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**POTENTIAL ROLE OF TCF7L2 GENE VARIANTS ON CARDIAC
SYMPATHETIC/PARASYMPATHETIC ACTIVITY**

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Running title: TCF7L2 gene variants and heart rate variability

ABSTRACT

Variants in Transcription Factor 7-like 2 gene (TCF7L2) have been found strongly associated with an increased risk of type 2 diabetes as well as with an impairment of GLP-1 (glucagon like peptide-1) signalling chain. In rats, stimulation of central GLP-1 receptors increases heart rate and activates autonomic regulatory neurons. We aimed to evaluate the potential role of TCF7L2 gene polymorphisms on sympathovagal response in relation to changes in plasma insulin and/or GLP-1 concentration following glucose ingestion. Genotyping was performed for *rs12255372* and *rs7903146* TCF7L2 gene variants in 250 non related healthy volunteers (mean age 27 ± 3 yrs). Consistent with previous reports, both SNPs were in strong linkage disequilibrium ($D'=0.87$, $r^2=0.76$). A subset of 167 patients underwent an oral glucose tolerance test while a continuous recording of Heart Rate Variability was performed. At baseline, no difference in fasting plasma insulin, in GLP-1 levels and in LF/HF ratio between the three genotypes were found. Along with glucose ingestion TT subjects had lower INS_{AUC} (insulin area under curve) as well as higher LF/HF_{AUC} (low frequency/high frequency area under curve) values. No difference in $GLP-1_{AUC}$ (GLP-1 area under curve) between TCF7L2 gene variants was found. A multivariate analysis including multiple covariates showed that only INS_{AUC} , $GLP1_{AUC}$ and TCF7L2 gene variants were independently associated with LF/HF_{AUC} . In conclusion TT genotype of *rs12255372* and *rs7903146* TCF7L2 gene variants is associated with lower insulin secretion and higher cardiosympathetic activity. Moreover such effect is independent of GLP-1 and insulin plasma concentrations suggesting a potential role of such gene variants in increasing cardiovascular risk via enhanced sympathetic nervous system activity.

Key words Gene Polymorphism - Glucose Metabolism – Cardiac Electrophysiology

INTRODUCTION

TCF7L2 plays a role in the wingless-type MMTV integration site family, member 1 (WNT) signalling pathway which influence the synthesis, and possibly secretion from intestinal L cells, of GLP1, an incretin hormone with insulinotropic and insulino-mimetic actions regulating glucose and energy homeostasis, food intake and cardiovascular functions (1-2). Variants in the transcription factor 7-like 2 (TCF7L2) gene have been found strongly associated with an impaired insulin secretion and with an increased risk of type 2 diabetes (T2D) (3-11).

Shafer *et al.* (11) have recently showed that the presence of T allele of *rs7903146* and *rs12255372* TCF7L2 gene single-nucleotide polymorphisms (SNPs) was associated with reduced GLP-1-induced insulin secretion probably due to an impairment of GLP-1 signalling chain (2,11).

Interestingly, previous animal data have revealed beneficial effects of GLP-1 and its analogues on cardiovascular system by improving endothelial function and increasing myocardial contractility (2-13). Moreover, GLP-1 is a neuropeptide synthesized by neurons in the caudal regions of the nucleus of the solitary tract, and GLP-1 receptors are also found in the heart and in areas of the central nervous system that govern autonomic control (14,15). In rats, stimulation of central GLP-1 receptors increases blood pressure and heart rate and activates autonomic regulatory neurons (16,17). In humans, only few data have been provided, showing that GLP-1 increases muscle sympathetic nerve traffic without affecting heart rate, plasma catecholamines, or cardiovagal and cardiosympathetic activity (18).

The potential role of TCF7L2 gene polymorphisms on sympathovagal response in relation to changes in plasma insulin and/or GLP-1 concentration following glucose ingestion is still unknown. In healthy young subjects, glucose ingestion and insulin infusion increases LF/HF ratio, an index of cardiac sympathovagal balance (19-21), producing a shift in cardiac autonomic nervous system activity toward sympathetic predominance (21,22).

Indeed, an alteration in the WNT signalling pathway related to TCF7L2 gene variant might result in an altered GLP-1 response and/or insulin secretion, which in turn could lead to an impaired cardiac sympathovagal balance and thus to an increased cardiovascular risk.

Thus, our study aims to: a) verify the effects of TCF7L2 gene polymorphisms on baseline and glucose ingestion stimulated plasma insulin and GLP-1 levels; b) evaluate the potential role of TCF7L2 gene variants in regulating cardiac sympathetic activity at baseline and after glucose ingestion.

MATERIALS AND METHODS

Subjects

Two hundred and fifty Caucasians non related non-diabetic subjects (115 men and 135 women), from 21-35 years of age (mean age 28 ± 3), living in Southern Italy, volunteered for the study. Clinical information was obtained by routine laboratory analyses, history and physical examination. All subjects were not hypertensive, had no evidence of metabolic or cardiovascular diseases and had liver, kidney, and thyroid function tests within the normal range. According to American Diabetes Association criteria, no subject was diabetic or affected by impaired fasting glucose (23). The participants did not take any drugs known to affect glucose tolerance. After a clear explanation of the potential risk of the study, all subjects provided written informed consent to participate in the study, which was approved by the Ethical Committee of our Institutions.

Analytical methods

Anthropometric determinations (weight, height and BMI) were measured by standard technique. Body mass index (BMI) was calculated as weight in kilograms divided by square of height expressed in meters (Kg/m^2). Waist circumference was measured at the midpoint between the lower rib margin and the iliac crest and hip circumference at the level of the greater trochanter. Waist/hip ratio was calculated. Plasma glucose was determined immediately by glucose oxidase method (Glucose Autoanalyzer, Beckman Coulter, Inc., Fullerton, CA).

Plasma fasting cholesterol and triglycerides were determined by routine laboratory methods (Roche Diagnostics, GmbH, Mannheim, Germany).

For estimation of circulating intact GLP-1, plasma immunologic active forms of GLP-1 was determined using a specific ELISA kit (LINCO Research U.S.A.). Active isoforms of GLP-1 include GLP-1(7-36) amide and glycine-extended GLP-1(7-37). After secretion from entero-endocrine L cells, GLP-1(7-36) amide is rapidly degraded by dipeptidyl peptidase-4 (DPP-4) to its N-terminally truncated metabolite GLP-1(9-36), which does not interact with the known GLP-1 receptor. Blood samples were collected in ice-cooled EDTA-plasma tubes and immediately, within 30 seconds, was added appropriate amount of DPP-IV inhibitor according to manufacture's directions. Tubes was centrifuged at 2500 rpm for 10 minutes in refrigerated centrifuge. Samples were stored at -80° C. Plasma insulin quantitative determinations were obtained using Mercodia Insulin ELISA kit.

Genotyping

A total of 250 individuals were genotyped for *rs12255372* (intron 3) G/T and *rs7903146* (intron 3 C/T) allelic variants of TCF7L2 gene on chromosome 10q25.3 (Genbankgi: 89161187). DNA was isolated from whole blood collected into EDTA-containing tubes using DNA extraction kits (Illustra Blood GenomicPrep Mini Spin Kit, GE Healthcare). The *rs12255372* polymorphism was identified using the following primers: upstream, 5'-GGC TTG CAG GTC AGA TTT TC-3', downstream 5'-ATT TGG CAT TCA AAT GGA GG-3'.

Polymerase chain reaction was carried out under the following conditions: 94°C for 4 minutes, followed by 35 cycles of 94°C for 30 seconds, 62°C for 30 seconds, 72°C for 30 seconds, with a final extension of 72°C for 10 minutes. Restriction fragment length polymorphism was carried out using *Tsp509I* enzyme. The resulting products were electrophoresed on a 4.5% agarose gel. The *rs7903146* was identified using the following primers: forward 5'-AAG AGA AGA TTC CTT TTT AAA TGG TG-3', reverse 5'- CCT CAT ACG GCA ATT AAA TTA TAC A-3'.

Polymerase chain reaction was carried out under the following conditions: 95°C for 5 minutes, followed by 34 cycles of 95°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds, with a

final extension of 72°C for 9 minutes. Restriction fragment length polymorphisms was carried out using *Hpy*-CH4III enzyme. The resulting products were electrophoresed on a 4.5% agarose gel.

In order to better assess the potential role of TCF7L2 gene polymorphism on HRV and on glucose ingestion induced GLP-1 levels, 167 subjects underwent an oral glucose tolerance test (OGTT) while a continuous recording of HRV by the Holter technique was performed.

OGTT

A subset of 167 patients underwent an oral glucose tolerance test (OGTT) for stimulating a secretion patterns of GLP-1. Tests were performed after an overnight fast of 12 h. At 08:00 hours, participants ingested, within five minute, a solution containing 75 g anhydrous glucose dissolved in 250 ml water. Venous blood samples were obtained at 0', 30', 60', 120' and 180' min for determination of plasma glucose, insulin and GLP-1 concentrations. All subjects were studied in a comfortable supine position.

Cardiovascular determinations

All cardiovascular measurements were carried out under quiet conditions in a room maintained at 21°C., in a supine position for at least 30 min before starting the baseline Holter recording. Subjects with a respiratory rate less than 10 breaths/min (i.e. <0.15 Hz) were excluded from the study, in order to avoid the overlap of oscillations of low and high frequency at spectral analysis. Blood pressure and heart rate were recorded starting at 08.00 hours for a 120 min period (30 min baseline and 90 min after glucose ingestion) by using respectively a standard mercury sphygmomanometer and a 2-channel 24-hour Holter ECG recordings (Del Mar Medical Systems, Irvine, Calif). All recordings were visually examined and manually over read to verify beat classification.

Abnormal beats and areas of artifact were automatically and manually identified and excluded from the analysis. The frequency domain analysis of HRV was performed (24). The mean heart rate was calculated, for a complete 10-minute segment of RR interval, 10 min before and 10 min after glucose ingestion (T0), for the time 20 to the time 30 (T1), for the time 50 to the time 60 (T2), for the time 110 to the time 120 (T3). Spectral components were identified and estimated by using the spectral decomposition algorithm proposed by *Johnsen* and *Andersen* and were then assigned (25), on the basis of their central frequency, to 1 of the 3 bands: very-low-frequency (VLF) (0–0.03 Hz), LF (0.04–0.15 Hz), and HF (0.16–0.45 Hz) (19). The low (LF) and high (HF) frequency components are normally considered (19) and reported in normalized units (20), which represent the relative value of the power of each component in proportion to the total power minus the VLF component. Because low-frequency power (LF) is modulated by baroreceptors and reflects both sympathetic and parasympathetic influences and high-frequency power (HF) reflects parasympathetic (vagally mediated) respiratory variation, LF/HF ratio, rather than LF, reflect relative sympathetic-parasympathetic activity and was used for calculating and reporting our data.

Calculations and statistical analysis

All data are presented as means \pm SD. Areas under the curve for insulin (INS_{AUC}), glucose (GLY_{AUC}) and GLP-1 ($GLP-1_{AUC}$) were calculated by trapezoidal method. To approximate normal distribution, plasma insulin and triglycerides were log-transformed for data analyses and back transformed for data presentation. Genotype frequencies were compared for investigating the Hardy-Weinberg equilibrium model by the χ^2 test. One-way ANOVA followed by Bonferroni multiple testing correction was applied to assess differences in clinical, biochemical and cardiovascular data among the genotype groups. Simple correlation analyses

allowed us to study the relationship between variables. Multivariate GLM (General Linear Model) analyses were used to evaluate the difference in plasma glucose and triglycerides among different genotype, after adjustment for gender, BMI and WHR. A multivariate linear regression analyses allowed us to investigate the association of TCF7L2 gene variants with LF/HF ratio independently of plasma GLP-1 levels, BMI, gender, INS_{AUC} and GLY_{AUC} . A $P < 0.05$ was chosen for levels of significance. Statistical analyses were performed using SPSS 15 software package (SPSS, Inc., Chicago, IL).

In order to investigate the difference in cardiovascular and metabolic parameters between the two genotype groups, sample size calculation was estimated on an IBM PC computer by *GPOWER* software. The resulting sample size, estimated according to a global effect size of 27 % with type I error of 0.05 and a power of 80% was 110 patients. Pairwise LD coefficients (D') between SNPs were calculated by Haploview software version 4.0.

RESULTS

Clinical Data

Clinical and biochemical characteristics of the study subjects are reported in Table 1. All subjects ($n=250$) were young (mean age= 28.1 ± 3.2), not obese, with no difference in gender ratio (135F/115M, $\chi^2=1.6$ $p=0.206$). According to gender ratio, female had a significantly lower BMI (21.8 ± 2.27 vs 25.5 ± 3.2 Kg/m^2 ; $p < 0.001$), WHR (0.75 ± 0.08 vs 0.85 ± 0.07 ; $p < 0.001$), plasma triglycerides (79.1 ± 12 vs 99.7 ± 27.2 mg/dl ; $p < 0.001$) levels and higher HDL-cholesterol (48.8 ± 2.2 vs 46.6 ± 2.2 mg/dl ; $p < 0.001$) levels compared to male subjects.

TCF7L2 gene variant analysis

The observed genotypes and allele distributions were in Hardy-Weinberg equilibrium ($p > 0.05$). The T allele frequencies was 0.41 for *rs12255372* and 0.39 for *rs7903146*. The frequency of *rs12255372* GG, GT and TT genotypes was 32.3%, 53.2% and 14.5%

respectively. As far as *rs7903146* gene variant is concerned the frequency of CC, CT and TT genotypes was 35.4%, 48.8% and 15.8% respectively. The genotypic frequencies observed were almost similar to that previously reported in European populations and consistent with previous reports, both SNPs were in strong linkage disequilibrium ($D'=0.87$, $r^2=0.76$). Because the two SNPs give the same information, results for SNP *rs7903146* are shown in as supplemental material.

Table 1 shows clinical and biochemical characteristics of the whole population (n= 250) stratified according to *rs12255372* (G/T) genotypes. No difference in genotype distribution between male and female subjects was found ($\chi^2=1.115$; $p=0.573$). Subjects carrying the TT genotype had significantly higher fasting plasma glucose levels compared to the GT and GG carriers, even after adjusting for gender, BMI and WHR ($p=0.04$). No differences in other clinical and biochemical parameters among the three genotypes were found.

Biochemical data and cardiovascular determinations over OGTT administration.

A subset of 167 subjects underwent an oral glucose tolerance test (OGTT) while a continuous recording of HRV by the Holter technique were performed. No subjects resulted affected by glucose intolerance.

At baseline, no significant correlations between LF/HF values and fasting plasma glucose ($r=0.08$, $p= .397$), insulin ($r=0.12$, $p= .398$) and GLP-1 ($r=0.12$, $p= .691$) levels were found.

In all subjects studied, oral glucose ingestion evoked a significant increase in plasma glucose (from 4.9 ± 0.5 to 7.1 ± 0.7 mmol/L $p= 0.038$) and insulin (from 49.6 ± 8 to 118.3 ± 10.2 pmol/L $p= 0.01$) levels at 60 minutes and in plasma GLP-1 (from 8.4 ± 1 to 17.6 ± 1 pmol/L $p= 0.02$) concentrations at 30 min compared to fasting values. The plasma peak of GLP-1 was transient and levels returned to near fasting within 90-120 minutes. Basal LF/HF ratio also rose

significantly after 30 min (from 2.07 ± 0.3 to 2.24 ± 0.5 $p=0.03$) reaching the maximum peak at 60 min and later declined without significant difference vs baseline.

Categorizing subjects in different genotype groups, no difference in baseline plasma insulin, GLP-1 levels and in LF/HF ratio between genotypes for either SNP were found, while fasting plasma glucose levels were significantly higher in TT carriers (Fig.1; Suppl. Fig 1).

After glucose ingestion, no significant difference in plasma glucose and GLP1 levels between the three genotype groups for each SNP was found. Subjects with TT genotype for both *rs12255372* and *rs7903146* variants had a significantly lower plasma insulin levels at 60' and higher LF/HF ratio at 30' and 60 minutes. No difference in LF/HF ratio at baseline and after 120 minutes of glucose ingestion between the three genotype groups for either SNP was found (Fig 1; Suppl Fig 1).

AUC for insulin (INS_{AUC}), GLP-1($GLP-1_{AUC}$) and LF/HF (LF/HF_{AUC}) were also calculated. LF/HF_{AUC} positively correlated with $GLP-1_{AUC}$ ($r= 0.39$, $p=0.03$) and INS_{AUC} ($r= 0.48$, $p=0.03$). A positive association between $GLP-1_{AUC}$ and INS_{AUC} ($r= 0.52$, $p=0.02$) was also found. All such association were still significant after adjusting for BMI (data not shown).

No difference in $GLP-1_{AUC}$ among TCF7L2 gene variants was found, while subjects carrying the TT genotype for both SNP had lower INS_{AUC} as well as higher LF/HF_{AUC} values compared the other genotype. No difference in INS_{AUC} and LF/HF_{AUC} between GG (or CC) and GT (or CT) genotype was also found. (Fig. 2; Suppl. Fig. 2)

The independent association of TCF7L2 gene variant with LF/HF ratio was tested in a multivariate analysis (Table 2). A model including LF/HF_{AUC} , as the dependent variable and BMI, gender, $GLP-1_{AUC}$, GLY_{AUC} , INS_{AUC} , TCF7L2 (G/T) gene polymorphism as covariates showed that only INS_{AUC} , $GLP-1_{AUC}$ and TCF7L2 (G/T) gene variant were independently associated with LF/HF_{AUC} .

DISCUSSION

The major findings of our investigation are: i) TT genotype of TCF7L2 variants is associated with lower insulin secretion and higher cardiosympathetic activity ii) TCF7L2 genotype effect on cardiosympathetic activity is independent of GLP-1 and insulin plasma concentrations.

TCF7L2 encodes a transcription factor playing an important role in the WNT signalling pathway (26) required for the normal development of the pancreas and pancreatic islets and it is also involved in GLP-1 production (27). Variants in TCF7L2 gene are strongly associated with an increased risk of type 2 diabetes (T2D) (1). Shafer *et al.* have recently demonstrated that the presence of the allele T of the rs12255372 TCF7L2 gene polymorphism is associated with reduced GLP-1-induced insulin secretion (11).

Accordingly, in our study subjects carrying the TT genotype of TCF7L2 (G/T) gene had reduced insulin secretion. Interestingly, a predominant cardiosympathetic activity (higher LF/HF ratio) in response to glucose ingestion in TT subjects was also found. The higher LF/HF ratio found in TT carriers might contribute to explain the association of TCF7L2 gene polymorphism with an increased risk of type 2 diabetes. In fact, it is generally accepted that impaired autonomic function is not only the consequence, but also a precursor of hyperglycemia (18,28). Chronically elevated levels of sympathetic activity may lead to gradual down regulation of the beta-adrenoceptor-mediated thermogenic and metabolic responses, which may be involved in the development of obesity and insulin resistance as seen in new onset type 2 diabetes mellitus (18,28,29).

Moreover, our results might also indicate TCF7L2 polymorphism as a new potential cardiovascular risk factor. In fact, an unbalanced sympathetic/parasympathetic tone, with a prevalence of sympathetic activity, is associated with higher cardiovascular mortality in type 2 diabetic patients (30,31).

Sympathetic nervous system is an important regulatory mechanism of metabolic and cardiovascular functions (32). In healthy young subjects, glucose ingestion enhances LF/HF

ratio, which is related to the insulin sensitivity, and negatively correlated with body fat content (21). It is well accepted that insulin stimulates sympathetic activity independently of changes in plasma glucose concentration (33,34) increasing the LF/HF ratio (35,36). Furthermore, in response to food intake (as well as glucose ingestion) intestinal enteroendocrine cells secrete multiple hormones, including Glucagon-like peptide-1 (GLP-1). GLP-1 given peripherally or centrally increases heart rate and blood pressure and activates autonomic regulatory neurons in rats thus suggesting that central GLP-1 regulates cardiovascular function by increasing the sympathetic system and inhibiting vagal activity (16,17).

Accordingly, in our study, the glucose induced increase in cardiosympathetic activity, was positively associated with increased insulin and GLP1 plasma levels. Furthermore, a multivariate analysis with LF/HF_{AUC} as the dependent variable, confirmed an independent role of both insulin and GLP1 levels in stimulating the cardiosympathetic activity.

Indeed, subjects carrying TT genotype had lower plasma insulin secretion and similar GLP1 levels compared to heterozygote and GG carriers. Furthermore, the multivariate analysis showed that TCF7L2 gene variant was significantly associated with AUC of LF/HF ratio independently of both insulin and GLP1 plasma levels, thus suggesting that an impaired GLP1 secretion is not likely to explain the increased sympathetic activity in carriers the risk allele. An altered GLP-1 signalling, rather than different GLP1 plasma levels, might explain the effect of TCF7L2 variant on cardio-sympathetic activity. Previous results, obtained by Shafer et al (11), showing that, despite similar basal and glucose induced total plasma GLP1 levels, the presence of the allele T was associated with reduced insulin secretion following GLP-1 infusion compared to allele G, seem to support our hypothesis.

In our study the active form of GLP-1 (peptide 7-36 and 7-37), instead of the total GLP1 (peptide 9-36) was evaluated. Indeed, systemic GLP-1 levels may not adequately reflect the level of the active hormone at tissue level. In fact, impaired TCF7L2 activity, through the TT genotype, might tissue specifically reduce or increase the GLP1 levels. Thus, the reduced efficiency of GLP1 in stimulating insulin secretion in pancreatic beta cell and the increased

activity on cardiac autonomic system, found in our study, might both reflect a functional defect in GLP signal (ie an impaired GLP1 receptor function).

In conclusion, our study demonstrate that TT genotype of *rs12255372* and *rs7903146* TCF7L2 gene variants are associated with reduced insulin secretion and with increased cardio sympathetic activity. Such results might contribute to explain the association of TCF7L2 gene polymorphisms with an increased risk of type 2 diabetes and suggest a potential role of TCF7L2 gene variants in increasing cardiovascular risk via enhanced sympathetic nervous system (SNS) activity.

The limited number of subjects and the lack of replication study are potential limitations of this study. Thus, further studies will be necessary for replicating our findings in a larger population group, with ethnical diversity to better elucidate molecular mechanisms linking TCF7L2 gene and cardiac sympathetic/parasympathetic activity.

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REFERENCES

1. Tianru Jin, Ling Liu . The Wnt signalling pathway effector TCF7L2 and Type II diabetes mellitus. *Molecular Endocrinology* 2008; **22** (11): 2383-2392
2. Yu M, Moreno C, Hoagland KM *et al.* Antihypertensive effect of glucagon-like peptide 1 in Dahl salt-sensitive rats. *J Hypertens* 2003; **21**: 1125-35.
3. Grant SF, Thorleifsson G, Reynisdottir I *et al.* Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* 2006; **38**: 320-3.
4. Groves CJ, Zeggini E, Minton J *et al.* Association analysis of 6,736 U.K. subjects provides replication and confirms TCF7L2 as a type 2 diabetes susceptibility gene with a substantial effect on individual risk. *Diabetes* 2006; **55**: 2640-4.
5. Florez JC, Jablonski KA, Bayley N *et al.* TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. *N Engl J Med* 2006; **355**: 241-50.
6. Damcott CM, Pollin TI, Reinhart LJ *et al.* Polymorphisms in the transcription factor 7-like 2 (TCF7L2) gene are associated with type 2 diabetes in the Amish: replication and evidence for a role in both insulin secretion and insulin resistance. *Diabetes* 2006; **55**: 2654-9.
7. Saxena R, Gianniny L, Burt NP *et al.* Common single nucleotide polymorphisms in TCF7L2 are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in nondiabetic individuals. *Diabetes* 2006; **55**: 2890-5.
8. Zhang C, Qi L, Hunter DJ, Meigs JB *et al.* Variant of transcription factor 7-like 2 (TCF7L2) gene and the risk of type 2 diabetes in large cohorts of U.S. women and men. *Diabetes* 2006; **55**: 2645-8.
9. Cauchi S, Meyre D, Dina C *et al.* Transcription factor TCF7L2 genetic study in the French population: expression in human beta-cells and adipose tissue and strong association with type 2 diabetes. *Diabetes* 2006; **55**: 2903-8.

10. Scott LJ, Bonnycastle LL, Willer CJ *et al.* Association of transcription factor 7-like 2 (TCF7L2) variants with type 2 diabetes in a Finnish sample. *Diabetes* 2006; **55**: 2649-53.
11. S. A. Schafer, O. Tscritter, F. M, F. Machicao *et al.* Impaired glucagons-like peptide-1-induced insulin secretion in carriers of transcription factor 7-lije 2 (TCF7L2) gene polymorphisms. *Diabetologia* 2007; **50**:2443-2450.
12. Nystrom T, Gutniak MK, Zhang Q *et al.* Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. *Am J Physiol Endocrinol Metab* 2004; **287**: 1209-15.
13. Ban K, Noyan-Ashraf MH, Hoefler J, Bolz SS, Drucker DJ and Husain M. Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. *Circulation* 2008; **117**: 2340-50.
14. Merchenthaler I, Lane M and Shughrue P. Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. *J Comp Neurol* 1999; **403**: 261-80.
15. Wei Y and Mojsov S. Tissue-specific expression of the human receptor for glucagon-like peptide-I: brain, heart and pancreatic forms have the same deduced amino acid sequences. *FEBS Lett.* 1995; **358**: 219-24.
16. Yamamoto H, Lee CE, Marcus JN *et al.* Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. *J Clin Invest* 2002; **110**: 43-52.
17. Yamamoto H, Kishi T, Lee CE *et al.* Glucagon-like peptide-1-responsive catecholamine neurons in the area postrema link peripheral glucagon-like peptide-1 with central autonomic control sites. *J Neurosci* 2003; **23**: 2939-46.

18. Bharucha AE, Charkoudian N, Andrews CN, Camilleri M, Sletten D, Zinsmeister AR and Low PA. Effects of glucagon-like peptide-1, yohimbine, and nitregeric modulation on sympathetic and parasympathetic activity in humans. *Am J Physiol Regul Integr Comp Physiol* 2008; **295**: 874-80.
19. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology Heart rate variability. Standards of measurements, physiological interpretation, and clinical use. *Eur Heart J* 1996; **17**: 354-381.
20. Malliani A, Lombardi F, Pagani M *et al.* Cardiovascular neural regulation explored in the frequency domain. *Circulation* 1991; **84**:1482- 1492.
21. Paolisso G, Manzella D, Ferrara N *et al.* Glucose ingestion affects cardiac ANS in healthy subjects with different amounts of body fat. *Am J Physiol* 1997; **273**: 471-8.
22. Paolisso G, Manzella D, Tagliamonte MR *et al.* M. Effects of different insulin infusion rates on heart rate variability in lean and obese subjects. *Metabolism* 1999; **48**: 755-62.
23. Aberti KGMM, Zimmet PZ, for the WHO Consultation. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of WHO consultation. *Diab Med* 1998; **15**: 539-53.
24. Kleiger RE, Phyllis K. Stein, J. Thomas Bigger. Heart rate variability: measurement and clinical utility. *Ann Noninvasive Electrocardiol* 2005; **10**: 88–101.
25. Jonsen SJ, Andersen N. On power estimation in maximum entropy spectral analysis. *Geophysics* 1978; **43**: 681–690.
26. Yi F, Brubaker PL and Jin T. TCF-4 mediates cell type-specific regulation of proglucagon gene expression by beta-catenin and glycogen synthase kinase-3beta. *J Biol Chem* 2005; **280**: 1457-1464.
27. Fehmann HC, Goke R and Goke B. Cell and molecular biology of the incretin

hormones glucagon-like peptide-I and glucose-dependent insulin releasing polypeptide. *Endocr Rev* 1995; **16**: 390-410.

28. Seals DR and Bell C. Chronic sympathetic activation: consequence and cause of age-associated obesity? *Diabetes* 2004; **53**: 276-284.

29. van Baak Marlen A. Meal-induced activation of sympathetic nervous system and its cardiovascular and thermogenic effects in man. *Physiology and Behaviour* 2007; **94**: 178-186.

30. Tsuji H, Venditti FJ, Jr., Manders ES, *et al.* Reduced heart rate variability and mortality risk in an elderly cohort. The Framingham Heart Study. *Circulation* 1994; **90**: 878-883.

31. Mancia G, Bousquet P, Elghozi JL *et al.* The sympathetic nervous system and the metabolic syndrome. *J Hypertens* 2007; **25**: 909-920.

32. Reaven GM, Lithell H and Landsberg L. Hypertension and associated metabolic abnormalities--the role of insulin resistance and the sympathoadrenal system. *N Engl J Med* 1996; **334**: 374-381.

33. Berne C, Fagius J, Pollare T *et al.* The sympathetic response to euglycaemic hyperinsulinaemia. Evidence from microelectrode nerve recordings in healthy subjects. *Diabetologia* 1992; **35**: 873-879.

34. Anderson EA, Hoffman RP, Balon TW *et al.* Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. *J Clin Invest* 1991; **87**: 2246-2252.

35. Schroeder EB, Chambless LE, Liao D *et al.* Diabetes, glucose, insulin, and heart rate variability: the Atherosclerosis Risk in Communities (ARIC) study. *Diabetes Care* 2005; **28**: 668-674.

36. Manzella D and G Paolisso. Cardiac autonomic activity and Type II diabetes mellitus. *Clinical Science* 2005; **108**: 93-99.

FIGURES

Fig. 1. Change in plasma glucose, insulin, GLP-1 and LF/HF during oral glucose tolerance test (n=167) stratified according to GG (n=81) GT (n=50) and TT (n=36) genotypes of *rs12255372* (G/T) TCF7L2 gene variant. Data are means \pm standard deviation. *p=0.02 TT vs GT; **p=0.01 TT vs GG by Bonferroni multiple testing correction.

Fig. 2. LF/HF_{AUC}, INS_{AUC} and GLP-1_{AUC} values according to *rs12233572* TCF7L2 gene polymorphism. Data are means \pm standard errors. *p= 0.01 TT vs GT and TT vs GG by Bonferroni multiple testing correction.

Suppl. Fig. 1. Change in plasma glucose, insulin, GLP-1 and LF/HF during oral glucose tolerance test (n=167) stratified according to CC (n= 83) CT (n=46) and TT (n=38) genotypes of *rs7903146* (C/T) TCF7L2 gene variant. Data are means \pm standard deviation. *p=0.02 TT vs CT ; **p=0.01 TT vs CC by Bonferroni multiple testing correction.

Suppl. Fig. 2. LF/HF_{AUC}, INS_{AUC} and GLP-1_{AUC} values according to *rs7903146* TCF7L2 gene polymorphism. Data are means \pm standard errors. *p=0.01.TT vs CT and TT vs CC by Bonferroni multiple testing correction.

TABLES

Table 1. Clinical characteristics of study population according to genotype at the rs12255372

	All (n=250)	GG (n=81)	GT (n=133)	TT (n=36)	p
Age (years)	28.1 ± 3.2	27.2 ± 3.4	27.6 ± 3.3	28.2 ± 2.7	.737
Gender	135F/115M	42F/39M	73F/60M	20F/16M	.573
BMI (Kg/m ²)	23.2 ± 3.3	22.7 ± 3.3	23.9 ± 3.5	24.0 ± 2.8	.395
WHR	0.77 ± 0.12	0.76 ± 0.10	0.80 ± 0.11	0.78 ± 0.06	.196
FPG (mmol/L)	5.1 ± 0.7	4.8 ± 0.4	4.7 ± 0.4	5.3 ± 0.4 [§]	.002
Total cholesterol (mg/dl)	158 ± 17	161 ± 17	156 ± 17	158 ± 21	.567
HDL cholesterol (mg/dl)	48 ± 2	48 ± 2	47 ± 3	48 ± 2	.455
Triglycerides (mg/dl)	87 ± 21	87 ± 14	91 ± 23	98 ± 17	.321
HR (beats/min)	71 ± 5	71 ± 6	72 ± 7	73 ± 6	.391
SBP (mmHg)	113 ± 9	114 ± 10	111 ± 10	115 ± 10	.550
DBP (mmHg)	72 ± 7	73 ± 8	71 ± 8	74 ± 7	.500

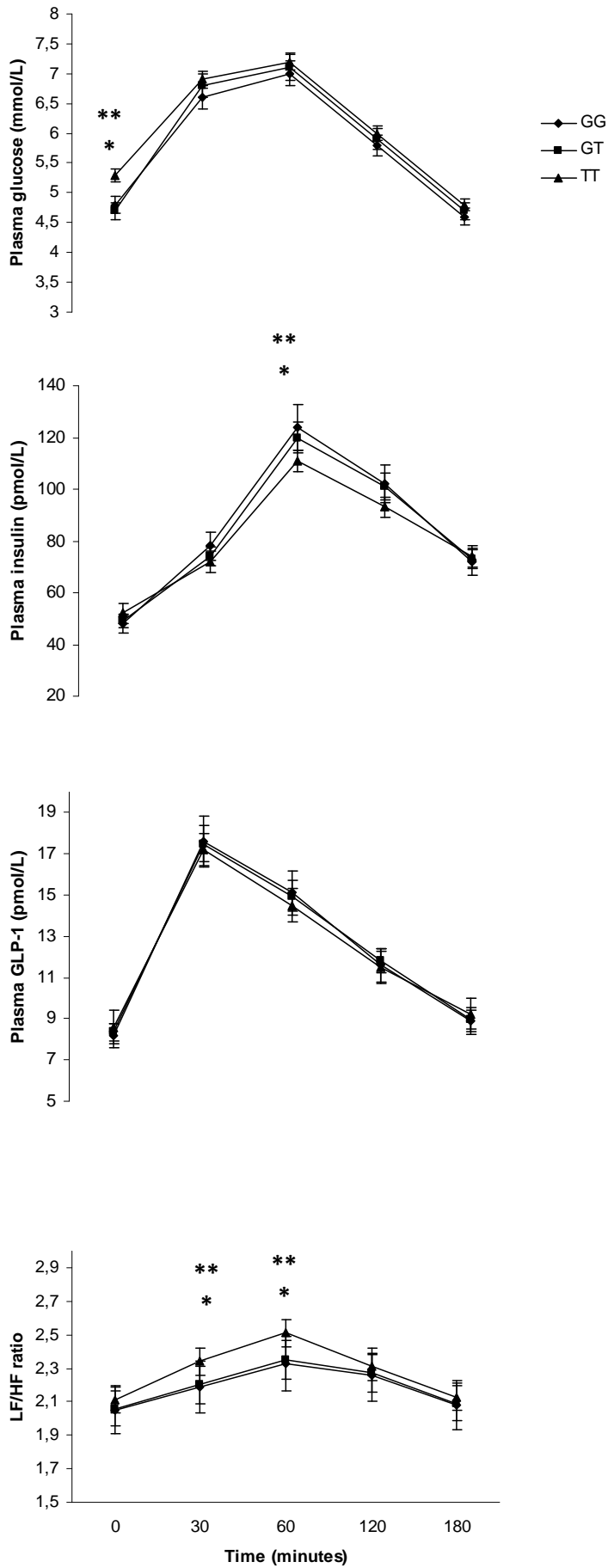
Data are means ± standard deviation. BMI, Body Mass Index; WHR, Waist/Hip Ratio; FPG, fasting plasma glucose; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure. *p* values between groups, [§] p=0.01 TT vs GT; p=0.02 TT vs GG by one-way ANOVA followed by Bonferroni multiple testing correction.

Table 2: Linear multiple regression analyses with AUC of LF/HF ratio as the dependent variable**(n = 167)**

	β	t	p	
Model 1				
BMI	-0.65	-0.621	.558	R ² = .554
Sex	0.03	0.027	.979	
AUC glucose				
(mmol/L x 180')	0.11	1.218	.259	
AUC insulin				
(pmol/L x 180')	0.32	1.041	.029	
AUC Glp1				
(pmol/L x 180')	0.18	1.953	.036	
TCF7L2				
polymorphism*	0.78	9.948	.022	

BMI, Body Mass Index; AUC, area under curve;* Calculated as GG carrier (=1) vs GT carrier (=2) vs TT carrier (=3). β = standardized regression coefficient; t = t "statistics computed by dividing the estimated value of the *beta* coefficient by its standard error.

FIGURE 1



SUPPLEMENTAL FIGURE 1

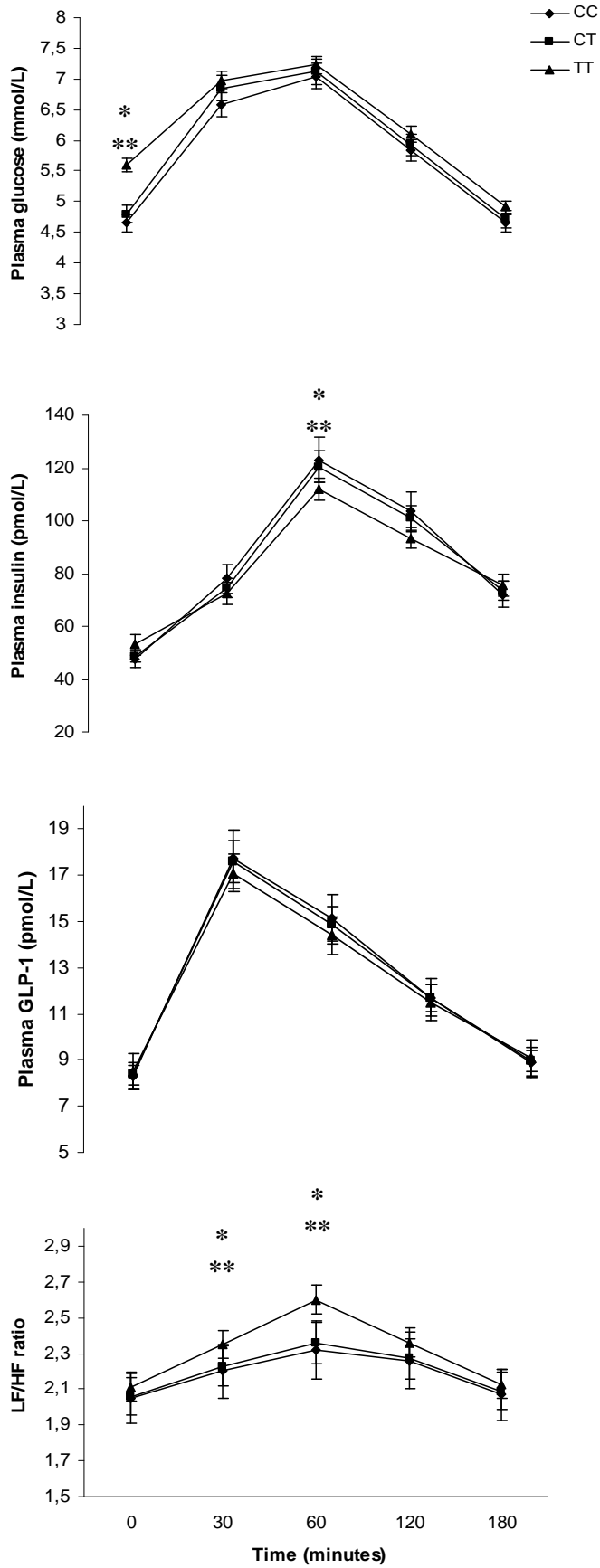
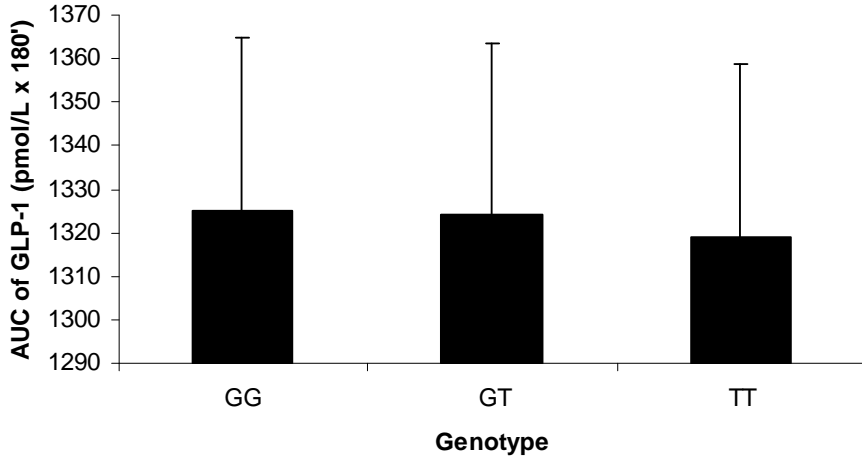
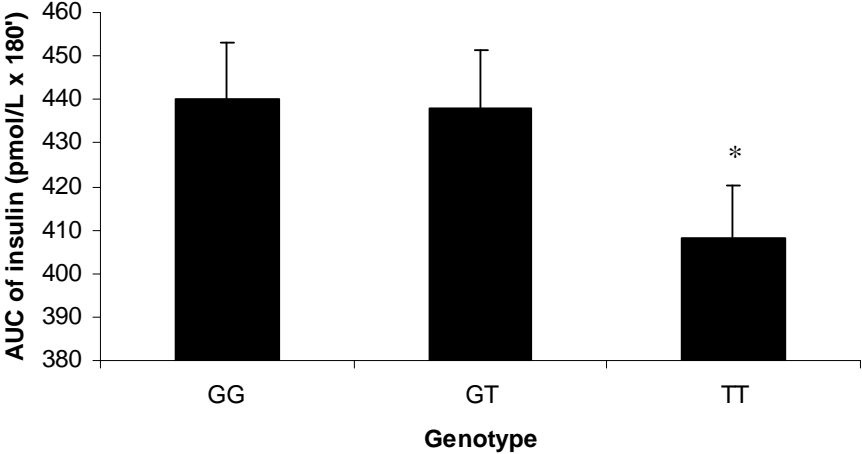
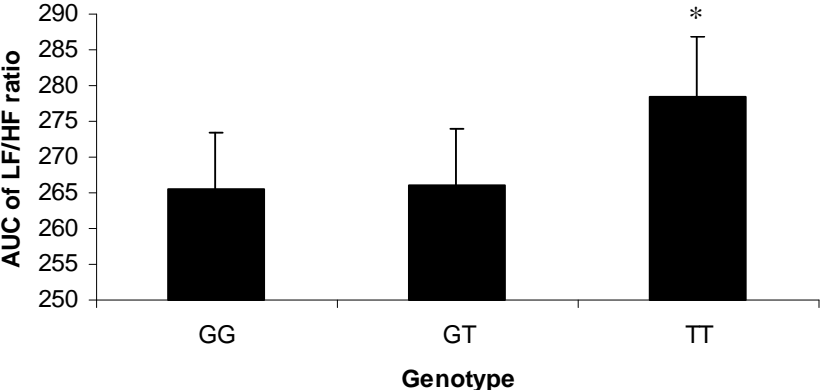


FIGURE 2



SUPPLEMENTAL FIGURE 2

