Endocrine, Metabolic & Immune Disorders - Drug Targets, 2020, *20*, 1719-1725

RESEARCH ARTICLE

Effects of a Low Carb Diet and Whey Proteins on Anthropometric, Hematochemical, and Cardiovascular Parameters in Subjects with Obesity

Giovanni De Pergola[1,*], Roberta Zupo[2], Luisa Lampignano[2], Silvia Paradiso[1], Isanna Murro[1], Annagrazia Cecere[3], Nicola Bartolomeo[4], Marco M. Ciccone[3], Gianluigi Giannelli[5] and Vincenzo Triggiani[6]

[1]Clinical Nutrition Unit, Medical Oncology, Department of Biomedical Science and Human Oncology, University of Bari, School of Medicine, Policlinico, Piazza Giulio Cesare 11, 70124 Bari, Italy; [2]National Institute of Gastroenterology "S. de Bellis", Research Hospital, Castellana Grotte, Italy; [3]Section of Cardiovascular Disease, Department of Organ Transplantation, University of Bari, School of Medicine, Policlinico, Piazza Giulio Cesare 11, 70124, Bari, Italy; [4]Medical Statistics, Department of Biomedical Science and Human Oncology, University of Bari, School of Medicine, Policlinico, Piazza Giulio Cesare 11, 70124 Bari, Italy; [5]Scientific Direction, National Institute of Gastroenterology "Saverio de Bellis", Research Hospital, Castellana Grotte, 70013 Bari, Italy; [6]Section of Endocrinology and Metabolic Diseases, Department of Emergency and Organ Transplantation University of Bari, School of Medicine, Policlinico, Piazza Giulio Cesare 11, 70124 Bari, Italy

ARTICLE HISTORY

Received: October 30, 2019
Revised: February 13, 2020
Accepted: May 04, 2020

DOI:
10.2174/1871530320666200610143724

Abstract: ***Background:*** The best way to lose body weight, without using drugs and/or suffering hunger and stress, has not yet been defined. The present study tested a low carbohydrate diet, enriched with proteins, in subjects with overweight and obesity.

Methods: The study enrolled 22 uncomplicated overweight and obese subjects. Several parameters were examined before and after 6 weeks of a low-carbohydrate diet, enriched with 18 g of whey proteins. Anthropometric (body mass index, waist circumference) variables, fasting hormones (insulin, TSH, FT3, FT4), and metabolic (glucose, prealbumin, and lipid levels) parameters were measured. 25-OH-vitamin D (25 (OH) D), parathyroid hormone (PTH) and osteocalcin, were also quantified. Body composition parameters (fat mass, fat-free mass, body cell mass, total body water) were measured by electrical bioimpedance analysis. As cardiovascular parameters, blood pressure, endothelium flow-mediated dilation (FMD), and common carotid artery intima-media thickness were also measured.

Results: The low-carbohydrate diet integrated with proteins induced a significant decrease in body weight ($P < 0.001$), waist circumference ($P < 0.001$), fat mass ($P < 0.001$), diastolic blood pressure ($P < 0.01$), triglycerides ($P < 0.001$), total cholesterol ($P < 0.001$), pre-albumin ($P < 0.001$), insulin ($P < 0.001$), HOMAIR ($P < 0.001$), FT3 ($P < 0.05$), and c-IMT ($P < 0.001$), and a significant increase in FMD ($P < 0.001$) and 25 (OH) D ($P < 0.001$) was also observed.

Conclusion: All these results suggest that a short-term non-prescriptive low carbohydrate diet, enriched with whey proteins, may be a good way to start losing fat mass and increase health.

Keywords: Low carbohydrate diet, whey proteins, endothelium, intima-media thickness, obesity, overweight.

1. INTRODUCTION

Obesity and abdominal obesity, in particular, are responsible for higher cardiovascular morbidity and mortality, posing a major health problem worldwide [1-3]. In fact, abdominal obesity is associated with a higher prevalence of insulin resistance, unfavorable lipid changes, hyperuricemia, a higher thrombotic and lower fibrinolytic activity, as well as greater risks of hypertension, diabetes, obstructive apnea syndrome, and cardiovascular disease [4-11].

Long-term control of body weight is fundamental to combat the high prevalence of overweight and obesity in both advanced and emerging countries, and the related health risks.

Modern management of obesity includes changes in lifestyle, physical activity, behavior, sleep quality, environment, *etc*., but diet is still the mainstay of obesity treatment [12]. In general terms, the optimal diet to treat obesity should provide adequate nutrition, should be safe, healthy, effective, economically sustainable, culturally acceptable, as well as ensuring the maintenance of long-term weight loss. Several different dietary approaches have been used, proposed for adult and young obese people, with different calorie intakes, macronutrient composition, duration of diet, and so on, but the best dietary strategy has not yet been established. Taking

*Address correspondence to this author at the Clinical Nutrition Unit, Medical Oncology, Department of Biomedical Science and Human Oncology, University of Bari, School of Medicine, Policlinic, Piazza Giulio Cesare 11, 70124 Bari, Italy; Tel: +39-080-5592909; Fax: +39-080-5478831; E-mail: giovanni.depergola@uniba.it

2212-3873/20 $65.00+.00

for granted that a diet inducing weight loss must be hypocaloric, best studied models include a) diets with a low lipid content, b) low carbohydrate (low carb) ketogenic or non-ketogenic diets, c) diets with a higher percentage of proteins, d) diets including meal replacements, e) Mediterranean diet, and, more recently, f) diets characterized by intermittent fasting, proposing that dieters should eat during 8 hours, and avoid food for the remaining 16 hours every day [13].

Low carb diets induce a 2-3-fold greater weight loss than low-fat standard diets, these are very effective for short-term programs, and have shown to increase energy expenditure during weight loss maintenance, thus improving the effectiveness of obesity treatment [14]. Low carb diets are also safe since they reduce some risk factors as well as being apparently devoid of negative side effects [15]. By contrast, recent studies have suggested that a high carbohydrate intake may increase CVD (cardiovascular disease) and mortality risks [16].

A higher dietary percentage or an absolute increase of proteins with or without lowering carbohydrates intake is a valid tool to reduce body weight [17-19]. The higher protein intake is responsible for body fat loss thanks to inhibiting hunger and stimulating satiety [20-22], thus decreasing calorie intake [19], increasing energy expenditure and fat oxidation [22-24], while maintaining FFM (fat free mass) and muscle mass [25, 26]. As compared to other kinds of proteins, whey proteins increase muscle growth [27], decrease blood pressure [28, 29], and reduce general oxidation and inflammation [27, 29, 30]. More information about ideal food composition is needed to obtain the best possible short and long term results, taking into account weight loss, insulin sensitivity, glycaemic control, and all other cardiovascular risk factors.

It is believed that a low carb diet enriched with whey proteins may be a very good dietary model to obtain weight loss while maintaining good health. However, to the best of our knowledge, no previous study has examined this possibility. Therefore, the aim of this study was to investigate whether six consecutive weeks of a protocol based on a low carbohydrate diet, enriched with whey proteins, without needing to weigh food and limit intake to a given number of kcal per day, together with moderate exercise, may be effective in inducing weight loss in subjects with overweight and obesity. It was also examined whether this protocol was associated with favorable changes of anthropometric, metabolic, hormonal, body composition, and cardiovascular parameters.

2. MATERIALS AND METHODS

2.1. Study Population

The study subjects were enrolled at the Outpatients Clinic of Clinical Nutrition, Medical Oncology, Department of Biomedical Science and Human Oncology, University of Bari School of Medicine. They had come to the Outpatients Clinic aiming to lose weight and receive advice on diet and lifestyle. The subjects were consecutively recruited from January 2018 to November 2018.

Inclusion criteria were a BMI (body mass index) over 25.0 kg/m^2, age over 18 years, and no drug prescriptions (including hormone replacement therapy for post-menopausal and oral contraception for pre-menopausal women).

Exclusion criteria were pregnancy, cancer, endocrine diseases, chronic inflammatory diseases, diabetes mellitus, stable hypertension, angina pectoris, stroke, transient ischemic attack, heart infarction, and congenital heart disease. All subjects had fasting blood glucose concentrations <126 mg/dl and HbA1c levels <6.5%. In total, 22 subjects, 14 women and 8 men, aged 19-70 years, were enrolled. Since patients were consecutively enrolled based on inclusion and exclusion criteria, strict study population selection was not performed.

Informed consent was obtained from all study participants prior to inclusion. The procedures followed were in accordance with the ethical standards for human experimentation (institutional and national), 1964 Helsinki declaration, and later amendments. Study approval was obtained from the local Ethics Committee, and all subjects gave informed consent to take part.

Based on routine blood tests, urinalysis, electrocardiogram, physical examination, and medical history, all subjects were apparently in good health. No participant was on a limited calorie diet nor had had done any intense physical activity before enrollment.

2.2. Diet Protocol

For six consecutive weeks, subjects were instructed to completely avoid all foods containing carbohydrates except for two portions of fruit, one at lunch, one at dinner, but subjects were not required to weigh food and count calories. Subjects were informed that one portion of fruit corresponds to 150 grams; all kinds of fruit were permitted, except for banana, persimmon, figs, and grapes. At breakfast, patients could eat nuts or dark chocolate, not more than 30 grams each, in addition to one glass of semi-skimmed milk or one jar of half-fat yogurt. Before starting the protocol, subjects had a meeting with the dieticians, who explained how to choose food based on the quality of fats and quantity and quality of proteins. No processed foods, processed meat, very fatty foods (meat or cheese or dairy products), fried foods, fatty sauces, and condiments, beverages with added sugar, were allowed. Extra-virgin olive oil, nuts, fish (any kind regardless of the quantity of fat) were permitted. Although patients were not obliged to weigh food, they were strongly encouraged to eat only when they were hungry, thus respecting physiological needs and limiting any superfluous food intake. In line with this concept, a regime was proposed of three meals per day, and snacks could be taken only if they consisted of fresh vegetables. Coffee and tea were not limited, whereas subjects could not drink alcohol. Weekly visits were made with the dietitians to monitor the food intake. Based on their investigations, it was shown that most patients had a mean daily energy intake of 1600 kcal, ranging between 1400 and 1800 kcal per day, which was actually an unconsciously hypocaloric diet. As to the percentage of macronutrients, lipids accounted for 50-55%, proteins for 25%, and carbohydrates for 15-20% of total calories.

At breakfast, all subjects took one sachet of a nutritional product called Myopenta (New Penta, Castelletto Stura, Italy). One sachet of Myopenta contains 18 g of whey proteins (4 g of L-leucine), 4 g of carbohydrates (with 1.9 g of fibers, fructose, and lactose), 1.4 g of lipids, 331 mg of cocoa polyphenols and several vitamins (with 5 mcg of vitamin D3). The product was dissolved in 200 ml of water at room temperature and drunk just before breakfast.

2.3. Physical Activity Protocol

Moderate exercise was encouraged, but no formal program was developed; this is an apparent limitation of the study. It was explained that the main goal was to expend more calories than before and subjects were encouraged to make simple changes, such as climbing stairs rather using an elevator, reducing the amount of time they spent driving, *etc*, in particular, doing at least 210 min per week of brisk walking, with a mean of 30 min per day.

2.4. General data and Anthropometric Measurements

BMI was calculated as the weight (kg rounded to nearest kg) divided by the square height (m rounded to nearest centimeter). Waist circumference (WC) was measured at the anatomic waistline, in the narrowest part of the abdomen, at the natural indentation between the iliac crest and the 10th rib (minimum waist). Blood pressure was recorded on at least three different occasions using a mercury manometer with the appropriate cuff size.

2.5. Metabolic and Hormonal Parameters

Blood samples were taken between 8 and 9 am after fasting overnight. Serum insulin concentrations were determined by radioimmunoassay (Behring, Scoppitto, Italy). Serum FT3, FT4, and TSH were measured using a competitive photometric method based on the solid phase antigen-linked technique (LIASON FT3, LIASON FT4, LIASON TSH, Dia-Sorin, Saluggia, Italy). Intact PTH determination was done by immune-chemiluminescence assay (Test LIAISON 1-84 PTH Dia-Sorin, Saluggia, Italy). Plasma glucose levels were determined by the glucose-oxidase method (Sclavo, Siena, Italy). Insulin resistance was calculated by the homeostasis model assessment (HOMAIR) [31]. Serum 25(OH)D was quantified by chemiluminescence method (Diasorin Inc, Stillwater, USA). The total (uncarboxylated and carboxylated) serum osteocalcin (OC) levels were determined by electrochemiluminescence using the Elecsys N-MID Osteocalcin kit (Roche Diagnostics, Mannheim, Germany), Modular Analytics E170 (Roche Diagnostics). Plasma lipids (triglycerides, total cholesterol and HDL-cholesterol) were determined by an automatic colorimetric method (Hitachi; Boehringer Mannheim, Mannheim, Germany). LDL cholesterol was calculated using the Friedewald equation. Prealbumin was measured by common laboratory methods. All samples were analyzed in duplicate.

2.6. Body Composition Parameters

Subjects underwent body composition evaluation. Body composition parameters (total body water [TBW], body cell mass [BCM], fat-free mass [FFM], fat mass [FM]) were measured by electrical bioimpedance analysis (BIA). In practice, a 50 kHz, tetra-polar phase-sensitive BIA (BIA-101 ASE; AKERN-Srl, Florence, Italy), injecting a sinusoidal alternating current of 400 μA, was used to measure resistance to nearest 1.0 Ω (R), reactance to nearest 0.1 Ω (Xc), and phase angle to nearest 0.1º (PA) [32].

2.7. Ultrasound Measurement of the Carotid Intima-Media Thickness (c-IMT)

All subjects underwent high-definition vascular echography according to the following protocols to measure c-IMT, a useful tool to evaluate early atherosclerosis. Ultrasonographic echo-color Doppler studies of left and right common carotid arteries were performed by the same physician with an Esaote MyLab™ Seven using a 7.5-MHz high-resolution probe. The patients were placed in a supine position, neck extended and rotated contralaterally by 45 °C, and the common carotid arteries were examined on the sagittal axis in lateral view. Pignoli *et al*. method was used to define c-IMT, as described in previous studies [33]: by focusing and freezing images on the distal common carotid artery wall, lengthwise axis during end-diastole, c- IMT was calculated as the distance between the leading borders of the first hyperechoic and second hyperechoic lines, separated by a hypoechoic space (“double-track pattern”). The measurements were performed bilaterally 1 cm proximally to the carotid bulb, three times, then the c-IMT value was calculated as the arithmetic means of each side. The c-IMT value considered for statistical analyses was the mean right and left measurements.For IMT detection, arterial segments with atherosclerotic plaques were excluded. All echo-color Doppler investigations were performed at the Cardiovascular Department of Bari Polyclinic.

2.8. Measurement of Flow-Mediated Dilation (FMD) of the Brachial Artery to Assess Endothelial Function

FMD was measured as previously described in a study [34]. Briefly, the probe was positioned 4-5 cm above the patient's elbow, in a supine position, to obtain a long-axis view projection of the brachial artery. After a 60-s resting period, the sphygmomanometer cuff was placed distally to the imaging transducer at the forearm level and inflated to 250 mmHg for 300 s. Rapid deflation of the blood pressure cuff was then performed to induce a brief increase in blood flow (reactive hyperemia), resulting in increased shear stress for brachial artery dilatation. Acquisitions of diameter and flow velocity were continued for 180 s. Normal maximum speeds were 50–70 cm/s and reactive hyperemia was calculated as the maximal velocity ratio during reactive hyperemia to the maximal velocity at baseline [34]. The maximum FMD recovery value was calculated as the ratio of diameter change to baseline value. FMD studies were performed using high-resolution ultrasonography (Esaote MyLab™ Seven). All ultrasound examinations were performed by the same physician to reduce variability. It was assumed that a reduced flow mediated dilation value was associated with a decreased endothelial function. This assessment was performed with subjects fasting for at least 8-12 hours, early in the morning, in a quiet, air-conditioned room (22-24°C). Subjects were asked not to do exercise or take exciting substances like chocolate or coffee/tea, which could affect endothelial function, for at least 4-6 hours before the examination.

2.9. Statistical Analysis

Continuous data were expressed as mean ± standard deviation (SD) for normally distributed parameters or the median ± interquartile range (IQR) in the case of skewed data distributions. Kolmogorov–Smirnov test was used to determine normal distribution. Comparison between before and after the six weeks of diet was made with Student's paired t-test or its non-parametric counterpart (Wilcoxon signed rank test). All tests of statistical significance were two-sided; the level of significance was set at $\alpha = 0.05$. Statistical analysis was performed with SAS software (version 9.4 for PC).

3. RESULTS

The results are shown in Table **1**. Patients mean age was 45 years (±13.9) and mean BMI before starting the protocol was 31.3 ± 6.2 kg/m^2. The study population general, anthropometric, metabolic, hormone, and cardiovascular parameters, before and after the diet period, are shown in Table **1**. Body weight, waist circumference (WC), diastolic blood pressure (DBP), insulin, HOMAIR, triglycerides, total cholesterol, LDL-cholesterol, FT3, pre-albumin, fat mass and c-IMT showed a significant decrease after dietary intervention, whereas FMD and vitamin D showed a significant increase. Systolic blood pressure (SBP), TSH, FT4, blood glucose, HDL-cholesterol, osteocalcin, PTH, FFM, BCM, and TBW did not show significant changes after the diet.

Table 1. Comparison of all parameters evaluated between Pre- and Post- dietary treatment.

Parameter	Pre-Treatment	Post-Treatment	P-value
Body weight (kg)[1]	84.8 ± 18.5	80.5 ± 16.3	<.0001[a]
WC (cm) [1]	102.6 ± 14.5	96.2 ± 14.5	<.0001[a]
SBP (mmHg)[1]	129.6 ± 15.6	125 ± 8.9	0.0756[a]
DBP(mmHg)[1]	83.5 ± 8.7	76.6 ± 8.4	0.0029[a]
Tot. cholesterol(mg/dl)[1]	199.4±39.8	182.3±36.8	0.0021[a]
HDL (mg/dl)[1]	58.5±16.8	60.4±21.8	0.4013[a]
LDL (mg/dl)[1]	121.5±35.9	112.7±35.6	0.0515[a]
TSH (µU/ml)[1]	1.8±0.8	1.6±0.7	0.2908[a]
FT3 (pg/ml)[1]	3±0.3	2.8±0.4	0.0196[a]
FT4 (pg/ml)[1]	10.4±1.3	10.8±1.3	0.1434[a]
PTH (pg/ml)[1]	30.3±12.1	28.3±16.5	0.4595[a]
FM (kg)[1]	28.8±12.6	25.4±11.7	<.0001[a]
FFM (kg)[1]	56.6±12.3	55.9±11.2	0.1536[a]
BCM (kg)[1]	31.7±7.9	31.3±8.9	0.5745[a]
FMD (%)[1]	8.8±3.4	12.1±4.7	0.0002[a]
25 (OH) D (ng/ml)[2]	22.5[12-26]	26[22-35]	<.0001[b]
Insulin(µUI/ml) [2]	8.7[5.4-10.9]	4.9[4.1-7.7]	<.0001[b]
Blood glucose(mg/dl)[2]	86.5[80-100]	85.5[79-90]	0.0728[b]
HOMAIR[2]	1.8[1.2-2.7]	1.1[0.8-1.6]	<.0001[b]
Triglycerides(mg/dl)[2]	99[66-148]	70.5[51-93]	<.0001[b]
Prealbumin(ng/ml)[2]	0.3[0.2-0.3]	0.2[0.2-0.3]	0.0004[b]
Osteocalcin (ng/ml)[2]	11.2[7.4-16.8]	10.2[5.4-14.2]	0.3097[b]
TBW(Lt)[2]	38.1[35.4-47.2]	38.3[34.4-46.6]	0.7497[b]
c-IMT(mm)[2]	0.7[0.6-0.8]	0.6[0.5-0.7]	0.0003[b]

Data are presented as mean ± SD[1] or median (IQR)[2]. Paired Student's t-test; b Wilcoxon signed rank test.
Bold entries denote $p<0.05$.

4. DISCUSSION

This study, performed in overweight healthy subjects and with obesity, shows that a very low carbohydrate diet, featuring foods with rich proteins and healthy lipids content, integrated with 18 g of whey proteins per day, induces weight loss and favorable changes in anthropometric, metabolic, hormonal, and cardiovascular variables.

These results were obtained without requiring subjects to weigh food and/or count calories, demonstrating that prescribing a set number of calories is not an obligatory step when prescribing a diet with the goal of obtaining weight loss. Subjects did not report hunger or stress, as commonly complained of by patients undergoing a hypocaloric diet and, therefore, the six weeks protocol seems to be well accepted by patients. It was believed that lack of hunger was possibly due to free intake of proteins and instructions were to prefer vegetables that are rich in fibers. In fact, both dietary proteins and fibers stimulate satiety [35-37]. Subjects were invited to increase their baseline physical activity, but no formal program was developed. Therefore, the success of this protocol cannot be mainly imputed to exercise. In our opinion, a very interesting result is that the diet, integrated with whey proteins, increased FMD, corresponding to a clear improvement of endothelium function. This is an important aspect since endothelial cells have a key role in the maintenance of functional capillaries and the endothelium is directly involved in peripheral vascular disease, heart disease, stroke, insulin resistance, diabetes mellitus, venous thrombosis, chronic kidney failure, tumor growth, and metastasis [38]. Therefore, vascular endothelium dysfunction is a disease hallmark. Moreover, the protocol decreased c-IMT, demonstrating that even a short period, as little as six weeks, may reduce the arterial thickness and change wall content. All these favorable modifications may be partly due to decreasing several cardiovascular risk factors, such as diastolic blood pressure, insulinemia, insulin resistance, total and LDL-cholesterol, and triglycerides. Another apparent, very interesting result is that this diet induced weight loss and a decrease of fat mass, without changing FFM and BCM, strongly suggesting that integration with 18 g of whey proteins per day has the capacity to save the protein content of FFM and BCM and, possibly, energy expenditure. In fact, it is well known that proteins may change body composition and promote energy expenditure in favor of a more fat-free body mass [35]. This is an important point if we consider that a lower energy expenditure is a typical effect and a weak point of traditional hypocaloric diets [39, 40]. A particular finding was that the dietary intervention was followed by a decrease of FT3 levels, without changes of TSH and FT4, suggesting a decreased conversion from FT4 to FT3. It was previously shown that FT3 serum levels and the FT3/FT4 ratio are directly associated with waist circumference, independent of insulin resistance, metabolic parameters and blood pressure levels in overweight and obese women, and suggested that this may be an adaptation mechanism to the thermogenic defect in obesity [41]. Therefore, the decrease of FT3 after dietary intervention might indicate the disappearance of this compensatory mechanism.

A significant decrease in pre-albumin levels was observed after dietary intervention. Since pre-albumin has a short half-life (2-3 days) and its levels express new synthesis in liver, prealbumin reduction would suggest a lower hepatic protein synthesis. Possibly, this phenomenon could balance a higher protein intake through spontaneous dieting and the addition of whey proteins.

An increase of 25 (OH) D levels was also obtained after dietary intervention, and this result may be due either to the presence of vitamin D3 in Myopenta or the common spontaneous increase of vitamin D that appears after the lowering of body fat mass following weight loss. Even though 25 (OH) D levels were higher after dietary intervention, osteocalcin and PTH levels were not different, suggesting that the protocol does not induce significant changes in bone metabolism and factors involved in bone metabolism.

The apparent weak point of this study is the lack of a control group not taking Myopenta but in actual fact, a control group could have reinforced the relevance of the findings. Moreover, the limited number of subjects enrolled in the study meant including both men and women, making it impossible to examine the effect of possible confounding factors such as physical activity, gender, and BMI.

Lastly, although this study is concentrated on the role of some specific aspects of dietary intervention in helping patients to lose weight, we should not forget that this needs to be integrated by overall changes in lifestyle and physical activity to treat obesity in the best way.

CONCLUSION

In conclusion, the present study, performed in healthy subjects with obesity and overweight but with no apparent clinical diseases, shows that 6 weeks of a non-prescriptive low carbohydrate diet, enriched with 18 g of whey proteins, induces several favorable effects such as a decrease in body weight, central fat accumulation, fat mass, diastolic blood pressure, triglycerides, total cholesterol, insulin, insulin resistance and c-IMT, and an increase in FMD and vitamin D. These results suggest that this kind of dietetic approach may be a good way to start the treatment of obesity.

AUTHORS' CONTRIBUTIONS

Conceptualization, G.D.P. and V.T.; methodology, G.D.P., R.Z. and L.L; software, N.B.; formal analysis, N.B..; investigation, G.D.P, R.Z., L.L., S.P. and I. M.; data curation, N.B.; writing - original draft preparation, G.D.P.; supervision: G.D.P., M.M.C. and V. T.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Local Ethics Committee (Comitato Etico Indipendente Azienda Ospedaliero-Universitaria "Consorziale Policlinico", Bari), according to a general statement of adherence to standards.

HUMAN AND ANIMAL RIGHTS

No animals were used in the study. All human procedures were performed in accordance with the ethical standards of institutional and/or national research committees and with the Helsinki Declaration of 1975, as revised in 2013 (http://ethics.iit.edu/ecodes/node/3931).

CONSENT FOR PUBLICATION

A written informed consent was obtained from all individuals included, prior to the publication of the study.

FUNDING

None.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analysed during the current study are available from the corresponding author, [GDP], on reasonable request.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Cornier, M.A.; Marshall, J.A.; Hill, J.O.; Maahs, D.M.; Eckel, R.H. Prevention of overweight/obesity as a strategy to optimize cardiovascular health. *Circulation,* **2011**, *124*(7), 840-850. http://dx.doi.org/10.1161/CIRCULATIONAHA.110.968461 PMID: 21844090

[2] Balagopal, P.B.; de Ferranti, S.D.; Cook, S.; Daniels, S.R.; Gidding, S.S.; Hayman, L.L.; McCrindle, B.W.; Mietus-Snyder, M.L.; Steinberger, J. American Heart Association Committee on Atherosclerosis Hypertension and Obesity in Youth of the Council on Cardiovascular Disease in the Young; Council on Nutrition, Physical Activity and Metabolism; Council on Epidemiology and Prevention. Nontraditional risk factors and biomarkers for cardiovascular disease: mechanistic, research, and clinical considerations for youth: A scientific statement from the American Heart Association. *Circulation,* **2011**, *123*(23), 2749-2769. http://dx.doi.org/10.1161/CIR.0b013e31821c7c64 PMID: 21555711

[3] Després, J.P. Body fat distribution and risk of cardiovascular disease: an update. *Circulation,* **2012**, *126*(10), 1301-1313. http://dx.doi.org/10.1161/CIRCULATIONAHA.111.067264 PMID: 22949540

[4] De Pergola, G.; De Mitrio, V.; Giorgino, F.; Sciaraffia, M.; Minenna, A.; Di Bari, L.; Pannacciulli, N.; Giorgino, R. Increase in both prothrombotic and anti-thrombotic factors in obese premenopausal women: relationship with body fat distribution. *Int. J. Obes. Relat. Metab. Disord.,* **1997**, *21*(7), 527-535. http://dx.doi.org/10.1038/sj.ijo.0800435 PMID: 9226481

[5] De Pergola, G.; Giagulli, V.A.; Guastamacchia, E. Platelet number is positively and independently associated with glycated hemoglobin in non-diabetic overweight and obese subjects. *Nutr. Metab. Cardiovasc. Dis.,* **2019**, *29*(3), P254-259. http://dx.doi.org/10.1016/j.numecd.2018.12.007

[6] Ciccone, M.M.; Cortese, F.; Gesualdo, M.; Donvito, I.; Carbonara, S.; De Pergola, G. A glycemic threshold of 90 mg/dl promotes early signs of atherosclerosis in apparently healthy overweight/obese subjects. *Endocr. Metab. Immune Disord. Drug Targets,* **2016**, *16*(4), 288-295. http://dx.doi.org/10.2174/1871530317666161205124955 PMID: 27919218

[7] De Pergola, G.; Cortese, F.; Termine, G.; Meliota, G.; Carbonara, R.; Masiello, M.; Cortese, A.M.; Silvestris, F.; Caccavo, D.; Ciccone, M.M. Uric acid, metabolic syndrome and atherosclerosis: The chicken or The egg,Which comes first? *Endocr. Metab. Immune Disord. Drug Targets,* **2018**, *18*(3), 251-259. http://dx.doi.org/10.2174/1871530318666180212101548 PMID: 29437024

[8] De Pergola, G.; Giagulli, V.A.; Bartolomeo, N.; Gaeta, F.; Petruzzella, A.; Guastamacchia, E.; Triggiani, V.; Silvestris, F. Independent relationship between serum osteocalcin and uric acid in a cohort of apparently healthy obese subjects. *Endocr. Metab. Immune Disord. Drug Targets,* **2017**, *17*(3), 207-212. http://dx.doi.org/10.2174/1871530317666170825164415 PMID: 28847266

[9] Manno, C.; Campobasso, N.; Nardecchia, A.; Triggiani, V.; Zupo, R.; Gesualdo, L.; Silvestris, F.; De Pergola, G. Relationship of para- and perirenal fat and epicardial fat with metabolic parameters in overweight and obese subjects. *Eat. Weight Disord.,* **2019**, *24*(1), 67-72. http://dx.doi.org/10.1007/s40519-018-0532-z PMID: 29956099

[10] De Pergola, G.; Nardecchia, A.; Guida, P.; Silvestris, F. Arterial hypertension in obesity: relationships with hormone and anthropometric parameters. *Eur. J. Cardiovasc. Prev. Rehabil.,* **2011**, *18*(2), 240-247. http://dx.doi.org/10.1177/1741826710389367 PMID: 21450671

[11] Vgontzas, A.N.; Papanicolaou, D.A.; Bixler, E.O.; Hopper, K.; Lotsikas, A.; Lin, H.M.; Kales, A.; Chrousos, G.P. Sleep apnea and daytime sleepiness and fatigue: relation to visceral obesity, insulin resistance, and hypercytokinemia. *J. Clin. Endocrinol. Metab.,* **2000**, *85*(3), 1151-1158. http://dx.doi.org/10.1210/jcem.85.3.6484 PMID: 10720054

[12] Tremblay, A. Obesity management: What should we do if fat gain is necessary to maintain body homeostasis in a modern world? *Front. Endocrinol.,* **2018**, *9*, 285. http://dx.doi.org/10.3389/fendo.2018.00285

[13] Patterson, R.E.; Sears, D.D. Metabolic effects of intermittent fasting. *Annu. Rev. Nutr.,* **2017**, *37*, 371-393. http://dx.doi.org/10.1146/annurev-nutr-071816-064634 PMID: 28715993

[14] Ebbeling, C.B.; Feldman, H.A.; Klein, G.L.; Wong, J.M.W.; Bielak, L.; Steltz, S.K.; Luoto, P.K.; Wolfe, R.R.; Wong, W.W.; Ludwig, D.S. Effects of a low carbohydrate diet on energy expenditure during weight loss maintenance: randomized trial *BMJ,* **2018**, *363*, k4583. http://dx.doi.org/10.1136/bmj.k4583

[15] Santos, F.L.; Esteves, S.S.; da Costa Pereira, A.; Yancy, W.S., Jr; Nunes, J.P.L. Systematic review and meta-analysis of clinical trials of the effects of low carbohydrate diets on cardiovascular risk factors. *Obes. Rev.,* **2012**, *13*(11), 1048-1066. http://dx.doi.org/10.1111/j.1467-789X.2012.01021.x PMID: 22905670

[16] Dehghan, M.; Mente, A.; Zhang, X.; Swaminathan, S.; Li, W.; Mohan, V.; Iqbal, R.; Kumar, R.; Wentzel-Viljoen, E.; Rosengren, A.; Amma, L.I.; Avezum, A.; Chifamba, J.; Diaz, R.; Khatib, R.; Lear, S.; Lopez-Jaramillo, P.; Liu, X.; Gupta, R.; Mohammadifard, N.; Gao, N.; Oguz, A.; Ramli, A.S.; Seron, P.; Sun, Y.; Szuba, A.; Tsolekile, L.; Wielgosz, A.; Yusuf, R.; Hussein Yusufali, A.; Teo, K.K.; Rangarajan, S.; Dagenais, G.; Bangdiwala, S.I.; Islam, S.; Anand, S.S.; Yusuf, S. Prospective Urban Rural Epidemiology (PURE) study investigators. Associations of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): a prospective cohort study. *Lancet,* **2017**, *390*(10107), 2050-2062. http://dx.doi.org/10.1016/S0140-6736(17)32252-3 PMID: 28864332

[17] Krieger, J.W.; Sitren, H.S.; Daniels, M.J.; Langkamp-Henken, B. Effects of variation in protein and carbohydrate intake on body mass and composition during energy restriction: A meta-regression 1. *Am. J. Clin. Nutr.,* **2006**, *83*(2), 260-274. http://dx.doi.org/10.1093/ajcn/83.2.260 PMID: 16469983

[18] Noakes, M.; Keogh, J.B.; Foster, P.R.; Clifton, P.M. Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women. *Am. J. Clin. Nutr.,* **2005**, *81*(6), 1298-1306. http://dx.doi.org/10.1093/ajcn/81.6.1298 PMID: 15941879

[19] Westerterp-Plantenga, M.S.; Nieuwenhuizen, A.; Tomé, D.; Soenen, S.; Westerterp, K.R. Dietary protein, weight loss, and weight maintenance. *Annu. Rev. Nutr.,* **2009**, *29*, 21-41. http://dx.doi.org/10.1146/annurev-nutr-080508-141056 PMID: 19400750

[20] Paddon-Jones, D.; Westman, E.; Mattes, R.D.; Wolfe, R.R.; Astrup, A.; Westerterp-Plantenga, M. Protein, weight management, and satiety. *Am. J. Clin. Nutr.,* **2008**, *87*(5), 1558S-1561S. http://dx.doi.org/10.1093/ajcn/87.5.1558S PMID: 18469287

[21] Bendtsen, L.Q.; Lorenzen, J.K.; Gomes, S.; Liaset, B.; Holst, J.J.; Ritz, C.; Reitelseder, S.; Sjödin, A.; Astrup, A. Effects of hydrolysed casein, intact casein and intact whey protein on energy expenditure and appetite regulation: A randomised, controlled, cross-over study. *Br. J. Nutr.,* **2014**, *112*(8), 1412-1422. http://dx.doi.org/10.1017/S000711451400213X PMID: 25191896

[22] Pal, S.; Radavelli-Bagatini, S.; Hagger, M.; Ellis, V. Comparative effects of whey and casein proteins on satiety in overweight and obese individuals: a randomized controlled trial. *Eur. J. Clin. Nutr.,* **2014**, *68*(9), 980-986. http://dx.doi.org/10.1038/ejcn.2014.84 PMID: 24801369

[23] Johnston, C.S.; Day, C.S.; Swan, P.D. Postprandial thermogenesis is increased 100% on a high-protein, low-fat diet versus a high-carbohydrate, low-fat diet in healthy, young women. *J. Am. Coll. Nutr.,* **2002**, *21*(1), 55-61. http://dx.doi.org/10.1080/07315724.2002.10719194 PMID: 11838888

[24] Veldhorst, M.A.; Westerterp-Plantenga, M.S.; Westerterp, K.R. Gluconeogenesis and energy expenditure after a high-protein, carbohydrate-free diet. *Am. J. Clin. Nutr.,* **2009**, *90*(3), 519-526. http://dx.doi.org/10.3945/ajcn.2009.27834 PMID: 19640952

[25] Frestedt, J.L.; Zenk, J.L.; Kuskowski, M.A.; Ward, L.S.; Bastian, E.D. A whey-protein supplement increases fat loss and spares lean muscle in obese subjects: a randomized human clinical study. *Nutr. Metab.,* **2008**, *27*, 5-8. http://dx.doi.org/10.1186/1743-7075-5-8

[26] Baer, D.J.; Stote, K.S.; Paul, D.R.; Harris, G.K.; Rumpler, W.V.; Clevidence, B.A. Whey protein but not soy protein supplementation alters body weight and composition in free-living overweight and obese adults. *J. Nutr.,* **2011**, *141*(8), 1489-1494.

[27] Kimball, S.R.; Jefferson, L.S. Signaling pathways and molecular mechanisms through which branched-chain amino acids mediate translational control of protein synthesis. *J. Nutr.,* **2006**, *136*(Suppl. 1), 227S-231S. http://dx.doi.org/10.1093/jn/136.1.227S PMID: 16365087

[28] FitzGerald, R.J.; Meisel, H. Lactokinins: whey protein-derived ACE inhibitory peptides. *Nahrung,* **1999**, *43*(3), 165-167. http://dx.doi.org/10.1002/(SICI)1521-3803(19990601)43:3<165::AID-FOOD165>3.0.CO;2-2 PMID: 10399349

[29] Pal, S.; Ellis, V. The chronic effects of whey proteins on blood pressure, vascular function, and inflammatory markers in overweight individuals. *Obesity (Silver Spring),* **2010**, *18*(7), 1354-1359. http://dx.doi.org/10.1038/oby.2009.397 PMID: 19893505

[30] Zhou, L.M.; Xu, J.Y.; Rao, C.P.; Han, S.; Wan, Z.; Qin, L.Q. Effect of whey supplementation on circulating C-reactive protein: A meta-analysis of randomized controlled trials. *Nutrients,* **2015**, *7*(2), 1131-1143. http://dx.doi.org/10.3390/nu7021131 PMID: 25671415

[31] Bonora, E.; Targher, G.; Alberiche, M.; Bonadonna, R.C.; Saggiani, F.; Zenere, M.B.; Monauni, T.; Muggeo, M. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care,* **2000**, *23*(1), 57-63. http://dx.doi.org/10.2337/diacare.23.1.57 PMID: 10857969

[32] De Pergola, G.; Martino, T.; Zupo, R.; Caccavo, D.; Pecorella, C.; Paradiso, S.; Silvestris, F.; Triggiani, V. 25-Hydroxyvitamin D levels are negatively and independently associated with fat mass in a cohort of healthy overweight and obese subjects. *Endocr. Metab. Immune Disord. Drug Targets,* **2019**, *19*(6), 838-844. http://dx.doi.org/10.2174/1871530319666190122094039 PMID: 30666920

[33] De Pergola, G.; Zupo, R.; Cecere, A.; Bartolomeo, N.; Triggiani, V.; Paradiso, S.; Lampignano, L.; Silvestris, F.; Ciccone, M.M. Platelet number is negatively and independently associated with carotid intima-media thickness in apparently healthy overweight/obese subjects. *Nutr. Metab. Cardiovasc. Dis.,* **2018**, *28*(12), 1217-1221. http://dx.doi.org/10.1016/j.numecd.2018.08.001 PMID: 30355470

[34] Ciccone, M.M.; Favale, S.; Scicchitano, P.; Mangini, F.; Mitacchione, G.; Gadaleta, F.; Longo, D.; Iacoviello, M.; Forleo, C.; Quistelli, G.; Taddei, S.; Resta, O.; Carratù, P. Reversibility of the endothelial dysfunction after CPAP therapy in OSAS patients. *Int. J. Cardiol.,* **2012**, *158*(3), 383-386. http://dx.doi.org/10.1016/j.ijcard.2011.01.065 PMID: 21353713

[35] Drummen, M.; Tischmann, L.; Gatta-Cherifi, B.; Adam, T.; Westerterp-Plantenga, M. Dietary protein and energy balance in relation to obesity and co-morbidities. *Front. Endocrinol. (Lausanne),* **2018**, *9*, 443. http://dx.doi.org/10.3389/fendo.2018.00443 PMID: 30127768

[36] Karl, J.P.; Meydani, M.; Barnett, J.B.; Vanegas, S.M.; Goldin, B.; Kane, A.; Rasmussen, H.; Saltzman, E.; Vangay, P.; Knights, D.; Chen, C.O.; Das, S.K.; Jonnalagadda, S.S.; Meydani, S.N.; Roberts, S.B. Substituting whole grains for refined grains in a 6-wk randomized trial favorably affects energy-balance metrics in healthy men and postmenopausal women. *Am. J. Clin. Nutr.,* **2017**, *105*(3), 589-599. http://dx.doi.org/10.3945/ajcn.116.139683 PMID: 28179223

[37] Al-Mana, N.M.; Robertson, M.D. Acute effect of resistant starch on food intake, appetite and satiety in overweight/obese males. *Nutrients,* **2018**, *10*(12), 1993. http://dx.doi.org/10.3390/nu10121993 PMID: 30558330

[38] Johannsen, D.L.; Knuth, N.D.; Huizenga, R.; Rood, J.C.; Ravussin, E.; Hall, K.D. Metabolic slowing with massive weight loss despite preservation of fat-free mass. *J. Clin. Endocrinol. Metab.,* **2012**, *97*(7), 2489-2496. http://dx.doi.org/10.1210/jc.2012-1444 PMID: 22535969

[39] Müller, M.J.; Bosy-Westphal, A. Adaptive thermogenesis with weight loss in humans. *Obesity (Silver Spring),* **2013**, *21*(2), 218-228. http://dx.doi.org/10.1002/oby.20027 PMID: 23404923

[40] De Pergola, G.; Ciampolillo, A.; Paolotti, S. Free triodothyronine and thyroid stimulating hormone serum levels are directly associated with waist circumference, independently of insulin resistance, metabolic parameters and blood pressure levels in overweight and obese women. *Clin. Endocrinol. (Oxf.),* **2007**, *67*, 265-269. http://dx.doi.org/10.1111/j.1365-2265.2007.02874.x PMID: 17547687

[41] Mallard, S.R.; Howe, A.S.; Houghton, L.A. Vitamin D status and weight loss: a systematic review and meta-analysis of randomized and nonrandomized controlled weight-loss trials. *Am. J. Clin. Nutr.,* **2016**, *104*(4), 1151-1159. http://dx.doi.org/10.3945/ajcn.116.136879 PMID: 27604772