

Body composition phenotype: Italian Mediterranean Diet and C677T MTHFR gene polymorphism interaction

L. DI RENZO^{1,5}, M. RIZZO¹, L. IACOPINO¹, F. SARLO¹, E. DOMINO¹, F. JACOANGELI², C. COLICA³, D. SERGI⁴, A. DE LORENZO^{1,5}

¹Division of Clinical Nutrition and Nutrigenomic, Department of Biomedicine and Prevention, University of Rome Tor Vergata, Rome, Italy

²Department of Internal Medicine, UOSD Service of Clinical Nutrition, Parenteral Therapy and Anorexia Nervosa, University Tor Vergata Rome, Rome, Italy

³CNR, ISN UOS of Pharmacology, Department of Pharmacology, University Magna Graecia, Roccelletta di Borgia, Catanzaro, Italy

⁴Department of Clinical Medicine, UOC of Cardiology, University of Rome Tor Vergata, Tor Vergata Hospital, Rome, Italy

⁵I.N.Di.M., National Institute for Mediterranean Diet and Nutrigenomic, Amantea, Italy

Abstract. – OBJECTIVES: Strategies to improve weight maintenance are focused on considering the genetic makeup and its interaction with dietary intake, with the aim to identify vulnerable individuals that will benefit from a variety of more personalized dietary recommendations. The aim of the study was to examine the impact of the C677T MTHFR gene Polymorphism on body composition changes induced by a balanced hypocaloric Italian Mediterranean diet (IMD).

SUBJECTS AND METHODS: Participation in the study included a complete screening of anthropometry and body composition by Dual-energy X-ray absorptiometry (DXA), and a genotyping for the C677T MTHFR polymorphism.

70 Italian Caucasian obese were enrolled and 56 of them completed the screening at baseline and 12 weeks after the nutritional intervention.

RESULTS: T(+) carriers had a higher content of Total Body Fat (TBFat), and Lean (TBLean), reflecting on higher weight and BMI, than T(-) carriers. After IMD, the 28.6% and 71.4% of total subjects decreased weight and TBFat (Kg), respectively. The relative changes were: delta % = -9.09 ± 3.85 for weight; delta % = -15.79 ± 8.51 for TBFat; delta % = -3.80 ± 5.60 for TBLean. The 5.3% of subjects who reached the end point of intervention, and the 8.9% who reduced TBFat (%) below the cut-off of preobesity, were T(-) carriers. A loss of TBLean (Kg) was observed in the 5.1% and 23.5% of T(-) and T(+) carriers.

CONCLUSIONS: MTHFR genetic variations analysis would be an innovative tool for the nutritional assessment, in order to predict the therapeutic response of obese subjects, in terms of fat and lean mass loss.

Key Words:

Lean Body Mass, Body Composition, Diet, Human Genetics, Genetic Susceptibility.

Introduction

A correct life style is necessary to preserve health status and wellbeing. The Mediterranean-like dietary pattern is known to be associated with health beneficial effects and lower incidence of various chronic diseases, as atherosclerosis, cardiovascular diseases, and cancer¹. The Italian Mediterranean Diet (IMD), typical diet rich in fruit, vegetables, legumes, whole grains, fish and low-fat dairy products, all coming from mediterranean place, is related to better management of body weight and cardiovascular risk factors^{2,3}. In particular, we demonstrated the effectiveness of IMD, the so called Nicotera Diet, in reducing fat mass and preserving lean mass in healthy and ill subjects⁴⁻⁶. Indeed, there are data suggesting the existence of significant gene-environment interaction effects that make some individuals more susceptible to body weight gain or loss than others because of genetic differences, when exposed to an obesogenic environment⁷. Moreover, body composition changes could be partially explained by genetic factors or by the interaction between genes and environment⁸.

A common polymorphism of methylenetetrahydrofolate reductase (MTHFR), the C677T mutation, results in a reduced specific MTHFR activity (~34% residual activity in T677T, ~71% residual activity in C677T relative to C677C). MTHFR by catalyzing the conversion of 5, 10 methylenetetrahydrofolate to 5-methyltetrahydrofolate, is a pivotal enzyme in folate metabolism and regulates the proportional usage of one-carbon units between

methylation reactions and nucleic acid synthesis. A direct consequence of MTHFR deficiency is hyperhomocysteinemia due to the lack of 5-methyl-tetrahydrofolate, the necessary methyl donor for homocysteine (tHcy) to be transformed into methionine. Recent meta-analyses showed an overall significantly higher cardiovascular disease risk in people with, compared to those without, C677T MTHFR polymorphism⁹.

Recently, the MTHFR gene polymorphisms were found to be associated with BMI-defined obesity and lean mass¹⁰, sustaining a linkage of body mass index (BMI) and lean mass to chromosome 1p36, where the *MTHFR* gene is located¹¹. It has been also demonstrated an association of C677T MTHFR polymorphism with BMI and central adiposity indices in healthy postmenopausal women. Moreover, epidemiological studies found that tHcy was correlated with lean mass and BMI^{12,13}.

However, BMI alone may not be accurate enough for assessing the extent of excessive fat accumulation and, thus, the obesity related disease risk, since body weight is composed of Total Body Lean (TBLearn), Total Body Fat (TBFat), and bone mass. Dual energy X-ray absorptiometry (DXA) can measure body composition with high precision and provides more reliable and precise measure for obesity^{14,15}.

Furthermore, we have shown a significant relationship between the Normal Weight Obese phenotype, a status of TBFat accumulation accompanied by TBLearn deficiency, and possession of wild type and heterozygous genotypes of C677T MTHFR enzyme polymorphism¹⁶. These results jointly imply that MTHFR may play a role in variation of TBLearn.

Up to now, there has been no study investigating the relationship between MTHFR gene polymorphism and the loss of TBLearn after an hypocaloric IMD.

Due to recent lines of evidence supporting the functional and genetic relevance of MTHFR and variation in TBLearn and TBFat, this study was designed to determine whether a balanced hypocaloric IMD differentially affected body composition and TBLearn of carrier T(+) or non carrier T(-) of C677T *MTHFR* gene polymorphism.

Subjects and Methods

Study Design

We used prospective, cohort design with repeated measures. After screening and subject selection

they entered a baseline period. After baseline measurements subjects were entered the intervention period and were followed at 4-week intervals. Final assessment was made at 12 weeks.

Subjects

Seventy Caucasian Italian obese subjects were consecutively recruited during three months from March 2009 by medical and nutritional staff of the Obesity Centre of Nicotera Hospital (VV, Italy). Subjects having serious chronic diseases/conditions that may have potential influence on endocrine and metabolism were excluded. Participation in the study included a complete medical history to gather informations about health status, current medications history, including supplements of vitamins and minerals, social habits, like alcohol drinking and smoking, food habits, physical activity and family history for chronic diseases, a complete screening of anthropometry and body composition, and a genotyping for the C677T MTHFR polymorphism.

The study group consisted of 56 subjects, 37 females (age: 46.54 ± 12.32) and 19 males (age: 43.84 ± 14.10), who completed the screening at baseline and 12 weeks after the nutritional intervention. The subjects were categorized in BMI-subgroups according to World Health Organization (WHO) criteria¹⁷. Moreover, gender, age and TBFat (%) cutoff points were also used to classify the total population^{14,18}.

A multidisciplinary team of dieticians and nutritionists met with each patient, and provided an educational session for nutrition and meal-planning guidance. A balanced hypocaloric IMD for 12 weeks was assigned to all eligible subjects. A loss of -10% of weight or TBFat (%) below the cut-off of obesity¹⁴ were considered the end-point of the intervention, and a treatment success.

A statement of informed consent was signed by all participants in accordance with principles of the Declaration of Helsinki. The study was approved by the Ethical Commission of the University of Rome "Tor Vergata", Italy. None of the subjects was receiving drug treatments at the time of the assessment.

Nutritional status assessment was conducted at the Obesity Centre of Nicotera Hospital (VV, Italy). Genetic analysis was performed at the University of Rome Tor Vergata, Human Nutrition Unit.

Physical Activity (PA) Assessment

Leisure time and PA habits were monitored using a validated questionnaire²⁹. Participants were

asked to maintain their usual exercise habits during 3 months of follow-up after the beginning of the nutritional intervention.

Dietary Assessment

Usual dietary intakes over the past 12 months were collected by a semiquantitative food-frequency questionnaire that included 127 food items and three portion-size pictures for 17 items. The alimentary diary and nutrient intake were analysed using diet analyser software IN-DALI. Daily and weekly food intake in g calculated from food intake frequency and portion sizes. Dietary intake of macronutrients (lipids, proteins and carbohydrates), folate, vitamin B6 and vitamin B12 was estimated. The dietary pattern of each subject was evaluated by nutritional indices: vegetable to animal protein ratio (V/A), Cholesterol/Saturated fat Index (CSI), Atherogenic Index (AI), Thrombogenic Index (TI); the Mediterranean Adequacy Index (MAI)^{4,20}.

Nutritional Intervention

The Italian Mediterranean Diet (IMD), the so called Nicotera Diet, was used^{3,4,20}. Total daily energy content of the diet was determined on an individual basis (max 2625 Kcal/day; min 2195 Kcal/day), taking into account resting metabolic rate (RMR), calculated using De Lorenzo et al. (2001) prediction equation for the Italian population, and level of PA²¹.

The macronutrient's composition of the dietary regimen was as follows: carbohydrates, 55% to 60%; proteins, 15% to 20% (of which about 60% was comprised of vegetable proteins); total fat, less than 30% (saturated fat < 10%; polyunsaturated fatty acids (PUFA) 6-10%: 5-6% of n-6 PUFA and 1-2% of n-3 PUFA; monounsaturated fatty acids (MUFA), about 15%; trans fatty acids < 1%; cholesterol consumption of 100 mg/day), sodium chloride less than 5 g and 30 g of fibers, *per die*. No alcoholic beverages were allowed. The daily intake of fruit and vegetables was more than 400 g. Extra-virgin olive oil was consumed daily in the amount of 20-25 g. The daily intake of carbohydrates was mainly derived from wheat (pasta and bread), other cereals and legumes (at least 3 times/week). The weekly frequency of consumption of animal foods was 3-4 times for fish, 1-2 for meat, 1 for eggs, 1 for cheese. No change in total energy intake (Kcal/day) was required during the experimental time.

The composition of the diet in terms of foods and food combinations was planned to obtain Veg-

etable/Animal (V/A) protein ratio as close to 1.5 as possible, monounsaturated/saturated fatty acid ratio close to 2, polyunsaturated/saturated fatty acid ratio close to 0.4-1, and low nutritional indices above mentioned⁴. The MAI index value was attended around 7²⁰. The IMD-based meal plan for each subject was obtained by a dietetic software package (Dietosystem, DS Medica SRL, Milan, Italy).

Anthropometric Measurements

After a 12-h overnight fast, all subjects underwent anthropometric evaluation. Anthropometric parameters of all the participants were measured according to standard methods (body weight, height, waist and hip circumferences)²². Subjects were instructed to take off their clothes and shoes before performing all the measurements. Body weight (kg) was measured to the nearest 0.1 kg, using a balance scale (Invernizzi, Rome, Italy). Height (cm) was measured using a stadiometry to the nearest 0.1 cm (Invernizzi, Rome, Italy). The waist and hip circumferences were measured with a flexible steel metric tape to the nearest 0.5 cm. Waist circumference was measured at the horizontal plane that corresponds with the narrowest point between the crest iliac and the bottom rib. Hip circumference was measured at the largest point when observed on a horizontal plane. BMI was calculated using the formula: BMI = body weight (kg)/height (m)².

Dual-Energy X-ray Absorptiometry (DXA)

Body composition analysis was assessed by DXA (i-DXA, GE Medical Systems, Milwaukee, WI, USA), according to the previously described procedure, for evaluating soft tissues, i.e. TBFat and TBLearn^{23,24}. The subjects were instructed not to exercise within 24 h from the test. The subjects were given complete instructions on the testing procedure. They wore a standard cotton t-shirt, shorts and socks. They laid supine on the DXA, without moving while the DXA scan recorded their results. The average measurement time was 20 min. The effective radiation dose from this procedure is about 0.01 mSv. The coefficient of variation (CV% = 100 × s.d./mean) intra- and intersubjects ranged from 1 to 5%. The coefficient of variation for bone mass measurements is < 1%; coefficient on this instrument for five subjects scanned six times over a 9-month period were 2.2% for TBFat and 1.1% for TBLearn. Subjects were classified as obese by using gender and age Percentage Body Fat (PBF) cutoff points¹⁴.

Analysis of Blood Samples

Blood samples (10 mL) were collected into sterile tubes containing EDTA (Vacutainer®). All materials were immediately placed on ice and plasma was separated by centrifugation at 1600 x g for 10 min at 4°C.

Homocysteine concentration was determined by a fully automated HPLC method, using reversed-phase separation and fluorescence detection, with reagents provided by the same company.

Analyses were carried out by the accredited Clinical Chemical Laboratories of the “Tor Vergata” Polyclinic (PTV) of Rome, Italy.

MTHFR Genotype Analysis

DNA was isolated from peripheral leukocytes by using the Gentra DNA isolation kit (Gentra Systems Inc, Minneapolis, MN, USA). According to a previously described procedure, genotyping for the *MTHFR* point polymorphism C677T was performed by polymerase chain reaction amplification with the primers 5'TGAAGGA-GAAGGTGTCTGCGGA3' (sense) and 5'AG-GACGGTGCGGTGAGAGTG3' (antisense). Thirty cycles (95°C for 45 s, 64°C for 30 s, and 72°C for 30 s) were used to amplify the 198-base pair (bp) product. Because the C-to-T transition at nucleotide 677 produces a *HinfI* digestion site, the amplified product derived from the mutant gene was cleaved into 175-bp and 23-bp fragments by *HinfI*, which leaves the wild-type gene unaffected. After electrophoresis through a 6%-polyacrylamide gel, the digestion products were visualized by staining with ethidium bromide.

Statistical Analysis

Data are presented as group means (or median) $\pm$ standard deviation (SD), percentage, or $< \Delta\% > (\Delta\%)$. $\Delta\%$ expresses the relative change, in percent, of a parameter respect to baseline. For the calculation we used the following formula:

$$\Delta\% = \frac{[(\text{Value at week 12}) - (\text{Value at baseline})]}{(\text{Value at baseline})} * 100$$

Data were analyzed to check assumptions about the distribution of the measured variables. Three genotype groups were first considered to check differences in considered variables between groups. Because a dominant or recessive effect existed, analysis was repeated comparing carriers T(+) vs. non-carriers T(-) groups. Com-

parisons among genotype groups were performed using independent t test and non parametric Mann Whitney test. Possible interactions between the C677T *MTHFR* polymorphism and gender were investigated with the general linear method. A paired t test or a non parametric Wilcoxon test were performed to evaluate differences before and after IMD nutritional intervention. All tests were considered significant at $p \leq 0.05$. Statistical analysis was performed using a computer software package (SPSS for Windows, version 13.0; SPSS Inc., Chicago, IL, USA).

Results

Dietary and Physical Assessment

Of all recruited subjects, 56 (F=39, M=17) completed the study and their results were eligible for data analysis. According to WHO criteria of obesity, based on TBFat (%) evaluation, all subjects were obese at the starting point, before the nutritional intervention. Dietary assessment is reported in Table I. A comparison of macronutrients, micronutrients, and nutritional indices between baseline and IMD standard meal is highlighted. PA assessment, at baseline both in men and women, is shown in Table II. The level of PA was classified into three categories (sedentary, moderate and vigorous) based on the time spent on life activity or programmed physical exercise. According to PA questionnaire, any subjects spent time for vigorous PA, the most spent 1-2 time/week for moderate PA, with a sedentary behavior ranging from 10 to 16 h/day.

Genotyping Assessment

The T allele frequency was 19.6%, and the CC, CT, and TT genotype frequencies were 69.6% ($n = 39$), 21.4% ($n = 12$), and 8.9% ($n = 5$), respectively. The T allele frequency in our sample was similar to that in other white populations. The *MTHFR* genotypes were similarly distributed in men and women (in men, CC: 63.2%, CT: 21.1%, TT: 15.7%, and in women, CC: 73%, CT: 21.6%, TT: 5.4%; $p = \text{ns}$). The study population was divided in 2 subgroups, i.e. T(+) carriers and T(-) non-carriers of the C677T *MTHFR* gene polymorphism.

Effects of IMD on Anthropometric and Body Composition Parameters

Anthropometric parameters, laboratory parameters and body composition characteristics by

Table I. Dietary assessment at baseline and after IMD nutritional intervention¹.

	Baseline	IMD nutritional intervention
Nutrients		
Energy (Kcal/day)	2950 ± 390	2410 ± 215
Carbohydrates (% en)	48 ± 2	53 ± 1
Lipids (% en)	35 ± 1	26 ± 1
Proteins (% en)	13 ± 1	17 ± 1
Fiber (g/die)	18 ± 4	33 ± 2
Folate (µg/die)	275 ± 50	466 ± 20
Vitamin B12 (µg/die)	3 ± 1	5 ± 1
Vitamin B6 (µg/die)	2 ± 1	2 ± 1
Nutritional quality indices		
Proteins V/A	1.2 ± 0.1	1.5 ± 0.1
CSI	27 ± 2	15 ± 1
AI	0.4 ± 0.1	0.2 ± 0.1
TI	0.8 ± 0.1	0.5 ± 0.1
MAI	2 ± 1	7 ± 1

¹Values expressed as mean ± SD. V/A, vegetable to animal protein ratio; CSI, Cholesterol/Saturated fat Index; AI, Atherogenic Index; TI, Thrombogenic Index; MAI, Mediterranean Adequacy Index.

DXA, at baseline and week 12 after IMD nutritional intervention, according to MTHFR genotypes, are given in Tables III and IV. Plasma tHcy concentrations according to MTHFR genotype are highlighted in Table III. At baseline, concentrations were higher in carrier T(+) patients than in non carrier T(-) (26.3±5.6 vs 18±4.5 mol/L, $p < 0.001$). After IMD we observed a statistically significant reduction of tHcy in T(+) patients (T0 vs T1 $p < 0.05$); any significant reduction of tHcy was observed in T(-) patients. After IMD, significant decreases in body weight, BMI ($p \leq 0.001$), waist, abdomen, hip circumferences, waist/hip ratio, total and regional fat (kg) and lean (kg), as well as TBFat (%), and TBLean/TBFat ratio, were observed in the total population. Of total subjects, the 28.6% decreased weight and the 71.4% decreased TBFat (kg): nobody increased weight and TBFat (kg). The relative changes were: $\Delta\% = -9.09 \pm 3.85$ [min: -4.13; max: -9.09] for weight; $\Delta\% = -15.79 \pm 8.51$ [min: -44.81; max: -2.22] for TBFat; $\Delta\% = -3.80 \pm 5.60$ [min: -20.44; max: +17.82] for TBLean, respectively.

Table II. Physical activity assessment¹.

Parameters	F (n=37)	M (=19)
Vigorous PA (time/week) ¹	—	—
Moderate PA (time/week)	1.1 ± 0.3	1.6 ± 0.2
Sedentary behaviour (h/day)	13.4 ± 1.5	12.1 ± 2.6

¹Values expressed as arithmetic ± SD. PA, Physical Activity.

The analysis of comparison between genotypes showed an association between the C677T MTHFR gene polymorphism and body composition variables before and after IMD. At baseline, T(+) carriers had higher body weight, BMI, waist, abdomen, hip, waist/hip, fat and lean at trunk and android level, and TBFat, even after checking for gender. After IMD, these differences remained statistically significant, except for waist/hip ratio and lean at trunk level. In addition, a difference in TBLean/TBFat ratio between genotypes was highlighted; the ratio was significantly lower in T(+) than T(-) carriers, particularly in men. In fact, the stratification both for gender and MTHFR genotypes revealed that T(-) carriers men had the highest increase in TBLean/TBFat ratio (baseline: 1.88±0.53 vs week 12: 2.35±0.68).

The relative changes according to genotypes are shown in Figure 1 [T(-) carriers vs T(+) carriers]: $\Delta\% = -9.31 \pm 4.01$ [CI: -10.61 ÷ -8.00] vs $\Delta\% = -8.60 \pm 3.51$ [CI: -10.40 ÷ -6.79] for weight; $\Delta\% = -17.96 \pm 8.63$ [CI: -19.77 ÷ -14.17] vs $\Delta\% = -13.09 \pm 7.82$ [CI: -17.10 ÷ -9.07] for TBFat; $\Delta\% = -2.86 \pm 5.94$ [CI: -4.79 ÷ -0.94] vs $\Delta\% = -5.94 \pm 4.15$ ($p \leq 0.05$, independent t -test) [CI: -8.08 ÷ -3.81] for TBLean, respectively. Moreover, a loss of TBLean (kg) was observed in the 10.7%, 5.1% and 23.5% of total group, T(-) and T(+) carriers, respectively. Only the 2.6% of subjects, i.e. one person, increased TBLean after IMD intervention and he was a T(-) carrier. The general linear model also revealed a difference in TBFat changes between

Table III. Anthropometric parameters and tHcy levels at baseline and week 12 after nutritional intervention, according to genotypes¹.

Parameters	Baseline			Week 12		
	Total (n=56)	T (-) (n=39)	T (+) (n=17)	Total (n=56)	T (-) (n=39)	T(+) (n=17)
Sex (F/M)	37/19	27/12	10/7	37/19	27/12	10/7
Weight (kg)	95.92 ± 16.36	99.23 ± 14.28	104.39 ± 18.04 ³	87.26 ± 15.91 ⁷	83.68 ± 13.92 ⁷	95.49 ± 17.52 ^{3,7}
BMI (kg/m ²)	36.59 ± 5.81	35.57 ± 5.50	38.91 ± 6.02 ²	33.26 ± 5.56 ⁷	32.24 ± 5.13 ⁷	35.60 ± 5.93 ^{3,7}
Waist (cm)	105.29 ± 12.65	102.02 ± 11.58	112.59 ± 12.15 ³	98.03 ± 12.90 ⁷	95.19 ± 12.15 ⁷	104.79 ± 11.99 ^{3,7}
Abdomen (cm)	115.49 ± 11.77	112.61 ± 10.85	121.93 ± 11.46 ³	108.47 ± 12.07 ⁷	105.73 ± 11.17 ⁷	115.47 ± 11.57 ^{3,7}
Hip (cm)	116.78 ± 10.35	115.28 ± 10.70	120.12 ± 8.92 ⁴	110.71 ± 9.67 ⁷	109.03 ± 9.48 ⁷	114.94 ± 8.88 ^{2,7}
Waist/Hip	0.90 ± 0.10	0.89 ± 0.10	0.94 ± 0.08 ²	0.89 ± 0.09 ⁷	0.87 ± 0.09 ⁵	0.91 ± 0.07 ⁶
tHcy (μmol/l)	22.15 ± 5.10	18.0 ± 4.50	26.30 ± 5.60 ²	20.85 ± 5.70	17.8 ± 6.15	23.90 ± 5.30 ^{2,7}

¹All values are mean ± SD. ²Reflects the significance of the differences between genotypes at baseline and week 12 determined with an independent t test ($p \leq 0.05$). ³Reflects the significance of the differences between genotypes at baseline and week 12 determined with an independent t test ($p \leq 0.01$). ⁴Reflects the significance of the differences between genotypes at baseline and week 12 determined with an independent t test ($p \leq 0.001$). ⁵Reflects the significance of the differences between baseline and week 12 determined with a paired t test ($p \leq 0.05$). ⁶Reflects the significance of the differences between baseline and week 12 determined with a paired t test ($p \leq 0.01$). ⁷Reflects the significance of the differences between baseline and week 12 determined with a paired t test ($p \leq 0.001$).

Table IV. Body composition parameters at baseline and week 12 after IMD nutritional intervention, according to genotypes¹.

Parameters	Baseline			Week 12		
	Total (n=56)	T (-) (n=39)	T (+) (n=17)	Total (n=56)	T (-) (n=39)	T(+) (n=17)
Fat trunk (kg)	22.86 ± 5.57	21.25 ± 4.51	26.56 ± 6.12 ³	18.99 ± 5.61 ⁷	17.44 ± 4.89 ⁷	22.52 ± 5.69 ^{4,7}
Fat android (kg)	4.16 ± 1.20	3.74 ± 0.94	5.12 ± 1.21 ⁴	3.39 ± 1.19 ⁷	3.01 ± 0.98 ⁷	4.26 ± 1.19 ^{4,7}
Fat gynoid (kg)	6.80 ± 1.81	6.60 ± 1.90	7.27 ± 1.57	5.72 ± 1.67 ⁷	5.45 ± 1.64 ⁷	6.35 ± 1.61 ⁷
TBFat (kg)	41.71 ± 9.55	39.55 ± 8.56	46.67 ± 10.08 ³	35.40 ± 9.93 ⁷	33.07 ± 8.82 ⁷	40.76 ± 10.52 ^{3,7}
Lean trunk (kg)	24.92 ± 5.24	23.84 ± 4.82	27.38 ± 5.47 ²	23.36 ± 4.69 ⁷	22.66 ± 4.44 ⁷	24.98 ± 5.00 ⁷
Lean android (kg)	3.66 ± 0.81	3.46 ± 0.70	4.11 ± 0.88 ³	3.41 ± 0.77 ⁷	3.24 ± 0.67 ⁷	3.79 ± 0.86 ^{2,7}
Lean gynoid (kg)	7.26 ± 1.50	7.02 ± 1.38	7.79 ± 1.66	6.93 ± 1.40 ⁷	6.79 ± 1.36 ⁵	7.26 ± 1.46 ⁷
TBLean (kg)	50.77 ± 10.41	49.16 ± 10.14	54.46 ± 10.39	48.74 ± 9.96 ⁷	47.66 ± 9.86 ⁷	51.21 ± 10.03 ⁷
PBF (%)	43.71 ± 6.54	43.29 ± 7.03	44.76 ± 5.07	40.43 ± 7.50 ⁷	39.40 ± 7.82 ⁷	42.71 ± 6.36 ⁶
TBLean/TBFat	1.28 ± 0.41	1.31 ± 0.47	1.20 ± 0.24	1.49 ± 0.57 ⁷	1.56 ± 0.63 ⁷	1.32 ± 0.36 ^{2,5}
BMC total body (kg)	2.80 ± 0.54	2.77 ± 0.51	2.87 ± 0.59	2.82 ± 0.53	2.76 ± 0.50	2.94 ± 0.59
BMD total body (g/cm ²)	1.22 ± 0.10	1.21 ± 0.10	1.24 ± 0.11	1.21 ± 0.10	1.20 ± 0.10	1.23 ± 0.12

¹All values are arithmetic ± SD. TBFat, Total Body Fat; TBLean, Total Body Lean; PBF, Percentage Body Fat; BMC, Bone Mineral Content; BMD, Bone Mineral Density. ²Reflects the significance of the differences between genotypes at baseline and week 12 determined with an independent t test ($p \leq 0.05$). ³Reflects the significance of the differences between genotypes at baseline and week 12 determined with an independent t test ($p \leq 0.01$). ⁴Reflects the significance of the differences between genotypes at baseline and week 12 determined with an independent t test ($p \leq 0.001$). ⁵Reflects the significance of the differences between baseline and week 12 determined with a paired t test ($p \leq 0.05$). ⁶Reflects the significance of the differences between baseline and week 12 determined with a paired t test ($p \leq 0.01$). ⁷Reflects the significance of the differences between baseline and week 12 determined with a paired t test ($p \leq 0.001$).

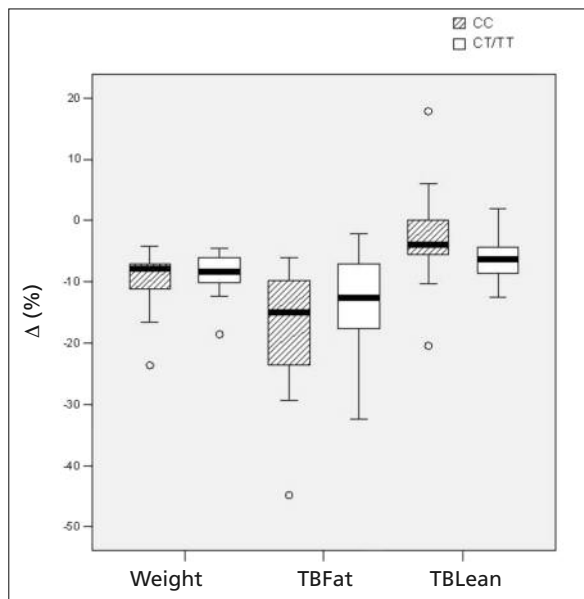


Figure 1. Relative change of TBLearn, TBFat and weight (%) in response to IMD nutritional intervention, according to genotypes¹. ¹All values are median $\pm$ SD (and CI). TBFat, Total Body Fat; TBLearn, Total Body Lean; CI; Interval of Confidence. [#]Reflects the significance of the differences between genotypes for changes after week 12 determined with general linear method ($p \leq 0.05$). [°]Reflects the outliers.

gender groups ($\Delta\%$ Female -14.65 ± 7.42 vs $\Delta\%$ -20.02 ± 10.12 ; $p \leq 0.05$). The stratification both for gender and MTHFR genotypes was significant for changes in TBFat and TBLearn ($p \leq 0.05$). Particularly, T(+) carriers lost more lean mass at trunk level respect to T(-) carriers ($\Delta\%$ -8.40 ± 6.71 vs -4.56 ± 7.96 ; $p \leq 0.05$) and relatively less fat mass at android region ($\Delta\%$ $+4.29 \pm 20.85$ vs -12.50 ± 23.64 ; $p \leq 0.05$). Among subjects who reached the end-point of intervention, the 5.3% ($n = 3$) became normal according to TBFat (%), and 8.9% ($n = 5$) reduced TBFat (%) below the cut-off of preobesity. All of them were T(-) carriers. The 28.6% of total subjects reached the end of point of the intervention with an equal distribution between genotypes (28.2%, and 29.4% of T(-) and T(+) carriers, respectively). Figure 2 highlighted differences in TBFat (%) changes respect to baseline values, in different genotypes, both in men and women.

Discussion

Strategies to improve weight maintenance are nowadays focused on considering together the ge-

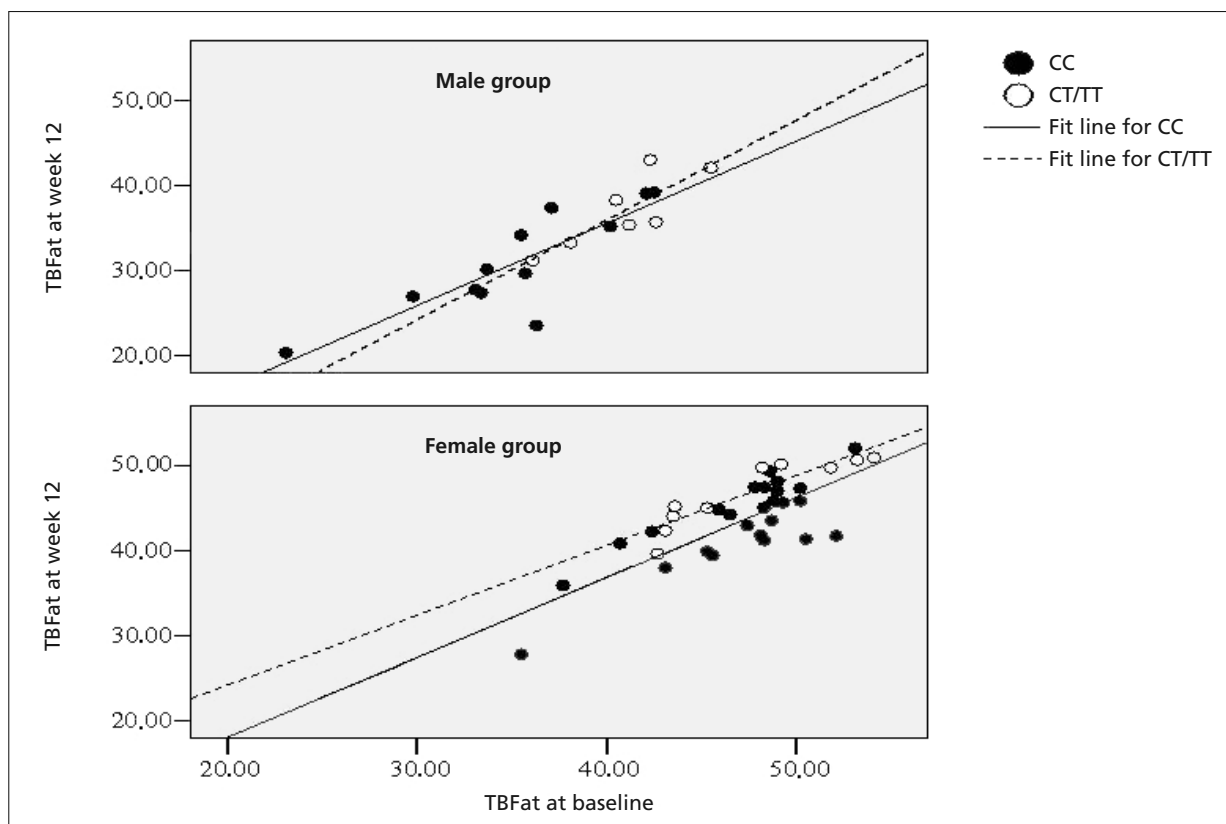


Figure 2. Correlation between TBFat (%) at baseline and at week 12, according to gender and genotypes.

netic makeup and its interaction with dietary intake, with the aim to identify vulnerable populations/individuals that will benefit from a variety of more personalized and mechanistic-based dietary recommendations⁸. It's an emerging area that genetic variants could be considered in obesity and disease management for their additive effects²⁵⁻²⁷.

It has long been suspected that "one size does not fit all" in terms of determining the optimal diet for an individual, and this has been demonstrated over the years in studies on gene-diet interactions and with the emergence of nutrigenetics²⁸.

The herein shown results demonstrate that IMD, at 3 months of intervention, is a valid dietary approach for losing weight and particularly TBFat, in a sample of Italian Caucasian obese subjects, but with inter-individual variability, a well-known phenomenon in nutrition research and practice.

The mechanisms responsible for the inter-individual differences in dietary response are very complex and poorly understood. The concept of gene-diet interaction describes the modulation of the effect of a dietary component on a specific phenotype, such as obesity, by a genetic variant²⁸. A role of genetic factors contributing to those differences in response to nutrients has been proposed for several decades and successfully demonstrated for rare inborn errors of metabolism²⁹.

It is unknown whether low compliance to dietary instructions, that play an important role in body composition, may account for the differences in the response to nutritional interventions. It is important to note that during the first months after the beginning of meal plan, subjects experienced the most dramatic weight loss, because they were very compliant to the administered diet. Thus, after 12 weeks, it is possible to reveal the true impact of given genetic polymorphisms or their combination on weight loss and body composition changes after a nutritional intervention.

Moreover, recent lines of evidence support the hypothesis regarding the heritability of body composition. Different genes may contribute to the expression of fat and lean mass³⁰. It has been well established that TBLean, TBFat, and BMI are under strong genetic control, with heritability of 0.52-0.72³⁰. All association signals for BMI were accompanied by even more convincing associations for TBLean. Some Authors did not

find association between bone mass and *MTHFR*, further suggesting that TBLean might be the major component accounting for the significant association of BMI with *MTHFR*³¹.

Body composition analysis is a pivotal strength of our study. Measures of body fat and lean distribution, could help to stratify individuals according to their level of body fat and preserved lean mass and to reveal the real impact of a nutritional intervention¹⁴.

To the best of our knowledge, this is the first study to indicate a link between C677T *MTHFR* genetic variant and body composition before and after IMD nutritional intervention. In particular, our findings suggest that C677T *MTHFR* polymorphism may play a significant role in body changes induced by IMD.

Several studies found that adherence to IMD, for its particular combination of micro- and macro-nutrients, was associated with beneficial effects on metabolic syndrome, cardiovascular diseases, several types of cancer and major chronic degenerative diseases, decreased overall and cardiovascular mortality, and weight management^{1-6,32,33}.

Because of the association of C677T *MTHFR* polymorphism with cardiovascular disease risk⁹, osteoporosis and sarcopenia^{10,31}, we considered the T(+) carriers as at risk subjects.

Before the nutritional intervention, we observed differences in body composition between *MTHFR* genotypes groups. T(+) carriers were fatter than T(-) carriers. Interestingly, the highest number of responders to IMD nutritional intervention with the lower TBLean (Kg) loss were T(-) carriers. T(+) carriers lost more lean mass, particularly at trunk level, and less fat mass, particularly at android level, than T(-) carriers. The stratification both for gender and *MTHFR* genotypes was significant for changes in TBFat and TBLean. In particular, T(+) carriers men were also characterized for a higher TBFat, at baseline, and lower increase in TBLean/TBFat ratio, respect to T(-) carriers men.

In our study, the presence of Ala222Val polymorphism in the *MTHFR* gene is associated with an higher fat mass at baseline and a decrease of lean mass after diet. In particular, reduction in lean body mass per se is an important risk factor for sarcopenia, a serious health problem that has not received sufficient attention yet. Sarcopenia is a syndrome characterised by progressive and generalised loss of skeletal muscle mass and strength with a risk of adverse outcomes including but not

limited to impaired protein turnover, mobility loss, osteoporosis, dyslipidemia, insulin resistance, overall frailty, and increased mortality³⁵.

Because it has been demonstrated that MTHFR gene is highly expressed in skeletal muscle, since the formation of muscles is associated with the simultaneous formation of homocysteine in connection with creatine/creatinine synthesis, the interaction between gender and MTHFR genotype, shown among men group, could be due to larger muscle mass in men³¹. Moreover, as proposed by Lambrinoudaki et al¹², the genetic variants affecting Hcy metabolism may result in impaired Hcy catabolism which, at a cellular level, may lead to tissutal dysfunction thus causing metabolic disturbances and related body composition modification.

Although further biochemical studies are needed to elucidate the molecular mechanisms that underlie and explain the relationship between MTHFR, TBFat and TBLean, we highlighted a significant reduction of tHcy levels in T(+) carriers, combined with a reduction of TBFat (% kg), and an increase of TBLean (% kg) after IMD nutritional intervention. However, the tHcy levels could be dependent also from other genetic variants and conditions.

Although larger study populations are needed to confirm this observation and clarify the possible role of MTHFR gene polymorphism on body composition regulation, our study highlights new findings. A criticism of this study is the relatively small sample size. However, it was large enough to provide us adequate statistical power and the associations have reached statistical significance in this context of limited sample size may be thought of as valid and reproducible in larger samples.

This study represents a pilot effort to explore the role of gene environment interaction in the body composition evaluation. It describes an important gene X environment interaction in Italian population, providing, for the first time, evidence of interaction between a genetic variant of MTHFR, the C677T polymorphism, and a balanced hypocaloric IMD nutritional intervention on anthropometric and body composition parameters. This new approach, based on genetic and body composition evaluations, defines the requirements to be assessed as guidelines to identifying a really effective diet and could improve long term weight management.

Moreover, our data provide the basis for personalized dietary recommendations based on the

individual's genetic makeup and information from other factors such as gender, in the context of negative energy balance, like hypocaloric nutritional intervention assigned to obese subjects.

In order to avoid the risk of sarcopenia induced by uncontrolled weight loss, we suggest that a tailored dietary protein intake, particularly for T(+) carrier men, who experienced the most dramatic loss of TBLean, could be a benefit in order to favour muscle mass preservation. Several important directions can be highlighted for future research.

Dietary proteins influence body weight by affecting four targets for body weight regulation: satiety, thermogenesis, energy efficiency, and body composition. Protein ingestion results in higher ratings of satiety than equicaloric amounts of carbohydrates or fat. During weight loss and decreased caloric intake, a relatively increased protein content of the diet maintained fat-free mass (i.e. muscle mass) and increased calcium balance.

Therefore, dietary proteins should receive more attention in nutrition recommendations in obesity than they have in the recent past (36). Diets with increased protein have now been shown to improve adult health with benefits for treatment or prevention of obesity, osteoporosis, type 2 diabetes, metabolic syndrome, heart disease, and sarcopenia³⁷.

Layman et al. proposed that the 2010 American Dietary Guidelines should emphasize more protein intake³⁸. Most weight loss diet with increased protein content (31% of total energy, i.e. 1.14 g/kg body weight/day versus 18%, or 0.64 g/kg body weight/day) showed not only decreased body weight but also an improved body composition and lower serum triglycerides. Beside the level of dietary proteins intake, the quality of the protein in the diet is an emerging area of research. Even though the dietary reference intake (DRI) give no recommendation about that, recent studies demonstrate that quality, in terms of consumption of a higher quality protein source, resulting in a higher essential amino acids (EAA) content per g of protein and distribution of dietary protein throughout the day are important³⁹.

Recently Loenneke et al⁴⁰ demonstrated that both quality protein intake and frequency are positively associated with lean mass, bone mineral density and bone mineral content, and had an inverse relationship with percentage of body fat.

Conclusions

We suppose that quality and distribution of protein intake are of particular interest for obese subjects under energy restriction, who might benefit from the consumption of a higher quality protein source, to increase and maintain lean mass, particularly in T(+) carriers.

Moreover, MTHFR genetic variations analysis would be an innovative tool for the nutritional assessment, in order to predict the therapeutic response of obese subjects, in terms of fat and lean mass loss, according to dietary protein quality intake.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

References

- 1) SOFI F, ABBATE R, GENSINI GF, CASINI A. Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. *Am J Clin Nutr* 2010; 92: 1189-1196.
- 2) DE LORENZO A, ANDREOLI A, SORGE RP, IACOPINO L, MONTAGNA S, PROMENZIO L, SERRANÒ P. Modification of dietary habits (Mediterranean diet) and cancer mortality in a southern Italian village from 1960 to 1996. *Ann N Y Acad Sci* 1999; 889: 224-229.
- 3) FIDANZA F, ALBERTI A, FRUTTINI D. The Nicotera diet: the reference Italian Mediterranean diet. *World Rev Nutr Diet* 2005; 95: 115-121.
- 4) DE LORENZO A, PETRONI ML, DE LUCA PP, ANDREOLI A, MORINI P, IACOPINO L, INNOCENTE I, PERRIELLO G. Use of quality control indices in moderately hypocaloric Mediterranean diet for treatment of obesity. *Diabetes Nutr Metab* 2001; 14: 181-188.
- 5) DI RENZO L, DI PIERRO D, BIGIONI M, SODI V, GALVANO F, CIANCI R, LA FAUCI L, DE LORENZO A. Is antioxidant plasma status in humans a consequence of the antioxidant food content influence? *Eur Rev Med Pharmacol Sci* 2007; 11: 185-192.
- 6) DE LORENZO A, NOCE A, BIGIONI M, CALABRESE V, DELLA ROCCA DG, DI DANIELE N, TOZZO C, DI RENZO L. The effects of Italian Mediterranean organic diet (IMOD) on health status. *Curr Pharm Des* 2010; 16: 814-824.
- 7) A TREMBLAY, L. PÉRUSSE, C. BOUCHARD. Energy balance and body-weight stability: impact of gene-environment interactions. *Br J Nutr* 2004; 92(Suppl. 1), S63-S66
- 8) GOYENECHEA E, DOLORES PARRA M, ALFREDO MARTÍNEZ J. Weight regain after slimming induced by an energy-restricted diet depends on interleukin-6 and peroxisome-proliferator-activated-receptor-gamma2 gene polymorphisms. *Br J Nutr* 2006; 96: 965-972.
- 9) LEWIS SJ, EBRAHIM S, DAVEY SMITH G. Meta-analysis of MTHFR 677C->T polymorphism and coronary heart disease: does totality of evidence support causal role for homocysteine and preventive potential of folate. *Br Med J* 2005; 331: 1053-1056.
- 10) LIU X, ZHAO LJ, LIU YJ, XIONG DH, RECKER RR, DENG HW. The MTHFR gene polymorphism is associated with lean body mass but not fat body mass. *Hum Genet* 2008; 123: 189-196.
- 11) NORRIS JM, LANGEFELD CD, SCHERZINGER AL, RICH SS, BOOKMAN E, BECK SR, SAAD MF, HAVNER SM, BERGMAN RN, BOWDEN DW, WAGENKNECHT LE. Quantitative trait loci for abdominal fat and BMI in Hispanic-Americans and African-Americans: the IRAS family study. *Int J Obes (Lond)* 2005; 29: 67-77.
- 12) LAMBRINOUDAKI I, KAPAROS G, PAPADIMITRIOU D, SERGENTANIS TN, CREATSA M, ALEXANDROU A, LOGOTHETIS E, CHRISTODOULAKOS G, KOUSKOUNI E. Methylene-tetrahydrofolate reductase C677T polymorphism is associated with central adiposity and increased androgenicity in healthy postmenopausal women. *Eur J Endocrinol* 2008; 159: 233-241.
- 13) JACQUES PF, BOSTOM AG, WILSON PW, RICH S, ROSENBERG IH, SELHUB J. Determinants of plasma total homocysteine concentration in the Framingham offspring cohort. *Am J Clin Nutr* 2001; 73: 613-621.
- 14) DE LORENZO A, BIANCHI A, MARONI P, IANNARELLI A, DI DANIELE N, IACOPINO L, DI RENZO L. Adiposity rather than BMI determines metabolic risk. *Int J Cardiol* 2011. [Epub ahead of print]
- 15) DI RENZO L, GALVANO F, ORLANDI C, BIANCHI A, DI GIACOMO C, LA FAUCI L, ACQUAVIVA R, DE LORENZO A. Oxidative stress in normal-weight obese syndrome. *Obesity (Silver Spring)* 2010; 18: 2125-2130.
- 16) DI RENZO L, BIGIONI M, BOTTINI FG, DEL GOBBO V, PREMROV MG, CIANCI R, DE LORENZO A. Normal Weight Obese syndrome: role of single nucleotide polymorphism of IL-1 5Ralpha and MTHFR 677C->T genes in the relationship between body composition and resting metabolic rate. *Eur Rev Med Pharmacol Sci* 2006; 10: 235-245.

- 17) WORLD HEALTH ORGANIZATION. Obesity: Preventing and Managing the Global Epidemic. Report Series 894 on a WHO Consultation on Obesity, WHO, Geneva, Switzerland, 2000.
- 18) PHYSICAL STATUS: the use and interpretation of anthropometry. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser, 1995.
- 19) E. PARODI, A. DE LORENZO. I disturbi del comportamento alimentare. www.enpam.it. Collana Università. Vol 6. 2009;
- 20) ALBERTI-FIDANZA A, FIDANZA F. Mediterranean Adequacy Index of Italian diets. *Public Health Nutr* 2004; 7: 937-941.
- 21) DE LORENZO A, TAGLIABUE A, ANDREOLI A, TESTOLIN G, COMELLI M, DEURENBERG P. Measured and predicted resting metabolic rate in Italian males and females, aged 18-59 y. *Eur J Clin Nutr* 2001; 55: 208-214.
- 22) LOHMAN TG, ROCHE AF, MARTORELL R. Anthropometric Standardization Reference Manual. Champaign, IL: Human Kinetics, 1998.
- 23) DI RENZO L, DEL GOBBO V, BIGIONI M, PREMROV MG, CIANCHI R, DE LORENZO A. Body composition analyses in normal weight obese women. *Eur Rev Med Pharmacol Sci* 2006; 10: 191-196.
- 24) A DE LORENZO, V DEL GOBBO, MG PREMROV, BIGIONI M, GALVANO F, DI RENZO L. Normal Weight Obese Syndrome: early inflammation? *Am J Clin Nutr* 2007; 85: 40-45.
- 25) DI RENZO L, BERTOLI A, BIGIONI M, DEL GOBBO V, PREMROV MG, CALABRESE V, DI DANIELE N, DE LORENZO A. Body composition and -174G/C interleukin-6 promoter gene polymorphism: association with progression of insulin resistance in normal weight obese syndrome. *Curr Pharm Des* 2008; 14: 2699-2706.
- 26) DI RENZO L, CARBONELLI MG, BIANCHI A, IACOPINO L, FIORITO R, DI DANIELE N, DE LORENZO A. Body composition changes after laparoscopic adjustable gastric banding: what is the role of -174G>C interleukin-6 promoter gene polymorphism in the therapeutic strategy? *Int J Obes (Lond)* 2012; 36: 369-378.
- 27) DI RENZO L, BIANCHI A, SARACENO R, CALABRESE V, CORNELIUS C, IACOPINO L, CHIMENTI S, DE LORENZO A. -174G/C IL-6 gene promoter polymorphism predicts therapeutic response to TNF- α blockers. *Pharmacogenet Genomics* 2012; 22: 134-142.
- 28) ORDOVAS JM. Genotype-phenotype associations: modulation by diet and obesity. *Obesity (Silver Spring)* 2008; 16(Suppl 3): 40-46.
- 29) HOLTZMAN NA. Genetic variation in nutritional requirements and susceptibility to disease: policy implications. *Am J Clin Nutr* 1988; 48: 1510-1516.
- 30) HSU FC, LENCHIK L, NICKLAS BJ, LOHMAN K, REGISTER TC, MYCHALECKYJ J, LANGEFELD CD, FREEDMAN BJ, BOWDEN DW, CARR JJ. Heritability of body composition measured by DXA in the diabetes heart study. *Obes Res* 2005; 13: 312-319.
- 31) LIVSHITS G, KATO BS, WILSON SG, SPECTOR TD. Linkage of genes to total lean body mass in normal women. *J Clin Endocrinol Metab* 2007; 92: 3171-3176.
- 32) KASTORINI CM, MILIONIS HJ, ESPOSITO K, GIUGLIANO D, GOUDEVENOS JA, PANAGIOTAKOS DB. The effect of Mediterranean diet on metabolic syndrome and its components: a meta-analysis of 50 studies and 534,906 individuals. *J Am Coll Cardiol* 2011; 57: 1299-1313.
- 33) ESPOSITO K, DI PALO C, MAIORINO MI, PETRIZZO M, BELLASTELLA G, SINISCALCHI I, GIUGLIANO D. Long-term effect of mediterranean-style diet and calorie restriction on biomarkers of longevity and oxidative stress in overweight men. *Cardiol Res Pract* 2010; 2011: 293916.
- 34) KAROLCHIK D, BAERTSCH R, DIEKHANS M, FUREY TS, HINRICH A, LU YT, ROSKIN KM, SCHWARTZ M, SUGNET CW, THOMAS DJ, WEBER RJ, HAUSSLER D, KENT WJ. The UCSC Genome Browser Database. *Nucleic Acids Res* 2003; 31: 51-54.
- 35) CRUZ-JENTOFT AJ, BAEYENS JP, BAUER JM, BOIRIE Y, CEDERHOLM T, LANDI F, MARTIN FC, MICHEL JP, ROLLAND Y, SCHNEIDER SM, TOPINKOVÁ E, VANDEWOUDE M, ZAMBONI M. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People Age Ageing 2010; 39: 412-423.
- 36) KELLER U. Dietary proteins in obesity and in diabetes. *Int J Vitam Nutr Res* 2011; 81: 125-133.
- 37) PADDON-JONES D, SHORT KR, CAMPBELL WW, VOLPI E, WOLFE RR. Role of dietary protein in the sarcopenia of aging. *Am J Clin Nutr* 2008; 87: 1562S-1566S.
- 38) LAYMAN DK. Dietary Guidelines should reflect new understandings about adult protein needs. *Nutr Metab* 2009; 6: 12.
- 39) CUTHBERTSON D, SMITH K, BABRAJ J, LEESE G, WADDELL T, ATHERTON P, RENNIE M. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J* 2005; 19: 422-424.
- 40) LOENNEKE JP, ABHISHEK BALAPUR, AUSTIN D. THROWER, GEORGANNE SYLER, MAUREEN TIMLIN, THOMAS J. Pujol Short report: Relationship between quality protein, lean mass and bone health. *Ann Nutr Metab* 2010; 57: 219-220.