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ORIGINAL ARTICLE

Natural vegan meal replacement: metabolic and oxidative modulation on overweight subjects

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Summary. *Background:* Obesity correlates with insulin resistance, dyslipidemia, oxidative stress, cardiovascular diseases. Meal replacement (MR) products offer a possible strategy for weight management and subsequent reduction in obesity-related risk factors. *Aims/Methods:* twenty-seven healthy overweight subjects (6M/21F, age range 21-70, BMI range 25.0-43.8 kg/m^2) were enrolled to test the efficacy of a MR supplement (GoJuvo®). Main aims: a) short-term: to compare glycaemic status and triglycerides levels at baseline (T0), after MR supplementation and after a standard Mediterranean meal taken 4 hours after MR; long-term: to evaluate, in 20 out of the 27 subjects, over three months (T1), the effects of the MR, b) taken together with a standard prescribed diet, and to compare it with the effects on the parameters of 20 controls only on the standard diet. Anthropometric parameters, lipid and glycaemia profile, oxidative status (oxidized LDL concentrations) and homocysteine metabolism were measured at T0 and T1. *Results:* Short-term aim: overweight/obese subjects' post-prandial MR insulin and triglycerides values were significantly lower than values after the standard meal (mean delta values p=0.0001 and p=0.006, respectively); long-term aim: at T1, subjects showed a significant improvement in anthropometric indices ($p<0.05$), a considerable decrease in ox-LDL levels and a significant improvement in Hcy metabolism ($p<0.001$). *Conclusions:* GoJuvo® modulated metabolic indices associated with the development of type 2 diabetes, possibly due to its fibre content. Subjects at T1 showed a significant decrease in homocysteinemia with an increase in folate levels and an anti-oxidative action on lipid peroxidation, most likely contributing to a reduction in cardiovascular risk.

Key words: obesity, oxidative status, meal replacement, fibre, glycaemic index, insulin resistance

Introduction

Abdominal obesity, an important cause of morbidity, disability and premature death, is implicated in the development of several chronic diseases (type 2 diabetes mellitus, insulin resistance, hypertension, dyslipidemia, inflammation, coronary heart disease) all leading to cardiovascular diseases (1–3).

Postprandial hyperglycaemia is an important risk factor of cardiovascular morbidity and mortality. Consistently, acute hyperglycaemia can have adverse effects on the arterial wall through a number of mechanisms, including increased Oxidative Stress (OS), endothelial dysfunction, and coagulation cascade activation (4, 5). However, an underlying condition of systemic oxidative stress may hamper weight loss despite diet and physical exercise (6). In fact, it is often reported that obesity is characterized by increased OS (an imbalance between Reactive Oxygen Species, ROS, not counterbalanced by an adequate Total Antioxidant Capacity, TAC), one of the possible mechanisms linking obesity to cardiovascular diseases (2, 7, 8).

Several components of the atherosclerotic plaque are themselves able to generate reactive oxygen species thus sustaining chronic oxidative damage to biological structures (8). Serum oxidized low-density lipoprotein (oxLDL), a marker of lipoprotein-associated oxidative stress, involved in atheromatous plaque formation (9) and associated with cardiovascular diseases, is an innovative additional cardiovascular risk factor (8, 10).

Moreover, also hyperhomocysteinemia (HHcy) has been associated with several diseases, e.g. cardiovascular disease (11), Alzheimer's disease and other forms of dementias, peripheral neuropathy (12), renal failure (13, 14) and hypothyiroidism (15) and, recently, with the enhanced inflammatory activation and mechanisms triggering autoimmunity (16, 17). Homocysteine (Hcy) metabolism is catalysed by enzymes requiring B vitamins as cofactors and, if levels of vitamins metabolically related are inadequate, HHcy can have a pro-oxidant effect causing or promoting oxidation (11). However, various polyphenolic antioxidants can inhibit the oxidative damage induced by Hcy or Hcy-thiolactone (18).

On the other hand, diet is the most important factor, which can have a direct impact on several pathological conditions such as obesity, diabetes and cardiovascular disease. Particularly, dietary fibre has notable health implications in weight control and has been long associated with a lower risk for chronic diseases (19–21). In fact, soluble dietary fibre influences postprandial glycaemia and insulinemia, has hypolipidemic effects and can affect food intake by modulating the production of gut hormones involved in signalling satiation (such as ghrelin, glucagon-like peptide-1, cholecystokinin, peptide YY) (20, 22).

Meal Replacement (MR), within a structured dietary plan, is a viable and potentially cost-effective solution for weight management and glycaemia status control, in order to reduce obesity-related risk factors (23, 24). MR is defined as a commercially available product, fortified with minerals and vitamins, designed to replace one or two meals per day with at least one meal consumed as normal food (25). The average calories per portion range from 200 to 300 kcal (837-1255 kJ) and the average composition is the following: 50% of carbohydrates, 20-25% of proteins and 25-30% of fat.

The present study had two aims.

Short-term aim: was achieved by comparing, in 27 overweight subjects, the glycaemia status and triglycerides levels at baseline (T0), 2 hours after MR supplementation (T0a) and 2 hours after a standard, isocaloric Mediterranean meal taken 2 hours after T0a (T0b) as shown in Figure 1.

Long-term aim: was achieved by evaluating, in 20 subjects (out of the 27), over three months (T1), the

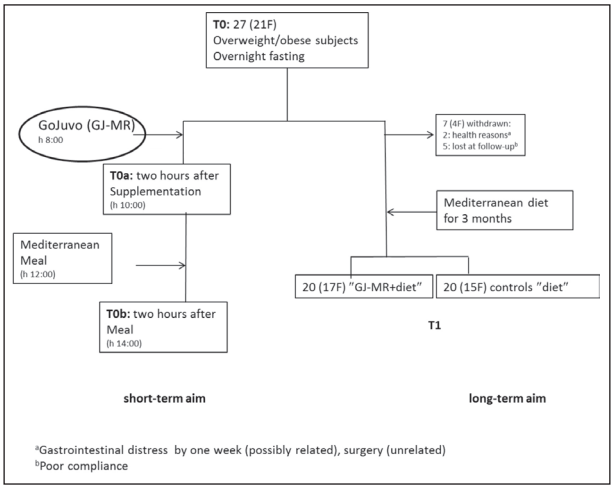

Figure 1. Trial participant flow. The attribution of adverse event association with subjects is specified within the parentheses

effects of the MR, taken together with a standard prescribed diet, on anthropometric parameters, biochemical indices (lipid and glycaemia profile), oxidative status and Hcy metabolism and by comparing them with the effects on the same parameters of 20 overweight controls only on the standard diet.

Materials and methods

Study population

Eligible participants were recruited at Ambulatorio Obesità e Lavoro, Clinica del Lavoro "Devoto", Fondazione IRCCS, Cà Granda Ospedale Maggiore Policlinico, Milano (Italy). In order to lose weight, 27 healthy overweight subjects (6M/21F, aged 48.3±11.3) were enrolled for the short-term aim of the study.

As shown in Figure 1, out of the 27 participants, 20 (4M/16F, aged 50.4±11.5) completed the 12-week supplementation period, while 7 dropped out (26%). Participants were instructed to take MR (GoJuvo, see details in the following section) together with a standard prescribed diet for weight management. Moreover, 20 healthy overweight subjects (5M/15F, aged 53±11) were enrolled as controls and told to take only the standard prescribed diet.

During an initial outpatient clinic examination, all participants were first interviewed about general health, habitual dietary intake, and lifestyle (i.e. family history, smoking, alcohol consumption, kind of work, educational and socioeconomic status, physical activity and exercise level) from a self-reported questionnaire. Exclusion criteria included: a history of Metabolic Syndrome, chronic or current health disorders, renal dysfunction, unstable psychiatric disorders (anorexia nervosa, bulimia nervosa), alcohol abuse, pregnancy, lactation, chronic use of prescription drugs or concurrent use of anti-obesity agents, a history of gastrointestinal tract surgery or very low calories diet (with the aim of losing weight) within the past 6 months and experiencing a hypersensitive reaction to certain foods or medications.

All participants, enrolled on a voluntary basis, followed the same study protocol and were asked to maintain habitual lifestyle and daily habits and provided written informed consent prior to participating in this investigation. This study was conducted according to the Good Clinical Practice guidelines and approved by the Human Ethic Committees of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan (2011, number: 852).

Product and intervention

GoJuvo® (GJ) (Erom Inc., La Mirada CA, USA) is a commercially available product consisting of plant-based powder concentrates made of 60 ingredients including whole grains (60% of brown rice), mushrooms, vegetables, seaweeds, fruits and berries. GJ, produced by freeze-drying, doesn't contain additives, animal proteins, or sweeteners; all the plants are organic non-GMO crops.

One GJ-MR (40 g mixed with 250 mL of plain water) provides 140 kcal (585 kJ) (85.7% of carbohydrates and 11.4% of proteins) and each portion has 1500 ORAC (Oxygen Radical Absorbance Capacity) which represents about 50% of the recommended daily intake (3000 ORAC/day).

Short-term aim: to compare the effects of GJ-MR on glycaemia status and triglycerides levels, the 27 subjects underwent venipuncture for blood collection at baseline, after over-night fasting (T0); then took one GJ-MR and underwent a second venipuncture two hours later (T0a). After 2 hours, they had a Mediterranean meal (60% carbohydrates, 25% fat, 15% proteins; 800-900 kcal; 3347-3765 kJ) and blood was collected again two hours later (T0b; 6 hours after GJ-MR).

Long-term aim: to evaluate over three months the effects of GJ-MR on body weight and biochemical indices, the compliant 20 subjects were told to take GJ together with the standard prescribed diet (52% carbohydrates, 28% fat, 18% proteins, 2% fibre, cholesterol<200 mg) according to a structured dietary plan ("GJ-MR plus diet"); each subject's caloric need was calculated as 25 kcal/Kg (105 kJ/Kg) per suitable weight. For 4 weeks, lunch consisted of one GJ-MR while, for the following 8 weeks, it was a partial GJ-MR (20 g mixed with 250 mL of plain water) together with one portion of fruit or vegetables (Tables 1 and 2). The consumption of fruit or vegetables at lunchtime was suggested to comply with fruit and vegetables intake WHO guidelines (≥ 400g/die) (26). The 20 controls were told to take only the standard prescribed diet, for the same period ("diet").

Table 1 Example of 1500 kcal classic diet prescribed for the first 4 weeks of GoJuvo meal replacement

Sweet breakfast: 250 g skimmed milk 40 g jam, 30 g wholemeal bread	Salted breakfast: 80 g wholemeal bread with 60 g Parma ham or bresaola
Break: skimmed yogurt 150 g	Break: 150 g skimmed yogurt
Lunch: 40 g GoJuvo® in 250 ml of orange juice	Lunch:40 g GoJuvo® in 250 ml of orange juice
Salted break: 30 g wholemeal bread with 30 g Parma ham or bresaola	Sweet break: 25 g biscuits
Dinner: 60 g whole wheat pasta, 160 g Fish or poultry or veal meat Vegetable without fixed quantity 400 g Fruit 50 g Wholemeal bread and 30 g olive oil	Dinner: 60 g whole wheat pasta, 160 g Fish or poultry or veal meat Vegetable without fixed quantity 400 g Fruit 50 g Wholemeal bread and 30 g olive oil

Table 2 Example of 1600 kcal Vegetarian diet prescribed for the first 4 weeks of GoJuvo® meal replacement

Breakfast: 250 g skimmed milk 40 g jam, 30 g wholemeal bread	Breakfast: 250 g skimmed milk 40 g jam, 30 g wholemeal bread
Break: 150 g skimmed yogurt and 200 g fruit	Break: 150 g skimmed yogurt and 200 g fruit
Lunch: 40 g GoJuvo® in 250 ml orange juice	Lunch: GoJuvo® in a orange juice
Break: 40 g wholemeal with 20 g jam without sugar	Break: 40 g wholemeal bread with 50 g cottage cheese
Dinner: 80 g whole wheat pasta,	Dinner: 70 g whole wheat pasta,
130 g Mozzarella cheese	Legumes: 150 g soja beans, beans, green peas ...
Vegetable without fixed quantity	Vegetable without fixed quantity
200 g Fruit	200 g Fruit
50 g Wholemeal bread and 15 g olive oil	50 g Wholemeal bread and 30 g olive oil

All participants' biochemical parameters were assessed at baseline (T0). As regards short-term aim, concentrations were assessed after GoJuvo® administration (T0a) and after a standard Mediterranean meal (T0b) while, for long-term aim, all subjects' levels were assessed after 12 weeks (T1).

Compliance with study protocol was self-reported and, more accurately, by controlling returned product at the final appointment.

Biochemical analysis

Peripheral blood samples were drawn at T0, T0a, T0b and T1, and collected into pre-evacuated and light-protected tubes, without any additive or with EDTA, to evaluate lipid panel, glycaemia status, oxidative status (ROS; TAC; oxLDL), and homocysteine metabolism (total Hcy, tHcy; vitamin B12, B12; serum folate, s-Fol; erythrocyte folate, ery-Fol). Serum aliquots were used to measure lipid profile (total cholesterol, t-C; high-density lipoprotein cholesterol, HDL-C; low-density lipoprotein cholesterol, LDL-C; triglycerides, TGs); insulin; ROS, TAC, oxLDL; B12 and s-Fol concentrations. EDTA whole blood was used to assess complete blood count (CBC), glycated haemoglobin (HBA1c), ery-Fol levels while a quota was put immediately in ice and centrifuged within 30 minutes to obtain plasma for tHcy determination. A sample with potassium oxalate and sodium fluoride EDTA ($KF+NA_2$ EDTA) was used to assess glucose concentrations.

All the aliquots, except for the one used for blood counting, were immediately frozen and stored at -80° C ready for assay.

Homeostasis model of assessment (HOMA) was used as a surrogate marker of insulin resistance (IR) and calculated as fasting serum insulin (μIU/mL)×fasting serum glucose (mmol/L)/22.5. HOMA index ≥2.5 was considered to provide an IR diagnosis, according to Bruneck study (27).

Lipid panel and glycaemic status were measured on the routine automated analyser Modular Analytics (Roche, Swiss), while HBA1c was assessed by using HPLC method (Variant™ II Turbo, BIORAD, CA, USA).

Serum ROS concentrations and TAC were measured by spectrophotometric method using a commercial kit (dROMs and OXY-Adsorbent test, respectively, Diacron International, Grosseto, Italy) on F.R.E.E. analyzer (Diacron), as previously described (12, 13).

Serum oxLDL levels were measured by a commercial Enzyme-Linked-Immunoassorbent Assay (Oxidized LDL ELISA, Mercodia, Uppsala, Sweden) on an EASIA reader (Medgenix Diagnostics, Fleurus, Belgium), according to the manufacturer's instructions (28). The NDA panel considers oxLDL, as measured by the Mercodia Oxidized LDL ELISA, a reliable marker of lipid oxidative damage (10, 17, 29).

Total plasma Hcy levels were measured using Homocysteine liquid enzymatic assay (Sentinel Diagnostics, Milan, Italy) on Modular P analyser (Roche Diagnostics, Indianapolis, IN, USA). Serum B12, s-Fol and ery-Fol concentrations were determined using the relevant Abbott Microparticle Enzyme Immunoassay (MEIA) kits (Abbott Laboratories, Abbott Park, IL, USA) on Architect analyser (Abbott) (13, 14).

Moreover, also at T0a and T0b triglycerides levels and glycaemic status were evaluated on the suitable blood samples.

Statistical analysis

Continuous variables are reported as mean ± standard deviation. For analysing T0 versus T0a and T0b, Kruskall-Wallis test for repeated measure was used. T0 versus T1 comparisons were conducted by Mann-Withney test. The correlations between some anthropometrical characteristics and biochemical parameters were calculated by regression analysis.

Statistical significance was settled at a p-value <0.05. Statistical analyses were performed using the statistical software MedCalc (30).

Results

Short-term aim. Table 3 shows the findings regarding the 27 overweight subjects: comparing a standard Mediterranean meal with GoJuvo® MR, significant differences were found in mean delta values percentage of glycaemic status parameters and triglycerides' levels; notably, while mean glucose levels did not differ significantly at T0a and T0b when compared to fasting levels, mean insulin concentrations were above the upper limit of reference value at T0b but within the reference interval at T0a. Moreover, mean triglycerides levels were significantly higher at T0b than at baseline and T0a.

Long-term aim. At baseline, both 20 subjects and 20 controls' anthropometric and biochemical parameters were almost comparable, as shown in Table 4 (T0). All participants had adequate hematologic status, based on the results of a standard CBC panel, and normal renal function (data not shown). Moreover, at baseline, both populations had very similar characteristics equally distributed, including:

- Lipid panel: a slight alteration in parameters was observed; particularly, both t-C and LDL-C concentrations were above cut-off value in 48% of the cases, TGs only in 4% but HDL-C levels were altered in 89% of the cases.
- Glycaemic status: even though only 7% of our population had both insulin and glucose concentra-

Table 3. Glycaemia status and triglycerides levels at baseline (T0), two hours after GoJuvo® administration (T0a) and two hours after a standard Mediterranean meal (T0b) taken two hours after T0a in the 27 subjects enrolled for the short-term aim of the study. Data are expressed as mean±SD [mean delta values percentage of parameters'changes].

Analyte (reference interval)	T0 (6M/11F)	T0a (6M/11F)	T0b (6M/11F)
glucose	92.2±19.6	88.3±11.4	95.5±22.3
(70-110 mg/dL)		[-4.3%]	[4.4%] *
insulin	10.9±7.3	11.2±13*	48.2±59.4#
(2.6-25 μIU/L)		[2.1%]	[306%]*
triglycerides	93.7±38.6	89.2±35.4°	119.2±47.5§
(<170 mg/dL)		[-5.8%]	[37.6%]*

**T0a vs T0b p=0.004; # T0 vs T0b p=0.006 ; °T0a vs T0b p=0.0001 ; §T0 vs T0b p=0.003 (Student's t test for pair data).*

Table 4. Anthropometrical characteristics and biochemical parameters of the 40 subjects enrolled for the long-term aim of the study: at baseline (T0) and after three months (T1) of GoJuvo meal replacement and prescribed diet ("GJ-MR plus diet") in 20 overweight subjects and after only the 3-months prescribed diet ("diet") in 20 overweight controls. Data are expressed as mean ±SD.

Parameters (reference interval or cut-off)	**Subjects (4M/16F)**		**Controls (5M/15F)**	
	T0	**T1**	**T0**	**T1**
weight (Kg)	83.88±13.2	79.17±11.9*	83.1±12.7	81.5±12.4
BMI (<25 Kg/m2)	30.98±5.0	29.01±4.3*	31.1±4.8	30.5±3.4
waist circumference (M<102 cm; F<88cm)	M: 111.5±4.9 F : 96.7±13.8	M: 108.0±8.5 F : 92.1±12.1§	M:103.8±12.3 F : 94.6±10.9	M:102.3±7.3 F : 93.3±9.0
glucose (70-110 mg/dL)	91.1±24.1	89.2±15.0	94.8±17.4	93.8±12.2
insulin (2.6-25 μIU/L)	12.2±8.5	12.5±6.3	10.9±7.3	10.28±4.03
glycated haemoglobin (20-42 mmol/mol)	33.48±4.34	33.48±3.72	35.96±3.1	35.89±3.2
HOMA (<2.5)	3.0±2.9	2.85±1.8	2.62±1.7	2.43±1.2
total Cholesterol (<200 mg/dL)	200.1±32.9	202.1±30.8	207.1±37.2	205.2±38.1
LDL Cholesterol (<130 mg/dL)	126.8±27.6	128.2±23.9	128.3±27.3	125.2±38.5
HDL Cholesterol (M>55mg/dL; F>65mg/dL)	M:40.0±3.5 F:53.8±12.5	M:38.7±4.2 F:55.2±11.4	M:43.1±2.3 F:50.0±10.4	M:42.4±4.2 F:51.3±13.2
triglycerides (<170 mg/dL)	98.1±35.7	99.1±47.4	104.07±45.8	102.11±58.4
d-ROMs (<300 UCarr)	362.6±72.0	365.5±54.4	373.6±60.5	378.5±75.5
TAC (>350μmolHClO/mL)	381.3±66.1	380.4±65.3	385.3±70.7	381.4±72.3
oxLDL (<70 U/L)	60.4±15.1	56.5±7.7#	68.86±21.1	67.04±16.2
s-Folate (7-28 nM)	11.05±2.33	14.48±3.40*	12.09±5.98	13.89±6.06
Ery-Folate (421-1462 nM)	514.75±158.62	692.28±225.21§	416.29±72.37	472.74±84.23
Homocysteine (<10,5 μM)	13.70±5.15	10.3±1.60*	13.34±5.52	12.77±4.51

**p<0.001, §p=0. 005, #p=0.09 T1 vs T0 (Student's t test for pair data)*

tions above the upper reference limit, 19% of subjects showed a pre-diabetic condition, (according to American Diabetes Association Consensus Statement), and 30% had HOMA above cut-off value. In both groups HOMA correlated significantly with BMI (r=0.55, p=0.01), WC (r=0.52, p=0.04) and HbA1c (r=0.58, p=0.01); oxLDL levels showed a positive correlation with t-C and TGs (r=0.49, p=0.03; r=0.60, p=0.008, respectively) and a positive trend only with LDL-C (r=0.43, p=0.07).

- Oxidative status: ROS levels were high in 73% of subjects despite adequate TAC in 67% of cases; 33% of subjects had oxLDL concentrations above the cut-off.
- Hcy metabolism: 30% of subjects showed slight hyperhomocysteinemia, although in the presence of a normal status of related vitamins.

After three months' "GJ-MR plus diet", subjects' anthropometric parameters improved significantly; in fact, BMI and females' WC decreased significantly (p<0.001 and p<0,005, respectively). No significant

change in glycaemic status was observed. Notably, after GJ-MR, 10% of subjects with baseline altered levels of glucose and HbA1c showed a significant ($p<0.001$) reduction in glucose (144.5±47.4 mg/dL *vs* 112.5±9.2 mg/dL, respectively), and about 35% showed a reduction in HbA1c (43.2±1.4 vs 40.9±2.1 mmol/mol, respectively). In addition, at T1, HOMA decreased, even if not significantly so, and did not correlate with anthropometric parameters and HbA1c. A significant decrease in BMI ($p=0.003$) was recorded in the control group ("diet") whereas all other metabolic parameters improved slightly, as expected from a well-planned Mediterranean diet.

As regards lipid panel, no significant differences were observed either after "GJ-MR plus diet" or "diet" because, at baseline, the majority of subjects showed t-C, LDL-C and TGs mean levels under cut-off value. Ninety % of subjects showed only altered HDL-C concentrations at T0 and unchanged at T1.

Interestingly, only in "GJ-MR plus diet" group, the percentage of subjects with oxLDL altered values decreased, even if not significantly so, from 30% to 5% ($p=0.09$); in addition, this group's oxLDL correlated only with TGs levels ($r=0.63$, $p=0.003$). Moreover, a significant improvement in Hcy metabolism was observed with a significant decrease in Hcy levels ($p<0.001$) and a significant increase in both folate concentrations ($p<0.001$). Vitamin B12 concentrations were within the reference interval in all the subjects at baseline and at T1 (data not shown).

All participants completed the 12-week supplementation period with high compliance (≥95%).

Discussion

Overweight/obesity is a common condition in the "western" world population and can lead to long-term health impairment (1-5,8).

Diets with relatively high glycaemic index (GI) and glycaemic load (GL) are associated with an elevated risk of coronary heart disease, stroke, and type 2 diabetes, particularly among overweight/obese subjects (31, 32). Therefore, the use of low-glycaemic index diets could be a preventive dietary strategy aimed at improving both diabetes control and cardiovascular risk factors.

The short-term aim of the present study was to evaluate and compare overweight subjects' changes in glycaemic status and triglycerides' levels after a GJ-MR (a plant-based powder concentrate primarily made of whole grains) and after a standard Mediterranean meal.

Our findings showed that MR metabolic modulations were better than post-meal ones as regards TGs, glucose and insulin levels. Interestingly, even if glucose values were within the reference interval after both GJ-MR and the standard Mediterranean meal, insulin concentrations were within the reference interval only after GoJuvo®.

Giacco *et al.* reported that lower plasma glucose and insulin responses have been observed in both diabetic and non-diabetic subjects after the ingestion of a low GI diet compared with a high GI diet (1, 21). In agreement with these findings, our study suggests the positive effect of GJ-MR, which, in a short time, mitigates the insulin resistance, and could prevent and/or reduce the risk of type 2 diabetes developing.

Our results could probably be explained by the low GI of brown rice contained in GoJuvo® (60%); in fact, whole-grain products, thanks to their physical form and high content of fibre (which binds water and forms a viscous solution) tend to be slowly digested and absorbed and, consequently, have relatively low GI (32–34). In fact, after GJ-MR consumption our study population, self- reported hunger control and a sensation of satiety.

The long-term aim of our study was to investigate the effects of 3 months' MR with GoJuvo® plus a standard prescribed diet ("GJ-MR plus diet") on overweight/obese subjects by evaluating anthropometric indices, glycaemic status, lipid panel, oxLDL levels and homocysteine metabolism at T0 and at T1. These findings were compared with those of controls, who took only the standard prescribed diet ("diet") for the same period.

Individuals with a central deposition of adipose tissue (visceral fat) can experience elevated cardiovascular morbidity and mortality (1, 2); weight loss has been proved to be essential for prevention, and treatment of obesity-related chronic diseases (35, 36).

Formula MR, designed for weight loss, represents a possible strategy for some individuals and is

ever more frequently prescribed in medical practice. Recently, meal replacements within a structured dietary plan were successful for short and long term weight reduction among overweight patients in order to mitigate diabetes and cardiovascular disease risk. In agreement with Hamdi *et al*. (24), after three months' "GJ-MR plus diet", we found a significant decrease in BMI and WC in about one third of the subjects (Table 2).

According to other authors (22–24), the significant improvement in anthropometric parameters was probably due to the presence of brown rice, which, because of its content of dietary fibre, influences body weight by multiple mechanisms depending on hormonal effects and intestinal fermentation.

Moreover, the consumption of low GI foods or meals has a higher satiating effect than those with high GI. The lower rate of nutrient digestion and absorption, typical of low GI foods, seems to stimulate the gastrointestinal receptors triggering satiety signals for a longer time and protracting the effects on hanger/satiety centres (21).

In the present study, no improvement was observed in glycaemic status, probably because all the participants' baseline glucose, insulin and HbA1c mean levels were within the reference interval or under the cut-off value. Notably, subjects with T0 altered levels of glycaemia and HbA1c (10% and 35%, respectively) showed that these parameters had really improved, even if not–fully normalized, after three months of "MR plus diet" compared to controls merely on the "diet".

HOMA was also calculated to delineate the balance between hepatic glucose output and insulin secretion (37). At baseline, GJ-MR subjects' HOMA correlated significantly with HbA1c; the positive association was however lost at T1 because GJ-MR (characterised by a low GI) can probably determine a reduced insulin demand and an improvement in glycaemia status.

In addition, baseline positive correlation between HOMA and anthropometric parameters confirmed obesity as a condition associated with insulin resistance, a precursor of type 2 diabetes and cardiovascular diseases (38-40). At T1, even if mean HOMA value decreased, no correlation was observed between HOMA and WC and BMI. The hormonal effects of fibre (decreasing insulin secretion, reducing the risk of reactive hypoglycaemia during the post-absorption period, promoting satiety, increasing fat oxidation and decreasing fat storage) could be a possible explanation according to previous data (21).

No significant differences in lipid profile were found because the majority of our subjects showed baseline t-C, LDL-C and TGs mean levels under cut-off value probably thanks to the Mediterranean diet (41, 42). However, the majority of subjects (89%) showed altered HDL-C concentrations at T0 and no improvement at T1 probably because of insufficient physical activity.

Recently, other less conventional risk factors have been postulated as different and/or additional mechanisms linking obesity to inflammatory condition (42). In our study, oxidative stress was thought to be a potential pathogenic mechanism linking obesity to endothelial dysfunction (2, 16, 17). As described in Table 2, most of these overweight subjects showed an oxidative stress condition due to elevated ROS concentrations not adequately balanced by a total antioxidant barrier. In the majority of cases (65%) TAC was adequate presumably thanks to the subjects' dietary habits (Mediterranean diet). Interestingly, as previously reported by us (42), oxidative stress seems to be an independent risk factor in obese subjects; in fact, ROS values correlated neither with BMI nor with lipid panel parameters.

Increased ROS concentrations contribute to enhanced oxidation of low-density lipoprotein, inactivation of endothelium-derived nitric oxide and vascular dysfunction (43). oxLDL, therefore, plays an important role in atherogenesis leading to increased risk of cardiovascular complications and can predict myocardial infarction in healthy elderly people (even after adjusting for age, gender, race smoking and metabolic syndrome) (43). At baseline 30% of our cases showed altered oxLDL levels, and lipid peroxidation was more advanced in subjects with serious dyslipidemia. In fact, oxLDL correlated with all lipid panel parameters except for HDL-C, as expected. At T1, 33% of subjects had increased oxLDL levels probably associated with an oxidative stress condition. Notably, the percentage of oxLDL altered values decreased, even if not

significantly so, only among the "GJ-MR plus diet" group showing an improvement in oxidative status. ROS production, a transient physiological condition of living organisms, can be neutralized by the cellular, endogenous and exogenous antioxidant system. ROS high levels damage biomolecules (nucleic acids, proteins and lipids) leading to a condition defined oxidative stress; therefore, a supplementation with antioxidant nutrients is required to prevent cellular modifications. If antioxidant systems do not deactivate ROS, they can react with cellular macromolecules and enhance the process of lipid peroxidation. Chronic oxidative stress condition, in fact, could cause irreversible damage to cellular homeostasis (5, 9). In this scenario, a diet rich in vegetables is naturally involved in weight control and prevention of several chronic diseases (41, 42). In particular, whole grains contain many antioxidants, including vitamins (e.g. vitamin E), trace minerals (e.g. selenium) and non-nutrient phytochemicals (e.g. phytic acid) (44). Particularly, vitamin E, an intracellular antioxidant removed in the refining process, prevents polyunsaturated fatty acid oxidation in cell membranes; selenium, a further component removed in the refining process, is a glutathione peroxidase cofactor; phytic acid chelates various metals by suppressing Fe-catalysed redox reaction damage (44–46).

In addition it has been reported that the total antioxidant activity of whole grain products and fruits and/or vegetables are similar (46). The ability of fruit and vegetables to protect against chronic disease is well documented (45). A diet rich in fruit and vegetables contains a variety of bioactive antioxidant components (vitamins, sterols, phenols, and flavonoids), micro- and macro-nutrients and fibers that may favor biological functions, help control, and prevent several chronic diseases (47, 48). Dietary intake of fruits and vegetables rich in nitrate/nitrite is an easily-practicable way to prevent insulin resistance and vascular endothelial dysfunction by increasing the NO availability. Accumulated evidence has shown that a NO-rich diet may also prevent other lifestyle-related diseases, including osteoporosis, chronic obstructive pulmonary disease (COPD), and cancer (45).

Jang *et al.* (33) reported the antioxidant properties of whole grain describing the significant decrease in malondialdehyde levels, a marker of lipid peroxidation, in patients with coronary artery disease after the consumption of whole grain and legume powder. In agreement with *Jang et al.* (33) the whole grain contained in GoJuvo® has shown antioxidant properties as illustrated by the ORAC score and the reduced rate of LDL oxidation.

In the present study, some findings supported the interesting differences between the "GoJuvo® MR plus diet" and the "diet" subjects. As expected, only subjects taking GoJuvo® supplement plus diet, but not controls, showed a significant increase in Folate levels (both serum and erythrocyte, $p<0,001$ and $p<0.005$, respectively) and a significant decrease in Hcy concentrations ($p<0,001$). Notably, high Hcy levels normalized only in subjects on the "GJ-MR plus diet", which helped control pro-oxidant molecule production. In fact, homocysteine, is the crucial aminothiol for biosynthesis of other aminothiols (methionine and cysteine) and for cell red-ox balance (glutathione biosynthesis, the most important intracellular antioxidant and most specific and sensible index of cell status). A lack of Vitamin B12 and/or Folate leads to HyperHcy, responsible for macrocytic anaemia and irreversible neurological damage, also implicated in the pathogenesis of atherosclerosis and considered an independent marker for ischaemic stroke (18).

A limitation of the present study is that obese subjects were recruited at Centro Obesità e Lavoro, where more women than men are examined as women are, in general, more concerned about their appearance than men seeking out personalized diet protocols (49).

In conclusion, our data showed that GoJuvo® MR, thanks to its high content of whole grain, plays an important role in controlling hunger, satiety and calories better than a traditional meal.

Moreover, the three months' "MR plus diet" (GoJuvo® associated with a structured dietary plan) acts on body weight regulation and mitigates metabolic indexes associated with the possible development of type 2 diabetes and cardiovascular diseases. Interestingly, the beneficial effect of whole grain consumption on lipid peroxidation and homocysteine metabolism through anti-oxidative action may represent a further protective mechanism against cardiovascular diseases.

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