, the levels of HMW adiponectin were higher in insulin-sensitive (ISI_{OGTT} ≥ 2.5) than insulin-resistant patients (ISI_{OGTT} < 2.5) (1.42 ± 0.47 vs. 0.97 ± 0.59, *p* < 0.05). For the other adiponectin components, similar values were observed between these obese subgroups.

After a 4-wk inpatient rehabilitation program, obese patients lost cumulatively by 7.2 ± 2.7% of their body weight (Table 3). Short-term weight loss did not alter total adiponectin levels and HOMA-IR values. However, the distribution pattern of multimeric adiponectin changed owing to significant increases of HMW adiponectin levels and the HMW-to-total adiponectin ratio, while MMW adiponectin significantly decreased. No change in LMW adiponectin levels were recorded.

In the obese population, simple regression analyses at baseline failed to provide associations between adiponectin components and measures of adiposity, such as BMI, waist circumference and percent fat mass, yet values of HMW-to-total adiponectin ratio were negatively related to waist (*r* = -0.51, *p* < 0.01). Baseline HMW adiponectin levels paralleled values of ISI_{OGTT} (*r* = 0.43, *p* < 0.05; Fig. 1a) and DI_{OGTT} (*r* = 0.42; *p* < 0.05), while LMW adiponectin levels were associated to HOMA-IR (*r* = -0.43, *p* < 0.05). Also negative was the association between total adiponectin and fasting insulin (*r* = -0.51, *p* < 0.01), P_{AUC} (*r* = -0.45, *p* < 0.05) and G_{AUC} (*r* = -0.43, *p* < 0.05).

At the end of the study, HMW adiponectin levels increased proportionately to baseline values of ISI_{OGTT} (*r* = 0.53, *p* < 0.01; Fig. 1) and final body weight (*r* = -0.46, *p* < 0.05). There was no association between percent change from baseline of HMW adiponectin and the change in body weight. While total adiponectin was correlated to final values of insulin and HOMA-IR (*r* = -0.46 and *r* = -0.44, *p* < 0.05 for both), HMW adiponectin did not appear to do so (*p* = 0.08). Baseline and final values of HMW (*r* = 0.71, *p* < 0.0001), MMW (*r* = 0.58, *p* < 0.01) and LMW adiponectin levels (*r* = 0.56, *p* < 0.01) were tightly correlated (Fig. 2).

By stepwise multivariate regression analysis, final HMW adiponectin levels were predicted by baseline ISI_{OGTT} values (β = 0.50, *p* = 0.01) and final body weight (β = -0.41, *p* = 0.02). As a whole, this model explained 41% of the variability of HMW adiponectin levels after weight loss.

Discussion

Adults with extreme obesity are a small proportion of the population, but account for a disproportionate amount of medical illnesses and

health care services⁸. Low-grade inflammation and insulin resistance explain the increased cardiovascular morbidity in this population. There is evidence supporting the role of adiponectin as an active link between obesity and metabolic dysfunction due to its ability to regulate glucose homeostasis and insulin sensitivity⁴. In animal studies, dysregulated expression of the adiponectin gene and hypo adiponectinaemia caused by overnutrition can promote insulin resistance prior to development of T2DM^{3,22}. In humans, adiponectin levels are inversely related to a number of metabolic disorders, i.e. obesity, visceral adiposity, insulin resistance, liver steatosis, and proatherogenic lipoproteins⁵⁻⁷. Noticeably, adiponectin circulates as multimeric components and the HMW isoform has been shown to control plasma glucose levels and hepatic insulin sensitivity more efficiently than MMW and LMW isoforms²³. It has been thus postulated that HMW adiponectin can promote antidiabetic effects by acting in the liver through intracellular activation of the AMP-activated protein kinase⁴.

In this study on extreme obesity, we found all adiponectin isoforms to be lower than in controls by a magnitude that was related to central fat. Especially, we observed that HMW adiponectin levels were well correlated to the OGTT-derived Matsuda index, which conventionally reflects the rate of disappearance of plasma glucose

Table 3 | Effect of short-term weight loss on anthropometric features, metabolic parameters, and adiponectin isoforms in the obese population. For abbreviation: FPG, fasting plasma glucose; FI, fasting insulin; HOMA-IR, homeostatic model of insulin resistance; HMW, high molecular weight; MMW, medium molecular weight; LMW, low molecular weight

Variable	Baseline	After weight loss	p
Weight (kg)	145.5 ± 25.5	134.9 ± 49.5	0.0001
Body mass index (kg/m ²)	51.6 ± 8.4	48.0 ± 7.9	0.0001
FPG (mg/dl)	99.4 ± 30.1	94.6 ± 9.2	ns
FI (mU/l)	18.9 ± 8.6	20.7 ± 11.8	ns
Homa-IR	4.77 ± 2.75	4.56 ± 2.23	ns
Total adiponectin (µg/ml)	2.98 ± 1.0	3.09 ± 1.02	ns
HMW adiponectin (µg/ml)	1.20 ± 0.57	1.54 ± 0.66	0.01
MMW adiponectin (µg/ml)	0.70 ± 0.26	0.57 ± 0.31	0.02
LMW adiponectin (µg/ml)	1.09 ± 0.64	1.05 ± 0.60	ns
HMW/total adiponectin (%)	39.5 ± 11.9	49.8 ± 15.0	0.0005

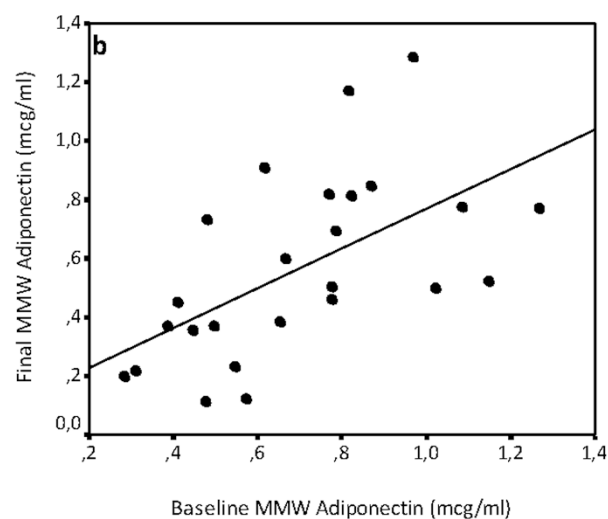
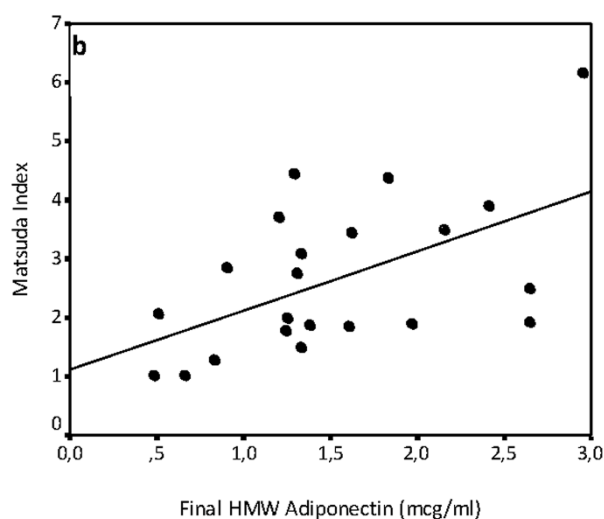
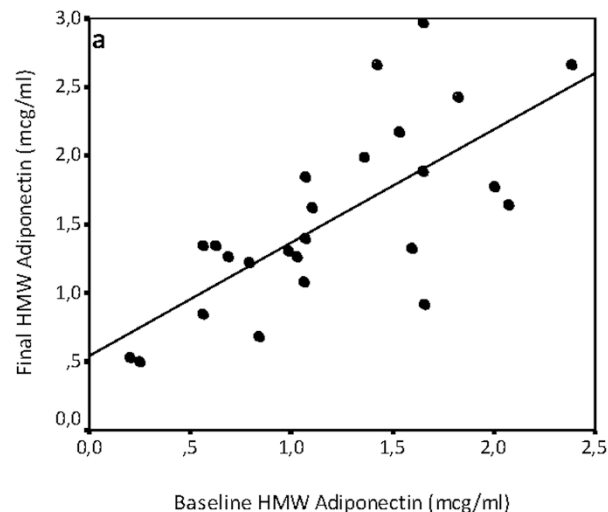
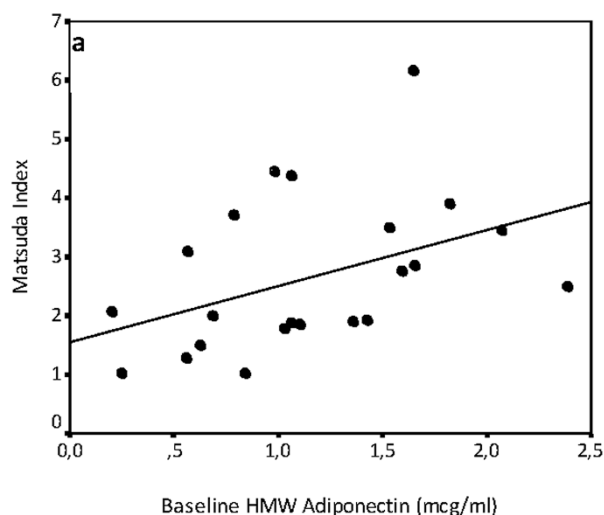


Figure 1 | Bivariate correlation between the Matsuda Index (ISI_{OGTT}) and baseline (Panel a) and final (Panel b) HMW adiponectin levels in the obese population. Coefficients are discussed in the Results section.

obtained during the insulin clamp and is thus a reliable measure of whole body insulin sensitivity^{19,20,24,25}, and were consequently greater in insulin-sensitive than insulin-resistant patients. Also, HMW adiponectin levels were related to the insulin disposition index, a surrogate marker of insulin secretion in relation to insulin sensitivity associated to the onset of type 2 diabetes mellitus²⁶. Therefore, current data may expand to extreme obesity the link previously shown between HMW adiponectin and glucose disposal rate in nondiabetic and diabetic obese subjects¹¹.

Main aim of this study was to investigate non adaptive responses of adiponectin isoforms to a short-term weight loss program in extreme obesity. Previous studies on total adiponectin had shown that stable weight reduction increases adiponectin⁷, and that >10% weight loss is required to significantly increase adiponectin levels²⁷. This event may imply also adaptive mechanisms. In analyses of adiponectin oligomers, previous studies showed no change^{14,28}, selective increases of HMW and MMW adiponectin¹⁵, or increases in all multimeric forms after stable weight loss¹⁶, with discrepancies possibly due to different study duration, timing of assessment since the beginning of diet-therapy, obesity categories under evaluation, and magnitude of lost weight. Opposed to these findings, the current study suggest that an even modest weight loss can modify HMW

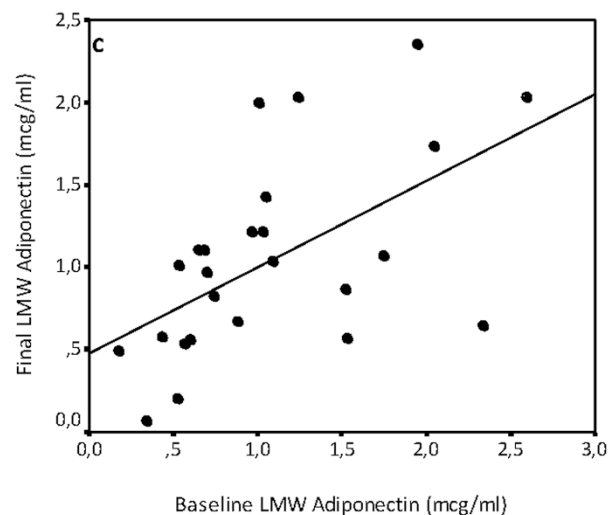


Figure 2 | Bivariate correlation between baseline and final levels of HMW adiponectin (Panel a), MMW adiponectin (Panel b) and LMW adiponectin (panel c) in the obese population. Coefficients are discussed in the Results section.



adiponectin (+42%), HMW-to-total adiponectin ratio (+35%) and MMW adiponectin levels (-18%) in the category of patients with extreme obesity (40–77 kg/m²). We observed that this event does not require modifications of insulin resistance and of total adiponectin, does allowing us to hypothesize that HMW adiponectin may undergo early secretory modifications from adipocytes in response to even small changes in fat mass. The magnitude of HMW adiponectin increase was greater in insulin-sensitive (58%) than -resistant patients (28%). Further, multivariate analysis revealed that baseline insulin sensitivity and final body weight were able to explain cumulatively 41% of HMW adiponectin variability caused by weight loss. While some argued that insulin and insulin sensitivity may act per se to regulate adiponectin multimerization^{29,30}, our data do not support this hypothesis. Rather, we speculate that HMW adiponectin changes are non-adaptive, and possibly dependent on post-translational modifications of the heavily glycosylated residues of adiponectin³¹, which are influenced by self-regulatory mechanisms³⁴ and modifications of proinflammatory adipocytokines³⁵ consequent to changes in fat accumulation^{32,33}.

The limited study sample and the lack of long term data are main study limitations, and thus neglect the role of long-term weight management on changes of insulin sensitivity via multimeric adiponectin actions. We are inclined to consider as potential points of strength both in the inclusion of patients with very high BMI ranges and the controlled inpatient weight management schedule, which allowed to detect short-term non-adaptive responses of multimeric adiponectin to weight loss.

Based on our findings, we suggest that HMW adiponectin may appropriately reflect insulin sensitivity and secretion in the context of extreme obesity, and that an even modest short-term weight loss influences the distribution pattern of adiponectin oligomers by privileging selective increments in HMW adiponectin, in proportion to inherent insulin sensitivity but prior to any metabolic improvements. Our data may, thus, suggest that HMW adiponectin acts as an early regulator of metabolic homeostasis in extreme obesity. Further studies should investigate long-term interaction between adiponectin components and insulin resistance in this setting.

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Author contributions

S.M. contributed to data analysis and wrote the manuscript; G.E.W., G.Gu. and G.Gr. contributed to patients recruitment and data collection; A.B. followed patients during dieting; A.O., G.A. and M.S. contributed to data interpretation and discussion; P.M. contributed to study plan, data analysis and manuscript writing. All authors read and approved the final version of the manuscript.

Additional information

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