

## Genotype $\times$ nutrient association of common polymorphisms in obesity-related genes with food preferences and time structure of energy intake

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Personal food preferences can either enhance or suppress the development of obesity and the selection and proportion of macronutrients in the diet seem to have a heritable component. In the present study, we therefore focused on dietary composition as a specific trait related to obesity and we determined whether genetic variations in leptin (LEP), LEP receptor (LEPR), adiponectin (ADIPOQ), IL-6 and pro-opiomelanocortin (POMC) underlie specific native food preferences and obesity-related anthropometric parameters. The total of 409 individuals of Czech Caucasian origin were enrolled into the present study and 7 d food records were obtained from the study subjects along with selected anthropometric measurements. In a subset of study subjects, plasma levels of ADIPOQ, LEP and soluble LEPR were measured. Independently of the BMI of the individuals, common variations in LEP and LEPR genes were associated with specific eating patterns, mainly with respect to timing of eating. The LEP + 19A/G polymorphism served as an independent predictor for BMI, percentage of body fat and skinfold thickness and significantly affected the time structure of the daily energy intake. The POMC *Rsa*I polymorphism was associated with percentage of body fat. The ADIPOQ 45 T/G polymorphism was associated with the thickness of the subscapular skinfold. The LEPR Gln223Arg polymorphism was associated with multiple parameters, including diastolic blood pressure, meal sizes during the day and plasma ADIPOQ levels. In a separate analysis, soluble leptin receptor (sObR) plasma levels and LEP:sObR ratio were significantly correlated with systolic blood pressure ( $\beta = -0.66$ ,  $P=0.002$ ;  $\beta = -1.23$ ,  $P=0.02$ ) and sObR plasma levels also served as an independent predictor for diastolic blood pressure ( $\beta = -0.50$ ;  $P=0.04$ ). To conclude, we report common allelic variants associated with specific feeding behaviour and obesity-related anthropometric traits. Moreover, we identified allelic variants that significantly influence the time structure of food intake during the day.

### Adipokines: Polymorphisms: Obesity: Nutrition: Feeding behaviour

In Western societies, the prevalence of obesity has been steadily increasing for the last few decades. Obesity (Online Mendelian Inheritance in Man® (OMIM) no. 601665) is generally associated with an increased risk for cardiovascular disorders, diabetes, lipid disorders and some types of cancer. The disease is generally associated with specific lifestyle and dietary habits that interfere with the given genetic background of the individual and several studies have focused recently on the genetic background of these characteristics<sup>(1,2)</sup>. However, resolution of the genetic factors underlying the susceptibility to certain feeding or lifestyle behaviour is far from being completed.

Previously, various studies reported a heritable component for specific feeding behaviour<sup>(3–8)</sup>. However, the underlying mechanisms that could explain credibly the inheritance of food preferences are yet to be elucidated. Various adipokines and their genetic variability have been found to be associated with obesity and its related traits; however, the results are often contradictory<sup>(9–13)</sup>.

Recently, it has been reported by de Krom *et al.*<sup>(14)</sup> that certain common allelic variants in leptin (LEP) and LEP receptor (LEPR) genes are specifically associated with distinctly different eating patterns, namely extreme snacking behaviour or excessive portion size<sup>(14)</sup>. On the other hand, Schulz *et al.*<sup>(15)</sup> propose strong evidence for lifestyle-based, i.e. environmental, influences based on their observations of Pima Indians and they suggest that the lifestyle associated with Westernisation plays a major role in the global epidemic of type 2 diabetes, independently of genetic background of an individual<sup>(15)</sup>.

Although it has been reported that genetic variations underlie specific eating patterns<sup>(4)</sup> and that specific food preferences are considered to be a risk factor for obesity, only a few reports have focused on these genotype  $\times$  nutrient associations<sup>(16)</sup>, and none has examined thoroughly the relationship between the single nucleotide polymorphisms (SNP) in genes encoding for adipokines and the time structure of the daily energy intake.

**Abbreviations:** ADIPOQ, adiponectin; LEP, leptin; LEPR, leptin receptor; POMC, pro-opiomelanocortin; SNP, single nucleotide polymorphism; sObR, soluble leptin receptor.

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Our previous study on 185 healthy Caucasian volunteers of Middle-European origin did not reveal any significant associations of selected SNP in LEP, LEPR, adiponectin (ADIPOQ), pro-opiomelanocortin (POMC) and ghrelin genes with specific food behaviour. However, in further analyses, distinct trends were observed towards specific nutritional behaviour and therefore the cohort sample was substantially extended for the purposes of the present study.

The aim of the present case-control study on 409 unrelated individuals of Czech (middle-European) Caucasian origin was to analyse the possible associations of eight selected SNP in obesity-related genes with selected lifestyle and dietary characteristics of studied individuals.

## Experimental methods

### Study subjects

A total of 409 unrelated Czech Caucasian individuals were recruited for the present study in a mass media campaign addressing the population of the south Moravia region of the Czech Republic<sup>(17)</sup>. The participants were divided into two groups: obese and lean subjects. The inclusion and exclusion criteria were derived from Ma *et al.*<sup>(18)</sup>. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Committee for Ethics of Medical Experiments on Human Subjects, Faculty of Medicine at Masaryk University (Brno, Czech Republic). Written informed consent was obtained from all subjects and it was archived.

The first subgroup consisted of 252 obese individuals ( $\text{BMI} \geq 30 \text{ kg/m}^2$ ; mean  $\text{BMI} 37.4$  (SD 6.25)  $\text{kg/m}^2$ ; median age 49.1 years; age range 18.6–73.9 years). The control group consisted of 157 healthy normal-weight control subjects with no history of childhood obesity or eating disorder (mean  $\text{BMI} 25.2$  (SD 3.1)  $\text{kg/m}^2$ ; median age 37.1 years; age range 18.1–67.5 years). A set of sixty-four morbidly obese patients was selected from the obese group ( $\text{BMI} \geq 40 \text{ kg/m}^2$ ; mean  $\text{BMI} 45.5$  (SD 5.6)  $\text{kg/m}^2$ ; median age 52.4 years; age range 18.6–73.2 years). These individuals were available for plasma LEP, soluble LEPR (sObR) and ADIPOQ analyses and were precisely matched for age and sex with another sixty-four subjects (control or non-morbidly obese) that underwent the same set of biochemical analyses.

### Anthropometric characteristics

All phenotypic measurements were performed by three specialists and included weight, height, BMI, lean body mass, fat mass, body fat, waist and hip circumferences, waist:hip ratio and skinfold thickness. Body composition was assessed by bioelectrical impedance analysis, using the single frequency bioimpedance analyser (BodyStat Ltd, Douglas, Isle of Man, UK), with the subject lying in a supine position. The measurement of height was performed with a calibrated stadiometer and weight (in light indoor clothes and without shoes) was measured with a precisely calibrated set of scales.

### Dietary intake

Participants were furthermore advised to complete standardised 7 d food records. Food intake data were obtained from the study subjects and were further analysed, whereas the percentage of daily energy intake from carbohydrates, fat and protein as well as total energy and macronutrient intake were calculated using the Nutrimaster Diet Analysis software modified for the Czech population (Abbott Laboratories, Abbott Park, IL, USA). Special attention was paid to extreme snacking behaviour (defined as higher daily energy intake from snacks than 25%), eventual dieting, extreme portion sizes and irregularity in eating. The structure of the daily energy intake was also investigated – a snacking index (established as a ratio of daily energy intake from snacks v. daily energy intake from the main meals) was calculated.

### Candidate genes

The selection of particular SNP was based on: (1) population frequency in the European Caucasian population; (2) their known or potential functional or regulatory impact on feeding behaviour or association in the case of synonymous SNP; and/or (3) a previously described association with obesity or feeding behaviour.

Genotyping was carried out for eight SNP in five genes related to the production of adipokines, control of energy homeostasis, appetite and satiety regulation: LEP (rs2167270); LEPR (rs1137101); ADIPOQ (rs2241766, + 94T/G); IL-6 (rs1800797, rs1800795); POMC (rs3754860, rs1009388).

### Genotyping

DNA for analyses was extracted from 5 ml of the patients' saliva using a standard technique employing proteinase K. Genotyping of each of eight investigated SNP in ADIPOQ, LEP, LEPR, IL6 and POMC genes was performed as described previously<sup>(19–26)</sup>, using standard PCR-based methodology followed by restriction fragment length polymorphism with subsequent electrophoresis on the agarose gel with ethidium bromide staining. The DNA fragments were visualised by UV illumination using Image Analyser (AlphaImager™ 1220; Alpha Innotech Corp., San Leandro, CA, USA).

All reactions were performed using the XP BIOER Cycler (Bioer Technology Co. Ltd, Hangzhou, China), the overall genotyping success varied between 83.3% (LEP +19A/G) and 99.7% (LEPR Gln223Arg); missing genotypes were due to either consistent PCR dropout or depletion of template DNA. To assess genotyping reliability we performed double sampling in more than 20% of the samples and found no differences. We always used quality control and negative controls were used to identify possible false-positives.

### Determination of plasma leptin, soluble leptin receptor and adiponectin

Blood samples for total LEP, ADIPOQ and sObR plasma analyses were collected after overnight fasting and were immediately centrifuged at 1700g for 20 min and then stored at  $-80^{\circ}\text{C}$  until analysis. Plasma LEP and sObR levels were

measured by commercially available sandwich ELISA (R&D Systems, Minneapolis, MN, USA) with a sensitivity of 7.8 pg/ml and 0.057 ng/ml, respectively. Plasma samples for LEP and sObR were 100-fold and 5-fold diluted with calibrator diluent immediately before the assay, respectively. The intra- and inter-assay CV were less than 3.3 and 5.4 % for the LEP assay, and 6.1 and 8.6 % for the sObR assay, respectively. Plasma ADIPOQ levels were measured by a commercially available ELISA (RayBiotech, Norcross, GA, USA) with a sensitivity less than 10 pg/ml. Samples were 50 000-fold diluted in singlet to assay range (4.1–1000 pg/ml) with standardised assay diluent. The intra- and inter-assay CV were less than 10 and 12 %, respectively.

### Statistics

The genotype distributions were tested for Hardy–Weinberg equilibrium by a set of  $\chi^2$  tests. Allelic frequencies were estimated by the ‘counting method’ and differences in allele frequencies between case and control subjects were tested by likelihood ratio  $\chi^2$  tests for 2  $\times$  2 tables (two alleles, case *v.* control subjects). Where applicable, it was first determined whether the variable presented a normal distribution using the Kolmogorov–Smirnov test, and in cases of skewed variables, logarithmic transformation was performed. For descriptive purposes, mean values are presented using untransformed values. Results are expressed as mean values and standard deviations unless otherwise stated.

To identify genetic as well as non-genetic variables that may contribute to predicting the anthropometric phenotype or nutritional phenotype, we carried out a forward stepwise logistic regression, a sequential procedure of adding one input variable at a time to build up a regression model in which the dependent variable (*i.e.* presence or absence of obesity) is represented as the linear combination of independent variables (anthropometric and nutritional parameters and genotypes of eight investigated SNP). In this analysis, the codes of genotypes were used as quantitative variables (AA = 0, AB = 1, BB = 2).

OR were calculated using the multiple logistic regression analysis models; we adjusted for covariates including age (continuous), BMI (<23, 23–24.9, 25–29.9, 30–34.9, or  $\geq 35 \text{ kg/m}^2$ ), sex, smoking (never, past, and current), alcohol intake (non-drinker and drinker (0.1–4.9, 5–10, or  $>10 \text{ g/d}$ ), family history of obesity and menopausal status in females.

Using sample tertiles, the nutrient variables were categorised in three groups of equal size (the upper third, the middle third and the lower third) as described by Santos *et al.*<sup>(16)</sup>. Each nutrient variable was then included in logistic regressions as binary indicators leaving one category as the reference.

The data analysis was performed using the Statistica v. 8.0 (Statsoft Inc., Tulsa, OK, USA) program package. The values of  $P < 0.05$  were considered statistically significant.

### Results

The baseline demographic, anthropometric, dietary and clinical characteristics of the study subjects in relation to sex are listed in Table 1. The allele frequencies of all examined

SNP exceeded 0.05. The Hardy–Weinberg equilibrium test showed that the examined polymorphisms, except for the ADIPOQ 45T/G and ADIPOQ 94T/G polymorphisms in the obese group, were in Hardy–Weinberg equilibrium. Moreover, no significant differences both in genotype distributions and allele frequencies were observed when comparing the morbidly obese cohort ( $\text{BMI} > 40 \text{ kg/m}^2$ ) with the controls and the obese cohort ( $30 < \text{BMI} \leq 40 \text{ kg/m}^2$ ) with the controls ( $\text{BMI} \leq 30 \text{ kg/m}^2$ ) (Table 2).

### Effect of single nucleotide polymorphisms on anthropometric characteristics (BMI, waist:hip ratio, total body fat, skinfold thickness)

In the next step, we tested whether these SNP had any effect on anthropometric characteristics related to obesity in the study subjects.

Univariate logistic regression analysis revealed that the subjects with the GG genotype of LEP + 19A/G had a 1.9 higher risk for the development of obesity compared with subjects with the LEP + 19 A allele (OR 1.9 (95 % CI 0.87, 3.02);  $P=0.002$ ). Moreover, the AA homozygotes of the LEPR Gln223Arg polymorphism carried in the univariate analysis approximately half the risk for the development of increased diastolic blood pressure when compared with the G allele carriers (OR 0.49 (95 % CI 0.12, 1.32);  $P=0.002$ ). In the multivariate regression modelling across all the study subjects that was based on the results of the univariate analysis, the LEP + 19A/G polymorphism expressed an independent prediction role for BMI ( $\beta = -0.15$ ;  $P=0.02$ ), while LEPR Gln223Arg was significantly correlated with diastolic blood pressure ( $\beta = -0.15$ ;  $P = 0.02$ ). When analysing the possible relationships between the SNP and the thickness of the skinfolds, LEP + 19A/G expressed a significant prediction role for the thickness of the triceptal skinfold ( $\beta = -0.14$ ;  $P=0.04$ ) and ADIPOQ + 94T/G was correlated with the thickness of the subscapular skinfold ( $\beta = 0.13$ ;  $P=0.04$ ). Moreover, LEP + 19A/G and POMC RsaI expressed an independent prediction role for percentage of body fat in the multivariate analysis ( $\beta = -0.17$ ,  $P=0.008$ ;  $\beta = 0.13$ ;  $\beta = 0.13$ ,  $P=0.03$ , respectively).

### Effect of single nucleotide polymorphisms on dietary characteristics and food preferences of the study subjects

In the univariate regression modelling, none of the examined polymorphisms served as an independent predictor for percentage of daily energy intake from macronutrients or abnormal eating patterns (extreme snacking behaviour, extreme portion sizes, night eating, irregular food intake; NS). When analysing the general eating behaviour patterns in the tertile analysis, the presence of extreme snacking behaviour was in the total studied cohort (obese plus non-obese individuals) associated with lower obesity risk (Table 3). This effect was also observed in females separately (OR 0.42 (95 % CI 0.23, 0.77);  $P=0.003$ ), but not in males (OR 1.26 (95 % CI 0.42, 3.74);  $P=0.44$ ).

When analysing the distribution of energy intake during the day, the ADIPOQ + 45T/G polymorphism was in the univariate regression modelling associated with the energy value of breakfast, defined as the first meal during the day ( $\beta = 0.15$ ;

**Table 1.** Descriptive statistics of the patients' baseline characteristics

(Mean values and standard deviations)

Group...	Obese (n 252)				Morbidly obese (n 64)*				Controls (n 157)			
	Female		Males		Females		Males		Females		Males	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Subjects (n)	188		64		51		13		120		37	
Body composition												
Age (years)	50.1	11.4	46.4	12.2	51.6	10.6	48.7	13.0	38.8	13.2	36.8	14.0
BMI (kg/m <sup>2</sup> )	37.5	6.3	37.0	6.0	45.3	5.2	46.9	5.3	25.0	3.3	25.7	2.5
Body fat (%)	46.3	5.9	32.8	6.7	52.4	4.1	41.9	3.8	31.5	7.0	19.5	6.0
Dietary intake												
Energy (kJ)	7848	2410	10 791	3201	7344	1885	10 357	3394	7799	1768	10 747	2425
Protein (% energy)	15.6	3.5	14.8	2.7	15.6	3.0	15.6	3.5	14.2	2.5	13.4	1.9
Carbohydrates (% energy)	49.4	5.4	49.6	5.4	49.6	5.7	48.8	4.9	51.1	5.1	51.6	6.1
Fat (% energy)	35.0	4.9	35.6	5.2	34.8	4.9	35.6	5.3	34.7	4.7	34.8	5.9
Hormonal status†												
Leptin (ng/ml)	45.6	25.8	37.3	17.7	45.1	25.6	38.1	18.5	29.6	28.1	NA	
sObR (ng/ml)	20.4	4.3	16.8	4.0	20.2	4.3	16.3	4.0	27.7	5.2	NA	
Leptin:sObR ratio	2.4	1.4	2.2	0.8	2.4	1.3	2.3	0.8	1.1	1.0	NA	
Adiponectin (μg/ml)	9.3	5.1	8.4	6.6	9.8	5.1	8.8	6.7	9.7	5.6	NA	
Anthropometry												
Waist circumference (cm)	103.9	8.9	116.0	9.2	124.5	10.2	141.9	11.2	82.1	9.7	90.1	11.6
Hip circumference (cm)	119.2	7.6	114.1	6.1	139.1	12.6	137.0	10.8	102.3	7.9	100.1	9.2
Waist:hip ratio	0.9	0.1	1.0	0.1	0.9	0.1	1.0	0.1	0.8	0.1	0.9	0.1
Skinfold thickness (mm)												
Supraspinal skinfold	26.0	7.9	23.1	8.8	30.3	12.5	32.0	16.4	19.4	24.4	15.3	5.8
Subscapular skinfold	30.1	21.6	28.4	8.7	35.4	10.0	31.0	14.6	19.1	10.6	22.4	26.9
Biceptal skinfold	22.1	6.4	16.9	5.6	26.3	8.2	25.1	8.0	14.5	5.1	11.3	6.3
Triceptal skinfold	29.6	5.6	22.9	7.0	31.5	6.9	29.1	7.4	21.5	5.5	16.5	5.3
Sum of all skinfolds	107.7	27.5	91.3	20.0	122.8	30.3	117.2	33.3	74.2	31.5	65.4	32.8
Systolic blood pressure (mmHg)	135.2	19.5	141.0	17.5	140.0	24.7	140.3	17.0	121.9	17.9	125.8	12.5
Diastolic blood pressure (mmHg)	89.0	11.0	92.3	13.6	93.1	17.8	95.7	12.8	81.7	11.1	79.7	11.2

NA, not analysed.

\*Subset of obese subjects.

† Analysed in a subset of individuals, consisting of the sixty-four morbidly obese subjects and sixty-four subjects from the other groups matched by age and sex.

Genotype X nutrient associations

**Table 2.** Distributions of genotypes and alleles of examined polymorphisms in the studied subpopulations\*

Polymorphism	Genotypes					Alleles	
	TT	TG	GG	P	T	G	P
ADIPOQ rs2241766 (+45T/G) (synonymous coding, GGT → GGG, Gly → Gly)							
Obese cases	149 (81)	28 (15)	7 (4)	0.36	326 (89)	42 (11)	0.42
Morbidly obese cases	51 (80)	12 (19)	1 (1)	0.90	114 (84)	14 (16)	0.64
Controls	126 (82)	25 (16)	2 (2)	—	277 (91)	29 (9)	—
ADIPOQ +94T/G (synonymous coding, GGT → GGG, Gly → Gly)							
Obese cases	97 (61)	45 (28)	16 (11)	0.26	239 (76)	77 (24)	0.74
Morbidly obese cases	35 (61)	19 (33)	3 (6)	0.96	89 (78)	25 (22)	0.79
Controls	82 (59)	48 (35)	8 (6)	—	212 (77)	64 (23)	—
LEP rs2167270 (+19A/G) (5' UTR)							
Obese cases	73 (40)	84 (46)	24 (14)	0.66	230 (63)	132 (37)	0.85
Morbidly obese cases	30 (45)	28 (42)	6 (13)	0.41	88 (69)	40 (31)	0.24
Controls	58 (37)	80 (52)	18 (11)	—	196 (63)	116 (37)	—
LEPR rs1137101 (+27265A/G) (synonymous coding, CAG → CGG, Gln → Arg)							
Obese cases	48 (26)	98 (53)	39 (21)	0.30	194 (52)	176 (48)	0.59
Morbidly obese cases	20 (31)	33 (51)	12 (18)	0.66	77 (57)	57 (43)	0.75
Controls	50 (32)	70 (45)	36 (23)	—	170 (54)	142 (46)	—
POMC rs3754860 (+1798C/T) (5' UTR)							
Obese cases	76 (41)	90 (49)	19 (10)	0.68	242 (65)	128 (35)	0.40
Morbidly obese cases	23 (35)	34 (52)	8 (13)	0.34	80 (62)	50 (38)	0.16
Controls	71 (45)	73 (46)	13 (9)	—	215 (69)	99 (31)	—
POMC rs1009388 (+1032C/G) (intronic)							
Obese cases	111 (59)	64 (34)	12 (7)	0.75	286 (76)	88 (24)	0.89
Morbidly obese cases	40 (62)	21 (32)	4 (6)	0.91	101 (78)	29 (22)	0.86
Controls	96 (62)	48 (31)	12 (7)	—	240 (77)	72 (23)	—
IL-6 rs1800797 (-596A/G) (5' UTR)							
Obese cases	56 (32)	90 (51)	31 (17)	0.94	202 (57)	152 (43)	0.75
Morbidly obese cases	22 (34)	28 (44)	14 (22)	0.54	72 (56)	56 (44)	0.94
Controls	46 (30)	80 (52)	28 (18)	—	172 (56)	136 (44)	—
IL-6 rs1800795 (-174G/C) (5' UTR)							
Obese cases	54 (31)	92 (52)	30 (17)	0.88	200 (57)	152 (43)	0.67
Morbidly obese cases	20 (31)	30 (47)	14 (22)	0.82	70 (55)	58 (45)	0.93
Controls	43 (30)	75 (51)	28 (19)	—	161 (55)	131 (45)	—

ADIPOQ, adiponectin; LEP, leptin; UTR, untranslated region; LEPR, leptin receptor; POMC, pro-opiomelanocortin.

\*Numbers in parentheses are the percentages of the genotypes present in the different groups. For some genotypes, only a 83–90 % success rate could be reached due to a less efficient PCR amplification.

**Table 3.** Association between the upper and lower tertiles of extreme snacking behaviour with obesity in the studied cohorts

	Obesity (+)		Obesity (-)		OR	95 % CI	P
	%	Total n	%	Total n			
Total					0.57	0.35, 0.95	0.019*
Upper tertile	75	248	59	155			
Lower tertile	93	248	42	155			
Male					1.26	0.42, 3.74	0.44
Upper tertile	13	61	7	36			
Lower tertile	28	61	19	36			
Female					0.42	0.23, 0.77	0.003*
Upper tertile	62	187	52	119			
Lower tertile	65	187	23	119			

\* $P < 0.05$ .

$P = 0.02$ ). In the multivariate modelling adjusted for age, sex and smoking, the LEPR Gln223Arg polymorphism was positively correlated with the energy value of the dinner ( $\beta = 0.13$ ;  $P = 0.04$ ), whereas the GG carriers expressed a trend toward higher energy intake later on, in their dinner. Moreover, the LEP + 19A/G polymorphism was correlated with the energy value of the supper ( $\beta = 0.13$ ;  $P = 0.05$ ), with the AG heterozygotes expressing a tendency toward the highest energy intake in their supper.

In the above-defined subset of patients, LEP, sObR and ADIPOQ plasma levels were analysed in relation to examined SNP. Neither ADIPOQ + 45T/G nor + 94T/G was associated with ADIPOQ plasma levels either in the obese or lean individuals. The LEPR Gln223Arg polymorphism was correlated with the ADIPOQ plasma levels ( $\beta = -0.28$ ;  $P = 0.03$ ), whereas the GG homozygotes were showing on average the lowest plasma ADIPOQ levels. The *Rsa*I polymorphism was associated with both plasma LEP levels ( $P = 0.007$ ) and the LEP:sObR ratio ( $P = 0.003$ ). Furthermore, the bivariate analysis was performed on examined polymorphisms to assess possible associations of LEP, sObR and the LEP:sObR ratio or ADIPOQ and dietary characteristics. To control for possible confounders, the results from the bivariate correlation analysis were consecutively explored using multivariate analysis using logarithmically transformed plasma LEP and sObR and LEP:sObR ratio regressed on total energy intake as well as on the energy intake provide by each macronutrient<sup>(19)</sup>. However, no significant associations were observed.

In a separate analysis, sObR plasma levels and the LEP:sObR ratio were significantly correlated with systolic blood pressure ( $\beta = -0.66$ ,  $P = 0.002$ ;  $\beta = -1.23$ ,  $P = 0.02$ ) and sObR plasma levels also served as an independent predictor for diastolic blood pressure ( $\beta = -0.50$ ;  $P = 0.04$ ).

## Discussion

The investigation of genotype  $\times$  nutrient interactions is a general base for a better understanding of the multifactorial pathogenesis of complex diseases as well as for the identification of obesity-related traits. Unfortunately, the evaluation of genotype  $\times$  nutrient associations seems to be extremely difficult, mainly because of the complicated epidemiology of obesity, too many candidate genes investigated and also

because of the small power of the reported observed findings, altogether making the investigation of genotype  $\times$  food preferences highly tangled and difficult to accomplish<sup>(16)</sup>.

In the present study, we investigated the associations of eight SNP in adipose tissue-related genes in a cohort of 409 individuals with precisely quantified anthropometric and dietary characteristics and we employed different statistical models to precisely assess interactions defined as departures from the multiplicative risk ratios<sup>(16)</sup>, thus strengthening our analysis.

The allele frequencies and genotype distributions within the present study closely resembled those previously reported in other Caucasian European populations<sup>(27–29)</sup>; no significant differences were observed both in allele and genotypes frequencies and these were highly similar to those reported for the HapMap CEU population (Utah residents with ancestry from northern and western Europe)<sup>(30)</sup>.

Moreover, the results of the present study refer to the possible interaction between the carriers of the genetic variant of the LEP + 19A/G polymorphism and various anthropometric parameters including BMI, percentage body fat and skinfold thickness. Our findings are partially in accordance with the findings of Mizuta *et al.*<sup>(31)</sup>, as we did not observe any association of the LEP + 19A/G polymorphism with plasma LEP levels either; however, we did not confirm any associations of LEP + 19A/G or LEPR Gln223Arg with sweet preference described by these Japanese investigators.

Analysis of the anthropometric parameters revealed a significant association of ADIPOQ + 94T/G with subscapular skinfold thickness. As reported by Yang *et al.*<sup>(32)</sup> the synonymous mutation ADIPOQ + 94T/G might affect steady-state mRNA levels by altering RNA splicing or stability<sup>(32)</sup>. This T/G or linked polymorphism nearby could therefore affect mRNA splicing or stability and lead to extensive metabolic consequences that might result in varying body fat distribution. The strong linkage disequilibrium with type 2 diabetes, measures of adiposity, and insulin levels found in the chromosomal region where the ADIPOQ gene is located<sup>(33–34)</sup> suggest that somewhere on this locus a common genetic variant should be located, such as the T-G polymorphism in the ADIPOQ gene, that expresses measurable effects on adiposity-related traits. As far as we know, the present study is the first to report an association of ADIPOQ + 94T/G with skinfold thickness.

Moreover, we observed a significant effect of the '−' allele of the POMC *Rsa*I polymorphism on the percentage of body fat, where the '−−' homozygotes presented with the highest percentage of body fat when compared with the '+' allele carriers. This is in contrast to the study by Baker *et al.*<sup>(22)</sup> who did not observe any effects of *Rsa*I on BMI or waist:hip ratio; moreover, we did not observe the association of the POMC C1032G polymorphism with the waist:hip ratio or BMI reported by these authors.

Surprisingly, the tertile analysis of the macronutrient intake revealed that the presence of extreme snacking behaviour was in the total studied cohort (obese cases plus non-obese individuals) associated with lower obesity risk, which contradicts the generally accepted model of maintaining steady body weight. The present results are in accordance with a study by Lioret *et al.*<sup>(35)</sup> on 748 French children, as the authors concluded that a combination of more frequent food intake occasions and lower contribution of the main meals to total daily energy intake is associated with a smaller risk of overweight in children. However, the observed patterns were not consistently expressed in males in the present study and thus conclusions on these eating patterns in relation to obesity risk require further investigation, possibly with a prospective design.

Furthermore, significant interactions with various dietary and circadian characteristics concerning the LEPR Gln223Arg polymorphism were observed. Recently, it has been reported by Guízar-Mendoza *et al.*<sup>(36)</sup> that adolescent individuals with the A allele (A/A and A/G) had higher heart sympathetic activity, body fat percentage and LEP levels. This is partially in contrast to our findings of higher plasma LEP levels in G allele carriers (GG + GA), also observed in our previous study<sup>(17)</sup>; however, the G allele carriers in the present study presented with lower body fat percentage, which is in accordance with the Mexican adolescent cohort. The finding of half the risk of AA carriers for diastolic blood pressure in our cohort is also in contrast to this Mexican study, where a sympathetic effect of the A allele was observed. The present study is so far the first to demonstrate any effect of LEPR Gln223Arg on the structuring of energy intake during the day, and thus it provides a basis for further investigation of the possible association of Gln223Arg with eating behaviour and involvement of this polymorphism in the regulation of circadian rhythmicity. In this context, the observed significant association of the LEPR Gln223Arg polymorphism with the blood pressure values is of particular importance.

We did not confirm the observations by Yannakoulia *et al.*<sup>(19)</sup> that the sObR is positively associated with energy intake from carbohydrates and negatively with energy intake from dietary fat, whereas the free LEP index was in this study on a Greek population negatively associated with energy intake from carbohydrates and positively with energy intake from dietary fat, thus contrasting with the present results. The present study also did not reveal any associations of free LEP and sObR with energy intake and the macronutrient composition of the diet reported by Yannakoulia *et al.*<sup>(19)</sup>.

The main strength of the present study is the use of state-of-the-art methodology including 7 d food records for evaluating the subjects' dietary intake. The 7 d food records

provide quantitatively accurate information on food consumed during the recording period by recording food while it is consumed, the problem of reporting bias or omission is lessened, whereas subjects are not restricted to selecting from a predetermined list of foods included in a FFQ<sup>(19)</sup>. Although confounding was appropriately controlled for through standard statistical procedures, there is always a possibility of residual confounding by other serum adipokines, genetic factors or unmeasured and unknown factors that have to be considered. Confirmation of the present results by future studies on different populations and above all the precise assessment of the adipokine genes in relation to the time structure of daily energy intake are warranted.

To conclude, the present population-based case-control study revealed significant associations of selected polymorphisms in genes encoding for adipokines both with percentage body fat, skinfold thickness and specific dietary composition and time patterns of feeding behaviour.

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