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

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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 Mediterannean 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 cancer[1]. The Italian Mediterranean Diet (IMD), typical diet rich in fruit, vegetables, legumes, whole grains, fish and low-fat diary 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 subjects[4-6]. 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 environment[7]. Moreover, body composition changes could be partially explained by genetic factors or by the interaction between genes and environment[8].

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

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methylation reactions and nucleic acid synthesis. A direct consequence of MTHFR deficiency is hyperhomocysteinemia due to the lack of 5-methyltetrahydrofolate, the necessary methyl donator 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 polymorphism[9].

Recently, the MTHFR gene polymorphisms were found to be associated with BMI-defined obesity and lean mass[10], sustaining a linkage of body mass index (BMI) and lean mass to chromosome 1p36, where the *MTHFR* gene is located[11]. 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 (TBLean), 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 TBLean deficiency, and possession of wild type and heterozygous genotypes of C677T MTHFR enzyme polymorphism[16]. These results jointly imply that MTHFR may play a role in variation of TBLean.

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

Due to recent lines of evidence supporting the functional and genetic relevance of MTHFR and variation in TBLean and TBFat, this study was designed to determine whether a balanced hypocaloric IMD differentially affected body composition and TBLean 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 nutrtional intervention. The subjects were categorized in BMI-subgroups according to World Health Organization (WHO) criteria[17]. 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 obesity[14] 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 questionnaire[29]. 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 INDALI. 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/Satured fat Index (CSI), Atherogenic Index (AI), Thrombogenic Index (TI); the Mediterranean Adeguacy 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 PA[21].

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%; poliunsaturated 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 Vegretable/Animal (V/A) protein ratio as close to 1.5 as possible, monounsatured/satured fatty acid ratio close to 2, polyunsatured/satured fatty acid ratio close to 0.4-1, and low nutritional indices above mentioned[4]. The MAI index value was attended around 7[20]. 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 TBLean[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 supin 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 TBLean. Subjects were classified as obese by using gender and age Percentage Body Fat (PBF) cutoff points[14].

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'TGAAGGAGAAGGTGTCTGCGGGA3' (sense) and 5'AGGACGGTGCGGTGAGAGTG3' (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 *Hin*fI digestion site, the amplified product derived from the mutant gene was cleaved into 175-bp and 23-bp fragments by *Hin*fI, 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) ± standard deviation (SD), percentage, or < Δ % > (Δ%). Δ% 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. Comparisons 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 = 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 intervention[1].

	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

[1]Values expressed as mean ± SD. V/A, vegetable to animal protein ratio; CSI, Cholesterol/Satured fat Index; AI, Atherogenic Index; TI, Thrombogenic Index; MAI, Mediterranean Adeguacy 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 circumpherences, 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: Δ% = –9.09 ± 3.85 [min: –4.13; max: –9.09] for weight; Δ% = –15.79 ± 8.51 [min: –44.81; max: -2.22] for TBFat; Δ% = –3.80 ± 5.60 [min: –20.44; max: +17.82] for TBLean, respectively.

Table II. Physical activity assessment[1].

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

[1]Values 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]: Δ% = –9.31±4.01 [CI: –10.61÷–8.00] vs Δ% = –8.60 ± 3.51 [CI: –10.40 ÷ –6.79] for weight; Δ% = –17.96 ± 8.63 [CI: –19.77 ÷ –14.17] vs Δ% = –13.09 ± 7.82 [CI: –17.10 ÷ –9.07] for TBFat; Δ% = –2.86 ± 5.94 [CI: –4.79 ÷ –0.94] vs Δ% = –5.94 ± 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 revelead a difference in TBFat changes between

Table III. Anthropometric parameters and tHcy levels at baseline and week 12 after nutritional intervention, according to genotypes[1].

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[2,7]
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 ± 0.99[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]

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

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

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

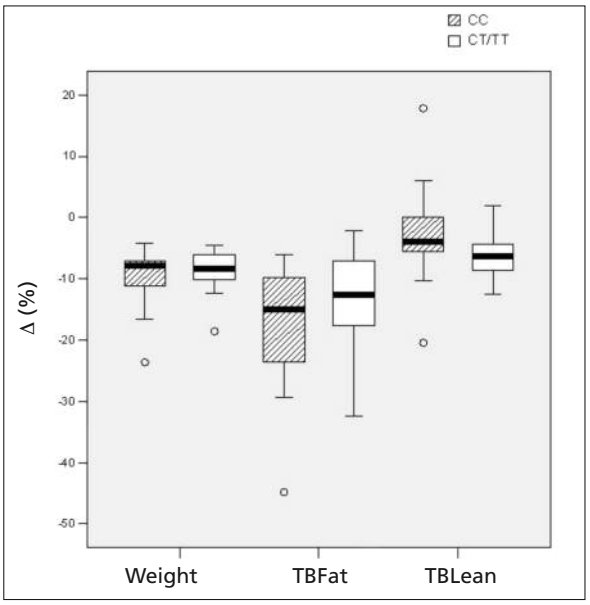

Figure 1. Relative change of TBLean, TBFat and weight (%) in response to IMD nutritional intervention, according to genotypes[1]. [1]All values are median ± SD (and CI). TBFat, Total Body Fat; TBLean, Total Body Lean; CI; Interval of Confidence. [#]Reflects the significance of the differences between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

gender groups (Δ% Female–14.65±7.42 vs Δ% –20.02 ± 10.12; $p \leq 0.05$). The stratification both for gender and MTHFR genotypes was significant for changes in TBFat and TBLean ($p \leq 0.05$). Particularly, T(+) carriers lost more lean mass at trunk level respect to T(-) carriers (Δ% –8.40 ± 6.71 vs –4.56 ± 7.96; $p \leq 0.05$) and relatively less fat mass at android region (Δ% +4.29 ± 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 highilighted differences in TBFat (%) changes respect to baseline values, in different genotypes, both in men and women.

Discussion

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

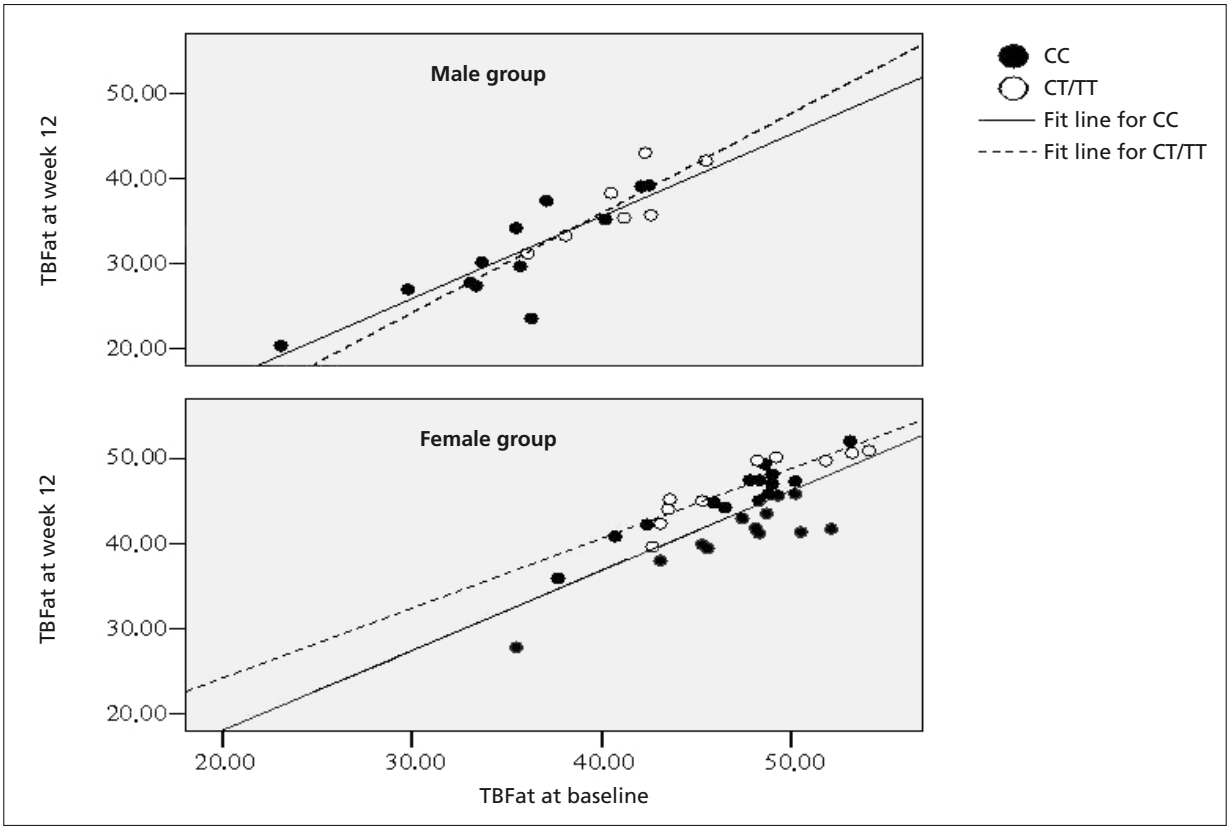

Figure 2. Correlation between TBFat (%) at baseline and at week 12, according to gender and genotypes.

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 recommendations[8]. It's an emerging area that genetic variants could be considered in obesity and disease management for their additive effects[25-27].

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 nutrigenetics[28].

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 variant[28]. 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 metabolism[29].

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 mass[30]. It has been well established that TBLean, TBFat, and BMI are under strong genetic control, with heritability of 0.52-0.72[30]. 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 intervention[14].

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 risk[9], 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 mortality[35].

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 men[31]. Moreover, as proposed by Lambrinoudaki et al[12], 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 critcism 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 sarcopenia[37].

Layman et al. proposed that the 2010 American Dietary Guidelines should emphasize more protein intake[38] 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 important[39].

Recently Loenneke et al[40] 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 maintaine 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.

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