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Augmented Muscle Vasodilatory Responses in Obese Children With Glu27 β_2-Adrenoceptor Polymorphism

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This study examined forearm vasodilatation during mental challenge and exercise in 72 obese children (OC; age = 10 ± 0.1 years) homozygous with polymorphism in the allele 27 of the β_2-adrenoceptors: Gln27 (n = 61) and Glu27 (n = 11). Forearm blood flow was recorded during 3 min of each using the Stroop color-word test (MS) and handgrip isometric exercise. Baseline hemodynamic and vascular measurements were similar. During the MS, peak forearm vascular conductance was significantly greater in group Glu27 (Δ = 0.35 ± 0.4 vs. 0.12 ± 0.1 units, respectively, p = .042). Similar results were found during exercise (Δ = 0.64 ± 0.1 vs. 0.13 ± 0.1 units, respectively, p = .035). Glu27 OC increased muscle vasodilatory responsiveness upon the MS and exercise.

Obesity is a major risk factor for developing vascular disorders (7). These alterations can also be found in children, and recent studies have reported vascular disorders in childhood obesity (19,37) observed as significantly impaired peripheral muscle blood flow (30).

The most frequent natural β_2-adrenoceptor polymorphisms are glycine for arginine at amino acid 16 (Gly for Arg) and glutamic acid to glutamine at amino acid 27 (Glu for Gln), and previous in vivo and in vitro studies have suggested that these variants of β_2-adrenoceptors (ADRB2) might affect functional responses to adrenergic stimulation differently, leading to distinct modulations in cardiovascular and metabolic phenotypes (2,8,12–14,17). In fact, the Glu27 β_2-adrenoceptor polymorphism is more markedly associated with obesity than with individuals not presenting this polymorphism (17,20,27). Green et al. (13) demonstrated that in Chinese hamster fibroblast cells, agonist-promoted down regulation is resistant in Glu27/Glu polymorphism compared with the wild type (Gln27/Gln). Further in vivo

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studies emphasized the clinical relevance of ADRB2 polymorphism on codon 27 in determining vascular reactivity in normotensive subjects (2,8). During continuous exposure of exogenous agonists, forearm vasodilatory response to intra-arterial infusion of isoproterenol was greater in subjects who were homozygous for the Glu27 β_2-adrenoceptor polymorphism than in subjects who were homozygous for the Gln27 β_2-adrenoceptor polymorphism (2). Under physiological conditions, the defense reaction, provoked by mental challenge or physical exercise, markedly increases sympathetic nerve activity and increases skeletal muscle blood flow in humans (29). The increase in muscle blood flow during mental challenge and exercise is, at least in part, ADRB2 mediated (11,18,21,28). Recently, Trombetta et al. (33) studied the vasodilatory response during physiological maneuvers and suggested that the allele Glu27 augments forearm vasodilatation during mental stress and exercise. In their study, forearm blood flow was lower in women who were homozygous for Gln27 than in women homozygous for Glu27, regardless of the mutation encoding for amino acids at position 16. It is unknown, however, whether this augmented vasodilatation in the presence of the allele Glu27 begins early in human life, which might confer to the Glu27 β_2-adrenoceptor polymorphism a more expanded protective effect against cardiovascular disorders during the life span in humans. In the current study, we describe the blood flow responsiveness during exercise and mental stress in children who were homozygous for Gln27 and children who homozygous for Glu27.

The aim of the current study was to test the hypothesis that obese children carrying the Glu27/Glu polymorphism of the ADRB2 have augmented muscle vasodilatation during exercise and mental stress compared with obese children with Gln27/Gln.

Materials and Methods

Participants

One hundred ten preselected obese child volunteers age 8–12 years from the Endocrinology Outpatient Clinic of the University of São Paulo were genotyped for Gln27 and Glu27 β_2-adrenoceptor alleles. These alleles were identified by single-strand conformation polymorphism analysis using polymerase chain reaction (PCR) as described in the literature (20). PCR amplification was performed on an automated device (Perkin Elmer Corporation, CA). A total of 38 participants were excluded from the study because they were heterozygous for the allele 27. Seventy-two subjects who were homozygous for the alleles 27 were included in the study. Sixty-one were homozygous for Gln27/Gln and 11 for Glu27/Glu. Children were screened for cardiovascular, endocrine, and metabolic disorders. The clinical examination, laboratory exams, and cardiopulmonary exercise test showed no evidence of disease in any children included in the study. Obesity was defined according to the age- and sex-specific BMI cutoffs criteria previously described (3), which defines obesity as BMI >95% for age and sex. Thus, our final sample comprised two subgroups: (1) Gln27/Gln group (n = 61; 26 boys and 35 girls) and (2) Glu27/Glu (n = 11; 02 boys and 09 girls). Participants had not taken any medications over the 3 months leading up to the study. The Human Subject Protection

Committees of the Heart Institute (InCor) and Clinicas Hospital, the University of São Paulo medical school, approved the study protocol whereby the parent or guardian of each child participant gave their written consent.

Measurements and Procedures

Anthropometric Measurements. Body weight was measured using electronic body weight scales while children wore light T-shirts and shorts. Height was measured by a Harpenden stadiometer. BMI and *z*-score of BMI was recorded using the LMS method (4).

Forearm Blood Flow. Forearm blood flow was measured by venous occlusion plethysmography (25). The nondominant arm was elevated above heart level to ensure adequate venous drainage. A mercury-filled silastic tube attached to a low-pressure transducer was placed around the forearm and connected to a plethysmograph (Hokanson, Bellevue, WA). Sphygmomanometer cuffs were placed around the wrist and upper arm. At 15-s intervals, the upper cuff was inflated above venous pressure for 7–8 s. Forearm blood flow (ml/min/100 ml tissue) was determined on the basis of a minimum of four separate readings. Forearm vascular conductance was calculated by dividing forearm blood flow by mean arterial pressure. Venous occlusion plethysmography is a valid and reproducible method for accessing the forearm blood flow in humans (35,36). This method has been extensively used for humans' blood flow measures in response to handgrip exercise in both adults and children (24,26,30,34,35). In adults, the reproducibility of forearm blood flow in two different measurements (interval of 1 week), expressed as ml/min/100ml, is r = .95. In children, the reproducibility of forearm blood flow in two different measurements (interval of 1 week), expressed as ml/min/100ml, is r = .90.

Blood Pressure and Heart Rate. During handgrip exercise, mean blood pressure was monitored noninvasively and intermittently from an automatic and oscillometric cuff (Dixtal, DX 2710; Brazil, Manaus) placed on the ankle, with cuff width adjusted to ankle circumference. Mean blood pressure was chosen because this parameter is accurately measured by the automatic and oscillometric device. The cuff inflated every 30 s. During mental stress, mean blood pressure was monitored noninvasively by a finger photoplethysmography device (FinaPress 2300; Ohmeda, Englewood, CO) on a beat-to-beat basis (AT/CODAS) at a frequency of 500 Hz. Heart rate was monitored continuously through lead II of the ECG.

Handgrip Exercise. After obtaining the maximal voluntary contraction (MVC, average of three trials), handgrip isometric exercise was performed at 30% of MVC with the dominant arm using a handgrip dynamometer. The force was controlled by visual feedback under investigator's supervision. The individuals were instructed to breathe normally during exercise and to avoid inadvertent performance of a Valsalva maneuver.

Mental Stress Testing. Mental stress was elicited by the Stroop color-word test (23). In the Stroop color-word test, participants were shown a series of names of colors written in a different color ink to the color stated. The participants were asked to identify the color of the ink, not reading the word.

Functional Capacity Testing. The peak oxygen uptake was considered at the end of the cardiopulmonary exercise test on treadmill (ramp protocol with increments every minute up to exhaustion (30).

Biochemical Analyses

Plasma Lipid and Glucose Concentrations. Fasting plasma concentrations of cholesterol and triglycerides in very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) fractions were determined by a combination of preparative ultracentrifugation, precipitation of apolipoprotein B-containing lipoproteins, and lipid analyses (COBAS MIRA - F. Hoffmann-La Roche, Basiléia, Suiça). Blood glucose was measured by a glucose oxidase method (COBAS- La Roche).

Leptin and Insulin Resistance. Plasmatic leptin levels were determined by immunofluorimetric assay (AutoDELFIA, Wallac, Turku, Finland). Plasmatic insulin levels were determined by radioimmunoassay. Insulin resistance was estimated by the homeostasis model assessment for insulin resistance (HOMA–*IR* score) and calculated using the following formula: fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/l)/22.5 (22). Previous study showed that HOMA-IR correlates strongly with the insulin sensitivity assessed by modified minimal model frequently sampled intravenous glucose tolerance test (5), which is an accurate and valid technique for the measurement of insulin sensitivity in adults, adolescents, and children (10,6). In addition, insulin resistance was estimated by mathematical model determining total insulin ($AUC_{insulin}$) and glucose ($AUC_{glucose}$) area under the curve during oral glucose tolerance testing (OGTT; 32). The area under the curve for insulin ($AUC_{insulin}$) and area under the curve for glucose ($AUC_{glucose}$) are insulin sensitivity indices in obese children (1,38).

Experimental Protocols

The children came to the hospital for the exams in 3 different days. In the first visit, blood samples were collected in the morning after a 12-hr overnight fast. One week later, they were submitted to the cardiopulmonary exercise test. Finally, in the third week, the children were submitted to handgrip exercise and mental stress protocols.

Handgrip Exercise Protocol. After a 15-min rest period, the arm was positioned for venous plethysmography. Then, forearm blood flow, blood pressure, and heart rate were recorded for 3 min of baseline followed by 3 min of handgrip exercise. Forearm blood flow was recorded continuously, and blood pressure was monitored noninvasively and intermittently from an automatic and oscillometric cuff placed on the ankle with cuff width adjusted to ankle circumference (22,28). The cuff inflated every 30 s. All studies were performed in a quiet, temperature-controlled (21°C) room at approximately the same time of day.

Mental Stress Protocol. After heart rate and blood pressure returned to baseline, the arm was again positioned for venous plethysmography. Baseline forearm blood flow, blood pressure, and heart rate were recorded for 3 min. Acute mental-stress testing was then performed for 3 min (Figure 1). Forearm blood flow, blood pres-

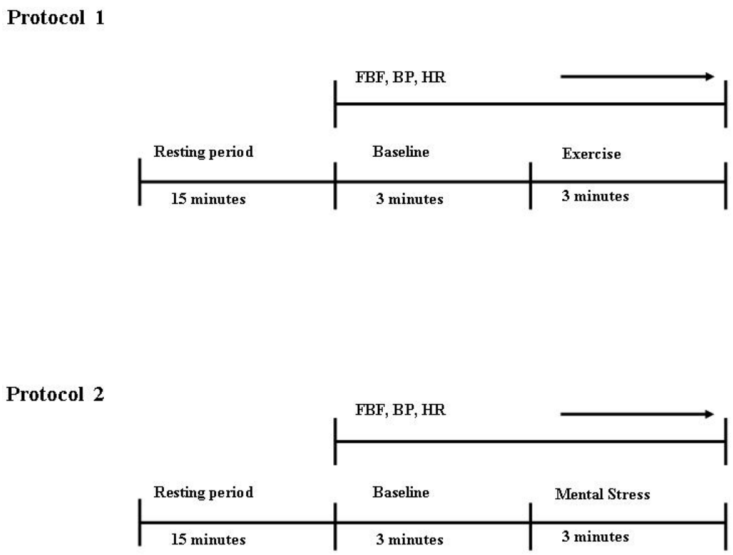

Figure 1 — Timeline of experimental protocols (see Experimental Protocol for explanation). FBF = forearm blood flow; BP = blood pressure; HR = heart rate.

sure, and heart rate were recorded continuously during mental stress. The task difficulty was determined on completion of the protocol, using a standard 5-point scale (0 = *not stressful*; 1 = *somewhat stressful*; 2 = *stressful*; 3 = *very stressful*; and 4 = *very very stressful*).

Statistical Analysis

The statistical procedures were carried out using SPSS version 13.0 (SPSS Inc.,Chicago, IL). Data are presented as mean ± SEM. Possible baseline differences between groups were compared by unpaired *t*-test. A chi-square (χ^2) test was used to assess the gender differences between Gln27/Gln and Glu27/Glu children. Forearm blood flow, forearm vascular conductance, mean blood pressure, and heart rate responses are presented as absolute change. They were submitted to two-way ANOVA analysis with repeated measures. Whenever significance was found, Scheffè's posthoc comparison was performed. Significant difference was considered to be $p < .05$.

Results

Baseline Measurements

There were no significant differences between groups in gender ($p = .12$), age ($p = .20$), body weight ($p = .18$), or height ($p = .22$; Table 1). Baseline metabolic and hemodynamic measurements in the two groups studied are shown in Table 2. Total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, and glucose levels

Table 1 Anthropometric Characteristics

	Gln27/Gln (*n* = 61)	Glu27/Glu (*n* = 11)	*p*
Physical Characteristics			
Gender (M/F)	26/35	02/09	.12
Age (years)	10 ± 0.1	10 ± 0.2	.20
Weight (kg)	68 ± 2.6	63 ± 3.7	.18
Height (m)	1.48 ± 0.01	1.44 ± 0.03	.22
BMI (kg/m^2)	31.1 ± 0.5	30.2 ± 1.6	.49
VO_2peak (ml/kg/min)	25 ± 0.3	24 ± 0.4	.41

Note. Values are mean ± SEM. BMI= body mass index.

Table 2 Metabolic Characteristics and Hemodynamic Measurements in Gln27/Gln and Glu27/Glu Groups

	Gln27/Gln (*n* = 61)	Glu27/Glu (*n* = 11)	*p*
Metabolic Measurements			
Total cholesterol (mg/dL)	171 ± 4	160 ± 7	.32
LDL-cholesterol (mg/dL)	108 ± 3	98 ± 8	.23
HDL-cholesterol (mg/dL)	41 ± 1.1	38 ± 2.1	.23
Triglycerides (mg/dl)	104 ± 4	123 ± 11	.15
HOMA (score)	4.3 ± 0.4	4.0 ± 0.4	.69
$AUC_{insulin}$ (μUI/ml/120 min)	11727.7 ± 999	14420.6 ± 2735	.33
$AUC_{glucose}$ (mg/dL/120 min)	13403.4 ± 323	13432.5 ± 364	.97
Leptin (μU/L)	54 ± 3.8	53 ± 9.4	.94
Hemodynamic Measurements			
MBP (mmHg)	80 ± 1.0	85 ± 3.7	.11
HR (bpm)	80 ± 1.5	87 ± 4.8	.10
Neurovascular Measurements			
FBF (ml/min/100ml)	2.69 ± 0.05	2.92 ± 0.14	.09
FVC (units)	3.28 ± 0.08	3.43 ± 0.25	.26

Note. Values are mean ± SEM. HOMA = homeostasis model assessment; AUC = area under the curve; MBP = mean blood pressure; HR = heart rate; FBF = forearm blood flow; FVC = forearm vascular conductance.

were similar in both the Gln27/Gln and Glu27/Glu groups. Insulin resistance by HOMA was also similar among groups. There were no significant differences in mean blood pressure, heart rate, forearm blood flow, and forearm vascular conductance among groups.

Response to Exercise

Maximal voluntary contraction was not different between groups (18 ± 0.4 vs. 17 ± 1.0 kg, p = .6). During handgrip exercise, mean blood pressure increased

progressively and were similar in the two groups studied (Table 3). Heart rate increased progressively and was similar in both the Gln27/Gln and Glu27/Glu groups (Table 3). Forearm blood flow was significantly greater in the Glu27/Glu group compared with the Gln27/Gln group (Figure 2a). Likewise, forearm vascular conductance was significantly greater in the Glu27/Glu group than in the Gln27/Gln group (Figure 2b).

Response to Mental Stress

During mental stress, mean blood pressure increased progressively and similarly in both the Gln27/Gln and Glu27/Glu groups (Table 3). With regard to heart rate, levels increased progressively and similarly in the two groups studied (Table 3). In contrast, forearm blood flow during mental stress was significantly greater in the Glu27/Glu group than in the Gln27/Gln group (Figure 3a). Likewise, forearm vascular conductance was significantly greater in the Glu27/Glu group than in the Gln27/Gln group (Figure 3b).

Discussion

The novelty of the current study is that, even in obese children, the presence of mutation encoding for amino acids Glu27/Glu of the β_2-adrenoceptor positively affected vasodilatory response to mental stress and exercise. In childhood obesity, a condition substantially impairing muscle vasodilatory responses (30), this polymorphism seems to protect against diminished vascular responses during challenge situations. Despite the fact that these children were obese, these findings remain

Table 3 Baseline and Absolute Changes in Mean Blood Pressure, Heart Rate During Mental Stress and Moderate Exercise at 30% of Maximal Voluntary Contraction in Obese Children Encoding Gln27/Gln and Glu27/Glu

Exercise (30% MVC)					
		Baseline	**1 min**	**2 min**	**3 min**
MBP	Gln27/Gln	81 ± 1	4 ± 1	9 ± 1	11 ± 1
(mmHg)	Glu27/Glu	75 ± 2	3 ± 2	11 ± 2	12 ± 2
HR	Gln27/Gln	80 ± 1	4 ± 1	8 ± 1	10 ± 1
(bpm)	Glu27/Glu	80 ± 2	4 ± 2	6 ± 2	10 ± 2
Mental Stress					
		Baseline	**1 min**	**2 min**	**3 min**
MBP	Gln27/Gln	80 ± 1	3 ± 1	7 ± 1	8 ± 1
(mmHg)	Glu27/Glu	85 ± 4	4 ± 2	8 ± 2	9 ± 2
HR	Gln27/Gln	80 ± 1	4 ± 1	5 ± 1	7 ± 1
(bpm)	Glu27/Glu	87 ± 5	2 ± 1	3 ± 1	4 ± 2

Note. Values are mean ± SEM. MVC = maximal voluntary contraction; MBP = mean blood pressure; HR = heart rate.

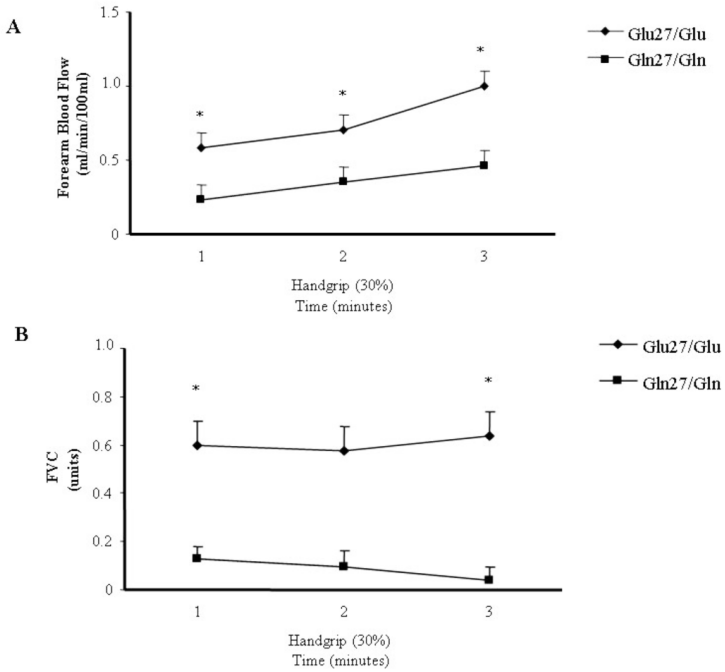

Figure 2 — Forearm blood flow (Panel A) and forearm vascular conductance (FVC, Panel B) responses (absolute change) during 30% of maximal voluntary contraction (MVC) handgrip exercise in children who are homozygous for Gln27/Gln and Glu27/Glu of the β_2-adrenoceptors. Note that, despite similar blood pressure and heart rate levels, increases in forearm blood flow and forearm vascular conductance were significantly higher in obese children who are homozygous Glu27/Glu than are Gln27/Gln. * = $p < .05$.

consistent with the modulation of Glu27 β_2-adrenoceptor polymorphism seen during physiological maneuvers in adulthood (32).

In fact, previous in vivo and in vitro studies have suggested that these variants of ADRB2 might affect functional responses to adrenergic stimulation differently, thereby possibly modulating cardiovascular and metabolic levels (12,15–17). Trombetta et al. (33) found augmented muscle blood flow during mental challenge and exercise in participants with Glu27 polymorphism of the ADRB2, whereas Heckbert et al. (15) observed that the Glu27 allele of the ADRB2 is associated with lower risk of coronary events in elderly participants. The current study extends our knowledge in obese children with the finding that the presence of Glu27/Glu β_2-adrenoceptor polymorphism is linked to greater muscle vasodilatation in response to exercise and mental stress compared with Gln27/Gln β_2-adrenoceptor polymorphism. These responses can attenuate the impairment caused by obesity.

Although we have gathered evidence for the genetic regulation of cardiovascular responses mediated by Glu27 β_2-adrenoceptors, the mechanisms involved in augmented muscle vasodilatation in humans who are homozygous for the Glu27 β_2-adrenoceptor remains unknown. It is unlikely that the increase in muscle blood

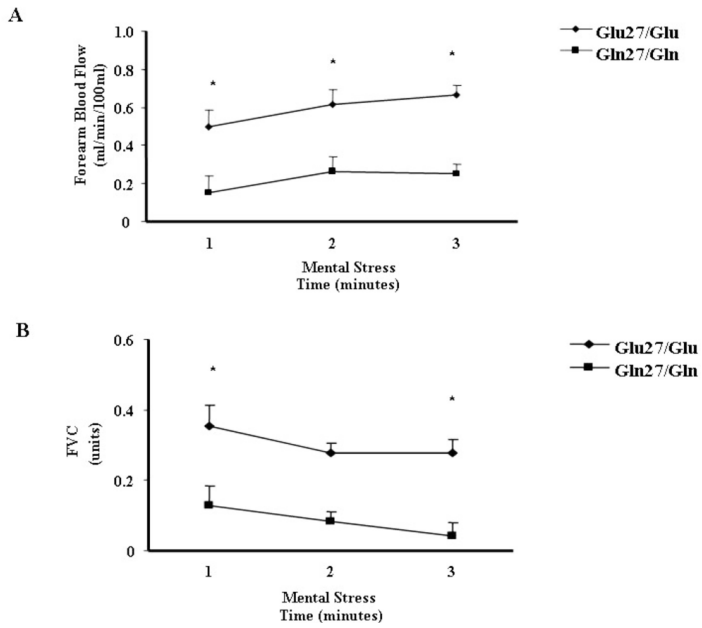

Figure 3 — Forearm blood flow (Panel A) and forearm vascular conductance (FVC, Panel B) responses (absolute change) during mental stress in children who are homozygous for Gln27/Gln and Glu27/Glu of the β_2-adrenoceptors. Note that, despite similar blood pressure and heart rate levels, increases in forearm blood flow and forearm vascular conductance were significantly higher in homozygous Glu27/Glu than in Gln27/Gln children. * = $p < .05$.

flow during mental stress and exercise is because of augmented levels of endogenous plasma ADRB2 agonist, because the plasma epinephrine concentrations at the peak of these two physiological maneuvers are no different between women who are homozygous for Glu27 and those who are homozygous for Gln27 (33). It seems more reasonable to suppose that mutation in the ADRB2 increases endogenous plasma β_2-adrenoceptor agonist sensitivity. In fact, previous studies have yielded evidence that subjects homozygous for Glu27 of the ADRB2 are more sensitive to intraarterial infusion of isoproterenol than participants homozygous for Gln27 of the β_2-adrenoceptor (2). On the other hand, one could question whether the increase in muscle vascular responsiveness in participants with ADRB2 polymorphism is indeed mediated by an increase in the sympathetic outflow, because results from a previous study do not support this argument. Muscle sympathetic nerve activity responses to mental stress and exercise were no different between participants who were homozygous for Glu27 and participants who were homozygous for Gln27 (33). These findings, however, do not rule out the possibility that the increased muscle vasodilatation in humans with Glu27 β_2-adrenoceptor polymorphism is because of reduced α-receptor sensitivity. Future studies should be conducted to clarify the mechanisms involved in the augmented muscle vasodilatory responses during physiological maneuvers in humans who are homozygous for the Glu27 β_2-adrenoceptors.

It has been demonstrated that metabolic disorders affect endothelium function in humans (9,31,39). Thus, someone could raise the question that the difference in forearm blood flow between Glu27/Glu and Gln27/Gln children was because of metabolic disorders. This seems to be unlikely, because there were no significant differences in lipid profile, leptin levels, and insulin resistance between the two groups studied.

Our results in obese children support the view that the homozygous for Glu27/Glu of the ADRB2 is conducive to greater vasodilatory responses during physiological maneuvers. Therefore, it is reasonable to assume that the risk of cardiovascular disorders is attenuated. Notably, Heckbert et al. (15) reported that the Glu27 allele of the ADRB2 was associated with lower risk of coronary events in elderly participants.

In conclusion, these findings might link the Glu27 β_2-adrenoceptor polymorphism to a protective effect against cardiovascular disorders during the life span in humans, and we can speculate that this genetic characteristic could make up part of a clustering of genes which explain, at least in part, the different predisposition to cardiovascular diseases in the presence of risk factors.

Acknowledgments

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