Cardiovascular Diabetology

BioMed Central

Original Investigation

Open Access

Metabolic and haemodynamic effects of oral glucose loading in young healthy men carrying the 825T-allele of the G protein β3 subunit

Jens Nürnberger*, Sandra Dammer, Thomas Philipp, Rene R Wenzel and Rafael F Schäfers

Address: Division of Nephrology & Hypertension, University of Essen, Hufelandstrasse 55, 45122 Essen, Germany

Email: Jens Nürnberger* - jens.nuernberger@uni-essen.de; Sandra Dammer - sandra.buehrmann@uni-essen.de; Thomas Philipp - thomas.philipp@uni-essen.de; Rene R Wenzel - rene@rrwenzel.de; Rafael F Schäfers - rafael.schaefers@uni-essen.de

* Corresponding author

Published: 25 June 2003

Cardiovascular Diabetology 2003, **2**:7

This article is available from: http://www.cardiab.com/content/2/1/7

Received: 21 April 2003
Accepted: 25 June 2003

© 2003 Nürnberger et al; licensee BioMed Central Ltd. This is an Open Access article: verbatim copying and redistribution of this article are permitted in all media for any purpose, provided this notice is preserved along with the article's original URL.

Abstract

Background: A C825T polymorphism was recently identified in the gene encoding the β3 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 polymorphism was identified in the gene *GNB3* which encodes the β3 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 laboratory 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 Immunosystems, 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 QS_2c [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-

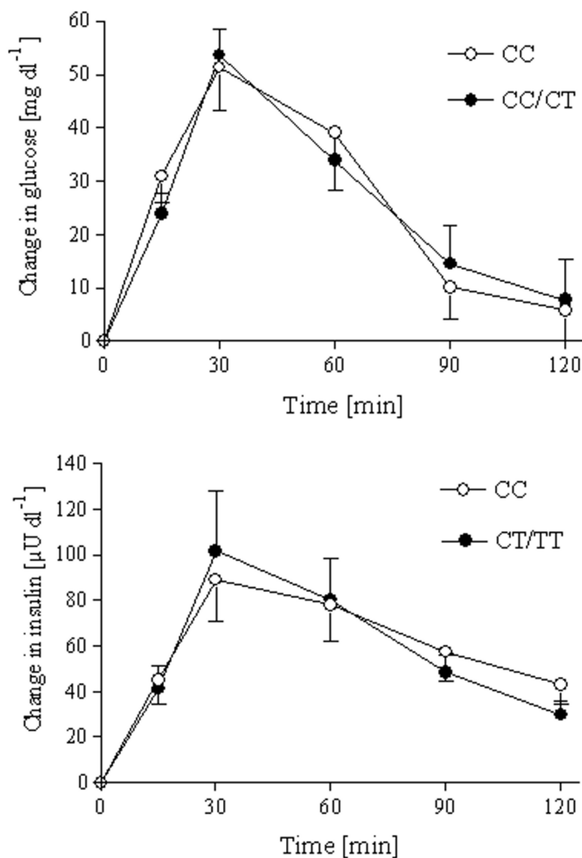

Figure 1
Changes in glucose and insulin following oral glucose load. Glucose and insulin responses to glucose loading are displayed as function of time. All values are mean ± SEM.

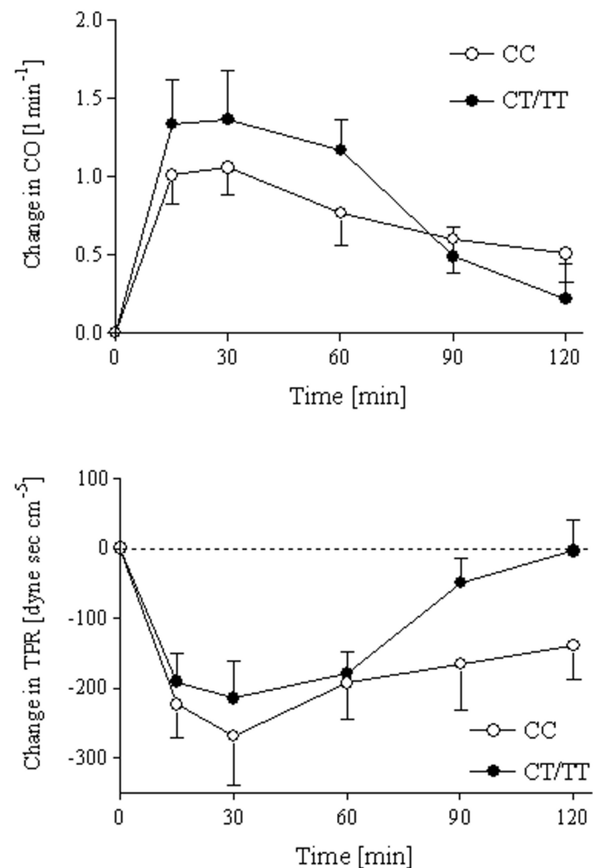

Figure 2
Changes in glucose and insulin following oral glucose load. Responses of cardiac output and total peripheral resistance to glucose loading are displayed as function of time. All values are mean ± SEM.

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 el al. n = 25 [30]; Virchow et al. n = 20 [31]).

A $P < 0.05$ was considered statistically significant. All values are shown as mean ± 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.

References

1. Timberlake DS, O'Connor DT and Parmer RJ: **Molecular genetics of essential hypertension: recent results and emerging strategies** *Curr Opin Nephrol Hypertens* 2001, **10:**71-79.
2. Siffert W, Rosskopf D, Siffert G, Busch S, Moritz A, Erbel R, Sharma AM, Ritz E, Wichmann H-E, Jakobs KH and Horsthemke B: **Association of a human G-protein beta3 subunit variant with hypertension** *Nat Genet* 1998, **18:**45-48.
3. Schunkert H, Hense HW, Döring A, Riegger GA and Siffert W: **Association between a polymorphism in the G protein beta3 subunit gene with lower renin and elevated diastolic blood pressure** *Hypertension* 1998, **32:**510-513.
4. Beige J, Hohenbleicher H, Distler A and Sharma AM: **G-Protein beta3 subunit C825T variant and ambulatory blood pressure in essential hypertension** *Hypertension* 1999, **33:**1049-1051.
5. Benjafield AV, Jeyasingam CL, Nyholt DR, Griffiths LR and Morris BJ: **G-protein beta3 subunit gene (GNB3) variant in causation of essential hypertension** *Hypertension* 1998, **32:**1094-1097.
6. Dong Y, Zhu H, Sagnella GA, Carter ND, Cook DG and Cappuccio FP: **Association between the C825T polymorphism of the G protein beta3- subunit gene and hypertension in blacks** *Hypertension* 1999, **34:**1193-1196.
7. Hegele RA, Anderson C, Young TK and Connelly PW: **G-protein beta3 subunit gene splice variant and body fat distribution in Nunavut Inuit** *Genome Res* 1999, **9:**972-977.
8. Siffert W, Naber C, Walla M and Ritz E: **G protein beta3 subunit 825T allele and its potential association with obesity in hypertensive individuals** *J Hypertens* 1999, **17:**1095-1098.
9. Ferrannini E, Buzzigoli G, Bonadonna R, Giorico MA, Oleggini M, Graziadei L, Pedrinelli R, Brandi L and Bevilacqua S: **Insulin resistance in essential hypertension** *N Engl J Med* 1987, **317:**350-357.
10. Lind L, Berne C and Lithell H: **Prevalence of insulin resistance in essential hypertension** *J Hypertens* 1995, **13:**1457-1462.
11. Morris AD, Petrie JR and Connell JM: **Insulin and hypertension** *J Hypertens* 1994, **12:**633-642.
12. Julius S and Jamerson K: **Sympathetics, insulin resistance and coronary risk in hypertension: the 'chicken-and-egg' question** *J Hypertens* 1994, **12:**495-502.
13. Li Q and Belz GG: **Systolic time intervals in clinical pharmacology** *Eur J Clin Pharmacol* 1993, **44:**415-421.
14. Boudoulas H: **Systolic time intervals** *Eur Heart J* 1990, **11:**93-104.
15. Lewis RP, Rittgers SE, Forester WF and Boudoulas H: **A Critical Review of the Systolic Time Intervals** *Circulation* 1977, **56:**146-158.
16. Schafers RF, Adler S, Daul A, Zeitler G, Vogelsang M, Zerkowski HR and Brodde OE: **Positive inotropic effects of the beta 2-adrenoceptor agonist terbutaline in the human heart: effects of long-term beta 1- adrenoceptor antagonist treatment** *J Am Coll Cardiol* 1994, **23:**1224-1233.
17. Schafers RF, Poller U, Ponicke K, Geissler M, Daul AE, Michel MC and Brodde OE: **Influence of adrenoceptor and muscarinic receptor blockade on the cardiovascular effects of exogenous noradrenaline and of endogenous noradrenaline released by infused tyramine** *Naunyn Schmiedebergs Arch Pharmacol* 1997, **355:**239-249.
18. Schafers RF, Nurnberger J, Herrmann B, Wenzel RR, Philipp T and Michel MC: **Adrenoceptors mediating the cardiovascular and metabolic effects of alpha-methylnoradrenaline in humans** *J Pharm Exp Ther* 1999, **289:**918-925.
19. Schafers RF, Nurnberger J, Rutz A, Siffert W, Wenzel RR, Mitchell A, Philipp T and Michel MC: **Haemodynamic characterization of young normotensive men carrying the 825T-allele of the G-protein beta3 subunit** *Pharmacogenetics* 2001, **11:**461-470.
20. Schafers RF, Piest U, von Birgelen C, Jakubetz J, Daul AE, Philipp T and Brodde OE: **Disodium cromoglycate does not prevent terbutaline-induced desensitization of beta 2-adrenoceptor-mediated cardiovascular in vivo functions in human volunteers** *J Cardiovasc Pharmacol* 1999, **33:**822-827.
21. Kubicek WG, Karnegis JN, Patterson RP, Witsoe DA and Mattson RH: **Development and evaluation of an impedance cardiac output system** *Aerosp Med* 1966, **37:**1208-1212.
22. Kubicek WG, From AH, Patterson RP, Witsoe DA, Castaneda A, Lillehei RC and Ersek R: **Impedance cardiography as a noninvasive means to monitor cardiac function** *J Assoc Adv Med Instrum* 1970, **4:**79-84.
23. Northridge DB, Findlay IN, Wilson J, Henderson E and Dargie HJ: **Non-invasive determination of cardiac output by Doppler echocardiography and electrical bioimpedance** *Br Heart J* 1990, **63:**93-97.
24. White SW, Quail AW, de Leeuw PW, Traugott FM, Brown WJ, Porges WL and Cottee DB: **Impedance cardiography for cardiac**

output measurement: an evaluation of accuracy and limitations *Eur Heart J* 1990, **11 Suppl I:**79-92.
25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF and Turner RC: **Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man** *Diabetologia* 1985, **28:**412-419.
26. Kosaka K, Kuzuya T, Yoshinaga H and Hagura R: **A prospective study of health check examinees for the development of non-insulin-dependent diabetes mellitus: relationship of the incidence of diabetes with the initial insulinogenic index and degree of obesity** *Diabet Med* 1996, **13:**S120-6.
27. Hegele RA, Harris SB, Hanley AJ, Cao H and Zinman B: **G protein beta3 subunit gene variant and blood pressure variation in Canadian Oji-Cree** *Hypertension* 1998, **32:**688-692.
28. Siffert W, Forster P, Jockel KH, Mvere DA, Brinkmann B, Naber C, Crookes R, Du P. Heyns A., Epplen JT, Fridey J, Freedman BI, Muller N, Stolke D, Sharma AM, Al Moutaery K, Grosse-Wilde H, Buerbaum B, Ehrlich T, Ahmad HR, Horsthemke B, Du Toit ED, Tiilikainen A, Ge J, Wang Y, Rosskopf D and et al.: **Worldwide ethnic distribution of the G protein beta3 subunit 825T allele and its association with obesity in Caucasian, Chinese, and Black African individuals** *J Am Soc Nephrol* 1999, **10:**1921-1930.
29. Grossmann M, Dobrev D, Siffert W and Kirch W: **Heterogeneity in hand veins responses to acetylcholine is not associated with polymorphisms in the G-protein beta3-subunit (C825T) and endothelial nitric oxide synthase (G894T) genes but with serum low density lipoprotein cholesterol** *Pharmacogenetics* 2001, **11:**307-316.
30. Wenzel RR, Siffert W, Bruck H, Philipp T and Schafers RF: **Enhanced vasoconstriction to endothelin-1, angiotensin II and noradrenaline in carriers of the GNB3 825T allele in the skin microcirculation** *Pharmacogenetics* 2002, **12:**489-495.
31. Virchow S, Ansorge N, Rübben H, Siffert G and Siffert W: **Enhanced fMLP-stimulated chemotaxis in human neutrophils from individuals carrying the G protein beta3 subunit 825 T-allele** *FEBS Letters* 1998, **436:**155-158.
32. Morris AD and Connell JM: **Insulin resistance and essential hypertension: mechanisms and clinical implications** *Am J Med Sci* 1994, **307 Suppl 1:**S47-52.
33. Rosskopf D, Frey U, Eckhardt S, Schmidt S, Ritz E, Hofmann S, Jaksch M, Muller N, Husing J, Siffert W and Jocke KH: **Interaction of the G protein beta 3 subunit T825 allele and the IRS-1 Arg972 variant in type 2 diabetes** *Eur J Med Res* 2000, **5:**484-490.
34. Beige J, Ringel J, Distler A and Sharma AM: **G-protein beta(3)-subunit C825T genotype and nephropathy in diabetes mellitus** *Nephrol Dial Transplant* 2000, **15:**1384-1387.
35. Shcherbak NS and Schwartz EI: **The C825T polymorphism in the G-protein beta3 subunit gene and diabetic complications in IDDM patients** *J Hum Genet* 2001, **46:**188-191.
36. Saller B, Nemesszeghy P, Mann K, Siffert W and Rosskopf D: **Glucose and Lipid Metabolism in Young Lean Normotensive Males with the G Protein beta3 825T-Allele** *Eur J Med Res* 2003, **8:**91-97.
37. Gambaro G, Anglani F and D'Angelo A: **Association studies of genetic polymorphisms and complex disease** *Lancet* 2000, **355:**308-311.
38. Kelbaek H, Munck O, Christensen NJ and Godtfredsen J: **Autonomic nervous control of postprandial hemodynamic changes at rest and upright exercise** *J Appl Physiol* 1987, **63:**1862-1865.
39. Scherrer U and Sartori C: **Insulin as a vascular and sympathoexcitatory hormone: implications for blood pressure regulation, insulin sensitivity, and cardiovascular morbidity.** *Circulation* 1997, **96:**4104-4113.
40. Dauzat M, Lafortune M, Patriquin H and Pomier-Layrargues G: **Meal induced changes in hepatic and splanchnic circulation: a non-invasive Doppler study in normal humans.** *Eur J Appl Physiol Occup Physiol* 1994, **68:**373-380.
41. Kooner JS, Peart WS and Mathias CJ: **The peptide release inhibitor, Octreotide (SMS201-995), prevents the haemodynamic changes following food ingestion in normal human subjects** *Q J Exp Physiol* 1989, **74:**569-572.
42. Gabow PA: **Autosomal dominant polycystic kidney disease** *N Engl J Med* 1993, **329:**332-342.
43. Eeles RA: **Future possibilities in the prevention of breast cancer: intervention strategies in BRCA1 and BRCA2 mutation carriers** *Breast Cancer Res* 2000, **2:**283-290.
44. Barbieri M, Rizzo MR, Manzella D and Paolisso G: **Age-related insulin resistance: is it an obligatory finding? The lesson from healthy centenarians** *Diabetes Metab Res Rev* 2001, **17:**19-26.
45. Wascher TC, Paulweber B, Malaimare L, Stadlmayr A, Iglseder B, Schmoelzer I and Renner W: **Associations of a human G protein beta3 subunit dimorphism with insulin resistance and carotid atherosclerosis** *Stroke* 2003, **34:**605-609.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp