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European Review for Medical and Pharmacological Sciences 2006; 10: 235-245

Normal Weight Obese syndrome: role of single nucleotide polymorphism of IL-15Rα and MTHFR 677C→T genes in the relationship between body composition and resting metabolic rate

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Abstract. – We have identified a subset of metabolically obese, but normal weight individuals, with potentially increased risks of developing the metabolic syndrome, despite their normal body mass index. We determined the relationship among body fat distribution, resting metabolic rate (RMR), total body water amount (%TBW), selected gene polymorphism on interleukin-15 receptor-alpha (IL-15Rα) and methylenetetrahydrofolate reductase 677C→T (MTHFR 677C→T), to distinguish normal weight obese (NWO) from nonobese with a normal metabolic profile and obese individuals. We analysed anthropometric variables, body composition by Dual energy X-ray Absorptiometry (DXA), RMR by indirect calorimetry, %TBW by bioimpedence analysis (BIA), MTHFR 677C→T and IL-15Rα genotypes of 128 clinically healthy Caucasian individuals.

We compared a group of female, defined as NWO and characterised by a BMI ≤ 25 kg/m² and FM ≥ 30% with groups of others female, and males, represented by nonobese with a BMI ≤ 25 kg/m² and FM ≤ 30%, and preobese-obese individuals with BMI ≥ 25 kg/m² and %FM ≥ 30%; none of the males was classified as NWO.

Significant correlations were found among body fat mass distribution, metabolic variables, percentage of total body water distribution and selected genetic variations. The variables that contributed significantly to the separation of classes were body tissue (Tissue), %TBW, RMR, the volumes of both oxygen (VO_2) and carbon dioxide (VCO_2). The distribution of MTHFR 677C→T and IL-15 genotypes was significantly different between classes.

Our data highlight that NWO individuals showed a significant relationship between the decrease in the basal metabolism (RMR), body fat mass increasing and total water amount. Possession of wild type homozygotes genotypes regarding IL-15Rα cytokine and 677C→T MTHFR enzyme characterised NWO individuals.

Key Words:

Body mass index, Body composition, Body fat mass, Metabolic diseases, MTHFR and interleukin-15Rα polymorphism.

Introduction

The World Health Organization (WHO) defines obesity as an excessive accumulation of fat to an extent that health may be impaired[1]. Central obesity is a major contributor to the development of metabolic syndrome (MS), which has been defined as the cluster of cardiovascular disease risk factors such as dyslipidaemia, hypertension, glucose intolerance and hyperinsulinaemia[2]. The term "metabolic syndrome" has been defined and institutionalized, principally by the World Health Organization (WHO)[3] and the Third Report of the National Cholesterol Education Program's Adult Treatment Panel (ATPIII)[4,5]. Ample data show that the presence of the metabolic syndrome may be effective in predicting the future risk of cardiovascular disease (CVD), although questions about how clear is the existing definition of the metabolic syndrome for diagnostic

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purposes are still opened. For example, microalbuminuria is listed in the WHO criteria but not in the ATP III; insulin resistance (as measured under hyperinsulinemic-euglycemic conditions) is relevant for WHO but not for ATP III, and while only an elevated fasting plasma glucose value is considered important in the ATP III definition, the WHO criteria recognize any measure whatsoever of insulin resistance[6,7]. According to current data, no review of the clinical evidence for inclusion or exclusion criteria for either of the two definitions of the syndrome has been published to date. However, there are a large body of evidence documenting the relationship between CVD risk and MS, diagnosed by either the ATP III or WHO definition or by their modifications[6,7].

In 2001, the National Cholesterol Education Program (NCEP) defined the criteria to diagnose MS[3]. The three or more of the following risk components are necessary: (1) waist circumference greater than 1.02 m for men and 0.88 m for women, (2) triglyceride (TG) levels of 150 mg/dl or more, (3) high density lipoprotein cholesterol (HDL-chol) levels less than 40 mg/dl for men and less than 50 mg/dl for women, (4) blood pressure of 130/85 mmHg or higher, (5) fasting glucose levels of 110 mg/dl or more. To date, three subtypes of obese individuals have been individualized: "at risk" of obesity with MS; metabolically healthy but obese individuals (MHO); metabolically obese but normal weight individuals (MONW)[8-11]. MONW represent a subset of individuals who have body mass index (BMI) of 18-25 kg/m^2, but display an excess of visceral fat[11,12] and a cluster of metabolic characteristics that may increase the development of MS. Interestingly, there is a group where genetic factors predispose to insulin resistance, dyslipidaemia, hypertension and cardiovascular diseases, as noted in syndrome X[13,14].

Methylenetetrahydrofolatereductase (MTHFR) is an important regulatory enzyme in the one carbon metabolism, because catalyzes the irreversible reduction of 5,10-methylenetetrahydrofolate (5,10-methyleneTHF) to 5-methyltetrahydrofolate (5-methylTHF) which serves as methyl donor for methionine, precursor of S-adenosyl-L-methionine (SAM)[15]. MTHFR activity is modulated by common polymorphism in the MTHFR gene, the C to T substitution at position 677 (alanine to valine), that encodes for a thermolabile variant with a reduced enzymatic function[16]. The alanine to valine mutation is known to cause lower levels of circulating folate and is postulated to lead to an accumulation of 5,10-methyleneTHF. Variant homozygous genotype affects genomic DNA methylation through an interaction with folate status[17] and it is associated with elevation in plasma homocysteine levels[17-20]. These metabolic changes are postulated to modify the risk of chronic diseases, including cardiovascular disease[21-24], colorectal cancer[25-28], and the risk of neural tube defects and dementia[29,30]. The metabolic basis for these observations has not been definitively established but may relate to the role of folate in nucleotide biosynthesis, DNA methylation[15] and/or maintenance of normal homocysteine concentration[20-24]. Therefore, the association of MTHFR 677C→T mutation with body composition and metabolism permits a further assessment of the role of folate levels in CVD risk in pre-obese and obese individuals, as in metabolically obese individuals.

One of the important key developments in obesity research over the past 5 years is the recognition that the disorder is characterised by chronic mild inflammation[32-34]. Several data indicated that there is an increased circulating level of inflammatory cytokines in the obese, given the observation that adipose tissue expresses and secretes a number of inflammation-related proteins, although, documenting the quantitative importance of adipose tissue to the circulating levels is difficult[6]. Systemic inflammation, in part via inadequately regulated pro-inflammatory cytokine, underlies autoimmunity, exacerbates presenile dementia, and promotes atherosclerosis. Atherosclerosis, an underlying cause of myocardial infarction, stroke, and other cardiovascular diseases, consists of a focal plaques characterized by cholesterol deposition, fibrosis and inflammation[34]. The presence of activated T lymphocytes and macrophages indicate a local immunologic activation in the atherosclerotic plaque that may be secondary to regulated pro-inflammatory cytokine too[35]. Interleukin-15 (IL-15) is a cytokine first identified in the supernatant of monkey epithelial cell line CV-1/EBNA as a factor that enhanced the antitumor response[36,37]. IL-15 is released from skeletal muscle to control fat deposition, white adipose tissue growth and mass, and it could play a decisive role in the suggested "conversation" between adipose tissue and skeletal muscle[38]. IL-15 seems to be involved in facilitating muscle proliferation and promoting fat depletion[38]. It has been speculated that a reciprocal balance of adipose and muscle-produced signals is necessary for a perfect body weight regulation:

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there are some evidences that the effects of IL-15 on white adipose tissue may be direct since the presence of IL-15 specific receptor subunit IL-15Rα detected in fat tissue (data not shown).

Recently, De Lorenzo et al[39,40], have described the normal weight obese (NWO) syndrome, called De Lorenzo syndrome, as a new syndrome present in young women, characterised by a particular body composition and presence of high values in CVD risk indexes. The purpose of this study was to define early indicators of disease as significant prognostic parameters for metabolic syndrome risks. We assessed whether MTHFR and IL-15 mutations were related to body composition, evaluating the relationships among body mass index, body fat mass percentage, resting metabolic rate and total body water percentage, in subject carrying NWO syndrome.

Participants and Methods

Participants

This study was comprised of 128 Caucasian subjects, subdivided into females (105) and males (23). Among the females, 35 were classified as NWO (BMI = 22.94 ± 1.38 kg/m^2 and FM% = 38.80 ± 6.1); 35 age-matched pre obese-obese (BMI 30.10 ± 5.75 kg/m^2 and FM% = 52.95 ± 5.01) and 35 nonobese age-matched women (BMI 20.33 ± 1.96 kg/m^2 and FM% = 20.01 ± 4.33). None of the males was age-matched NWO; 13 were pre obese-obese (BMI 30.10 ± 5.75 kg/m^2 and FM% = 52.95 ± 5.01) and 10 nonobese (BMI 20.33 ± 1.96 kg/m^2 and FM% = 20.01 ± 4.33).

None of the individuals had impaired either glucose tolerance and diabetes or clinical history of CDV risks. No individuals were taking any medication. Participants were randomly selected among all the participants to the studies on the basis of body composition and energy metabolism at the Unit of Human Nutrition of Tor Vergata University (Rome, Italy).

Informed consent was obtained from all the participants before the beginning of the study, according to Medical Ethics Committee Guidelines of the University.

Anthropometric Measurements

We measured anthropometric variables of all participants according to standard methods[41]. Individuals were instructed to remove their shoes and undress before any measurements were taken. Body weight (kg) was measured to the nearest 0.1 kg, using a balance scale (Invernizzi, Rome, Italy). Height (m) was measured using a stadiometer to the nearest 0.01 m (Invernizzi, Rome, Italy). Two circumferences (waist and hip) were measured to the nearest 0.05 m using a flexible steel metric tape. Abdominal circumference was defined as the horizontal distance around the abdomen at the umbilicus. Hip circumference (H) was measured as the distance passing horizontally through the two superior iliac bones. Body mass index (BMI) was calculated using the formula: BMI = body weight (kg)/height (m^2).

Bioelectrical Impedance Analysis (BIA)

Resistance, reactance, impedance and phase angle at 50 kHz frequency were measured using a BIA phase sensitive system (BIA 101S, Akern/RJL Systems-Florence, Italy). Body composition analysis was assessed with estimates of fat mass (FM), free mass (FM), total body water (TBW). Measurements were taken on left side of the body, with injection and sensor electrodes placed on the hand and foot in reference position. To calculate %TBW, it was used the formula according to De Lorenzo et al[42]. Percent fat mass (%FM) was also calculated[43].

Dual X-ray Absorptiometry (DXA)

Total body composition was assessed by dual-energy x-ray absorptiometry (DXA) (Lunar DPX – Florence, Italy). Standard DXA quality control and calibration measures were performed prior to each testing session. Individuals were required to remove all clothing including shoes, socks and jewels except undergarments, prior to being positioned on the DXA table. Scans were performed with individuals in a supine position. The entire body was scanned beginning from the top of the head and moving in a rectilinear pattern. Mean measurement time was 15 min. Radiation exposure was < 8 μSv.

Resting Metabolic Rate (RMR) Measurement

Resting Metabolic Rate (RMR) was measured by indirect calorimetry after a 12 hr fast. The volumes of both oxygen (VO_2) and carbon dioxide (VCO_2) were measured using a canopy system for 30 minutes (Sensormedic 2900, California, USA). The first 10 min was considered a period of acclimatisation, while the latest 20 min

were used for analysis. RMR was calculated from oxygen consumption and carbon dioxide production according to Weir's equation[44]:

$$RMR = 1.44 \times [3.91 \times VO_2\ (ml) + 1.106 \times VCO_2\ (ml)]$$

For the calculation of RMR, only data of individuals with seemingly steady-state conditions (i.e. VO_2 and VCO_2 did not vary more than 5% from the mean value of the 20 min measurement period) were used.

Individuals were instructed to drink only water, consume no alcohol, no proteins for 12 hrs before testing and refrain from smoking and engaging in physical activities for 24 hrs before testing. Prior to the RMR measurements, individuals were supine for 25-30 minutes in a quiet room. All tests were performed on individuals while in a supine position. Room temperature of the room was set at an average of 22°C. For additional quality control two different certified oxygen/carbon dioxide gas mixtures (SIAD Ltd Co, Rome, Italy) were used.

Basal Insulin

Serum insulin level (BI) was assayed using an immunoenzymometric assay (Medgenix Ins-EASIA, Biosource, Belgium).

Genetic Analysis

Genomic DNA was extracted from peripheral whole blood using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA).

MTHFR genotype. There are three MTHFR 677 genotypes: wild type homozygote (CC), variant heterozygote (CT), and variant homozygote (TT). Genotyping for MTHFR 677C→T was assessed by polymerase chain reaction, which was followed by restriction enzyme analysis with Hinf I, according to a modification of PCR-RFLP method of Frosst et al[16]. Briefly, amplification was performed using initial denaturation at 94°C for 4 min followed by 29 cycles of 94°C for 1 min, 62°C for1 min, and 72°C for1 min with a final extension at 72°C for 10 min. The buffer for PCR reaction contained 2.5 mM $MgCl_2$, 9.9 mM Tris-HCl (pH 8.8), 50 mM KCl, 0.1% Triton-X100, 0.50 µM deoxyribonucleotide triphosphate (dNTPs), 1 U of *Taq* DNA polymerase, and 0.2 µM of each primers. The primer sequences are:

5'-TGAAGGAGAAGGTGTCTGCGGGA-3' and 5'-AGGACGGTGCGGTGAAGATG-3'

IL-15 exon 5 intron-exon border (IL-15Rα IVS-5) genotype. Subjects were categorized as exhibiting wild type homozygote AA, variant heterozygote AG and variant homozygote GG genotypes. The exon 5 intron-exon border variant (A to G), 20 bp from the border in intron 5 (SNP accession rs 3136618), was genotyped by using the enzyme HpaII. The fragment containing HpaII site was amplified according to modified method by Riechman et al[45]. Amplification was performed using initial denaturation at 94°C for 4 min followed by 34 cycles of 94°C for 1 min, 50°C for1 min, and 72°C for 1 min with a final extension at 72°C for 10 min. The buffer for PCR reaction contained 2.5 mM $MgCl_2$, 9.9 mM Tris-HCl (pH 8.8), 50 mM KCl, 0.1% Triton-X100, 0.50 µM deoxyribonucleotide triphosphate (dNTPs), 1 U of *Taq* DNA polymerase, and, 0.2 µM of each primers. The primer sequences are:

5'-ATCTGATGGAGGCCTTCTGAG-3' and 5'-GCTTCATGAGGAACTAGGACC-3'

Statistical Analysis

Descriptive values are expressed as mean ± standard error (SE). Canonical discriminate analysis, chi-square test of independence and variance analysis were performed using the SPSS statistical package (SPSS Chicago, IL). A value of $p < 0.05$ was considered significant.

Results

Overall 128 Caucasian individuals were examined. The participants were classified as nonobese, NWO and pre obese-obese individuals according to the National Cholesterol Education Program (NCEP) criteria[4], on the basis of their FM distribution using DXA, adopting the percentage of FM as criteria of classification.

Due to a special interest in NWO, female subjects showing a BMI ≤ 25 kg/m^2 and FM ≥ 30% were classified as NWO and indicated as F1 group; female nonobese showing a BMI ≤ 25 kg/m^2 and FM ≤ 30%, and pre obese-obese individuals with BMI ≥ 25 kg/m^2 and FM ≥30% were indicated as F2 group. All males were represented by nonobese and obese subject. None of the males subjects on the basis of their BMI and %FM was recorded as NWO.

Table I. Descriptive characteristics of body composition variables and respiratory parameters in female and male subjects.

	Age	Tissue	W	H	%TBW	VO_2	VCO_2	RMR	BI
Females									
F_1									
Mean	28.7	55.8	73.0	98.5	50.4	173.7	166.5	1.25	6.2
S.E.	1.4	0.9	1.3	1.1	0.6	4.3	4.2	0.03	0.8
F_2									
Mean	33.3	66.1	79.6	102.5	47.1	192.6	182.0	1.38	8.5
S.E.	1.4	1.9	1.7	1.9	0.7	3.7	3.3	0.02	0.8
Variance analysis (P)	0.002	0.001	0.01	n.s.	0.001	0.003	0.007	0.002	0.056
Males									
Mean	45	90.6	98.2	106.3	53.4	252.0	225.3	1.78	12.7
S.E.	2.3	0.4	4.2	2.2	0.9	13.1	12.3	0.09	3.4

Mean values of body and respiratory parameters in female and male subjects. Categories: F1 = NWO females with BMI ≤ 25 kg/m^2 and FM > 30%; F_2 = female nonobese with a BMI ≤ 25 kg/m^2 and FM ≤ 30%, and preobese-obese individuals with BMI ≥ 25 kg/m^2 and FM ≥ 30%. Males: all males subject nonobese and obese. None of the males subjects was recorded with a value of BMI ≤ 25 kg/m^2 and FM > 30%. Parameters: age, Tissue = tissue body composition, W = waist and H = hip circumferences, %TBW = percent of total body water, VO_2 = volume of oxygen consumption and VCO_2 = volume of carbon dioxide production, RMR=resting metabolic rate, BI = basal insulin level. SE = Standard Error.

Descriptive characteristics of body composition variables and respiratory parameters were compared among the two F1 and F2 groups (Table I). The means of age, tissue body composition (Tissue), waist (W) and hip (H) circumferences, percent of total body water (%TBW), oxygen consumption (VO_2) and carbon dioxide (VCO_2) production, resting metabolic rate (RMR), and basal insulin level (BI) were compared. Significant differences ($p < 0.05$) were obtained comparing parameters of NWO individuals with others. However, no significant differences between classes were observed for hip parameter. The mean values of all variables, with the exception of %TBW in F_2, were lower in F_1 than F_2 and males.

The frequency of MTHFR 677C→T genotypes in females were shown in Table II. The distribution of MTHFR genotypes was significantly different between classes F_1 and F_2. In particular there was a strong decrease of variant homozigote TT genotype corresponding to 4.2% in F_1 as compared to 28.8% in F_2 and to 20.0 in males.

Table III shows the frequency of IL-15Rα (IVS-5) genotypes in females and males. The distribution of IL-15 Rα (IVS-5) genotypes was significantly different between classes. In particular, there was a strong decrease of GG genotype in F_1 down to 14.3% as compared to the 36.8% in F_2 group and to the 48.0% in males.

In Table IV we showed the result of a discriminant analysis by method enter. Grouping dependent variable was sex, RMR, %FM with two categories: females with BMI ≤ 25 and %FM > 30% (F1), other females (F2) and males (M). Independent variables were represented by body, polymorphic and respiratory parameters. For MTHFR 677C→T polymorphism we have considered two genotypic classes: TT and (CT+CC) and for IL-15Rα (IVS-5)

Table II. Genotypes frequency of MTHFR 677C→T variant in the studied population.

	Genotypes frequency (%)		
	CC	CT	TT
Females			
F_1	16.7	79.1	4.2
F_2	16.9	54.3	28.8
Males	20.0	60.0	20.0

Chi square test of independence: CC vs CT vs TT genotypes, (P) < 0.04; TT vs other genotypes (P) < 0.02. Categories: F_1 = NWO females with BMI ≤ 25 kg/m^2 and FM > 30%; F_2 = female nonobese with a BMI ≤ 25 kg/m^2 and FM ≤ 30%, and preobese-obese individuals with BMI ≥ 25 kg/m^2 and FM ≥ 30%. Males: all males subjects nonobese and obese. None of the males subject was recorded with a value of BMI ≤ 25 kg/m^2 and FM > 30%.

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Table III. Genotypes frequency of IL-15Ra IVS-5 variant in the studied population.

	Genotypes frequency (%)		
	AA	AG	GG
Females			
F_1	34.3	51.4	14.3
F_2	23.5	39.7	36.8
Males	16.0	36.0	48.0

Chi square test of independence: AA vs AG vs GG genotypes, (P) < 0.06; GG vs other genotypes (P) < 0.02. Categories: F_1 = NWO females with BMI ≤ 25 kg/m^2 and FM > 30%; F_2 = female nonobese with a BMI ≤ 25 kg/m^2 and FM ≤ 30%, and preobese-obese individuals with BMI ≥ 25 kg/m^2 and FM ≥ 30%. Males: all males subjects nonobese and obese. None of the males subject was recorded with a value of BMI ≤ 25 kg/m^2 and FM > 30%.

polymorphism two genotypic classes: GG and (AG+AA). Two significant discriminant functions were identified. The first function separates males from females. Tissue, %TBW, VO_2, VCO_2 and RMR contribute significantly to separation. The second function separates the two classes of females. Besides the variables contributing to the first function, also MHTFR TT genotype and waist contribute significantly to separation.

The results of a discriminant analysis by method enter with two categories as dependent variable were summarised in Table V: F_2 and M. Independent variables are body, respiratory and polymorphic parameter. The variables contributing significantly to separation were: tissue, %TBW, VO_2,VCO_2 and RMR.

Table VI shows the results of a discriminant analysis by method enter with two categories as dependent variable: F_1 and M. Independent variables were represented by body, respiratory and polymorphic parameters. The variables contributing significantly to separation were: tissue, %TBW, VO_2, VCO_2, RMR and IL-15Rα (IVS-5) GG genotype. Table VII shows the results of a discriminant analysis by method enter with two categories as dependent variable: F_1 and F_2. Independent variables were the body, respiratory and polymorphic parameters. The variables contributing significantly to

Table IV. Discriminant analysis by method enter. Standardized Canonical Discriminant Function Coefficients.

	Function	
	1	2
Tissue	1.436	0.796
H	-0.154	0.705
W	0.005	0.640
%TBW	1.402	0.884
VO_2	3.178	-0.075
VCO_2	1.446	0.421
RMR	-4.294	-0.486
MTHFR (TT)	-0.149	-0.716
IL-15Rα IVS 5 (GG)	0.067	0.262
Age	0.195	0.145

Independent variables: body, respiratory and polymorphic parameters. Dependent variable SEX, RMR, %FM. Categories: F_1 = NWO females with BMI ≤ 25 kg/m^2 and FM > 30%; F_2 = female nonobese with a BMI ≤ 25 kg/m^2 and FM ≤ 30%, and preobese-obese individuals with BMI ≥ 25 kg/m^2 and FM ≥ 30%. Males: all males subject nonobese and obese. None of the males subjects was recorded with a value of BMI ≥ 25kg/m^2 and FM > 30%. Parameters: Tissue = tissue body composition, H = hip and W = waist circumferences, %TBW = percent of total body water, VO_2 = volume of oxygen consumption and VCO_2 = volume of carbon dioxide, RMR = resting metabolic rate, MTHFR and IL-15Rα IVS 5 genotypes TT and GG respectively, age.
Summary of Canonical Discriminant Functions

Function	Eigen value	% variance	Canonical correlation	χ^2	df	p
1	3.738	93.8	0.89	150.240	20	< 0.001
2	0.249	6.2	0.45	18.790	9	< 0.03

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Table V. Discriminant analysis by method enter. Standardized Canonical Discriminant Function Coefficients.

	Function 1
Tissue	1.441
%TBW	1.603
VO_2	2.046
VCO_2	1.031
RMR	-2.851
MTHFR (TT)	-0.260
IL-15Rα IVS 5 (GG)	0.058
Age	0.274

Independent variables: body, respiratory and polymorphic parameters. Dependent variable SEX, RMR, %FM. Categories: F_1 = NWO females with BMI ≥ 25 kg/m² and FM > 30%; F_2 = female nonobese with a BMI ≤ 25 kg/m² and FM ≤ 30%, and preobese-obese individuals with BMI ≥ 25 kg/m² and FM ≥ 30%. Males: all males subjects nonobese and obese. None of the males subjects was recorded with a value of BMI ≥ 25 kg/m² and FM > 30%.
Parameters: Tissue = tissue body composition, %TBW = percent of total body water, VO_2 = volume of oxygen consumption and VCO_2 = volume of carbon dioxide, RMR = resting metabolic rate, MTHFR and IL-15Rα IVS 5 genotypes TT and GG respectively, age.
Summary of Canonical Discriminant Functions

Function	Eigen value	% of variance	Canonical correlation	χ^2	df	p
1	3.841	100	0.89	115.131	8	< 0.001

Table VI. Discriminant analysis by method enter. Standardized Canonical Discriminant Function Coefficients.

	Function
Tissue	1.131
%TBW	1.046
VO_2	3.266
VCO_2	1.079
RMR	-4.121
MTHFR (TT)	0.193
IL-15Rα IVS 5 (GG)	0.385
Age	0.235

Independent variables considering body, respiratory and polymorphic parameters. Dependent variable SEX, RMR, %FM. Categories: F_1 = NWO females with BMI ≤ 25 kg/m² and FM > 30%; F_2 = female nonobese with a BMI ≤ 25 kg/m² and FM ≤ 30%, and preobese-obese individuals with BMI ≥ 25 kg/m² and FM ≥ 30%. Males: all males subjects nonobese and obese. None of the males subjects was recorded with a value of BMI ≤ 25 kg/m² and FM > 30%.
Parameters: age, Tissue = tissue body composition, %TBW = percent of total body water, VO_2 = volume of oxygen consumption and VCO_2 = volume of carbon dioxide production, RMR = resting metabolic rate, MTHFR and IL-15Rα IVS 5 genotypes TT and GG respectively.
Summary of Canonical Discriminant Functions

Function	Eigen value	% of variance	Canonical correlation	χ^2	df	p
1	5.408	100	0.92	68.727	8	< 0.001

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Table VII. Discriminant analysis by method enter. Standardized Canonical Discriminant Function Coefficients.

	Function
Tissue	0.192
%TBW	-0.445
VO_2	-0.827
VCO_2	-0.186
RMR	1.232
MTHFR (TT)	0.504
IL-15Rα IVS 5 (GG)	0.343
Age	0.051

Independent variables: body, respiratory and polymorphic parameters. Dependent variable SEX, RMR, %FM. Categories: F_1 = NWO females with BMI ≤ 25 kg/m^2 and FM > 30%; F_2 = female nonobese with a BMI ≤ 25 kg/mm^2 and FM ≤ 30%, and preobese-obese individuals with BMI ≥ 25 kg/m2 and FM ≥ 30%. Males: all males subject nonobese and obese. None of the males subject was recorded with a value of BMI ≤ 25 kg/mm^2 and FM > 30%. Parameters: age, Tissue = tissue body composition, W = waist and H = hip circumferences, RMR = resting metabolic rate, %TBW = percent of total body water, VO_2 = volume of oxygen consumption and VCO_2 = volume of carbon dioxide production, MTHFR and IL-15 IVS 5 genotypes TT and GG respectively.
Summary of Canonical Discriminant Functions

Function	Eigen value	% of variance	Canonical correlation	χ^2	df	p
1	0.252	100	0.45	16.620	8	< 0.03

separation between groups were the %TBW, VO_2, RMR, MTHFR (TT) genotype and IL-15Rα (IVS-5) GG genotype.

Discussion

Data from observational studies in general populations indicate that the MTHFR 677C→T polymorphism is associated with a lower folate and a higher plasma total homocysteine concentration, which is most pronounced in subjects with the TT genotype who have a marginal folate status[46]. These metabolic changes are associated with an increased risk of chronic degenerative disease such as CVD and cancer[21-30].

IL-15 is a cytokine first identified as a factor that enhances T-cell proliferation, NK cell cytotoxicity, and up-regulates the production of NK cell-derived cytokines, including IFN-gamma, GM-CSF, and TNF-alpha, and may protect T-cells and neutrophils from apoptosis. Some authors have proposed a very important anabolic function for IL-15 in skeletal muscle as a stimulator of muscle fibers accumulation, moreover this cytokine seems to act as metabolic regulator between skeletal muscle and adipose tissue. In particular, the effect of IL-15 on adipose tissue seem to be direct since the presence of the three types of receptor subunits (IL-15Rα, IL-2Rβ, and IL-2 Rγc) that has been demonstrated in fat tissue[37,38]. Our study assessed both the MTHFR C677T and IL-15Rα polymorphisms and their possible joint effect in body composition and metabolic disorders. As described by De Lorenzo et al[39,40], the NWO syndrome in young women is described by a particular body composition, analysed by DXA according previous to De Lorenzo et al.[47], and presence of high values in CVD risk indexes. However, a predictive evaluation based not only on anthropometric and biochemical, but also by molecular biology methods may be usefully for a correct classification of these subjects. The analysis in this paper showed that a considerable number of subjects, with a classification of NWO, present a significant and characteristic genotype.

This study suggests that body fat distribution, RMR, %TBW amount and single nucleotide polymorphism of IL-15Rα IVS-5 and MTHFR 677C→T mutations are associated to distinguish NWO women from controls with a normal metabolic profiles and obese individuals.

The first observation on this population of 128 subjects showing that no males subjects with a BMI ≤ 25 kg/m^2 and % FM ≥ 30% are

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present and the NWO classification is a distinctive characteristic of the 33.7% females subjects. A second consideration, regarding the two classes of females, is that in the NWO women population there is a significant relationship among the decrease in the basal metabolism, body fat mass increasing and total water amount. As indicated in the Table II, the mean values of all variables, with the exception of %TBW, are lower in NWO than in others, suggesting that these descriptive characteristics of body composition variables and respiratory parameters may be associated to a different genotype. The analysis of a common genetic variant in the MTHFR gene involving a cytosine to thymidine (C→T) transition at nucleotide 677, showed a significant difference between NWO individuals and the others. In particular, there is a strong decrease of variant homozygous TT genotype in NWO women compared to other groups. A similar result is also obtained by IL-15Rα IVS-5 polymorphism analysis, where the variant genotype i.e. GG homozygous genotype is significantly reduced in the NWO women. Thus, regarding the NWO women we observe in both genes a significant reduction in the variant homozygous genes, supporting that the NWO phenotype is not associated with a variant form of these genotypes, although a different pattern of genetic polymorphism between NWO and other subjects is found. On the basis of these results a statistical analysis, performed according to method enter, identified two functions that contribute significantly to separate different groups, in particular in the first function: tissue, %TBW, VO_2, VCO_2 and RMR contribute significantly to separate males from females whereas in the second function the variables that contributing significantly to separation between the groups were: %TBW, VO_2, RMR, MTHFR 677C→T (TT) genotype and IL-15Rα IVS-5 (GG) genotype.

The relationship between basal metabolism and variant genotype is likely to be a complex biological sum of genetic and nutritional differences. Further studies will be performed to readily explain the association between MTHFR 677C→T (TT) and IL-15Rα IVS-5 (GG) genotypes and these findings need to be re-evaluated in future studies. On the basis of our analysis we may consider two genotypic classes for MTHFR 677C→T polymorphism: (CC+CT) and TT and two genotypic classes for IL-15 IVS-5 polymorphism: (AA+AG) and GG. Thus, as a whole these results showed a significant relationship between the NWO phenotype and possession of wild type and heterozygous or mutated genotypes associated with MTHFR enzyme and IL-15Rα.

Conclusion

As reported previously[21], there were no genotype-specific Dietary Reference Intakes (DRI) and up to now data are insufficient to conclude that consumption of the current, Recommended Dietary Allowance (RDA) for folate is sufficient to maintain normal folate status in individuals with the MTHFR 677C→T polymorphism.

To be noted that a limit of the present study is the lacking of the folate and homocystein plasma level in the studied subject, and further studies will be performed addressing these questions.

However, it is important to have detailed information on the consequences of the different genotypes in the basal metabolism and further evaluation regarding body composition and variant genotypes will be necessary in the future, especially for the introduction of new molecular assays in the clinical practice as diagnostic tools and criteria other than body fat percentage for the prevention and to avoid the misclassification of obesity.

In summary, the findings of the current study indicate that on the basis of our analysis we may consider two genotypic classes for MTHFR 677C→T polymorphism: (CC+CT) and TT and two genotypic classes for IL-15Rα IVS-5 polymorphism: (AA+AG) and GG. Thus, as a whole these results showed a significant relationship between the NWO phenotype and possession of wild type and heterozygous genotypes associated with MTHFR enzyme and IL-15Rα.

In conclusion, these two polymorphisms might be useful tools to predict disease risks related to metabolic syndrome and obesity, and suggest the importance to evaluate the adequacy of folate intake and influence of the MTHFR 677C→T polymorphism on folate and homocysteine status. This study provides data that may be important in future revisions of the folate RDA. Treatment with adequate amount of folate, in which MTHFR genotype is considered, according the potential effects of age and sex, might be important in the prevention of NWO syndrome and related diseases.

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References

1) WHO, OBESITY. Preventing and managing the global epidemic. Report on a WHO Consultation of Obesity. 1997; Geneva, 3-5 June, WHO/NUT/NCD/98.1 Geneva, Switzerland.

2) VALDEZ R, SEIDELL JC, AHN YI, WEISS KM. A new index of abdominal adiposity as indicator of risk for cardiovascular diseases; a cross population study. Int J Obesity 1993; 17: 77-82.

3) WORLD HEALTH ORGANIZATION. Definition, diagnosis, and classification of diabetes mellitus and its complications: report of a WHO consultation. Geneva, World Health Org., 1999.

4) EXPERT PANEL ON DETECTION, EVALUATION, AND TREATMENT OF HIGH BLOOD CHOLESTEROL IN ADULTS 2001. Executive Summary of the third Report of the National Cholesterol Education Program (NCPE) Expert Panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA 2001; 285: 2486-2497.

5) GRUNDY SM, BREWER HB Jr, CLEEMAN JI, SMITH SC JR, LENFANT C. National Heart, Lung, and Blood Institute, American Heart Association: definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004; 109: 433-438.

6) RICHARD K, J BUSE, E FERRANNINI, M STERN. The Metabolic Syndrome: time for a critical appraisal. Joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 2005; 28: 2289-2304.

7) GRUNDY SMG, BREWER B, CLEEMAN JI, SMITH SC, LENFANT C. Definition of metabolic syndrome. Circulation 2004; 109: 433-438.

8) KARELIS AD, ST PIERRE DH, CONUS F, LHORET RR, POEHLMAN ET. Metabolic and body composition factors in subgroups of obesity: what do we do? J Clin Endocr Metab 2004; 89: 2569-2575.

9) RUDERMAN NB, SCHNEIDER SH, BERCHTOLD P. The metabolically-obese, normal weight individual. Am J Clin Nutr 1981; 34: 1617-1621.

10) RUDERMAN NB, BERCHTOLD P, SCHNEIDER S. Obesity associated disorder in normal weight individual: some speculation. Int J Obesity 1982; 6: 151-157.

11) RUDERMAN N, CHISHOLM D, PI-SUNYER X, SCHNEIDER S. The metabolically obese normal weight revisited. Diabetes 1998; 47: 699-713.

12) YOU T, RYAN AS, NICKLAS J. The metabolic syndrome in obese postmenopausal women: relationship to body composition, visceral fat, and inflammation. J Clin Endocrinol Metab 2004; 89: 5517-5522.

13) WAICHEMBERG B, LEO M, DOMINGOS A, LERARIO AC, SANTOMAURO A, TEREZA MG. Syndrome X: a syndrome of insulin resistance: epidemiological and clinical evidence. Diabetes Metab Rev 1994; 10: 19-29.

14) WAICHEMBERG B. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev 2000; 21: 697-738.

15) RAZIN A, RIGGS AD. DNA methylation and gene function. Science 1980; 210: 604-610.

16) FROSST P, BLOM HJ, MILOS R, GOYETTE P, SHEPPARD CA, MATTHEWS RG, BOERS GJ, DEN HEIJER M, KLUIJTMANS LA, VAN DEN HEUVEL LP, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995; 10; 111-113.

17) FRISO S, CHOI SW, GIRELLI D, MASON JB, DOLNIKOWSKI GG, BAGLEY PJ, OLIVIERI O, JACQUES PF, ROSENBERG IH, CORROCHER R, SELHUB J. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Natl Acad Sci U S A 2002; 99: 5606-5611.

18) GUINOTTE CL, BURNS MG, AXUME JA, HATA H, URRUTIA TF, ALAMILLA A, MCCABE D, SINGGIH A, COGGER EA, CAUDILL MA. Methylenetetrahydrofolate reductase 677C→T variant modulates folate status response to controlled folate intakes in young women. J Nutr 2003; 133: 1272-1280.

19) VESELA K, PAVLIKOVA M, JANOSIKOVA B, ANDEL M, ZVAROVA J, HYANEK J, KOZICH V. Genetic determinants of folate status in central Bohemia. Physiol Res 2005; 54: 295-303.

20) JACQUES PF, BOSTOM AG, WILLIAMS RR, ELLISON RC, ECKFELDT JH, ROSENBERG IH, SELHUB J, ROZEN R. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation 1996; 93: 7-9.

21) DE BREE A, VERSCHUREN WMM, BJORKE-MONSEN AL, VAN DER PUT NMJ, HEIL SG, TRIJBELS FJM, BLOOM HJ. Effect of the methylenetetrahydrofolate reductase 677C→T mutation on the relations among folate intake and plasma folate and homocysteine concentration in general population sample. Am J Clin Nutr 2003; 77: 687-693.

22) MA J, STAMPFER MJ, HENNEKENS CH, FROSST P, SELHUB J, HORSFORD J, MALINOW MR, WILLETT WC, ROZEN R. Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physicians. Circulation 1996; 94: 2410-2416.

23) BRATTSTROM L,WILCKEN DE. Homocysteine and cardiovascular disease: cause or effect? Am J Clin Nutr 2000; 72: 315-323.

24) UELAND PM, REFSUM H, BERESFORD SA, VOLLSET SE. The controversy over homocysteine and cardiovascular risk. Am J Clin Nutr 2000; 72: 324-332.

25) KIM JI. Methylenetetrahydrofolate reductase polymorphisms, folate, and cancer risk: a paradigm of gene-nutrient interactions in carcinogenesis. Nutr Rev 2000; 58: 205-217.

26) BAYLEY LB. Folate, methyl-related nutrients, alcohol, and MTHFR 677C→T polymorphism affect cancer risk: intake recommendations. J Nutr 2003; 133: S3748-3753.

235-245/Art. 1.1458 16-10-2006 15:14 Pagina 245

27) MA J, STAMPFER J, GIOVANNUCCI E, *et al*. Methylenetetrahydrofolate reductase polymorphisms, dietary interactions, and risk of colorectal cancer. Cancer Res 1997; 57: 1098-1102.

28) MA J, STAMPFER MJ, GIOVANNUCCI E, ARTIGAS C, HUNTER DJ, FUCHS C, WILLETT WC, SELHUB J, HENNEKENS CH, ROZEN R. A methylenetetrahydrofolate reductase polymorphism, and the risk of colorectal cancer. Cancer Res 1996; 56: 4862-4864.

29) VAN DER PUT NM, STEEGERS-THEUNISSEN PM, FROSST P, TRIJBELS FJM, ESKES BLOM HJ. Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. Lancet 1995; 346: 1070-1071.

30) YOO JH, CHOI GD, KANG SS. Pathogenicity of thermolabile methylenetetrahydrofolate reductase for vascular dementia. Arterioscler Thromb Vasc Biol 2000; 20: 1921-1925.

31) AL SUWAIDI J, HIGANO ST, HOLMES DR, LENNON R, LERMAN A. Obesity is independently associated with coronary endothelial dysfunction in patients with normal or mildly diseased coronary arteries. J Am Coll Cardiol 2001; 37: 1523-1528.

32) SUNDELL J, LAINE H, LUOTOLAHTI M, KALLIOKOSKI K, RAITAKARI O, NUUTILA P, KNUUTI J. Obesity affects myocardial vasoreactivity and coronary flow response to insulin. Obes Res 2002; 10: 617-624.

33) TREMBLAY AJ, DESPRES JP, PICHE ME, NADEAU A, BERGERON J, ALMERAS N, TREMBLAY A, LEMIEUX S. Association between the fatty acid content of triglyceride, visceral adipose tissue accumulation and components of the insulin resistance syndrome. Metabolism 2004; 53: 310-317.

34) BROD SA. Unregulated inflammation shortens human functional longevity. Inflamm Res 2000; 49: 561-570.

35) BURTON JD, BAMFORD RN, PETERS C, GRANT AJ, KURYS G, GOLDMAN CK, BRENNAN J, ROESSLER E, WALDMANN TA. A lymphokine, provisionally designated interleukin T and produced by a human adult T-cell leukemia line, stimulates T-cell proliferation and the induction of lymphokine-activated killer cells. Proc Natl Acad Sci USA 1994; 91: 4935-4939.

36) GRABSTEIN KH, EISENMAN J, SHANEBECK K, RAUCH C, SRINIVASAN S, FUNG V, BEERS C, RICHARDSON J, SCHOENBORN MA, AHDIEH M. Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor. Science 1994; 264: 965-968.

37) CARBO N, LOPEZ-SORIANO J, COSTELLI P, ALVAREZ B, BUSQUETS S, BACCINO FM, QUINN LS, LOPEZ-SORIANO FJ, ARGILES JM. Interleukin-15 mediates reciprocal regulation of adipose and muscle mass: a potential role in body weight control. Biochim Biophys Acta 2001; 1526: 17-24.

38) ARGILES JM, LOPEZ-SORIANO J, ALMENDRO V, BUSQUETS S, LOPEZ-SORIANO FJ. Cross-talk between skeletal muscle and adipose tissue: a link with obesity. Med Res Rev 2005; 25: 49-65.

39) DI RENZO L, DEL GOBBO V, BIGIONI M, PREMROV MG, CIANCI R, DE LORENZO A. Body composition analyses in normal weight obese women. Eur Rev Med Pharmacol Sci 2006; 10: 191-196.

40) DE LORENZO A, MARTINOLI R, VAIA F, DI RENZO L. Normal Weight Obese (NWO) Women: an evaluation of candidate new syndrome. Nutr Metab Cardiovasc Dis 2006 (in press).

41) LOHMAN TG, ROCHE AF, MARTORELL R. Anthropometric standardization reference manual: Human Kinetics, Champaing, IL, 1998.

42) DE LORENZO A, CANDELORO N, ANDREOLI A, DURENBERG P. Determination of intracellular water by multifrequency bioelectrical impedance. Ann Nutr Metab 1995; 39: 177-184.

43) FORBES GB. Human body composition: growth aging, nutrition and activity. Springer-Verlag, Berlin Heidelberg, New York, 1987.

44) WEIR JB. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol 1949; 109: 1-9.

45) RIECHMAN SE, ROTH SM, FERRELL RE. Association of interleukin-15 receptor genetic variation with resistance exercise training responses. J Appl Physiol 2004; 97: 2214-2219.

46) SHELNUTT KP, KAUWELL GP, CHAPMAN CM, GREGORY JF 3RD, MANEVAL DR, BROWDY AA, THERIAQUE DW, BAILEY LB. Folate status response to controlled folate intake is affected by the methylenetetrahydrofolate reductase 677C→T polymorphism in young women. J Nutr 2003; 133: 4107-4111.

47) DE LORENZO A, DURENBERG P, PIETRANTUONO M, DI DANIELE N, CERVELLI V, ANDREOLI A. How fat is obese?. Acta Diabetol 2003; 40: S254-257.