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Metabolic and haemodynamic effects of oral glucose loading in young healthy men carrying the 825T-allele of the G protein $\beta 3$ subunit

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Abstract

Background: A C825T polymorphism was recently identified in the gene encoding the $\beta 3$ subunit of heterotrimeric G-proteins (*GNB3*). The T-allele is significantly associated with essential hypertension and obesity. In order to further explore a possible pathogenetic link between the T-allele and impaired glucose tolerance we studied metabolic and haemodynamic responses to oral glucose loading in young, healthy subjects with and without the 825T-allele.

Methods: Twelve subjects with and 10 without the 825T-allele were investigated at rest and following glucose ingestion (75 g). Blood glucose, serum insulin and haemodynamics were determined prior to and over 2 hours following glucose ingestion. We non-invasively measured stroke volume (SV, by impedance-cardiography), blood pressure (BP), heart rate (HR), and systolic-time-intervals. Cardiac output (CO) was calculated from HR and SV. Total peripheral resistance was calculated from CO and BP. Metabolic and haemodynamic changes were quantified by maximal responses and by calculation of areas under the concentration time profile (AUC). Significances of differences between subjects with and without the T-allele were determined by unpaired two-tailed t-tests. A $p < 0.05$ was considered statistically significant.

Results: Metabolic and haemodynamic parameters at baseline were very similar between both groups. The presence of the T-allele did not alter the response of any metabolic or haemodynamic parameter to glucose loading.

Conclusions: In conclusion, this study does not support the hypothesis that the C825T polymorphism may serve as a genetic marker of early impaired glucose tolerance.

Background

The pathogenesis of hypertension is characterized by a strong genetic background [1]. Recently a C825T poly-

morphism was identified in the gene *GNB3* which encodes the $\beta 3$ subunit of heterotrimeric G proteins and an association of the T-allele of this polymorphism with

hypertension has been demonstrated [2]. The prevalence of the 825T-allele was found to be significantly increased in middle-European Caucasian subjects with essential hypertension, with an odds ratio for homozygous 825T-allele carriers of approximately 1.8 [2]. Association of the 825T-allele with hypertension has been confirmed in four additional independent patient samples, i.e. a population-based, cross-sectional study from southern Germany [3], a group of patients with established essential hypertension from a German hypertension clinic [4], a cohort of white Australian hypertensives with a strong family background of essential hypertension [5] and in a population-based sample of black people of African origin [6]. The mechanisms linking the presence of the T-allele to the development of hypertension in later life are, however, unclear. Interestingly, the T-allele is not only associated with hypertension but a consistent association with obesity has also been shown in different and independent samples both in subjects with normal blood pressure [7] and in hypertensive patients [8].

It has long been recognized that both hypertension and obesity are associated with impaired insulin resistance and glucose intolerance [9,10]. However, it is a matter of ongoing debate, whether this disturbance of glucose metabolism contributes pathogenetically to the development of enhanced total peripheral resistance as the haemodynamic hallmark of hypertension or whether, vice versa, impaired glucose tolerance is a mere consequence of the haemodynamic changes that take place during the development of hypertension [11,12].

If impaired glucose tolerance is a contributory cause and not only a sequelae of hypertension we hypothesized that young, normotensive men who differed only with respect to genotype at the *GNB3* locus, i.e. with respect to their genetic predisposition to develop hypertension should be characterized by impaired glucose tolerance. In order to further explore a possible pathogenetic link between the C825T polymorphism and impaired glucose tolerance we have therefore compared blood glucose and serum insulin responses following an oral glucose load in young, healthy, normotensive, non-obese men with and without the 825T-allele. In order to assess the possible contribution of haemodynamic alterations we also non-invasively measured haemodynamics at rest and in response to oral glucose loading.

Methods

Study Population and Protocol

The study was performed in 22 young, male volunteers after informed, written consent had been obtained. All subjects were drug free and judged to be healthy on the basis of medical history, physical examination, electrocardiogram and routine laboratory screening. Routine labo-

ratory screening included measurements of serum electrolytes, white and red blood cell count, thrombocyte count, hemoglobin, hematocrit, fasting glucose, cholesterol, uric acid, creatinine, urea, lactate dehydrogenase, bilirubin, gamma glutamyl transpeptidase, glutamic pyruvic transaminase, thromboplastin time, and partial thromboplastin time. Subjects and investigators were blinded with regard to genotype at the *GNB3* locus. The study protocol had been approved by the Ethics Committee of the University of Essen Medical School and was in accordance with the principles laid down in the Declaration of Helsinki.

12 subjects were carriers of the 825T-allele (9 hetero- and 3 homozygous), while 10 subjects without this allele served as controls. On the study day, subjects were reported to the laboratory at 7–8 a.m. after an overnight fast and remained in the supine position during the entire investigation. One indwelling catheter was placed into an antecubital forearm vein, which was used for blood withdrawals. Each subject was instrumented with a blood pressure cuff, circular tape electrodes for measurement of transthoracic impedance and a microphone for phonocardiographic recordings.

After a resting period of 30 min, baseline haemodynamic measurements were performed and blood for baseline measurements of whole blood glucose and serum insulin was taken. Thereafter, each subject drank a standardized commercially available solution containing 75 g of glucose (Dextro® O.G.T., Boehringer Mannheim, Milano, Italy) over five minutes. Fifteen, 30, 60, 90 and 120 minutes min after intake of the glucose solution haemodynamic measurements were repeated and blood was drawn for determination of blood glucose and serum insulin.

Biochemical, Anthropometric and Haemodynamic Measurements

Whole blood glucose was determined by the glucose oxidase method (glucose autoanalyser, EBIO 6666, Eppendorf, Hamburg, Germany). Serum insulin levels were assessed by radioimmunoassay (Biochem Immunossystems, Freiburg, Germany).

Height and weight were measured and body mass index (BMI) was calculated as weight to height squared. Systolic (SBP) and diastolic blood pressure (DBP) [mm Hg] was measured with a standard mercury sphygmomanometer with the disappearance of Korotkow's sound defined as diastolic blood pressure (DBP).

We measured systolic time intervals (STI) to characterize left ventricular performance. Systolic time intervals provide an accurate and sensitive measure of left ventricular function [13,14]. STI were measured according to

Table 1: Study population: Demographic characteristics, metabolic and haemodynamic parameters at rest.

	CC	CT/TT	P-value	95% CI
Age [years]	25.8 ± 1.1	26.3 ± 0.6	0.711	-2.9/2.0
Height [cm]	181.7 ± 2	186.7 ± 2	0.121	-11.4/1.4
Weight [kg]	78.2 ± 2	79.2 ± 2.5	0.783	-8.4/6.4
Body mass index [kg m ⁻²]	23.7 ± 0.6	22.7 ± 0.4	0.174	-0.5/2.5
Fasting blood glucose [mg dl ⁻¹]	86.8 ± 2.3	86.8 ± 1.5	0.991	-5.5/5.5
Fasting serum insulin [μU dl ⁻¹]	10.4 ± 2.8	7.2 ± 1.4	0.287	-2.9/9.3
Relative insulin resistance HOMA IR	30.4 ± 5.6	28.0 ± 5.4	0.766	-18.9/14.2
Heart rate [per min]	62.5 ± 2.7	57.1 ± 2.7	0.175	-2.6/13.4
Systolic blood pressure [mm Hg]	116.9 ± 2.7	118.6 ± 1.9	0.597	-8.4/4.9
Diastolic blood pressure [mm Hg]	75.3 ± 1.9	73.7 ± 2.4	0.632	-5.1/8.2
Stroke volume [ml]	89.5 ± 8.2	107.1 ± 5.6	0.083	-37.7/2.5
Cardiac output [l min ⁻¹]	5.6 ± 0.5	6.1 ± 0.4	0.441	-1.8/0.8
Total peripheral resistance [dyne sec cm ⁻⁵]	1383 ± 122.4	1216 ± 77.3	0.249	-125/458
Total electromechanical systole [ms]	480.9 ± 6.1	489.4 ± 5.4	0.306	-25.5/8.4
Number of subjects	10	12		

Data are mean ± SEM. Parameters were determined in the supine position at the end of a resting period of 30 minutes. P-values were determined in a two-tailed unpaired t-test between CC-genotypes (data column 1) and combined CT/TT-genotypes (data column 2). 95% CI: 95% confidence interval. (* P < 0.05).

standard techniques [13,15] from simultaneous recordings of an electrocardiographic lead, a phonocardiogram and a carotid pulse tracing at high paper speed (100 mm × s⁻¹) using a Siemens-Cardirex[®] multichannel ink jet recorder (Siemens Medizintechnik, Erlangen, Germany) as previously described [16–19]. From these recordings we determined the duration of the RR-interval to calculate heart rate (HR) [b.p.m.] and the duration of the electromechanical systole which was corrected for heart rate to yield QS₂c [20].

Stroke volume (SV) [ml] was measured by impedance cardiography using the standard approach with circular tape electrodes and graphical signal analysis according to Kubicek's equation [21,22]. A 'Kardio-Dynagraph' was used to record changes in transthoracic impedance (Heinz Diefenbach Elektromedizin, Frankfurt, Germany). Impedance cardiography yields measurements that agree closely with those obtained by Doppler echocardiography or thermodilution [23], and is acceptable for clinical use particularly in studies investigating young subjects free from any cardiovascular disease [24]. In our laboratory, we have determined that the variability of stroke volume measured by impedance cardiography is less than 5.5 % (expressed as a percentage of the coefficient of variation) [18].

Cardiac output (CO) [l × min⁻¹] was calculated as CO = HR × SV/1000. Total peripheral resistance (TPR) [dyne × sec × cm⁻⁵] was calculated as mean arterial pressure × 80 divided by CO, where mean arterial pressure was defined

as DBP plus one third of pulse pressure. Pulse pressure was calculated by subtracting DBP from SBP.

Insulin Sensitivity and Statistical Analysis

The following indices of glucose tolerance or insulin sensitivity, respectively, were evaluated: i) fasting insulin levels, ii) relative insulin resistance expressed by the homeostatic model assessment HOMA IR (fasting insulin (μU dl⁻¹) × fasting glucose (mg dl⁻¹)/22.5) [25], iii) the maximum change in insulin and glucose from baseline following oral glucose loading, iv) the area under the insulin response profile (AUC) as a marker of total insulin secretion, and v) the insulinogenic index as a marker of early phase insulin secretion [26]. The insulinogenic index was measured as ratio of increment of insulin to that of glucose at 30 minutes after glucose ingestion [26].

The metabolic and haemodynamic response to oral glucose loading was described by the changes from fasting baseline values. Maximum change and area under the response profile (AUC) following glucose loading were determined for all parameters.

Based on population studies [2–4,6,27], our primary hypothesis was that the presence of the T-allele (homozygous or heterozygous) would affect metabolic and cardiovascular function relative to homozygous C-allele carriers. Therefore, our primary statistical comparisons have determined the significance of differences between T-allele carriers and non-carriers by unpaired, two-tailed t-tests, i.e. CT and TT subjects were pooled for analyses. This was also necessary for epidemiological rea-

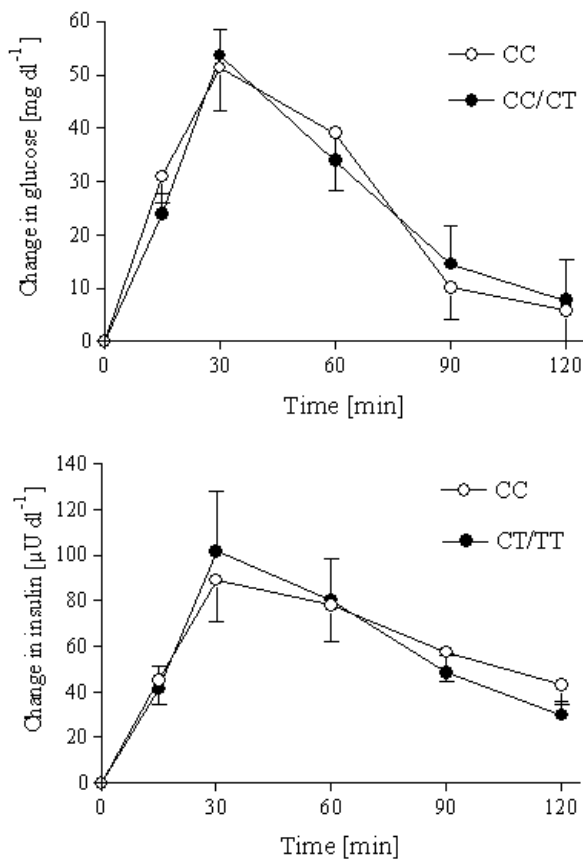


Figure 1
Changes in glucose and insulin following oral glucose load. Glucose and insulin responses to glucose loading are displayed as function of time. All values are mean \pm SEM.

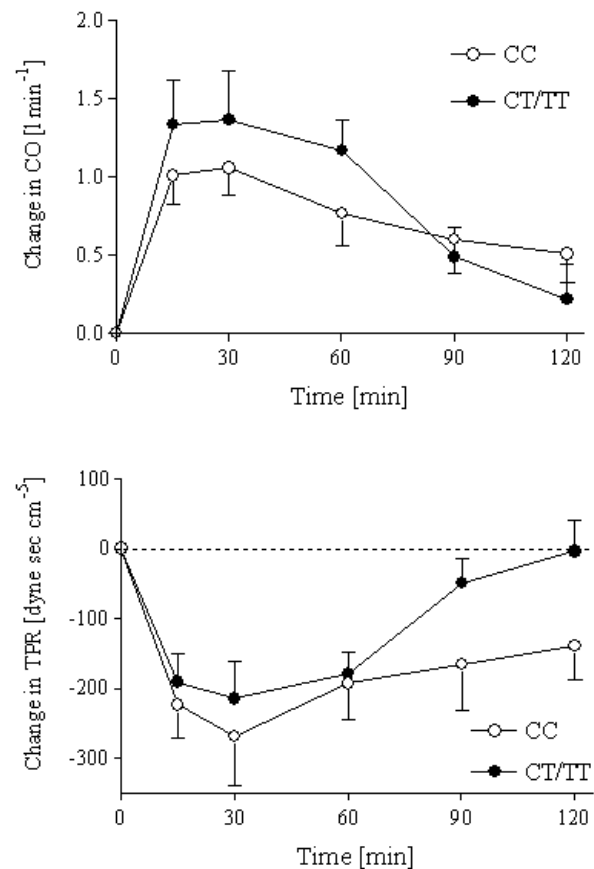


Figure 2
Changes in glucose and insulin following oral glucose load. Responses of cardiac output and total peripheral resistance to glucose loading are displayed as function of time. All values are mean \pm SEM.

sons, since only about 10% of young, German males are homozygous T-allele carriers [28]. The number of subjects in this study ($n = 22$) was within the range found in the literature (Grossmann et al. $n = 26$ [29]; Wenzel et al. $n = 25$ [30]; Virchow et al. $n = 20$ [31]).

A $P < 0.05$ was considered statistically significant. All values are shown as mean \pm SEM. 95% confidence intervals (CI) are provided for better assessment of the precision of group comparisons.

Results

Subjects were matched for anthropometric measures (Table 1). Blood glucose, serum insulin, relative insulin resistance (HOMA IR), and haemodynamic parameters at baseline were also very similar between carriers and non-carriers of the 825T-allele (Table 1).

The rise in glucose and insulin after oral glucose loading was not different between 825T-allele carriers (T) and control (C) subjects and there was no significant difference in any of the determined indices of insulin sensitivity (Table 2, Figure 1). Oral glucose loading produced profound haemodynamic changes characterized by an increase in HR, systolic blood pressure and CO, a fall in TPR and DBP and a shortening of QS_2c . (Figure 2) However, there was no significant difference in the haemodynamic responses to oral glucose loading between T-allele carriers and non-carriers (Table 2, Figure 2).

Discussion

The epidemiologic associations among essential hypertension, obesity and non-insulin-dependent diabetes mellitus are well recognized. To date, an underlying common pathophysiological mechanism has not been identi-

Table 2: Changes in glucose, insulin and haemodynamic parameters following an oral glucose load. Responses to glucose loading were quantified either as the maximum response during the 120 minutes observation period, as the area under the response profile (AUC) from 15 to 120 minute (comp. Figure 1, 2) or as insulinogenic index.

Max response	CC	CT/TT	P-value	95% CI
Blood glucose [mg dl ⁻¹]	58.6 ± 6.6	57.0 ± 5.3	0.849	-15.7/18.9
Serum insulin [μU dl ⁻¹]	95.9 ± 16.9	118.3 ± 27.1	0.510	-92.4/47.4
Heart rate [per min]	8.7 ± 1.4	9.0 ± 0.7	0.834	-3.4/2.7
Systolic blood pressure [mm Hg]	6.5 ± 1.0	8.3 ± 0.8	0.173	-4.5/0.9
Diastolic blood pressure [mm Hg]	-4.2 ± 1.3	-3.8 ± 0.9	0.793	-3.6/2.8
Stroke volume [ml]	15.2 ± 0.8	21.0 ± 3.0	0.090	-12.7/1.0
Cardiac output [l min ⁻¹]	1.2 ± 0.2	2.0 ± 0.2	0.071	-1.3/-0.2
Total peripheral resistance [dyne sec cm ⁻⁵]	-300 ± 64.4	-289 ± 33.8	0.880	-159/137
Total electromechanical systole [ms]	-16.3 ± 2.2	-17.1 ± 1.8	0.772	-5.0/6.7
AUC response	CC	CT/TT	P-value	95% CI
Blood glucose [mg dl ⁻¹]	3184 ± 740	3136 ± 452	0.954	-1694/1792
Serum insulin [μU dl ⁻¹]	7388 ± 1425	7223 ± 1219	0.930	-3722/4052
Heart rate [per min]	425 ± 122	582 ± 71	0.259	-439/125
Systolic blood pressure [mm Hg]	521 ± 90	752 ± 73	0.058	-472/8
Diastolic blood pressure [mm Hg]	367 ± 116	302 ± 87	0.346	-435/159
Stroke volume [ml]	836 ± 195	764 ± 202	0.800	-517/662
Cardiac output [l min ⁻¹]	88 ± 19	104 ± 12	0.478	-63/31
Total peripheral resistance [dyne sec cm ⁻⁵]	1383 ± 122.4	1216 ± 77.3	0.273	-21877/6536
Total electromechanical systole [ms]	-1242 ± 259	-1499 ± 172	0.408	-376/888
	CC	CT/TT	P-value	95% CI
Insulinogenic index	2.43 ± 0.87	1.96 ± 0.49	0.628	-2.468/1.525

Data are mean ± SEM. P-values determined in a two-tailed unpaired t-test between CC-genotypes (data column 1) and combined CT/TT-genotypes (data column 2). 95% CI: 95% confidence interval.

fied [32]. The rationale of the present study was based on the assumption that the C825T polymorphism in the gene *GNB3*, which is associated with obesity and hypertension [2,8], may be also associated with an impaired glucose tolerance in young, healthy subjects.

In this study, we did not find that subjects carrying the T-allele exhibited an altered response of any metabolic or haemodynamic parameter to oral glucose load. Several indices of insulin sensitivity were evaluated, including fasting insulin levels, maximal and total insulin secretion and the insulinogenic index that estimates early insulin secretion. None of these indices was significantly influenced by the presence of the 825T-allele. Hence, these data do not support the hypothesis that the C825T polymorphism serves as an early genetic marker of an impaired glucose tolerance in young, normotensive men.

There is conflicting evidence in the literature whether the *GNB3* gene may serve as a candidate gene for diabetes mellitus type 2. In a case-control study (n = 1284), Rosskopf et al. found a significant association between the 825T-allele and type 2 diabetes [33]. In contrast, Beige et

al found no increased frequency of this polymorphism in a cohort of 1008 diabetic patients [34]. Moreover, there was no association between the 825T-allele with diabetic complications, including nephropathy, retinopathy and neuropathy [34,35]. The results of the present study are consistent with a recent report from Saller et al., who did not find differences between TC and CC genotypes with regard to time-courses for glucose and insulin concentrations after an oral glucose tolerance test [36]. As in the study by Saller et al., our study does not provide any evidence for a pathogenetic link between the C825T polymorphism and impaired glucose tolerance.

In light of the extensive use of association studies, identifying and characterizing physiological relationships is needed to validate the potential relevance of gene polymorphisms [37]. This is the first study elucidating a potential pathophysiological mechanism between the 825T-allele and hemodynamic changes following oral glucose loading.

Glucose loading produced marked haemodynamic changes. Although there was no statistical significance, the

overall change (AUC) in systolic blood pressure tended to be larger in carriers of the T-allele ($p = 0.058$). Observed increases in stroke volume, cardiac output, heart rate and shortening of the QS_2c demonstrate a strong activation of the sympathetic nervous system [38]. Insulin is a centrally acting hormone and is known to stimulate sympathetic nerve activity [39]. Given that the rises of these haemodynamic parameters were closely timed to the insulin increase further suggests that insulin induced activation of the sympathetic nervous system is responsible for the observed haemodynamic changes. Consistent with previous reports [40], we also observed a fall in total peripheral resistance and diastolic blood pressure that may be attributed to splanchnic vasodilatation during intestinal resorption [41]. Together, the pronounced haemodynamic changes following oral glucose loading illustrated the tight coregulation of the metabolic and cardiovascular systems.

Age is a major determinant of genotype penetrance and phenotype expression in many inherited disorders including cystic kidney disease and cancer [42,43]. Similarly, impaired glucose tolerance occurs more frequently with advancing age [44], and an association between the *GNB3* 825T-allele and reduced insulin sensitivity in middle-aged men with abdominal fat distribution has been recently described [45]. The subjects in our study ranged from 21 to 32 years of age. Hence, it is unclear whether or not our results can be extended to older individuals, i.e. whether the 825T locus may modify glucose handling in the elderly. However, a homogeneous study population is advantageous, particularly when anticipated differences may be small. It is possible that the small population size used may be a potential limitation in this study.

In this study we employed systolic time intervals and impedance cardiography. Even though these techniques are less frequently used than other modern techniques such as echo-doppler calculations, they provide accurate and reproducible measures of systemic hemodynamics. In particular systolic time intervals are highly reproducible as determined by the coefficient of correlation [18].

In conclusion, these findings do not support the hypothesis that the *GNB3* 825T-allele is associated with impaired glucose tolerance, or serves as an early genetic marker of impaired glucose tolerance in young, normotensive men.

Competing interests

None declared.

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