

Relationship of the Trp64Arg polymorphism of the beta3-adrenoceptor gene to central adiposity and high blood pressure: interaction with age. Cross-sectional and longitudinal findings of the Olivetti Prospective Heart Study

Pasquale Strazzullo^a, Roberto Iacone^a, Alfonso Siani^b,
 Francesco P. Cappuccio^c, Ornella Russo^a, Gianvincenzo Barba^b,
 Antonio Barbato^a, Lanfranco D'Elia^a, Maurizio Trevisan^d and
 Eduardo Farinaro^e

Methods The association of the Trp64Arg polymorphism of the beta3-adrenoceptor (beta3-AR) gene with high blood pressure, central adiposity and other features of the metabolic syndrome was investigated in a large unselected sample of a white male working population in Southern Italy ($n = 979$).

Results In the whole population, subjects heterozygous for the Trp64Arg mutation (11.2%) were not different from the homozygous Trp64Trp for any of the variables investigated. However, upon stratification for age, among men in the upper tertile of age (> 53 years), the Trp64Arg genotype was associated with higher waist:hip ratio (0.992 ± 0.021 versus 0.982 ± 0.037 , $P < 0.05$), serum uric acid (6.34 ± 1.50 versus 5.75 ± 1.30 $\mu\text{mol/l}$, $P < 0.05$) and systolic blood pressure (144.3 ± 19.4 versus 136.9 ± 18.9 mmHg, $P < 0.05$) compared with the wild-type homozygotes. Accordingly, in the same age group, the carriers of Trp64Arg genotype were more often in the upper tertile of abdominal adiposity (69.7 versus 43.7%, $P < 0.02$) and serum uric acid (56.3 versus 34.8%, $P < 0.02$) and were more often hypertensive (68.6 versus 57.6%, $P < 0.058$) than the Trp64Trp homozygotes. No such differences were observed in younger age groups. No association was found with fasting serum insulin and the homeostasis model assessment (HOMA) index of insulin resistance. Furthermore, in a subgroup of 457 men for

whom retrospective 20-year follow-up data were available, the variant genotype was associated with a higher probability of developing overweight (44.7 versus 27.0%, $P < 0.05$) and a trend to higher blood pressure (52.6 versus 38.4%, $P = 0.09$) over 20 years.

Conclusion We conclude that the Trp64Arg variant of the beta3-AR receptor predicts a greater tendency to develop abdominal adiposity and high blood pressure with advancing age. *J Hypertens* 19:399–406 © 2001 Lippincott Williams & Wilkins.

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Keywords: beta3-adrenergic receptor, gene polymorphism, hypertension, central adiposity, metabolic syndrome

^aDepartment of Clinical and Experimental Medicine, ^bDepartment of Preventive Medical Sciences, 'Federico II' University of Naples Medical School, Naples, Italy; ^cInstitute of Food Science, CNR, Avellino, Italy; ^dDepartment of General Practice and Primary Care, St. Georges' Hospital Medical School, University of London, UK and ^eDepartment of Social and Preventive Medicine, State University of New York at Buffalo, New York, USA.

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Correspondence and requests for reprints to P. Strazzullo, M.D., Department of Clinical and Experimental Medicine, 'Federico II' University of Naples, via S. Pansini 5 80131 Naples, Italy.
 Tel: +39 081 7463686; fax: +39 081 5466152; email: strazzul@unina.it

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Introduction

Overweight, in particular visceral adiposity, and high blood pressure tend to cluster in the same individuals [1,2]. Both disorders have a high degree of heritability and share a polygenic model of inheritance [3,4]. A high sympathetic nervous system activity has been associated with both overweight and hypertension [5–7] and is believed to have pathogenetic relevance [8,9]. Therefore the genes involved in adrenergic regulation are good candidates to improve our understanding of the etiology of this association, as one or more functional genetic abnormalities at this level could have a

multiplicity of metabolic and cardiovascular effects. Among the genes involved in catecholamine activity, the one encoding for the beta3-adrenergic receptor (beta3-AR) is of particular interest given the demonstration of functional beta3-adrenergic receptors in human adipocytes [10,11] and of mRNA and protein expression in human tissues [12–14]. The main effect of beta3-AR stimulation appears to be the acceleration of lipolysis *in vitro* [10,15] and *in vivo* [16]. A mutation in the codon 64 of the beta3-AR gene leads to replacement of a tryptophane with an arginine residue in the receptor molecule (Trp64Arg) and appears to affect at

least some of the receptor functions both *in vivo* [17–19] and *in vitro* [20]. A number of studies have explored the possible association between this variant and various features of the so-called metabolic syndrome, including overweight and the tendency to gain weight over time, insulin resistance and high blood pressure, but the results have been controversial and firm conclusions on the possible role of this mutation have not yet been reached [21]. Most of the studies carried out so far have involved selected patients with one or more metabolic disorders and their normal counterparts; very few have been population-based investigations, with adequate sample size and there is no longitudinal observation available with long-term follow-up.

We thus decided to investigate the possible association of the beta3-AR Trp64Arg polymorphism with blood pressure and other features of the metabolic syndrome in a large sample of middle-aged male workers attending the 1994–95 follow-up examination of the Olivetti Prospective Heart Study. In addition to the findings of this cross-sectional survey, we report longitudinal findings from a subset of participants who were seen for the first time in 1975 and were then followed up for 20 years.

Methods

Population and field procedures

The study was performed at the Olivetti factories of Pozzuoli (Naples) and Marcianise (Caserta) and was part of an investigation on the prevalence of cardiovascular risk factors in southern Italy initiated in 1975 and involving the participation of the Olivetti factory male workforce. The methodology of the study has been described in detail previously [22]. The study protocol was approved by the local Ethics Committee and participants gave their informed consent to participate.

Between May 1994 and December 1995, 1075 men in the age range 25–75 years were examined. The beta3-AR Trp64Arg polymorphism was characterized in 979 participants who were included in the present analysis. A subgroup of these subjects ($n = 457$) had been seen for the first time in 1975 and was thus the object of the 20-year follow-up analysis.

The examinations were performed in the morning, in a quiet and comfortable room within the medical centre of the Pozzuoli and Marcianise factories. The participants underwent physical examination, anthropometry, blood pressure measurements and blood tests; a fixed sequence questionnaire was given, including demographic information and past medical history.

Blood pressure and anthropometric measurements

Blood pressure was measured between 0800 and

1100 h, after the subject had been sitting upright for at least 10 min. Systolic and diastolic (phase V) blood pressure were taken three times 2 min apart with a random zero sphygmomanometer (Gelman Hawksley Ltd., Sussex, England). The first reading was discarded and the average of the second two readings was recorded for systolic and diastolic blood pressure.

Body weight and height were measured on a standard beam balance scale with an attached ruler. Body weight was measured to the nearest 0.1 kg and height was measured to the nearest cm, with subjects wearing only light indoor clothing without shoes. The body mass index was calculated as weight in kg divided by the square of the height in metres.

At the 1994–1995 (but not at the 1975 examination) the waist circumference was measured at the umbilicus level and the hip circumference was measured at the widest circumference over the trochanters, with the subject standing erect with the abdomen relaxed, the arms at the sides and the feet together. The measurements were performed at the nearest 0.1 cm, with a flexible inextensible plastic tape. The ratio of waist:hip circumference was calculated and was taken as an estimate of the pattern of fat distribution.

Both anthropometric and blood pressure measurements were performed by trained observers who had attended training sessions for standardization of the procedures. The operator code was recorded in order to check for possible measurement biases.

Blood sampling and biochemical assays

After the blood pressure measurements, a fasting venous blood sample was taken in the seated position and without stasis. The blood specimens were immediately centrifuged and stored at -70° until analysed. Serum cholesterol, triglyceride, glucose and uric acid levels were measured with automated methods (Cobas-Mira, Roche, Italy). Serum insulin concentration was measured only in 1994–1995 by radioimmunoassay (Insulina Lisophase, Technogenetics, Milan, Italy) and insulin resistance was estimated by homeostasis model assessment (HOMA) using the formula: fasting serum insulin (pmol/l) \times fasting serum glucose (mmol/l)/22.5, as described by Matthews *et al.* [23].

Beta3-AR gene polymorphism

Genomic DNA was isolated from leukocytes with a non-enzymatic, salting-out procedure [24]. The DNA, free of RNA and protein contamination as shown by 260/280 absorbance ratios, was amplified by the polymerase chain reaction (PCR), carried out according to Widén *et al.* [25], with few modifications. PCR conditions were: initial denaturation at 94°C for 5 min; then, 35 cycles at 94°C for 30 s, at 61°C for 30 s and at 72°C

for 30 s; final extension step at 72°C for 5 min, using a GeneAmp PCR System 9600 (Perkin Elmer, Milan, Italy). The PCR product, a 210 base pair (bp) fragment, was checked on a 1.5% agarose gel with a Gel Electrophoresis Apparatus GNA-200 (Pharmacia Biotech, Milan, Italy). Restriction fragment length polymorphism analysis (RFLP) was performed by adding 5 units of BstNI (New England Biolabs, USA) in the appropriate buffer to the PCR product and by incubating at 60°C for 2 h: this restriction enzyme is specific for the sequence CC/(A or T)GG. The digested samples were separated by electrophoresis on 3% agarose gel, ethidium bromide stained and analyzed under UV-light. Digestion of PCR products with BstNI produced various fragments of different size: 99, 62, 30, 12 and 7 bp (the last three fragments are not visible on agarose gel), but in the presence of a T→C mutation, shifting the codon TGG^{Trp} to CGG^{Arg}, one of these restriction sites is missing and consequently an additional band (161 bp) corresponding to the undigested mutant allele becomes apparent. A 10% random sample of the study population and all the heterozygotes were double genotyped in a blinded fashion.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS-8.0, Chicago, Illinois, USA). As the triglyceride and insulin values did not follow a Gaussian distribution, log-transformation was executed and log-transformed values used for the analyses. The Student *t*-test for unpaired observations was used to assess differences between group means and analysis of covariance (ANCOVA) was used to control for possible confounders. Interactions in the reciprocal relationships between different variables were explored by factorial analysis. Cross-tabulation with chi-square calculation was used to estimate differences in the frequency of occurrence of categorical variables. Results are expressed as means and standard deviation, unless otherwise indicated. Two-sided $P < 0.05$ values were considered statistically significant.

Results

Cross sectional study

Descriptive statistics of the Olivetti study population at the 1994–1995 examination is given in Table 1. Participants (979) were characterized for the beta3-AR gene Trp64Arg polymorphism. There were 159 (16.5%) obese individuals (body mass index, BMI > 30), and 301 (33.8%) had a HOMA index above the value reportedly identified as the 80% percentile for a non-obese non-diabetic normotriglyceridemic sample of male population [26]. Subjects numbering 169 (17.2%) were currently taking antihypertensive medications while 29 (3%) were being treated for diabetes mellitus.

The Trp64Arg variant was present in 110 individuals, a

Table 1 Characteristics of the Olivetti study population at the 1994–1995 examination (n = 979)

	Mean ± SD	Range
Age (years)	51.8 ± 7.5	25–75
BMI (kg/m ²)	27.0 ± 3.1	18.8–37.0
Waist: hip ratio	0.973 ± 0.031	0.852–1.220
Serum cholesterol (mmol/l)	5.7 ± 1.05	1.73–9.97
Serum triglyceride (mmol/l)	1.74 ± 1.03	0.47–11.05
Serum uric acid (μmol/l)	339 ± 77	95–690
Serum glucose (mmol/l)	5.65 ± 1.3	3.9–16.9
Serum insulin (pmol/l)	67.7 ± 36.4	15.1–357.3
Systolic BP (mmHg)	130.3 ± 17.4	89–225
Diastolic BP (mmHg)	84.1 ± 10.0	44–130

BMI, body mass index; BP, blood pressure.

proportion (11.2%) similar to the one found in other Caucasian populations. There was only one subject homozygous for the variant and he was excluded from the analysis. The allele distribution followed the Hardy–Weinberg equilibrium.

A comparison of anthropometric, metabolic and haemodynamic findings in homozygous ‘wild-type’ individuals and those carrying the Trp64Arg variant is shown in Table 2. No significant difference was detected in any of the variables investigated; however, the waist:hip ratio and serum uric acid tended to be higher in the heterozygotes ($P = 0.07$ and $P = 0.08$, respectively).

As both abdominal adiposity and systolic blood pressure increase with age, the possible interaction between age and the beta3-AR polymorphism was evaluated by factorial analysis. This revealed a strong interaction between the beta3-AR polymorphism and age ($F = 6.42$, $P = 0.002$). Thus, the comparison between homozygous wild-type and heterozygous individuals was carried out upon stratification of the population by tertiles of age (i.e. < 49, 49–53, and > 53 years). No between-group difference was observed in the two lower tertiles. However, significant differences were detected among older individuals. The Trp64Arg subgroup had significantly higher waist:hip ratio, serum uric acid and systolic blood pressure (Table 2). The differences in body mass and diastolic pressure were smaller and did not reach statistical significance.

After exclusion of treated hypertensive participants, the difference in systolic pressure persisted (8.9 mmHg) and remained statistically significant ($P < 0.05$).

As abdominal adiposity relates to blood pressure, the ANCOVA was applied to the data with the beta3-AR genotype as the fixed factor and the waist:hip ratio as a covariate. This model indicated that the waist:hip ratio was strongly associated with systolic pressure ($P <$

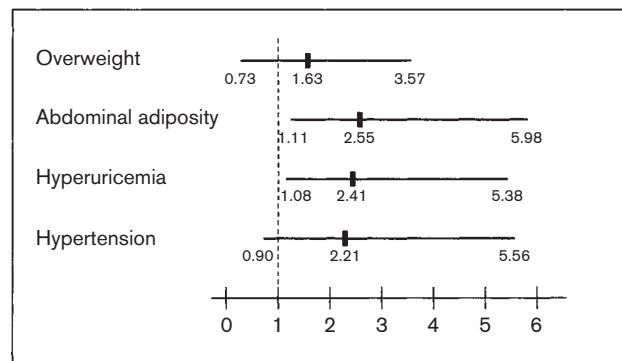
Table 2 Selected anthropometric, biochemical and hemodynamic variables by beta3-AR genotype in the whole study population and in older participants

	Whole study population		Older age group (54 years or more)	
	Trp64Trp (n = 869)	Trp64Arg (n = 110)	Trp64Trp (n = 277)	Trp64Arg (n = 33)
Age (years)	51.7 ± 7.6	52.8 ± 6.6	59.9 ± 4.7	61.3 ± 5.0
BMI (kg/m ²)	27.0 ± 3.1	26.8 ± 2.9	26.7 ± 3.4	27.0 ± 3.3
Waist-to-hip ratio	0.972 ± 0.031	0.977 ± 0.025	0.982 ± 0.037	0.992 ± 0.021*
Serum CHOL (mmol/l)	5.7 ± 1.0	5.7 ± 1.1	5.8 ± 1.1	5.7 ± 1.0
Serum TG (mmol/l)	1.7 ± 0.9	2.0 ± 1.5	1.7 ± 1.0	1.8 ± 0.9
Uric acid (μmol/l)	339 ± 74	353 ± 84	342 ± 77	377 ± 89*
Glucose (mmol/l)	5.6 ± 1.4	5.6 ± 1.0	6.0 ± 1.8	6.0 ± 1.0
Serum insulin (pmol/l)	68.0 ± 37.3	64.9 ± 29.1	68.7 ± 36.7	64.9 ± 36.2
Systolic BP (mmHg)	130.3 ± 17.2	130.2 ± 19.6	136.9 ± 18.9	144.3 ± 19.4*
Diastolic BP (mmHg)	84.2 ± 9.8	83.5 ± 11.3	85.9 ± 10.6	88.9 ± 10.0

* $P < 0.05$. BMI, body mass index; CHOL, cholesterol; TG, triglyceride; BP, blood pressure.

0.001) and that, controlling for the difference in waist : hip ratio, the effect of the beta3-AR polymorphism on blood pressure was attenuated ($P = 0.086$). Given the small age difference (1.4 years) between the Trp64Trp and the Trp64Arg subgroups among subjects in the upper tertile of age (Table 2), multivariate analysis was again carried out using age as a covariate in the relationship between the beta3-AR polymorphism and systolic pressure. As expected, age was found to be strongly associated with blood pressure ($P < 0.001$) and the relationship between the beta3-AR polymorphism and systolic pressure was also attenuated ($P = 0.070$).

In order to examine the association of the allelic variant of the beta3-AR gene with the main features of the metabolic syndrome, the frequency of overweight (BMI > 27), abdominal adiposity (waist : hip ratio in the upper tertile of the distribution), insulin resistance (HOMA index in the upper tertile), hypertriglyceridemia (TG > 200 mg/dl), hyperuricemia (upper tertile) and hypertension (BP > 140 and/or 90 mmHg or ongoing antihypertensive treatment) was examined in the Trp64Trp and the Trp64Arg groups. No difference was found for any of these variables when the analysis was carried out on the whole study population. Upon stratification by age, no difference again was detected among subjects in the two lower tertiles (< 49 and 49–53 years-old, respectively), whereas in participants aged 54 years or more the frequency of abdominal adiposity (69.7 versus 43.7%, $P < 0.02$) and of hyperuricemia (56.3 versus 34.8%, $P < 0.02$) was significantly greater in the heterozygous group. The prevalence of hypertension also tended to be higher in this group, but the difference was not statistically significant (68.6 versus 57.6%, $P < 0.058$). Figure 1 gives the odds ratio for overweight, abdominal adiposity, hyperuricemia and hypertension accord-

Fig. 1

Odds ratio (with 95% confidence interval) for overweight, abdominal adiposity, hyperuricemia and hypertension according to beta3-AR genotype (age-group > 53 years-old, $n = 308$). Overweight, body mass index ≥ 27 ; Abdominal adiposity, waist : hip ratio in the population upper tertile; Hyperuricemia, serum uric acid in the population upper tertile; Hypertension, SBP ≥ 140 and/or DBP ≥ 90 and/or on current drug treatment.

ing to beta3-AR genotype for individuals aged 54 years or higher.

Follow-up study

Of the participants examined in 1994–1995, 457 had been seen for the first time in 1975; 405 of them had a Trp64Trp and 52 a Trp64Arg genotype, a proportion similar to that found in the entire population. At baseline, the two groups were comparable for all the variables being investigated (Table 3). Time changes in body weight, serum lipids, glucose, uric acid and blood pressure over the 20-year interval between the initial and the last examination were evaluated according to beta3-AR genotype. Looking at the entire cohort, between-group differences were small and did not reach statistical significance. Nevertheless, there was a

Table 3 Baseline characteristics of the cohort ($n = 457$) undergoing 20-year follow-up observation by beta3-AR genotype

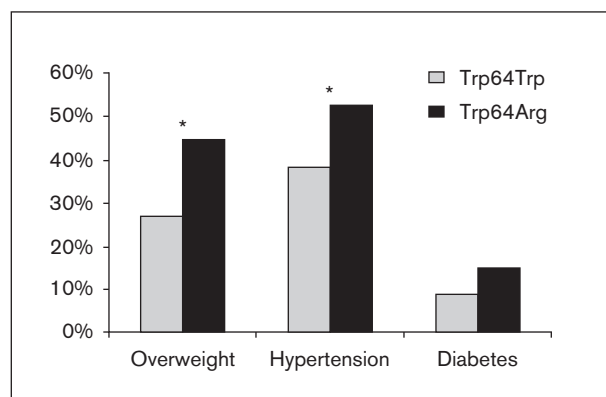
	Trp64Trp ($n = 405$)	Trp64Arg ($n = 52$)
Age (years)	36.6 \pm 6.51	36.5 \pm 6.84
BMI (kg/m ²)	25.5 \pm 3.0	25.5 \pm 2.4
Serum CHOL (mmol/l)	4.9 \pm 1.0	4.8 \pm 0.9
Serum TG (mmol/l)	1.5 \pm 0.9	1.5 \pm 0.7
Serum uric acid (μ mol/l)	327 \pm 77	345 \pm 71
Serum glucose (mmol/l)	4.4 \pm 0.8	4.4 \pm 0.7
Systolic BP (mmHg)	124.8 \pm 15.5	123.2 \pm 12.9
Diastolic BP (mmHg)	82.2 \pm 10.4	81.3 \pm 10.3

BMI, body mass index; CHOL, cholesterol; TG, triglyceride; BP, blood pressure.

general trend for all these variables to increase more in the heterozygous compared to the homozygous wild-type group, with particular regard to body weight (+ 0.91 kg, $P = 0.10$) and systolic blood pressure (+ 4.0 mmHg, $P = 0.10$). There were significant correlations between the changes in systolic or diastolic pressure and the respective changes in body weight ($r = 0.144$ and $r = 0.261$, respectively, $P < 0.01$). As in the cross-sectional analysis, the data were stratified by age. Given the relatively small sample size, two groups were defined using the median of age (35 years) at the time of the first examination in 1975. Again, greater between-group differences were observed in older participants, although none attained statistical significance. In particular, Trp64Arg carriers gained more weight (2.63 ± 1.21 versus 1.68 ± 0.45 kg), had larger increases in serum uric acid (30.9 ± 19.6 versus 4.2 ± 5.9 μ mol/l), systolic (17.6 ± 3.2 versus 11.1 ± 1.4 mmHg) and diastolic pressure (6.2 ± 2.5 versus 3.6 ± 0.9 mmHg).

The incidence of overweight in the entire cohort over 20 years was investigated using a BMI > 27 or ≤ 27 as a categorical variable (Fig. 2). Among 323 participants who had a BMI < 27 at baseline, overweight was detected at final examination in 17 of 38 Trp64Arg individuals versus 77 of 285 Trp64Trp participants (44.7 versus 27.0%, chi-square = 5.10, $P = 0.02$). The relative risk of becoming overweight for the heterozygous group was 1.97 (CI = 1.09–3.57).

With a similar procedure, the incidence of hypertension (final BP ≥ 140 and/or 90 mmHg or currently treated for hypertension) was investigated among those who were normotensive (BP $< 140/90$ and untreated) at baseline. There were 20 of 38 new cases of hypertension among carriers of the mutant beta3-AR allele and 114 of 297 cases among homozygous wild-type individuals (52.6 versus 38.4%, chi-square = 2.85, $P = 0.09$). The relative risk of becoming hypertensive was 1.67 for the heterozygous group (CI = 0.93–3.03).

Fig. 2

Incidence of overweight, hypertension and diabetes in 20 years according to beta3-AR genotype (the grey bars refer to Trp64Trp and the black bars to Trp64Arg genotype). * $P < 0.05$. Overweight, body mass index ≥ 27 ; Hypertension, systolic blood pressure ≥ 140 and/or diastolic blood pressure ≥ 90 and/or on current drug treatment; Diabetes, fasting blood glucose ≥ 126 mg/dl.

There was no significant difference in the incidence of diabetes (fasting glucose above 126 mg/dl at final examination) by beta3-AR genotype (14.9 versus 8.7%, chi-square = 1.88, $P = 0.17$).

Discussion

As recently pointed out [21], very few studies that explored the association between the Trp64Arg polymorphism of the beta3-adrenergic receptor gene and obesity were population-based investigations [27–29]. Our report deals with the largest sample of male population so far examined and, in addition to the relation with body weight and obesity, explores the association of the beta3-AR polymorphism with several other features of the metabolic syndrome. It is also the first study to provide long-term follow-up findings.

In the analysis of the whole study population, the comparison of the characteristics of participants with different beta3-AR genotype unravelled a trend for greater abdominal adiposity and higher serum uric acid levels in heterozygous compared to 'wild-type' homozygous participants. The differences were small, however, and not statistically significant, indicating overall, a weak association. As the prevalence of abdominal adiposity, insulin resistance and high blood pressure increases with increasing age, the data were analyzed upon stratification of the participants in three groups identified by tertiles of age. Significant associations of the Trp64Arg genotype with abdominal adiposity, serum uric acid and blood pressure were seen in the older age group while they could not be detected in younger groups. This is to our knowledge the first report of an interaction between this beta3-AR polymorphism and

age, nevertheless this finding may not surprise. It is conceivable that a small effect of the genetic variant may become apparent only later in life at a time when other factors cooperate to produce similar effects. In a recent study in young clinically healthy individuals, those who carried the variant, even at a single allele, were found to have lower resting autonomic nervous system activity compared with 'wild type' homozygotes, albeit in the absence of metabolic or other clinical disorder [30]. It is possible that under the influence of ageing on autonomic nervous system activity [31], the effects of the variant beta3-AR become obvious on clinical grounds.

Abdominal adiposity is a risk factor for hypertension: therefore it is appropriate to control for the effect of the waist:hip ratio on the relationship between the beta3-AR genotype and blood pressure. When taking the waist:hip ratio into account, the association of the variant allele of the beta3-AR gene with blood pressure was indeed attenuated.

The analysis of the subgroup of participants, for whom 20-year follow-up information was available, showed an association of the beta3-AR polymorphism with the tendency to gain weight over time with concomitant greater increases in uric acid and blood pressure. Again, these effects were more evident among older individuals. In this longitudinal evaluation, the Trp64Arg genotype predicted a greater incidence of overweight over 20 years. A limitation of this analysis was the lack of anthropometric measures at baseline examination other than body weight and height. There was also a trend to higher incidence of hypertension in the carriers of the variant allele that, however, did not attain statistical significance.

A recent meta-analysis of the association of the Trp64Arg variant with body mass index showed that, based on existing data from 23 studies including a total of 7399 subjects, no association could be detected [32]. The studies included in the meta-analysis were heterogeneous as to the characteristics of the subjects involved and very few were population-based. On the other hand, our data are supportive of an effect of the Trp64Arg variant on the accumulation of abdominal fat and indicate that the effect becomes more pronounced with ageing. While many studies investigated the association between this genetic polymorphism and body weight or obesity, very few analyzed its effect on the body fat pattern. Our finding of a more important and significant association between the Trp64Arg polymorphism and the waist:hip ratio suggests a selective or prevalent effect of the variant on the rate of upper body fat accumulation. This is compatible with the results of studies on the differential expression of mRNA for beta3-adrenergic receptors in various human

tissues, showing that beta3-AR mRNA levels were high in deep fat depots such as perirenal and omental, lower in subcutaneous fat and detectable in the gallbladder and the colon, thus suggesting possible effects on the rate of fat mobilization and also lipid assimilation during digestion [12].

The finding of a significant association between the beta3-AR polymorphism and serum uric acid levels in both the cross-sectional and the longitudinal evaluation is not surprising in view of the known interrelations between uric acid, overweight and obesity [22,33]. A recent study of the effects of visceral fat accumulation on uric acid metabolism concluded that visceral adiposity is linked to uric acid overproduction [34]. We and others have previously reported that overweight and obesity are also associated with reduced renal urate clearance together with a trend to hyperuricemia, thus suggesting an alternative or additional mechanism for the increased serum uric acid levels [22,35].

Few studies so far have reported a relationship between the Trp64 Arg polymorphism of the beta3-AR and blood pressure [25,36–38]. In the 1994–95 cross-sectional analysis of the Olivetti study population, the Trp64Arg genotype was associated with a consistent trend to higher blood pressure values in the carriers of the variant allele. This trend achieved statistical significance in older participants who were also affected by more pronounced abdominal adiposity. These findings are in agreement with the reports by Widén *et al.* in Finns [25] and by Tonolo and co-workers in a Sardinian population [38]. It has been reported that, in addition to lipolysis and thermogenesis in adipocytes, the beta3-AR also modulates the peripheral vascular tone in the dog inducing vasodilation predominantly in skin and fat [39,40]. In our study, multivariate analysis showed that the difference in adiposity had a significant effect on the difference in blood pressure, raising the possibility that the higher blood pressure could be at least partly secondary to the effect of the variant allele on body fat mass and distribution. In the studies by Widén *et al.* [25] and by Tonolo *et al.* [38], this possible explanation for the association between the beta3-AR polymorphism and hypertension was not ruled out.

In the longitudinal analysis, there was a similar trend to a more pronounced increase in blood pressure together with greater increase in body weight in older heterozygous participants. While the occurrence of overweight was significantly more common in this group, the trend to higher incidence of hypertension did not reach statistical significance. No association was observed in our study population between the beta3-AR polymorphism, fasting serum insulin and insulin sensitivity, at least as assessed by the HOMA index. Following the report by Widén *et al.* suggesting an association of the

beta3-AR mutation with reduced insulin sensitivity measured by euglycemic hyperinsulinemic clamp [25], most epidemiological and clinical studies thereafter have not confirmed this association [29,37,41–53]. Since these studies, however, including our own, have used less sensitive measures of insulin sensitivity, they may not have had the power to unveil a relatively small effect.

In conclusion, this investigation of a large sample of unselected male population indicates that the Trp64Arg variant of the beta3-AR receptor predicts a greater tendency to develop overweight, abdominal adiposity and high blood pressure with advancing age. Although these effects are relatively small, they are relevant to the pathogenesis of these common disorders and may contribute to cardiovascular risk.

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