An observational study of sequential protein-sparing, very low-calorie ketogenic diet (Oloproteic diet) and hypocaloric Mediterranean-like diet for the treatment of obesity

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\textbf{ABSTRACT}

The impact of a rehabilitative multi-step dietary program consisting in different diets has been scantily investigated. In an open-label study, 73 obese patients underwent a two-phase weight loss (WL) program: a 3-week protein-sparing, very low-calorie, ketogenic diet (\textlti\textless;500 kcal/day; \textit{Oloproteic\textsuperscript{R} Diet}) and a 6-week hypocaloric (25–30 kcal/kg of ideal body weight/day), low glycemic index, Mediterranean-like diet (hypo-MD). Both phases improved visceral adiposity, liver enzymes, GH levels, blood pressure and glucose and lipid metabolism. However, the hypo-MD was responsible for a re-increase in blood lipids and glucose tolerance parameters. Changes in visceral adiposity and glucose control-related variables were more consistent in patients with metabolic syndrome. However, in these patients the hypo-MD did not result in a consistent re-increase in glucose control-related variables. A dietary program consisting in a ketogenic regimen followed by a balanced MD appeared to be feasible and efficacious in reducing cardiovascular risk, particularly in patients with metabolic syndrome.

\textbf{Introduction}

Being a major contributor to several chronic diseases, obesity is a serious health problem worldwide (Skidmore & Yarnell 2004; Kosti & Panagiotakos 2006; Prentice 2006; ISTAT 2015). Management of obesity-related complications is possible and effective but brings to a high costs burden (Wang et al. 2011). On the other hand, several studies have demonstrated that diet-induced weight loss (WL) could be a cheap and effective intervention to improve or even solve many of associated comorbidities (Franz et al. 2007; Jensen et al. 2014). In respect with this, different dietetic strategies are available and, in the short term, all appear to result in a significant WL (Dansinger et al. 2005; Tsai & Wadden 2005; Franz et al. 2007; Gardner et al. 2007). However, some of these could be preferred to others due to more consistent benefits in both the short and long term (Bravata et al. 2003; Wycherley et al. 2012; Paoli et al. 2013a). Very low-calorie diets (VLCDs) providing adequate amount of proteins and micronutrients appear to result in more consistent short-term WL and improvements in metabolic profile (mainly glucose control and insulin resistance), in better body composition due to muscle protein sparing as well as in higher long-term WL maintenance (Bravata et al. 2003; Wycherley et al. 2012; Paoli et al. 2013a).

In the last decades, interest in WL diets has evolved and attention has been focused much more on their composition, particularly the carbohydrate content. Among dietary strategies, low-carbohydrate and low-carbohydrate-ketogenic diets have attracted several researchers. The positive effects of ketogenic diets go beyond the promotion of WL and substantially rest on the positive impact on metabolism, particularly on glucose homeostasis (Hussain et al. 2012; Schugar & Crawford 2012; Wycherley et al. 2012; Paoli et al. 2013a).

However, long-term maintenance on a WL diet in obese patients is often difficult and it is not infrequent assisting to treatment failures and weight regain after few months of intervention. Particularly, adherence to VLC, low-carbohydrate ketogenic diets could be even much more difficult mainly due to the high carbohydrate restriction and should be limited to few weeks.
Therefore, after having benefited from their metabolic impact, patients also need to undergo a dietary rehabilitation program, which should consider the progressive reintroduction of high-quality carbohydrates and switching to a Mediterranean-like dietary pattern.

Although the short-term efficacy of different WL dietary interventions has been object of investigation in several comparative and non-comparative studies, the effects of adherence to two different WL diet in sequence have been scantily addressed by previous research. As far as we are aware, there are only two studies addressing the feasibility and efficacy of a biphasic ketogenic and balanced Mediterranean diet in obese subjects (Paoli et al. 2013b; Leonetti et al. 2015). Besides, although short-term VLCDs have been associated with improved pancreatic β cell sensitivity (Wing et al. 1994; Lim et al. 2011; Malandrucco et al. 2012), a specific focus on the impact of a biphasic dietary intervention on insulin resistant patients has been scantily addressed. Nowadays, there is only one study conducted by Leonetti et al. (2015) in a small group of type-2 diabetes patients – scheduled for laparoscopic bariatric surgery – showing a significant decrease and re-increase in blood glucose after 10 d of a VLC ketogenic and low-carbohydrate diet, respectively (Leonetti et al. 2015). As appropriate data from observational studies enable the design of appropriate randomized trial, the aim of this study was to evaluate the feasibility and the efficacy of adhering to a hypocaloric, low glycemic index, Mediterranean-like diet when this follows a protein-sparing, very low-calorie, ketogenic diet.

**Methods**

**Study subjects and design**

Consecutive adult obese outpatients attending the Clinical Nutrition Unit, A.O.R.N. “San Giuseppe Moscati” for weight concerns between December 2014 and May 2015 were evaluated for inclusion in an open-label interventional study. All patients were considered eligible, regardless of a history of multiple failures. However, a very low-calorie, ketogenic dietetic regimen can be achieved through the use of normal foods. Accordingly, during the OD phase patients were asked to consume a very low-calorie (<500 kcal/day) protein-based diet providing approximately 10–20 g of carbohydrates and lipids per day. The sources of these macronutrients were vegetables, olive oil (for seasoning) and high protein foods. Total daily protein content of the OD was set to 1.4 g per kilogram of ideal body weight calculated by Lorentz’s equations (WHO 1995). Half of this protein intake was provided through the administration of a liquid formula containing a fixed proportion (1:6:5) of essential amino acids (arginine, ornithine-alpha-ketoglutarate, taurine, cysteine, tryptophan, hydroxyproline and citrulline) and high-quality (milk whey) proteins (Ghamin®; Gefaldiet Service srl, Italia). This intervention phase was also complemented by the daily administration of alkalizing substances (Olobasic® – Gefaldiet Service srl, Italia; calcium carbonate, 1500 mg daily; magnesium carbonate, 850 mg daily; potassium bicarbonate, 500 mg daily; sodium bicarbonate, 1500 mg daily; potassium citrate, 500 mg daily), herbal remedies generally prescribed for their diuretic, antioxidant, anti-inflammatory, and hepatoprotective properties (Olodren® – Gefaldiet Service srl, Italia; containing equisetum, hawthorn, milk thistle, nettle, orthosiphon and fucoxanthin; see Table S1) (Ferenci et al. 1989; Abidov et al. 2010; Carneiro et al. 2014; Liu et al. 2014; Maeda 2015; Namazi et al. 2011; Namazi et al. 2012; Trimarco et al. 2012) and a complete (100% of recommended dietary allowances)
multivitamin-multimineral supplement. Patients were allowed to drink water or unsweetened drinks freely (not tea or coffee) during the day recommending minimum intake of 2 L/day. In patients with a history of kidney stones, the amount was increased to 3 L. To avoid unintended hypoglycemia and electrolyte imbalance, all treatments with hypoglycemic agents and diuretics were discontinued before starting the course. Anti-hypertensive medications and uric acid and lipid-lowering drugs were left unchanged.

Phase II (hypo-MD). During the hypo-MD phase, patients were prescribed a balanced hypocaloric diet providing 25–30 kcal/kg of ideal body weight per day (54% from carbohydrate, 18% of energy from protein and 28% from fat [saturated, <7%]). Particularly, the consumption of low glycemic index foods (www.glycemicindex.com) was encouraged by switching as much as possible to the use of whole foods. Patients were also asked to add more vegetables to their meals and to consume no more than two portions of fruit per day in order to increase fiber intake up to 30–35 g per day, without exceeding with the intake of simple sugars. The use of sources of vegetable proteins was also encouraged. Finally, olive oil was the main source of fats, whilst the intake of alcohol was not allowed. In addition, patients continued the administration of herbal remedies, as well as that of amino acids and whey proteins (only at breakfast; one 15 g sachet) in view of their emerging role in maintaining muscle mass during WL (Devries & Phillips 2015).

Assessments

Data on the following parameters were collected at baseline (day 0) and at the end of both DO and MD intervention phases (day 22 and day 64, respectively), before any pharmacological treatment was reintroduced.

Anthropometry

Body weight and standing height were measured by the same calibrated flat scale equipped with a telescopic, vertical steel stadiometer according to standard procedures (WHO 1995). Waist and hip circumferences were measured using a plastic flexible tape at the midpoint between the lowest rib and the iliac crest and around the largest portion of the buttocks, respectively. The BMI (weight [kg] and height [m] squared; kg/m²) and the waist/hip ratio (WHR) were calculated accordingly (WHO 1995).

Abdominal ultrasonography

We assessed the aorto-mesenteric fat thickness (AMFT), as surrogate measure of visceral adiposity, according to a previously validated procedure (Monaco et al. 2014). Patients were also screened for the presence of cholestatic liver disease (overt cholelithiasis or biliary sludge).

Hematology and Biochemistry

After an over-night fast (8–12 h), venous blood samples were drawn for the evaluation of the following parameters: glucose, insulin, C-peptide, glycated hemoglobin, growth hormone (GH), insulin-like growth factor 1 (IGF-1), blood urea nitrogen (BUN), creatinine, uric acid, total cholesterol, high-density and low-density lipoprotein cholesterol (HDL and LDL, respectively), triglycerides, aspartate and alanine aminotransferases (AST and ALT, respectively), gamma glutamyl transferase (γ-GT), and electrolytes (sodium and potassium). Insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) (Matthews et al. 1985). The triglyceride/HDL cholesterol ratio was also considered (McLaughlin et al. 2003).

Blood pressure

After having the patient seated for at least 5 minutes in a chair, systolic and diastolic blood pressure (SBP and DBP, respectively) were measured using appropriately sized sphygmomanometers with feet on the floor, and arm supported at heart level. Heart rate was also recorded. The average of three measurements, obtained at 2-minute intervals, was used for the analysis (Chobanian et al. 2003).

Urine sample spot checks

Patients were asked to check ketosis status (Accu-Chek Ketur-Test®; Roche Diagnostics, Indianapolis, IN) daily during the OD phase.

Metabolic syndrome diagnosis

The National Cholesterol Education Program’s Adult Treatment Panel III criteria were used to define metabolic syndrome (NCEP-III 2001). Accordingly, subjects had to have ≥3 of the following traits: (1) waist circumference >102 cm in men and >88 cm in women; (2) serum triglycerides ≥150 mg/dL and/or the use of lipid lowering medications; (3) HDL-cholesterol <40 mg/dL in men and <50 mg/dL in women and/or the use of lipid lowering medications; (4) blood
pressure ≥130/85 mmHg and/or the use of antihypertensive agents; and (5) fasting plasma glucose level ≥110 mg/dL.

**Study endpoints**

Changes (increase or reduction as appropriate) in the following study parameters were considered as efficacy outcome measures: body weight, BMI, WC, HC, WHR, AMFT, uric acid, glucose, insulin, HOMA-IR, C-peptide, glycosylated hemoglobin, GH, IGF-1, total cholesterol, LDL, triglycerides, triglyceride/HDL ratio, AST, ALT, γ-GT, blood pressure (SBP and DBP) and heart rate. The frequency of the following side effects was also recorded daily using a self-administered questionnaire: asthenia, headache, dizziness, fainting, orthostatic hypotension, heartburn, nausea, vomiting, palpitations, muscle cramps, hunger and constipation.

**Statistical analysis**

Due to the lack of preliminary data on a similar study design, the sample size was based on the change in efficacy outcome measures and was calculated to a priori according to a statistical power of 80%, an effect size of 0.5 (medium entity; considered as clinically meaningful) (Cohen 1992) and a one-tailed type-I error <1.7% (to account for the number of comparisons). Accordingly, at least 72 patients were required in the efficacy population to be analyzed.

Descriptive statistics of study variables were reported as mean and standard deviation or counts and percentage, as appropriate. Comparisons between genders at baseline were performed using the Fisher’s exact test (categorical variables) and the Student’s t-test (continuous variables). In primary analysis, changes in study parameters during each phase of the intervention were initially compared over time using a mixed model for repeated measure adjusted for age, gender, current smoking, menopause and pharmacological therapy (where appropriate). Then, we compared changes between patients presenting with metabolic syndrome versus those not presenting it (secondary analysis). Accordingly, we estimated fully-adjusted changes from baseline of the different study parameters using an analysis of covariance model and compared them using a mixed model for repeated measure. The interaction between time and group (presence of metabolic syndrome) was also investigated. All statistical analyses were performed using the software STATA version 12.0 statistical software (Stata Corporation, College Station, TX).

**Results**

In total, 73 patients (BMI [mean ± DS], 33.4 ± 6.3 kg/m² – range, 30.2–51.3 kg/m²) out of 82 screened were recruited. The main reasons of exclusion were: recent history of diet-induced WL, n = 5; and refusal, n = 3. Among the 32 female patients enrolled, 17 were in menopause. None of them was receiving oral contraceptives or hormone-replacement therapy. Active smoking status was reported by 16 patients. The baseline features of the study population are presented in Table 1. Particularly, male patients were characterized by higher abdominal adiposity, serum glucose, creatinine, uric acid, ALT, triglycerides and triglycerides-HDL.

**Table 1. Features of the study population by gender.**

| Characteristic                  | Overall* (N = 73) | Females* (N = 32) | Males* (N = 41) | p Valueb |
|--------------------------------|-------------------|-------------------|-----------------|----------|
| Age, year                      | 50.1 (10.8)       | 50.0 (9.9)        | 50.1 (11.5)     | 0.972    |
| Body weight, kg                | 91.2 (18.0)       | 83.2 (14.9)       | 97.4 (17.9)     | <0.001   |
| BMI, kg/m²                     | 33.4 (6.3)        | 34.1 (6.9)        | 32.8 (5.7)      | 0.385    |
| Waist circumference, cm        | 106.9 (13.2)      | 102.8 (12.8)      | 110.0 (12.7)    | 0.019    |
| Hip circumference, cm          | 112.0 (11.7)      | 114.9 (12.5)      | 109.8 (10.6)    | 0.061    |
| Waist-Hip ratio                | 0.95 (0.07)       | 0.89 (0.05)       | 1.00 (0.05)     | <0.001   |
| AMFT, mm                       | 18.1 (6.2)        | 14.0 (4.2)        | 21.3 (5.7)      | <0.001   |
| Urea, mg/dL                    | 34 (9)            | 35 (9)            | 32 (8)          | 0.091    |
| Creatinine, mg/dL              | 0.83 (0.16)       | 0.74 (0.12)       | 0.89 (0.15)     | <0.001   |
| Uric acid, mg/dL               | 5.7 (1.4)         | 5.0 (1.4)         | 6.2 (1.2)       | <0.001   |
| Glucose, mg/dL                 | 110 (29)          | 101 (16)          | 117 (35)        | 0.012    |
| Insulin, μU/mL                 | 13.5 (8.4)        | 12.7 (7.4)        | 14.2 (9.1)      | 0.472    |
| HOMA-IR                        | 3.8 (2.9)         | 3.3 (2.2)         | 4.3 (3.2)       | 0.115    |
| C-peptide, ng/mL               | 3.5 (1.2)         | 3.4 (1.3)         | 3.6 (1.2)       | 0.327    |
| Potassium, mEq/L               | 4.4 (0.3)         | 4.4 (0.3)         | 4.4 (0.4)       | 0.749    |
| Na, mmol/L                     | 147 (79)          | 123 (73)          | 165 (80)        | 0.024    |
| Total cholesterol, mg/dL       | 218 (32)          | 218 (33)          | 218 (33)        | 0.941    |
| HDL cholesterol, mg/dL         | 48 (12)           | 54 (12)           | 43 (10)         | <0.001   |
| Triglycerides, mg/dL           | 141 (30)          | 139 (31)          | 143 (30)        | 0.663    |
| Triglycerides - HDL ratio      | 3.5 (2.5)         | 2.6 (1.9)         | 3.6 (1.9)       | 0.003    |
| Sodium, mEq/L                  | 139 (18)          | 139 (20)          | 139 (16)        | 0.427    |
| Potassium, mEq/L               | 4.4 (0.3)         | 4.4 (0.3)         | 4.4 (0.4)       | 0.749    |
| AST, U/L                       | 22.4 (11.8)       | 19.9 (7.4)        | 24.3 (14.1)     | 0.106    |
| ALT, U/L                       | 28.6 (24.3)       | 21.4 (12.9)       | 34.2 (29.4)     | 0.015    |
| γ-GT, U/L                      | 28.1 (19.7)       | 26.7 (15.0)       | 29.1 (22.8)     | 0.586    |
| SBP, mmHg                      | 133 (10)          | 136 (110)         | 132 (10)        | 0.113    |
| DBP, mmHg                      | 80 (8.3)          | 81 (8.2)          | 79 (8.0)        | 0.346    |
| Heart rate, bpm                | 72.8 (2.5)        | 73.2 (2.6)        | 72.6 (2.4)      | 0.305    |

**Ethics**

The study was performed in agreement with the principles of the Declaration of Helsinki and the protocol was approved by the Ethics Committee of the A.O.R.N. “San Giuseppe Moscati”. Written informed consent was obtained from every patient.

**Table 1. Features of the study population by gender.**

Abbreviations: BMI: body mass index; AMFT: aorto-mesenteric fat thickness; BUN: blood urea nitrogen; HOMA-IR: homeostasis model assessment of insulin resistance; HbA1C: glycosylated hemoglobin; IGF-1: insulin-like growth factor 1; HDL: high density lipoprotein; LDL: low density lipoprotein; AST: aspartate amino-transferase; ALT: alanine amino-transferase; γ-GT: gamma glutamyl transferase; SBP: systolic blood pressure; DBP: diastolic blood pressure.

*Data are reported as mean and standard deviation (between parentheses).

bBetween gender at baseline.
Table 2. Anthropometric, clinical and metabolic features during the intervention period.

| Characteristic                  | Baselinea | Day 22a | Day 64a | p Valueb,c | p Valueb,d | p Valueb,e |
|--------------------------------|-----------|---------|---------|------------|------------|------------|
| Body weight, kg               | 91.2 (18.0) | 85.7 (17.3) | 81.0 (16.5) | <0.001     | <0.001     | <0.001     |
| BMI, kg/m²                     | 33.4 (6.3)  | 31.4 (6.0)  | 29.7 (5.5)  | <0.001     | <0.001     | <0.001     |
| Waist circumference, cm        | 106.9 (13.2) | 101.9 (12.6) | 97.6 (12.5) | <0.001     | <0.001     | <0.001     |
| Hip circumference, cm          | 112.0 (11.7) | 108.3 (11.3) | 104.6 (10.9) | <0.001     | <0.001     | <0.001     |
| Waist-Hip ratio                | 0.95 (0.07)  | 0.94 (0.07)  | 0.93 (0.07)  | <0.001     | <0.001     | <0.001     |
| AMFT, mm                       | 18.1 (6.2)   | 14.2 (5.3)   | 12.6 (4.7)   | <0.001     | <0.001     | <0.001     |
| BUN, mg/dL                     | 34 (9)       | 33 (9)       | 33 (8)       | 1.000      | 1.000      | 1.000      |
| Creatinine, mg/dL              | 0.83 (0.16)  | 0.85 (0.16)  | 0.81 (0.15)  | 0.075      | 0.649      | <0.001     |
| Uric acid, mg/dL               | 5.7 (1.4)    | 5.9 (1.3)    | 4.4 (1.1)    | 0.034      | <0.001     | <0.001     |
| Glucose, mg/dL                 | 110 (29)     | 94 (14)      | 100 (14)     | <0.001     | <0.001     | <0.001     |
| Triglycerides, mg/dL           | 147 (79)     | 95 (46)      | 110 (53)     | <0.001     | <0.001     | <0.001     |
| Potassium, mEq/L               | 4.4 (0.3)    | 4.4 (0.3)    | 4.0 (0.3)    | 1.000      | <0.001     | <0.001     |
| Sodium, mEq/L                  | 139 (1.8)    | 139 (1.9)    | 140 (1.9)    | 0.901      | <0.001     | <0.001     |
| ALT, UI/dL                     | 22.4 (11.8)  | 20.9 (7.7)   | 16.3 (5.1)   | 0.316      | <0.001     | <0.001     |
| γ-GT, UI/dL                    | 28.6 (24.3)  | 24.3 (13.8)  | 18.5 (7.5)   | 0.044      | <0.001     | <0.001     |
| LDL cholesterol, mg/dL         | 141 (30)     | 124 (28)     | 141 (30)     | <0.001     | 1.000      | <0.001     |
| HDL cholesterol, mg/dL         | 112.0 (11.7) | 108.3 (11.3) | 104.6 (10.9) | <0.001     | <0.001     | <0.001     |
| Total cholesterol, mg/dL       | 218 (32)     | 187 (31)     | 195 (34)     | <0.001     | <0.001     | <0.001     |
| Triglycerides - HDL ratio      | 3.5 (2.5)    | 2.3 (1.4)    | 2.4 (1.5)    | <0.001     | 0.339      | <0.001     |
| AST, UI/dL                     | 135 (8.4)    | 81 (4.8)     | 104 (4.4)    | <0.001     | <0.001     | <0.001     |
| ALAT, UI/dL                    | 174 (97)     | 95 (46)      | 110 (53)     | <0.001     | <0.001     | <0.001     |
| γ-GT, UI/dL                    | 28.1 (19.7)  | 20.2 (9.8)   | 21.0 (11.6)  | 0.002      | 0.004      | 0.659      |
| SBP, mmHg                      | 133 (10.5)   | 122 (7.5)    | 122 (7.4)    | <0.001     | <0.001     | 0.476      |
| DBP, mmHg                      | 80 (8.3)     | 72 (7.4)     | 72 (7.3)     | <0.001     | <0.001     | 1.000      |
| Heart rate, bpm                | 72.8 (2.5)   | 71.8 (2.3)   | 72.0 (3.0)   | 0.013      | 0.195      | 0.962      |

Abbreviations: BMI: body mass index; AMFT: aorto-mesenteric fat thickness; BUN: blood urea nitrogen; HOMA-IR: homeostasis model assessment of insulin resistance; HbA1C: glycosylated hemoglobin; IGF-1: insulin-like growth factor 1; HDL: high density lipoprotein; LDL: low density lipoprotein; SBP: systolic blood pressure; DBP: diastolic blood pressure.

aData are reported as mean and standard deviation (between parentheses).
bFor paired comparison of day 22 versus baseline.
cFor paired comparison of day 22 versus baseline.
dFor paired comparison of day 64 versus baseline.
eFor paired comparison of day 64 versus day 22.

ratio and lower HDL levels. Accordingly, we observed a slightly higher prevalence of metabolic syndrome (58.5% versus 50%; p value = 0.487) and a trend to more frequent diabetes (24.4% versus 9.4%; p = 0.128). A more evident impairment of GH-IGF-1 axis was also observed.

The intervention was well tolerated and no serious adverse event occurred. Throughout the treatment period, constipation and mild muscle cramps were the most frequently reported side effects (during OD, 19.2 and 17.8%, respectively). However, their frequencies were significantly reduced during the second phase of the study (11.0 and 2.7%, respectively). Also hunger was reported (12.3 and 2.7% during OD and hypo-MD, respectively). Other less frequent (<5%) side effects reported only during the first intervention phase were headache and palpitations. Overall, “no side effects” during OD and hypo-MD was reported by 39 (53.4%) and 63 (86.3%) patients, respectively.

As shown by results on body WL, compliance to the diet was high in both intervention phases, with all and 64 (87.7%) patients losing at the end of the 2-phase treatment more than 7.5 and 10% of the initial body weight, respectively. Biochemical parameters also confirmed the substantial safety of the intervention proposed. Particularly, no alteration (value outside the normal range of our laboratory) occurred in principal serum electrolytes, liver enzymes and kidney function parameters, although a slight increase in uric acid and serum was observed at group level during the OD phase (Table 2).

The OD phase was associated with a significant improvement in visceral adiposity, γ-GT, GH levels, blood pressure and in most metabolic parameters, particularly of those pertaining glucose and lipid metabolism; although an expected decrease in HDL cholesterol was recorded. Likewise, OD was responsible for a significant decrease in IGF-1. A complete restoration of IGF-1 to baseline levels was observed at the end of the hypo-MD phase, during which a reduction in GH to values in any case higher than baseline was also recorded. Hypo-MD was also responsible for a persisting positive effect on visceral adiposity and liver function enzymes and for a consistent reduction in uric acid. On the other hand, although the progressive
reintroduction of carbohydrates brought to the restoration of HDL, this phase of dietary intervention was associated with a significant re-increase in parameters of both lipid and glucose metabolism, while blood pressure was substantially stabilized.

Overall, the prevalence of metabolic syndrome at study entry was 54.8%. Both interventions resulted in a progressive, significant reduction in its frequency (versus baseline, $p = 0.017$ and $p = 0.007$ for OD and hypo-MD, respectively) and that of related diagnostic criteria (Figure 1). Particularly, the most relevant changes were observed during the OD phase although a significant trend was still observed for the hypo-MD phase. Interestingly, in secondary efficacy analyses (Table 3), it was observed that, independently of age, gender, current smoking, menopause, and pharmacological therapy the reduction in visceral adiposity and the improvement in glucose control-related variables (glucose, insulin, HOMA-IR, C-peptide, glycosylated hemoglobin, triglycerides, triglyceride/HDL ratio) and transaminases were even higher in patients with metabolic syndrome. Compared to those without, patients with metabolic syndrome showed a less evident increase and a higher decrease in uric acid during OD and hypo-MD, respectively. Furthermore, in patients with metabolic syndrome we observed a less evident decrease and a higher increase in IGF-1 and HDL during OD and hypo-MD, respectively.

Finally, in respect to the improvement of cardiometabolic profile, we report also that the use of oral hypoglycemic agents and the diuretic therapy was no longer deemed necessary. Likewise, most patients (79.5%, 35 out of 44 patients) required a consistent reduction in the dosage (pills/day) of anti-hypertensive therapy: baseline, 0.86 ± 0.84; end of OD, 0.55 ± 0.66 ($p < 0.001$ versus baseline); end of study, 0.45 ± 0.58 (versus baseline, $p < 0.001$; versus phase-1, $p = 0.016$).

**Discussion**

In this study, we investigated the effects of the adherence in sequence to two WL diets differing in macronutrients composition in obese patients. The intervention consisted in an initial 3-week protein-sparing, very low-calorie, ketogenic diet and a subsequent 6-week hypocaloric, low glycemic index, Mediterranean-like diet. Despite the different duration, the two phases of the dietary program resulted in a similar WL. This could be reasonably ascribed to the different calorie content of the diets (Dansinger et al. 2005; Franz et al. 2007; Gardner et al. 2007; Wycherley et al. 2012). However, we observed also different effects on body fat distribution and cardiometabolic parameters in the overall population and in patients suffering from metabolic syndrome.

Indeed, a positive outcome of this study was the compliance to the intervention with about 90% of patients losing at least 7.5% of initial body weight and none withdrawing the consent or lost to follow-up. The dietary program was also safe and well-accepted as a limited number of patients reported common side effects, particularly during the OD phase. This is in agreement with previous studies addressing also the safety and the feasibility of a protein sparing modified fast (PSMF) dietary intervention (Blackburn et al. 1975; Cappello et al. 2012; Sukkar et al. 2013; Paoli et al. 2013b; Leonetti et al. 2015; Castaldo et al. 2016, 2015a). No emotional disturbances were reported but previous studies of intensive WL have described an intensification of them, particularly of depression and anxiety (Stunkard & Rush 1974; Halmi et al. 1980).
Indeed, VLCDs have raised reasonable concerns in the past as dietary regimens with a calorie content below the basal metabolic rate could be responsible for malnutrition, in minerals and micronutrient in the short term and in all nutrients in the long term (Blackburn et al. 1975; Gardner et al. 2010). Besides, ketogenic regimens are known to potentially result in electrolyte imbalances and to increase uric acid (Blackburn et al. 1975; Atkinson 1986). However, these complications could be avoided using appropriate supplements and through the shortening of PSMF phase. Therefore, a sequential dietary rehabilitation program as the one proposed herein enables obtaining the metabolic benefits of ketogenic dietary regimens (Blackburn et al. 1975; Lim et al. 2011; Cappello et al. 2012; Malandrurcero et al. 2012; Sukkar et al. 2013; Paoli et al. 2013b; Leonetti et al. 2015; Castaldo et al. 2016, 2015a) but also to limit their potential adverse effects by early switching to a balanced and healthy dietary pattern which considers carbohydrates as the main source of calories.

OD resulted in a higher reduction in visceral adiposity than hypo-MD, which is consistent with available evidence on VLCD (Chaston & Dixon 2008). In addition, although a significant improvement of all cardiometabolic parameters was achieved at the end of the study, the reintroduction of about a 10-fold higher amount of high-quality carbohydrates was responsible for a relevant re-increase of blood glucose, insulin resistance and blood lipids. A higher intake of carbohydrates was also responsible for a reduction of GH. However, we observed a positive restoration of IGF-1 and HDL levels and a maintenance of improved blood pressure. Similar trends in blood glucose, lipids and pressure were recently observed by Paoli et al. (2013b) and Leonetti et al. (2015) when applying a ketogenic diet followed by a Mediterranean diet or a low-calorie diet with increasing content in carbohydrates. These observations clearly emphasize that in obese patients; weight excess-related complications not only depend on visceral fat distribution but also on diet composition and how macronutrients are metabolized even in the presence of a negative energy balance.

Nonetheless, the whole intervention resulted in a progressive reduction in the frequency of metabolic syndrome and the number of its criteria in both phases. In line with this, an interesting finding was the different effects produced by the two dietary regimens according to the presence of metabolic syndrome. In patients with metabolic syndrome, we observed a higher improvement of all cardiometabolic parameters and ALT and a reduction in visceral adipose tissue during both phases. These changes were even more consistent during OD. In addition, during

### Table 3. Changes in anthropometric, clinical and metabolic features according to presence of metabolic syndrome.

| Characteristic | Phase 1 – intervention | Phase 2 – intervention |
|---------------|------------------------|------------------------|
|               | MetS Change | MetS Change | p Value | MetS Change | MetS Change | p Value | p Value |
| Body weight, kg | $-5.6$ (0.2) | $-5.3$ (0.2) | $-10.5$ (0.76) | $-10.0$ (0.6) | <0.001 | 0.084 | 0.883 |
| Waist circumference, cm | $-4.8$ (0.3) | $-5.0$ (0.3) | $-9.3$ (0.4) | $-9.1$ (0.3) | <0.001 | 0.019 | 0.633 |
| AMFT, mm | $-3.4$ (0.3) | $-4.3$ (0.2) | $-4.8$ (0.3) | $-6.2$ (0.3) | <0.001 | 0.018 | <0.001 |
| Uric acid, mg/dL | 0.5 (0.1) | 0.03 (0.1) | $-1.1$ (0.1) | $-1.4$ (0.1) | <0.001 | 0.004 | 0.011 |
| Glucose, mg/dL | $-6.7$ (2.6) | $-22.8$ (2.4) | $-0.8$ (2.7) | $-17.6$ (2.4) | <0.001 | <0.001 | <0.001 |
| Insulin, µU/mL | $-3.7$ (0.9) | $-6.9$ (0.8) | $-1.0$ (1.0) | $-4.9$ (0.9) | <0.001 | <0.001 | 0.001 |
| HOMA-IR | $-0.9$ (0.3) | $-2.7$ (0.3) | $-0.2$ (0.3) | $-1.9$ (0.2) | <0.001 | <0.001 | <0.001 |
| C-peptide, ng/mL | $-0.6$ (0.1) | $-0.9$ (0.1) | $-0.4$ (0.1) | $-0.8$ (0.1) | <0.001 | <0.001 | 0.043 |
| HbA1C, % | $-0.2$ (0.1) | $-0.8$ (0.1) | $-0.1$ (0.1) | $-0.5$ (0.1) | <0.001 | <0.001 | <0.001 |
| Growth hormone, ng/mL | 1.02 (0.11) | 0.75 (0.10) | 0.19 (0.08) | 0.10 (0.07) | <0.001 | 0.117 | 0.124 |
| IGF-1, ng/mL | $-88.1$ (20.0) | $-36.5$ (18.1) | $-45.2$ (21.4) | $14.8$ (19.4) | <0.001 | 0.277 | 0.032 |
| Total cholesterol, mg/dL | $-28.8$ (2.9) | $-33.4$ (2.6) | $-18.6$ (3.6) | $-27.3$ (3.3) | <0.001 | 0.498 | 0.331 |
| HDL cholesterol, mg/dL | $-5.6$ (1.2) | $-2.2$ (1.1) | $-1.0$ (1.3) | $1.9$ (1.3) | <0.001 | 0.001 | 0.026 |
| LDL cholesterol, mg/dL | $-18.8$ (2.8) | $-16.4$ (2.5) | $-0.3$ (3.0) | $-0.4$ (2.7) | <0.001 | 0.934 | 0.645 |
| Triglycerides, mg/dL | $-23.2$ (6.7) | $-74.4$ (6.1) | $-13.3$ (6.4) | $-57.1$ (5.8) | <0.001 | <0.001 | <0.001 |
| Triglycerides - HDL ratio | $-0.4$ (0.2) | $-1.8$ (0.2) | $-0.4$ (0.2) | $-1.6$ (0.2) | 0.023 | <0.001 | <0.001 |
| AST, U/IUL | 0.9 (1.3) | $-3.4$ (1.2) | $-4.2$ (1.7) | $-7.7$ (1.5) | <0.001 | 0.038 | 0.123 |
| ALT, U/IUL | $-0.4$ (21.4) | $-7.6$ (2.2) | $-3.1$ (3.3) | $-15.9$ (2.7) | <0.001 | 0.088 | 0.008 |
| γ-GT, U/IUL | $-9.2$ (3.3) | $-6.6$ (3.0) | $-9.1$ (3.2) | $-5.4$ (2.9) | <0.001 | 0.081 | 0.872 |
| SBP, mmHg | $-13.5$ (1.1) | $-9.3$ (2.2) | $-19.2$ (3.1) | $-8.9$ (2.0) | <0.001 | 0.212 | 0.120 |
| DBP, mmHg | $-7.7$ (1.8) | $-8.9$ (1.7) | $-7.6$ (1.8) | $-8.8$ (1.7) | <0.001 | 0.118 | 0.830 |
| Heart rate, bpm | $-1.1$ (0.6) | $-1.0$ (0.5) | $-0.6$ (0.7) | $-1.1$ (0.6) | 0.009 | 0.425 | 0.632 |

Abbreviations: MetS: metabolic syndrome (MetS−: absent; MetS+: present); AMFT: aorto-mesenteric fat thickness; HOMA-IR: homeostasis model assessment of insulin resistance; HbA1C: glycosylated hemoglobin; IGF-1: insulin-like growth factor 1; HDL: high density lipoprotein; LDL: low density lipoprotein; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Data (changes versus baseline) are reported as mean and standard error (between parentheses) according to analysis of covariance model and compared between groups using a mixed model for repeated measure (models adjusted for age, gender, current smoking, menopause and concomitant therapy (as appropriate)).

p Value for time.

p Value for group (metabolic syndrome + versus metabolic syndrome).

p Value for interaction (time × group).
this phase, in contrast to all other participants, we assisted to a less evident increase in uric acid and decrease in HDL and IGF-1. Also the results sustains the metabolic benefit of a ketogenic diet as uric acid has been considered as an additional feature of metabolic syndrome (Billiet et al. 2014) and treatment with GH (resulting in higher IGF-1) has been associated with the reduction in visceral adiposity (Berryman et al. 2013).

Our results are consistent with the recent findings (Wing et al. 1994; Lim et al. 2011; Malandrucco et al. 2012) that short-term metabolic benefits of WL in patients characterized by insulin resistance are mainly related to calorie restriction rather than to an improvement of peripheral insulin sensitivity. Accordingly, all WL diets, regardless of their composition, could be effective in reducing cardiovascular risk both in the short and in the long term (Douyon & Schteingart 2002; Dansinger et al. 2005; Gardner et al. 2007; Wycherley et al. 2012; Kashyap et al. 2013). However, the more consistent improvements of obesity-related complications in patients with metabolic syndrome suggest that also extent of calorie deficit and how this is realized can actively play a role. A short initial cycle of ketogenic diet in insulin resistant patients exerts a positive effect on GH-IGF-1 axis, inducing lipolysis within visceral adipose tissue without impairing insulin sensitivity (Douyon & Schteingart 2002; Schugar & Crawford 2012; Kashyap et al. 2013). On the other hand, evidence from bariatric surgery studies suggest that also disruption of carbohydrates absorption also plays a role in the restoration of pancreatic β-cell function (Kashyap et al. 2013). Accordingly, a short-term protein-sparing, very low-calorie, ketogenic diet has to be preferred as first-line strategy at least in insulin resistant patients. Then, a dietary rehabilitation to a balanced diet could and should be considered in order to avoid a detrimental “yo-yo” effect (Cereda et al. 2011). Rehabilitation may take advantage from the transient post-diet hypophagia induced by ketosis (Honors et al. 2009; Gibson et al. 2015). Indeed, the necessity to achieve long-term WL maintenance is a priority and VLCDs have been found responsible for higher initial WL and, despite a more rapid weight regain, for a slightly better long-term result (Franz et al. 2007; Hemmingsson et al. 2012).

Some limitations of our study should be recognized. First, our study does not provide an answer to the still open question of how to achieve long-term WL maintenance, although behavioral interventions dealing with both diet and physical activity likely result in better outcome (Dombrowski et al. 2014). Second, it could be argued that the use of herbal remedies has biased the results. However, although based on the preliminary findings of some randomized trials (Lirussi et al. 2002; Asgary et al. 2004; Abidov et al. 2010; Cicero et al. 2012; Kianbakht et al. 2013) a contribution cannot be fully excluded, as reported above, changes in efficacy parameters were consistent with previous findings in terms of both direction and extent. Nonetheless, remedies have been used continuously during both the two phases of the study. Accordingly, a confounding effect on the independent impact of dietary interventions was unlikely but deserves further investigation.

Third, we have conducted a prospective observational study with no control group. A proof of concept study is an important step for designing new therapeutic strategies but a randomized trial is required to support the present findings. On the other hand, in this perspective, our study represents a valuable proof-of-concept study and has offered important suggestion on which WL strategy should be considered in patients with metabolic syndrome.

In conclusion, a dietary rehabilitation program consisting in a VLC ketogenic regimen followed by a balanced Mediterranean diet appeared to be a feasible, safe and efficacious program in reducing cardiovascular risk in obese patients, particularly in those suffering from complicated visceral adiposity and insulin resistance dyslipidemic syndrome. This study could be the basis for designing appropriate randomized trials, which should consider also long-term trends in body weight as relevant outcome.

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