Effects of the *C825T* polymorphism of the *GNB3* gene on body adiposity and blood pressure in fertile and menopausal women: a population-based study

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Objectives The 825T allele of the *GNB3* gene is implicated in adipose distribution, predisposing to obesity and hypertension. Menopause is also considered a condition leading to excess adiposity and hypertension. The aim of the present study was to clarify whether the effects of menopause on body weight and blood pressure are influenced by the *C825T* polymorphism of the *GNB3* gene.

Methods The study involved 1339 subjects (43% men) aged 18–95 years, undergoing, in an epidemiological population-based frame, questionnaire, anthropometrics, blood examinations, genotyped at the *GNB3* 825 locus.

Results Mean skinfold thickness (MST), truncal obesity and excess subcutaneous adiposity (MST greater than median) were higher in women than in men. A significant interaction was detected between menopausal status and the *C825T* polymorphism ($P_{int} > 0.0001$). MST, truncal obesity and excess subcutaneous adiposity were lower in *CC* fertile than menopausal women, but were comparable in *TT* fertile and menopausal women. In a multivariate logistic model for excess subcutaneous adiposity, the relative risk of menopause was 4.12 (95% confidence interval 2.35–7.22) in *CC* women but was insignificant in the other two genotypes. In fertile women only, higher systolic blood pressure (SBP) was detected in *TT* than in *CC* genotypes.

Conclusion An interaction exists between the *C825T* polymorphism and menopause in controlling body adiposity and blood pressure in women. Adiposity and SBP are higher in menopausal than in fertile women, provided they have the *CC* genotype. *TT* fertile women show the same adiposity as those in menopause. Men have the same excess adiposity as menopausal women, independent of the *GNB3* genotype. *J Hypertens* 25:000–000 © 2007 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Introduction

There is evidence that the β₃-subunit of heterotrimeric G-proteins (located on chromosome 12p13) [1] is implicated in adipogenesis, adipose distribution and body weight [2,3]. In particular, the *825T* mutation in exon 10 has been found to be positively associated with overweight and postpregnancy weight retention [4]. Studies in stem cells overexpressing Gαi2 show increased adipogenesis [5], whereas rats knocked out for Gαi2 display reduced fat mass [6]. The guanine nucleotide binding protein b3 (GNB3) is localized in the plasma membrane and mediates the response to receptor agonists. Although its physiological role in the development of obesity is not fully understood, it is known that signal transduction alteration may modulate disease susceptibility [7].

Studies in humans have also demonstrated an association between the *C825T* mutation and high blood pressure

[8–11]. The current hypothesis is that the *825T* allele predisposes to obesity possibly via hyperinsulinemia [12], by Gαi2 signal transduction [5] or a lower lipolytic response of adipose tissue to catecholamines [13,14] and to hypertension possibly by overweight, hyperinsulinemia or haemorheological changes [8,15]. A strongly significant interaction between the *C825T* polymorphism of the *GNB3* gene and adiposity for systolic blood pressure (SBP) has actually been demonstrated by Dong *et al*. [16] in twins.

Menopause is also considered a condition leading to excess body adiposity and arterial hypertension [17]. The aim of the present epidemiological study based on men and women from the general population is to clarify whether the effects of menopause on body weight and blood pressure are influenced by the *C825T* polymorphism of the *GNB3* gene.

Methods
Determination of phenotype
The protocol of the study was described elsewhere [18–20]. Briefly, all the inhabitants of two small towns in the Leogra valley in northern Italy were invited by letter to refer at fast to the local general hospital; 73% of them responded, gave written informed consent to the study, and were enrolled. This Caucasian population is located in a secluded area with little or no immigration and shares a very homogeneous lifestyle. The study was approved by the local ethics committee and was conducted according to the Declaration of Helsinki. The data obtained from the cross-sectional initial survey conducted over 4 years are described herein.

Before clinical examination, all subjects answered a detailed questionnaire concerning lifestyle, smoking and drinking habits, nutrition, physical activity, quality of life, medication, personal and familial medical history.

Body height and weight were recorded without shoes with the subjects wearing light indoor clothing. Body mass index (BMI, in kg/m^2) was computed as weight divided by squared height. Waist circumference was determined by means of a tape measure during normal breathing.

Subscapular, suprailiac and triceps skinfolds were measured at the right side of the body to the nearest 0.1 mm with a Harpenden Skinfold Caliper (Baty, Burgess Hill, UK) providing a constant pressure of 0.01 kg/mm^2 (0.098 N/mm^2) ± 10% at all openings of the 90 mm^2 anvils. Before the skinfolds were measured in triplicate, the subject sat quietly, so that the muscles of the shoulder and upper arm were relaxed. The subscapular skinfold was measured at the inferior angle of the scapula, the suprailiac skinfold at the intersection of a line joining the spinal and the anterior part of the axilla and a horizontal line at the level of the iliac crest and the triceps skinfold at the midportion of the muscle. According to previous data [21,22], truncal obesity was defined as a ratio of subscapular to triceps skinfold thickness of 2.24 or more in men and 1.32 or more in women.

The average of three measurements was taken for each skinfold; the average of all three skinfolds (mean skinfold thickness; MST) was also calculated.

Subjects were labelled as having arterial hypertension when SBP was 140 mmHg or greater or diastolic blood pressure (DBP) was 90 mmHg or greater, or current antihypertensive treatment was detected; as being overweight when the BMI was 25 kg/m^2 or greater; as having diabetes mellitus when fasting blood glucose was 126 mg/dl or greater or current antidiabetic treatment was detected; and as having excess subcutaneous adiposity when the measured MST was greater than the median MST.

Daily alcohol intake was calculated in grams of ethanol from a detailed questionnaire asking for the daily consumption of wine (ethanol 10–12%), beer (ethanol 3–7%), aperitifs (16–24%) and drinks (ethanol 33–46%). SBP and DBP (Korotkoff phase 5) were taken in triplicate at 10-min intervals in the lying posture by trained doctors using a mercury sphygmomanometer, taking special care to avoid any terminal digit preference. In order to minimize white-coat effects, the average of the last two measurements (in mmHg) was taken. Pulse heart rate (in beats per minute; bpm) was also taken in triplicate contextually, and averaged.

Blood was drawn after an overnight fast with subjects resting in the supine position for 30 min. Blood components, summarized in Table 1, were measured by standard methods.

For each individual, the homeostasis model assessment (HOMA) index [23] was calculated from HOMA $=$ circulating insulin (in μU/ml) × blood glucose (in mmol/l)/22.5.

Menopausal status was determined in women by a detailed questionnaire asking for the age of cessation of menses.

Determination of genotype
Genomic DNA was isolated from whole blood collected in ethylenediamine tetraacetic acid tubes using a blood

Table 1   General characteristics of the study population

Items	Whole population (n = 1339)	Men (n = 591)	Women (n = 748)
Age (years)	60.4 ± 17.2	59.5 ± 15.9	62.6 ± 16.6***
BMI (kg/m^2)	26.5 ± 4.5	26.9 ± 3.8	26.2 ± 4.8
Overweight (%)	61.0	67.1	59.3***
Smoking habits (cigarettes/day)	1.6 ± 4.6	2.3 ± 5.8	1.6 ± 3.3***
Ethanol intake (g/day)	31.5 ± 36.3	51.5 ± 44.7	16.4 ± 16.8***
Waist circumference (cm)	92.5 ± 12.9	97.4 ± 10.7	89.2 ± 13.1***
Skinfold thickness (mm)			
Triceps	15.9 ± 7.3	11.6 ± 4.9	19.2 ± 7.1***
Subscapular	18.4 ± 7.4	16.8 ± 6.5	19.2 ± 8.3***
Suprailiac	12.0 ± 5.8	10.4 ± 4.5	13.7 ± 6.1***
Mean	15.6 ± 6.2	12.6 ± 4.5	18.0 ± 6.2***
Excess subcutaneous adiposity[a] (%)	47.1	43.2	50.1*
Truncal obesity (%)	16.1	12.8	19.0**
SBP (mmHg)	158.1 ± 26.0	158.3 ± 24.9	158.9 ± 27.4
DBP (mmHg)	89.3 ± 11.2	91.2 ± 10.6	88.2 ± 11.4***
Heart rate (bpm)	70.2 ± 10.6	68.1 ± 10.4	71.9 ± 9.5***
Serum cholesterol (mg/dl)			
Total	220.0 ± 45.3	215.3 ± 42.9	225.7 ± 46.0***
HDL-cholesterol	45.4 ± 12.4	41.9 ± 11.4	48.3 ± 12.3***
LDL-cholestorl	151.1 ± 38.2		147.2 ± 38.0***
Serum triglycerides (mg/dl)	116.5 ± 70.5	124.9 ± 78.8	111.9 ± 65.6*
Blood glucose (mg/dl)	104.1 ± 25.7	107.3 ± 28.4	102.2 ± 23.4**
Diabetes mellitus (%)	19.8	21.9	18.1*
Blood insulin (mg/dl)	8.2 ± 6.8	8.0 ± 6.6	8.4 ± 7.1
HOMA index	2.13 ± 1.90	2.13 ± 1.90	2.13 ± 1.90

BMI, Body mass index; DBP, diastolic blood pressure; HDL, high-density-lipoprotein; HOMA, homeostasis model assessment; LDL, low-density-lipoprotein; SBP, systolic blood pressure. *$P < 0.01$; **$P < 0.001$; ***$P < 0.0001$ versus men. [a] Mean skinfold thickness greater than median.

DNA Prep Plus spin-column system, according to the protocol provided by the manufacturer (A&A Biotechnology, Gdansk, Poland). For the determination of the *C825T* polymorphism of the *GNB3* gene, DNA was extracted from cellular blood components by the salting-out method and amplified using the following primer pair: forward 5′–GCAGTGAOOCACTT GCCACCCG TG–3′, reverse 5′–GCAGCAGCCCAGGGCTGGC–3′. The polymerase chain reaction was carried out in a final volume of 25 μl containing 2 mmol magnesium chloride, 0.2 mmol deoxynucleoside triphosphate (Boehringer Mannheim, Mannheim, Germany), 0.2 mmol each primer, and 1 U Taq DNA polymerase (Biotherm, Gene Craft, Munster, Germany). After an initial denaturation for 5 min at 94°C, the samples were subjected to 35 cycles at 94°C for 1 min, 69.3°C for 45 s, and 72°C for 1 min, with a final extension of 5 min at 72°C. The 286-basepair product represents the T allele, whereas a C allele was cut into 116 and 152-bp fragments. The three genotypes were scored after running on a 2.5% agarose gel with 10 μg/ml ethidium bromide [24].

The analysis was performed in the 1354 subjects (591 men and 763 women) who had available data on questionnaire, *C825T* polymorphism and skinfold thickness; women who went into menopause less than 6 months before or received substitutive hormonal therapy were not included. The general characteristics of subjects with incomplete data did not differ from those included in the analysis.

Statistical methods
For database management and statistical analysis, SAS version 8.2 (SAS Institute, Cary, North Carolina, USA) was used. The distributions of circulating insulin, postload blood glucose, serum triglycerides, and HOMA index were normalized by log transformation, and log-transformed values were used in the analysis. Fasting blood glucose distribution was normalized as in Ranade *et al.* [25] (i.e. glucose values were raised to the power of -1.4 and multiplied by $-100\,000$) to obtain a normal distribution.

Proportions were compared using Fisher's exact test. Hardy–Weinberg equilibrium and allele frequencies were evaluated by chi^2 statistics. Continuous variables were expressed as mean ± standard deviation and compared across genotypes with analysis of variance or, when proper, of covariance and the Tukey's posthoc test, also adjusting for confounders and calculating 95% confidence intervals when proper. Correlations were evaluated using the Pearson method. In logistic regression, genotypes were represented by dummy variables defined according to the deviation from the mean coding approach, not implying any genetic hypothesis. Odds ratios and 95% confidence intervals were calculated for the *TT* and *CT* versus *CC* genotypes with logistic models including age and other covariables when appropriate.

We searched for possible covariates of the MST, using stepwise multiple regression with the *P* value for independent variables to enter and stay in the model set at 0.10. The covariates considered for entry into the model were sex, age, alcohol consumption, glycaemia, total cholesterol and triglycerides. We added to the model the sex×*C825T* polymorphism interaction term. The analysis was then repeated in both sexes separately. Furthermore, as hormonal status significantly altered the model, the analysis was further repeated in fertile and menopausal women separately.

In all statistics, the null hypothesis was rejected when obtaining two-sided values below 0.05.

Results
Whole population
The general characteristics of the study population are summarized in Table 1. The prevalence of arterial hypertension (76.4%) was in line with previous findings recorded by the same group in similar north Italian population cohorts.

The frequencies of the *C* and *T* alleles were 66.7 and 33.3%, respectively, similar to those previously described in other Caucasians [8,24]. The *CC*, *CT* and *TT* genotypes were found in 45% ($n = 612$), 43.3% ($n = 587$) and 11.7% ($n = 160$), respectively, of the study population. These frequencies were in agreement with those predicted by the Hardy–Weinberg equilibrium ($P = 0.22$).

The prevalence of arterial hypertension (80.2% in *CC*, 81.9% in *CT* and 81% in *TT*) and diabetes (20.6, 18.4 and 22.1%, respectively) was no different across genotypes.

Triceps skinfold thickness was 15.6 ± 7.2 mm in *CC*, 15.9 ± 7.0 mm in *CT* and 17.1 ± 8.3 mm in *TT* subjects ($P < 0.03$ *TT* versus *CC*), subscapular was 17.9 ± 7.6, 18.6 ± 7.6 and 19.4 ± 7.9 mm, respectively ($P = 0.05$), and suprailiac 11.7 ± 5.6, 12.0 ± 6.0 and 12.8 ± 5.7 mm, respectively ($P = 0.05$). MST was 15.3 ± 6.1 mm in *CC*, 15.7 ± 6.1 mm in *CT* and 16.2 ± 6.4 mm in *TT* subjects ($P = 0.003$).

In stepwise multiple regression, MST was significantly and independently associated ($F = 38.5$, $r = 0.38$, $P < 0.0001$) with sex, age, alcohol intake, serum triglycerides, total serum cholesterol and blood glucose. A significant interaction was found between the *C825T* polymorphism and sex in relation to MST ($P < 0.0001$). When menopausal status was added to the model ($F = 43.2$, $r = 0.40$, $P < 0.0001$), a significant interaction ($P < 0.0001$) between the *C825T* polymorphism and hormonal status was detected. A sex-specific analysis adjusted for the aforementioned covariates was therefore performed.

Sex-specific analysis

In comparison with men, women were 6% older and had higher values of heart rate (+3.5%), serum total and high-density lipoprotein cholesterol (+5% and +15%, respectively), lower values of blood glucose (−4.6%) and triglycerides (−24%) and a lower prevalence of overweight and diabetes mellitus. Ethanol intake (−68%) and the number of cigarettes smoked per day (−30%) were significantly lower in women than in men (Table 1).

After adjusting for the above-mentioned confounders, triceps, subscapular, suprailiac and mean skinfold thickness were significantly lower in men than in women (11.6 ± 6.8 versus 19.2 ± 6.6, $P < 0.05$; 16.1 ± 7.8 versus 20.0 ± 7.8, $P < 0.0001$; 10.0 ± 5.9 versus 13.7 ± 5.7, $P < 0.0001$; and 12.7 versus 17.7 $P < 0.0001$, respectively), whereas BMI was no different between the sexes. Excess subcutaneous adiposity (MST greater than the median) and truncal obesity were reported less in men than in women (26.6 versus 52.9, $P < 0.0001$, and 10.6 versus 18.4, $P < 0.001$, respectively).

Age, blood lipids, glycaemic indices and ethanol intake were different in fertile and menopausal women (Table 2), so that statistics were adjusted for these confounders when comparing the adiposity indices of the two groups of women. Adjusted values are shown in Table 3. MST was 14% higher in menopausal than in fertile women, mainly because of the difference in subscapular skinfold. BMI was 10% higher and waist circumference 12% higher in menopausal than in fertile women. Excess subcutaneous adiposity and truncal obesity were also reported more in the former than in the latter.

In fertile women, but not in menopausal women, triceps, subscapular and suprailiac skinfold thickness, and more generally MST, were higher in the *TT* than in the *CC* genotype (Table 4). Excess body adiposity was reported more in *TT* [relative risk (RR) 3.12, 95% confidence interval (CI) 1.25–7.78] and in *CT* (RR 2.13, 95% CI

Table 2   General characteristics of the 748 women according to hormonal status

Items	Fertile ($n = 173$)	Menopausal ($n = 575$)
Age (years)	36.5 ± 8.0	70.5 ± 8.6[***]
Smoking habits (cigarettes/day)	1.5 ± 3.6	1.0 ± 3.2
Waist circumference (cm)	79.5 ± 12.7	92.1 ± 11.7[***]
Ethanol intake (g/day)	12.7 ± 16.3	17.5 ± 16.8[***]
Serum cholesterol (mg/dl)		
Total	196.8 ± 38.0	234.4 ± 44.3[***]
HDL-cholesterol	49.7 ± 14.6	47.9 ± 11.5[**]
LDL-cholesterol	131.7 ± 33.5	162.0 ± 38.8[***]
Serum triglycerides (mg/dl)	76.7 ± 45.7	122.4 ± 64.4[***]
Blood glucose (mg/dl)	91.7 ± 19.3	105.3 ± 23.7[***]
Diabetes mellitus (%)	6.1	21.9[***]
Blood insulin (mg/dl)	6.4 ± 3.8	9.0 ± 7.7[***]
HOMA index	1.49 ± 1.12	2.33 ± 2.02[***]

HDL, High-density-lipoprotein; HOMA, homeostasis model assessment; LDL, low-density-lipoprotein. [*] $P < 0.05$; [**] $P < 0.005$; [***] $P < 0.0001$ versus fertile.

1.12–4.05) compared with *CC* fertile women, whereas there was no difference between genotypes in menopausal women or in men. BMI was also higher among fertile women in the *TT* than in the other two genotypes, but was no different across genotype in menopausal women (Table 4). In fertile women only, significantly higher SBP values were detected in the *TT* than in the *CC* genotype also after adjusting for confounders, whereas in menopausal women blood pressure was uniform across genotypes (Table 4).

In women with the *TT* genotype, BMI, skinfold thicknesses, the prevalence of excess subcutaneous adiposity and the prevalence of truncal obesity were similar in those who were fertile and in those who were menopausal (Table 4). On the contrary, in *CC* women, BMI, overweight, truncal obesity, MST and excess subcutaneous adiposity were significantly greater in those who were menopausal compared with those who were fertile. In a multivariate logistic model for excess subcutaneous adiposity, the relative risk of being in menopause was 4.12 (95% CI 2.35–7.22) in *CC* women, but was insignificant in the other two genotypes.

Table 3   Indices of adiposity, blood pressure and heart rate in the 748 women according to hormonal status

	Fertile ($n = 173$)	Menopausal ($n = 575$)	Fertile ($n = 173$)	Menopausal ($n = 575$)
	Crude values		Adjusted for confounders[a]	
BMI (kg/m^2)	23.6 ± 4.6	27.0 ± 4.5[***]	24.4 ± 6.2	26.8 ± 6.2[**]
Overweight (%)	25.9	66.0[***]	43.1	52.7[***]
Waist circumference (cm)	79.5 ± 12.7	92.1 ± 11.7[***]	83.7 ± 21.6	90.7 ± 15.6[***]
Skinfold thickness (mm)				
Triceps	18.7 ± 7.1	19.4 ± 7.1[*]	18.2 ± 9.5	19.5 ± 9.4
Subscapular	18.5 ± 8.1	20.5 ± 8.2[***]	17.1 ± 11.4	21.2 ± 11.4[*]
Suprailiac	11.6 ± 5.0	14.7 ± 6.0[***]	12.7 ± 8.4	14.2 ± 8.4
Mean	16.3 ± 6.1	18.5 ± 6.1[***]	16.2 ± 8.9	18.4 ± 8.9[*]
Excess subcutaneous adiposity[b] (%)	34.0	55.2[***]	35.5	49.0[*]
Truncal obesity (%)	11.9	22.5[***]	9.6	24.2[***]
SBP (mmHg)	132.7 ± 15.8	166.8 ± 25.0[***]	155.3 ± 28.8	159.3 ± 28.8
DBP (mmHg)	82.3 ± 9.3	89.9 ± 11.3[***]	85.5 ± 14.2	88.8 ± 14.2
Heart rate (bpm)	72.6 ± 9.6	71.7 ± 10.7[*]	77.6 ± 18.5	70.0 ± 13.4[***]

BMI, Body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure. [*] $P < 0.05$; [**] $P < 0.005$; [***] $P < 0.0001$ versus fertile. [a] Age, serum total cholesterol and triglycerides, blood glucose, ethanol daily intake. [b] Skinfold thickness greater than median.

Table 4 General characteristics of women according to hormonal status and *C825T* polymorphism of *GNB3* gene

	Fertile women ($n = 173$)			Menopausal women ($n = 575$)		
	CC ($n = 81$)	CT ($n = 48$)	TT ($n = 24$)	CC ($n = 270$)	CT ($n = 235$)	TT ($n = 70$)
Age (years)	36.1 ± 7.9	36.7 ± 8.4	35.4 ± 8.9	$70.8 \pm 8.8^{†††}$	$70.2 \pm 8.6^{†††}$	$69.8 \pm 8.2^{†††}$
BMI (kg/m^2)	22.9 ± 4.3	23.6 ± 4.4	$25.3 \pm 5.5^{**}$	$27.1 \pm 4.5^{†††}$	27.2 ± 5.0	26.4 ± 4.2
Overweight (%)	23.2	23.6	40.7	$66.4^{†††}$	$67.8^{†††}$	$58.6^{†††}$
Skinfold thickness (mm)						
Triceps	17.2 ± 6.6	19.0 ± 6.6	$22.9 \pm 8.8^{***}$	$19.4 \pm 6.7^{†}$	19.3 ± 6.7	19.6 ± 8.2
Subscapular	17.0 ± 7.9	18.9 ± 7.7	$22.0 \pm 9.0^{***}$	$20.4 \pm 8.0^{††}$	20.4 ± 8.4	21.1 ± 8.4
Suprailiac	10.1 ± 4.2	12.8 ± 6.7	$13.3 \pm 5.8^{**}$	$14.8 \pm 6.2^{†}$	14.8 ± 6.2	14.6 ± 5.5
Mean	14.8 ± 5.4	19.4 ± 6.9	19.4 ± 6.9	18.5 ± 6.1	18.5 ± 6.1	18.4 ± 6.1
Truncal obesity (%)	10.3	9.9	28.2	$20.8^{†}$	$23.7^{†}$	25.0
SBP (mmHg)	131.6 ± 14.5	131.3 ± 36.5	$139.5 \pm 17.5^{***}$	169.2 ± 25.9	165.5 ± 23.9	164.2 ± 25.3
DBP (mmHg)	81.7 ± 9.7	82.1 ± 9.6	83.7 ± 8.3	90.3 ± 11.7	89.7 ± 11.4	89.5 ± 9.5
Total cholesterol (mg/dl)	193.5 ± 36.3	198.5 ± 39.6	202.5 ± 39.1	$232.3 \pm 43.8^{†††}$	$236.3 \pm 43.8^{†††}$	$235.4 \pm 48.1^{††}$
HDL-cholesterol (mg/dl)	49.5 ± 13.3	50.8 ± 16.9	47.3 ± 14.2	47.2 ± 11.1	48.6 ± 12.1	47.7 ± 11.1
Triglycerides (mg/dl)	78.4 ± 51.7	72.5 ± 39.5	81.6 ± 40.2	$123.6 \pm 69.2^{†††}$	$119.5 \pm 60.6^{†††}$	$128.2 \pm 57.3^{†}$
Blood glucose (mg/dl)	90.7 ± 24.4	92.0 ± 12.4	92.7 ± 15.7	106.3 ± 26.0	104.2 ± 21.3	102.6 ± 16.9
Diabetes mellitus (%)	7.2	4.4	7.4	$22.6^{††}$	$19.9^{††}$	25.7
Blood insulin (mg/dl)	6.3 ± 3.7	5.9 ± 3.5	7.9 ± 4.2	$9.5 \pm 9.0^{†}$	8.4 ± 6.6	8.8 ± 5.4
HOMA index	1.48 ± 1.18	1.39 ± 1.02	1.79 ± 1.59	$2.26 \pm 2.07^{†}$	2.22 ± 2.00	2.13 ± 1.57

BMI, Body mass index; DBP, diastolic blood pressure; HDL, high-density-lipoprotein; HOMA, homeostasis model assessment; SBP, systolic blood pressure. $^* P < 0.05$; $^{**} P < 0.001$; $^{***} P < 0.0001$ versus *CC* of fertile women; $^† P < 0.01$; $^{††} P < 0.001$; $^{†††} P < 0.0001$ versus the same genotype of fertile women. Adjustment was made for the confounders listed in Table 3 within the *C825T* polymorphism, not within hormonal status.

Blood pressure was apparently higher in menopausal than in fertile women, a difference that was no longer present after adjusting for age (Table 3). In fertile women only, significantly higher SBP values were detected in the *TT* than in the *CC* genotype also after adjusting for confounders, whereas in menopausal women blood pressure was uniform across genotype (Table 4). A positive correlation of MST with SBP was detected in *CC* ($r = 0.20$, $P = 0.001$) and *CT* ($r = 0.17$, $P = 0.004$) women, but not in *TT* women or in men.

Among men, no significant association with the *C825T* polymorphism was found either for BMI, truncal obesity, MST or excess subcutaneous adiposity.

Discussion

The G-proteins are signal transducers that communicate information from hormones to intracellular signalling pathways [26,27]. Their structure has been clarified and described [28,29]. The *GNB3* gene, codifying for the G-protein subunit β_3, has been characterized by the group of Siffert *et al.* [1,27]. Apart from a few exceptions [1,30–32], the *825T* mutation of this gene leading to the occurrence of the splice variant Gβ3s [1] has been observed to be associated in humans with obesity [27,33,34], higher values of BMI or skinfold thickness [8,34] and a higher risk of postpregnancy obesity [8].

Obesity *per se* predisposes to hypertension, but it has also been suggested that the *825T* mutation directly participates in pressor control after adjusting for BMI [1,11] of for skinfold thickness [8], being significantly associated with essential hypertension [1] and accounting for 15% of cases [1].

This effect of the *C825T* polymorphism on body fat and blood pressure is similar to that commonly attributed to menopause, and it is simply natural to wonder whether an interaction between hormonal status and the *GNB3* gene occurs in humans. Menopause could even be the (or one of the) intermediate phenotype(s) leading to the final effects of the *825T* mutation. The present study was conceived to check systematically for this hypothesis. A general population of men and women [18–20] was chosen for this purpose, so avoiding any bias leading to results not applicable to the whole population. Our data indicated that triceps, subscapular and suprailiac skinfold thickness (and more specifically MST) as well as BMI were higher in menopausal than in fertile women; although the latter were older than the former; such differences, although less pronounced, were also detectable and statistically significant after adjusting for age. This supports the belief that menopause, independent of age, partly determines body adiposity.

A further analysis showed that this trend towards greater adiposity in menopausal women was confined to the *CC* genotype (when the relative risk of menopause in this respect was greater than 4), and absent in the *T* carriers; women in menopause were more adipose than fertile women, provided they carried the *CC* polymorphism of the *GNB3* gene. When showing the *TT* genotype, their skinfold triceps, subscapular and suprailiac skinfold thickness (and more specifically MST) were no different according to hormonal status. Not only this, but in contrast, higher values of skinfold thickness in *TT* than in *CC* women were found in fertile women only, but were absent in menopausal women or in men.

With regard to blood pressure, significantly higher values were detected in TT than in CC women, provided they were fertile, whereas no difference in blood pressure according to genotype was found in menopausal women. Menopause therefore blunts the effects of the T allele on blood pressure.

In conclusion, our data indicate that the expected increase in body adiposity with menopause, which is taken for granted for all women, takes place only in those who are *CC* for the *C825T* polymorphism of the *GNB3* gene. In contrast, higher blood pressure should be expected in *TT* women, provided they are fertile, whereas in menopausal women blood pressure values are uniform across the *C825T* genotype.

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