Diabetes-associated genetic variation in *TCF7L2* alters pulsatile insulin secretion in humans

Marcello C. Laurenti 1, Chiara Dalla Man PhD 2, Ron T. Varghese MBBS 1, James C. Andrews MD 3, Robert A. Rizza MD 1, Aleksey Matveyenko PhD 1,4, Giuseppe De Nicolao PhD 5, Claudio Cobelli PhD 2, Adrian Vella MD 1*.

1Division of Endocrinology, Diabetes & Metabolism, Mayo Clinic, Rochester, MN, USA
2Department of Information Engineering, University of Padova, Padova, Italy
3Vascular and Interventional Radiology, Mayo Clinic, Rochester, MN, USA
4Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, USA
5Department of Computer Engineering and Systems Science, University of Pavia, Pavia, Italy

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*Address for Correspondence:  Adrian Vella MD
Endocrine Research Unit
Mayo Clinic College of Medicine
200 First ST SW, 5-194 Joseph
Rochester, MN 55905
(T) 507-255-6515
(F) 507-255-4828
Email: vella.adrian@mayo.edu

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Abstract

Background: Metabolic disorders such as type 2 diabetes have been associated with a decrease in insulin pulse frequency and amplitude. We hypothesized that the T-allele at rs7903146 in TCF7L2, previously associated with β-cell dysfunction, would be associated with changes in these insulin pulse characteristics.

Methods: 29 nondiabetic subjects (age = 46 ± 2, BMI = 28 ± 1 Kg/M²) participated in this study. Of these, 16 were homozygous for the C allele at rs7903146 and 13 were homozygous for the T allele. Deconvolution of peripheral C-peptide concentrations allowed the reconstruction of portal insulin secretion over time. This data was used for subsequent analyses. Pulse orderliness was assessed by Approximate Entropy (ApEn) and the dispersion of insulin pulses was measured by a Frequency Dispersion Index (FDI) applied to a Fourier Transform of individual insulin secretion rates.

Results: During fasting conditions, the CC genotype group exhibited decreased pulse disorderliness compared to the TT genotype group (1.10 ± 0.03 vs. 1.19 ± 0.04, p = 0.03). FDI decreased in response to hyperglycemia in the CC genotype group, perhaps reflecting less entrainment of insulin secretion during fasting.

Conclusion: Diabetes-associated variation in TCF7L2 is associated with decreased orderliness and pulse dispersion unchanged by hyperglycemia. Quantification of ApEn and FDI could represent novel markers of β-cell health.

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Introduction

Type 2 diabetes is a common, but complex, metabolic disorder characterized by defects in islet function during the fasting and postprandial states which result in hyperglycemia. The disorder arises out of a complex interaction between genetic predisposition and environmental exposures (chiefly those affecting nutritional intake and energy expenditure). To date, genome-wide association studies have identified more than 200 loci harboring common genetic variants which predispose to type 2 diabetes (1), although the effect size of many is small (1-3). Most of these variants influence β-cell function and insulin secretion (4). Of these common variants, the greatest predisposition to disease is arguably conferred by the T-allele at rs7903146 in TCF7L2 (1, 3) which is associated with a subtle defect in insulin secretion (5). This results in impaired compensation for a decrease in insulin action as evident by comparison of the hyperbolic relationship between β-cell responsivity and insulin action in subjects with and without 2 copies of the diabetes-associated (T) allele (6). Impaired post-prandial suppression of glucagon secretion (6) is also likely to contribute to postprandial hyperglycemia, at least in situations where insulin secretion is impaired (7).

The secretion of insulin by β-cells is pulsatile and arises from a complex interplay between cellular metabolism and electrophysiology (8). Although individual β-cells have a pulse periodicity of ~ 5 minutes, the pulses of insulin secretion seen in the portal circulation represent the summation of multiple asynchronous clusters of β-cells entrained to secrete synchronously by metabolic (9, 10) or other stimuli (11, 12). After insulin is secreted into the portal circulation it undergoes hepatic extraction (13) so that by the time they appear in the systemic circulation, insulin pulses are attenuated and difficult to discern (14). The degree of hepatic extraction of portal insulin seems to be influenced by the amount of insulin secreted whether in response to an oral challenge (15), or the size of the pulse mass (16). Therefore, accurate identification of insulin pulses needs to account for hepatic insulin extraction.
The study of insulin pulse characteristics in humans, to date, has suggested that in people with type 2 diabetes both pulse amplitude and pulse frequency are decreased compared to healthy subjects (17). Indeed, defective insulin pulsatility has been observed in 1st degree relatives of people with diabetes prior to the development of overt hyperglycemia (18). Similar defects have been associated with obesity and with aging (19). Insulin secretagogues can increase pulse amplitude in people with type 2 diabetes. However, pulse frequency is unchanged suggesting that decreased pulse periodicity is a feature of diseased islets (20, 21). Given the complexity of the events leading to insulin synthesis and packaging (22) as well as pulse generation in the β-cell (8) it is possible that abnormal pulse characteristics may serve as an early marker of β-cell dysfunction that precedes the development of hyperglycemia, especially in people genetically predisposed to type 2 diabetes by family history (18), or more specifically due to genetic variation associated with impaired insulin secretion (as is the case for the T-allele at rs7903146 (6, 23)). We therefore sought to determine whether nondiabetic subjects homozygous for the diabetes-associated allele at rs7903146 (TT genotype) exhibited detectable abnormalities in insulin pulse secretion.

To do so, we developed a novel method that utilizes nonparametric stochastic deconvolution applied to peripheral C-peptide concentrations after determination of C-peptide clearance in each subject (24, 25). The reconstructed insulin secretion, used for this analysis, demonstrated pulse characteristics comparable to those obtained from hepatic vein insulin concentrations, while avoiding the confounding effects of hepatic extraction (24).

Although the basal and above-basal components of the data series did not exhibit significant differences between genotype groups, we observed increased disorderliness of insulin pulses (26) in subjects with diabetes-associated variation at rs7903146. These differences were only present during the fasting phase of the experiment.

To better understand the underlying frequencies and amplitudes of the pulse series that together produce the minute to minute variation in insulin secretion (18, 27-29) we used a Fourier
transform of the insulin secretion rate over the duration of the two phases of the experiment. In the CC, but not in the TT, genotype group we noted a decrease in the dispersion of pulse frequencies in response to hyperglycemia, as measured by a Frequency Dispersion Index (FDI) (30). In contrast, that in the TT group was relatively unchanged by hyperglycemia. This data suggests that diabetes-associated variation in TCF7L2 is associated with decreased pulse orderliness before overt defects in β-cell function are apparent. Pulse disorderliness and (n unchanging) dispersion of pulse frequencies during fasting may be a novel marker of impaired β-cell health.
Results

Volunteer Characteristics (Table 1)

Subject characteristics grouped by genotype at rs7903146 are summarized in Table 1. There were no significant anthropometric differences between genotype groups. Minimal model indices of insulin secretion and action also did not differ between groups. Note that in one subject due to loss of venous access we only have data from the fasting phase of the experiment (Supplementary Figures 1 – 6).

Plasma Glucose, Insulin, C-peptide concentrations and Insulin Secretion Rate during the experiment (Figure 1)

Mean glucose (Panel A) during the fasting phase of the experiment did not differ between groups. Although mean glucose during the hyperglycemic clamp was slightly and significantly increased (Table 1) in the TT group, these differences were not sustained.

Hepatic vein insulin concentrations (Panel B) and C-peptide concentrations (Panel C) did not differ during either phase of the experiment (Table 1). Nonparametric deconvolution of peripheral C-peptide concentrations allowed the reconstruction of individualized insulin secretion rates used for this analysis.

The mean rate of insulin secretion did not differ during either phase of the experiment for the two groups (Panel D).

Insulin Secretion Rate from representative subjects and Individual Basal Insulin Secretion Rate and Insulin Pulse Amplitude during fasting and during hyperglycemia in each genotype group (Figure 2)

Representative individual insulin secretion rates (obtained by deconvolution of peripheral C-peptide concentrations) of those analyzed are shown during the fasting (Panel A) and hyperglycemic (Panel B) phases of the experiment. Basal, above basal and pulse amplitude were calculated for each individual (Supplementary Figures 1 – 2).
The non-pulsatile component (Basal) of insulin secretion (Panel C) did not differ during fasting (142 ± 13 vs. 132 ± 13 pmol/min, CC vs. TT respectively, \( p = 0.71 \)). Basal secretion rose significantly in response to hyperglycemia but did not differ between groups (394 ± 36 vs. 411 ± 35 pmol/min, \( p = 0.68 \)).

Insulin pulse amplitude (Panel D) did not differ during fasting (59 ± 8 vs. 60 ± 6 pmol/min, \( p = 0.88 \)). Pulse amplitude also rose significantly in response to hyperglycemia but did not differ between groups (164 ± 21 vs. 185 ± 20 pmol/min, \( p = 0.27 \)).

*Individual ApEn during fasting and during hyperglycemia in each genotype group (Figure 3)*

Pulse orderliness as measured by Approximate Entropy (ApEn) differed significantly during fasting (1.10 ± 0.03 vs. 1.19 ± 0.04, \( p = 0.03 \)), with a higher score (increased disorderliness) in the TT group (Panel A). No differences in ApEn were apparent during hyperglycemia (Panel B – 1.18 ± 0.03 vs. 1.17 ± 0.03, \( p = 0.92 \)).

*Periodogram analysis, Individual Pulse Interval and Frequency Dispersion Index during fasting and during hyperglycemia in each genotype group (Figure 4)*

The Fourier power spectra shown are representative of those obtained during fasting (Panel A), and during hyperglycemia (Panel B) which were then used to calculate pulse interval and Frequency Dispersion Index (FDI – see Supplementary Figures 3 – 6).

Subjects with the CC genotype showed a non-significant trend towards slower pulse intervals during fasting (Panel C – 6.2 ± 0.6 vs. 4.9 ± 0.3 min, \( p = 0.06 \)). In response to hyperglycemia, pulse interval did not change significantly within (CC, \( p = 0.09 \) and TT, \( p = 0.41 \)) genotype groups.

The frequency dispersion index (Panel D) did not differ significantly between genotype groups during fasting. However, in response to hyperglycemia, the dispersion of pulse frequencies decreased significantly in the CC group (0.48 ± 0.02 vs. 0.41 ± 0.01, \( p = 0.02 \)) but not in the TT group (\( p = 0.41 \)). The between-group change in FDI (fasting vs. hyperglycemia) was not significant (\( p = 0.25 \))
Pulse characteristics attributable to glucose tolerance status (Table 2)

To ensure that differences in pulse characteristics could not be explained by increased numbers of impaired glucose tolerance in one genotype group versus the other, we compared pulse characteristics in those with normal glucose tolerance (NGT) versus those with impaired glucose tolerance (IGT). Despite the presence of impaired β-cell function in response to a 75g OGTT at the time of screening, no significant differences in pulse characteristics were associated with glucose tolerance status.
Discussion

This is the first application of our recently described methodology which utilizes peripheral C-peptide concentrations to deconvolve pulsatile insulin secretion into the portal vein without the potential confounding effects of hepatic extraction (24). The ability to measure high frequency pulsatile insulin secretion without hepatic vein catheterization raises the possibility of performing larger studies across the spectrum of glucose tolerance to follow up and to replicate the findings of the present study. Diabetes-associated variation in \textit{TCF7L2} is associated with a mild impairment in insulin secretion for a given degree of insulin action (6). However, in this small sample size when subjects were matched for age, sex, weight and fasting glucose we likely lacked the statistical power to discern differences in insulin secretion and action in response to a 75g OGTT. Nevertheless, pulse analysis revealed that the diabetes-associated allele in \textit{TCF7L2} was associated with a decrease in pulse orderliness as measured by ApEn (31). This could not be explained by changes in $\beta$-cell responsivity ($\Phi$) and insulin action ($S_i$) measured during the OGTT as evidenced by a lack of correlation with these indices (data not shown) and the absence of similar differences between people with NGT and IGT (Table 2).

What is the mechanism underlying these changes? Multiple, well-powered, \textit{in vivo} studies – including ours – measuring $\beta$-cell function in response to an oral challenge, demonstrate an association of the diabetes-associated allele (T) at rs7903146 with impaired insulin secretion (6, 23, 32-34). While it is possible that diabetes-associated variation in \textit{TCF7L2} is not the etiologic variant but is in linkage disequilibrium with an etiologic variant in another locus, or controls another locus (35), this seems unlikely (36) as multiple experiments would implicate TCF7L2 in the pathogenesis of type 2 diabetes. For example, decreasing TCF7L2 expression in mature mouse islets impaired glucose-stimulated insulin secretion (37). Intriguingly, a subsequent study where TCF7L2 expression was decreased demonstrated normal secretion in response to K$^+$ or Ca$^{2+}$-induced depolarization suggesting a defect in glucose-sensing (38). No decrease in insulin granules was observed, although the subcellular distribution was changed in a manner suggesting
that TCF7L2 knockdown altered granule recruitment and tethering to the cell membrane (38). Similar results were observed in islets from humans heterozygous or homozygous for the diabetes-associated allele at rs7903146 (39).

In contrast, TCF7L2 knockout resulted in decreased islet mass and islet coordination as well as a corresponding decrease in β-cell responsiveness to glucose (40). TCF7L2 activates β-catenin. Overexpression of TCF7L2, as observed in type 2 diabetes (33), sequesters β-catenin in the nucleus disrupting the actin cytoskeleton and insulin vesicle trafficking (41). While diabetes-associated genetic variation in TCF7L2 is unlikely to have as dramatic effects on β-cell function as those observed with gene silencing or overexpression experiments, the experimental data would support a role of TCF7L2 in coordinating insulin vesicle trafficking and docking with the cell membrane prior to release.

Another surprising finding is that the diabetes-protective allele showed a tendency to longer pulse intervals during fasting compared to that observed in those individuals homozygous for the T-allele at rs7903146. Decreased pulse frequency has been thought to represent impairment in β-cell health (17, 42, 43), however in this case, while not statistically significant, during the fasting state the opposite was observed; people with diabetes-protective variation in the TCF7L2 locus had a tendency to a longer main pulse interval. On the other hand a mean measurement of time interval between pulses may not fully characterize the contribution of different islets secretion rates and pulse frequencies to net insulin secretion. We therefore applied a Frequency Dispersion Index, to estimate the dispersion of insulin frequencies contributing to insulin secretion in a given individual. With this in mind, we measured frequency dispersion. It is notable that FDI is positively correlated with lengthening pulse interval in the fasting state, increasing as the average pulse interval lengthens (Supplementary Figure 7). We also observed that pulse frequency dispersion decreased in response to hyperglycemia in the CC, but not in the TT, genotype group. This suggests that in health, if multiple, small asynchronous pulses
contribute to insulin secretion, the main contribution(s) to insulin secretion can occur at a lower frequency.

During the hyperglycemic clamp, there was a slight, but significant, tendency to higher glucose concentrations in the TT group. While this may have masked between-group differences in insulin pulsatility in response to glucose elevation, this cannot explain the differences present in the fasting phase of the experiment. Also, net insulin secretion increased similarly between groups while pulse characteristics (other than amplitude) were essentially unchanged in the TT group in response to hyperglycemia. Future studies manipulating insulin action (6) or fasting insulin secretion (43) will be required to determine if the relatively fixed FDI in the group with diabetes-associated variation in TCF7L2, reflects a necessity to entrain more islets in the basal fasting state to maintain euglycemia.

It is also important to note that this study was powered to detect the effect of diabetes-associated genetic variation in TCF7L2 – focusing on the common variant (rs7903146) with the greatest effect on disease-predisposition. To maximize our power to detect an effect we recruited by genotype, selecting subjects homozygous for the disease-associated (TT) and the disease-protective (CC) variants. Unfortunately, this sample size and recruitment method means that we will have insufficient power to reliably detect an effect of other common variants with lesser effect sizes on insulin pulse characteristics (3). Fortunately, our methodology should allow the development of larger cohorts where these questions can be addressed.

Prior experiments using the oral minimal model to measure β-cell function (44) have suggested that while β-cell responsivity to glucose is relatively unchanged across the spectrum of glucose tolerance, it is inappropriate for the degree of insulin action that is present as glucose tolerance worsens (45, 46). Despite the inclusion of people with some degree of glucose intolerance, none of the between-group differences in pulse characteristics could be explained by their glucose tolerance status. Pulse characteristics also did not correlate with the standard indices of insulin secretion and action obtained from the minimal model. This may be in part due to the
experimental design where efforts were made to ensure that fasting glucose concentrations at the
time of screening did not differ between genotype groups in the fasting state. Similarly, a
hyperglycemic clamp was utilized to ensure that glucose concentrations did not differ between
groups during hyperglycemia. None of the subjects studied had impaired fasting glucose so that
future experiments will be required to determine if people with impaired fasting glucose, with or
without IGT, have altered insulin pulse characteristics compared to people with normal fasting
glucose.

In conclusion, people predisposed to type 2 diabetes because of diabetes-associated
genetic variation at rs7903146 exhibit subtle differences in insulin pulse characteristics compared
to those without risk alleles. Increased pulse disorderliness occurred independently of changes in
more conventional measures of β-cell function. This raises the possibility that pulse
characteristics of β-cell insulin secretion may serve as additional, independent measures of β-cell
health. Since this will require additional experiments to determine their significance and utility,
the use of our methodology which eschews the need of a hepatic vein catheter, is a significant
advantage. Future studies in larger numbers of subjects are now feasible and may help validate
these novel measures of β-cell (dys-)function.
Materials and Methods

Study Subjects

The subject (10 males and 19 females) characteristics are described in Table 1. They underwent a screening exam to ensure they were healthy and had no active illness, no prior history of diabetes mellitus and were not taking medications that might alter glucose metabolism. A 2-hour, 7-sample Oral Glucose Tolerance Test (OGTT) was used at the time of screening to measure insulin secretion and action as before (47). Body composition was measured by dual energy X-ray absorptiometry (iDXA scanner; GE, Wauwatosa, WI). Participants met with a nutritionist and followed a weight maintenance diet (55% carbohydrate, 30% fat and 15% protein) for a minimum of three days prior to the study visit.

Experimental Design

As previously outlined in (24), subjects were admitted to the Clinical Research and Translation Unit (CRTU) at 1700 on the day prior to study. They then consumed a standard meal and fasted overnight. The following morning (at approximately 0630), an 18g cannula was inserted retrogradely into a dorsal hand vein. This was then placed in a heated Plexiglas box maintained at 55°C to allow sampling of arterialized venous blood. Subjects were then moved to the radiology suite where a hepatic vein catheter was placed via the femoral vein under fluoroscopic guidance (16, 48). Following their return, at 0800 (0 min) blood was sampled at 1 minute intervals from the arterialized hand vein and from the hepatic vein at 2 minute intervals over a 45 minute period (Fasting Phase). At 0846 (46 min) glucose infusion commenced and the infusion rate was adjusted to rapidly achieve and maintain peripheral glucose concentrations of ~9.8mmol/l. Following this 30 minute (0915), blood was sampled during the Hyperglycemic Phase for an additional 45 minutes. At 1000 (120 min), all cannulas and the hepatic vein catheter were removed. Participants consumed a meal and left the CRTU.

Analytic Techniques
Plasma samples were placed on ice, centrifuged at 4°C, separated, and stored at −20°C until assayed. Glucose concentrations were measured using a glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was measured using a chemiluminescence assay (Access Assay; Beckman, Chaska, MN). C-peptide was measured using a 2-site immunoenzymatic sandwich assay (Roche Diagnostics, Indianapolis, IN 46250) in accordance with the manufacturer’s instructions.

Assessment of Pulsatile Insulin Secretion by Stochastic Nonparametric Deconvolution and Individualized C-peptide Kinetics

Deconvolution was utilized to determine the insulin secretion rate over time (ISR(t)) derived from peripheral C-peptide concentrations after assessment of the Coefficient of Variation (CV) of our C-peptide assay as previously described (24). To reconstruct the ISR signal, we relied on nonparametric deconvolution (49), which does not require any a priori assumption of the unknown input signal. However, critical to accurate estimation of secretion is knowledge of hormone kinetics. This was directly calculated from experimental data of C-peptide decay in each individual using a Maximum A Posteriori estimation (25, 50), with a two-compartment model under the assumption that the C-peptide kinetic is linear (51).

Assessment of Insulin Secretion Pulsatility and Pulse Characteristics by Temporal and Frequency Analysis

Once the pulsatile ISR(t) was reconstructed, we analyzed the euglycemic and hyperglycemic portion of the signal separately. In each portion, we first calculated the average (basal) ISR during fasting and hyperglycemia (ISR_{basf} and ISR_{bhyper}, respectively) and the above basal ISR (ISR_{abf} and ISR_{abhyper}, respectively). ISR_{ab} for each phase of the experiment was taken as ISR_{total} – ISR_{b}. The standard deviation (ISR_{SD}) of ISR_{ab} in each phase of the experiment was used as a measure of pulse amplitude. The reconstructed ISR(t) for each individual is shown in Supplementary Figures 1 & 2.
We then calculated the Approximate Entropy (ApEn) for the insulin secretion rates (31). ApEn is a validated measure of the regularity and complexity of a temporal series that has been used previously to measure disorderliness in pulsatile insulin secretion (21, 52). We subsequently used the FFT algorithm implemented in MATLAB R2017b to obtain the signal power spectra and estimate the main pulse interval. The resulting periodogram for each individual is shown in Supplementary Figures 3 – 6. The main pulse interval was calculated as the inverse pulse period of the fastest harmonic in the spectrum that had an amplitude of $\geq 40\%$ of the peak amplitude. This threshold was chosen empirically, under the assumption that spectrum peaks with amplitude less than 40% of peak amplitude do not contribute significantly to the main pulsatile pattern observed peripherally. The pulse interval thus calculated represents an estimate of the average time interval between two pulses.

In order to provide a more rigorous measurement of pulsatility, and better characterize the contribution of different pulse frequencies to overall secretion, we first applied a high-pass filter with a cut-off frequency of $8.3 \times 10^{-4}$ Hz in order to remove the effect of slow frequencies (those with a pulse interval longer than 20 minutes). This enabled us to focus on pulses with a frequency that contributes to minute-to-minute pulsatility (0 to 20 minutes, as previously reported (53)). To quantify the variability of the frequencies we used a Frequency Dispersion Index (FDI). This index (between 0 and 1) increases as the signal bandwidth increases and it is defined by:

$$FDI = \sqrt{1 - \frac{\mu_1^2}{\mu_0\mu_2}}$$

where $\mu_n$ is the $-n$th statistical moment of the spectrum (30).

**Statistical Analysis**

Previously Meier et al. measured pulsatile insulin secretion in 6 healthy adults (16). In that experiment pulses measured in the peripheral vein occurred with a periodicity of $4.20 \pm 0.24$ Min/pulse and an amplitude of $59 \pm 20$ pmol/l (Mean ± SD). Assuming similar variation, with 13
subjects in each genotype group we would have 80% power to detect a 6% and 39% change in Mean pulse frequency and amplitude respectively at an \( \alpha \) of 0.05.

Our data and results are presented as Mean ± SEM. Between group comparisons were performed using an unpaired, two-tailed Student \( t \)-test for normally distributed variables; conversely, when samples were not normally distributed, a two-tailed Wilcoxon test was used. A paired, 2-way student \( t \)-test (parametric) or Wilcoxon matched-pairs signed rank test (non-parametric) was used to examine within group changes (fasting vs. hyperglycemia). A \( p \)-value < 0.05 was considered statistically significant. Analysis was performed in Prism 5 (GraphPad Software, San Diego, CA).

Study approval

After approval by the Mayo Clinic Institutional Review Board, 29 non-diabetic subjects, recruited from among subjects who had previously participated in a series of published experiments (6, 25, 54), provided informed, written consent to participate in this study. All were recruited on the basis of genotype at rs7903146 as outlined previously (6).
Acknowledgments

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Author Contributions

M.C.L and C.D.M. developed the method, analyzed the data and reviewed/edited manuscript. R.T.V. researched data and ran the studies. R.A.R, A.M. G.D.N contributed to the discussion and reviewed/edited manuscript. C.C. developed the method and reviewed/edited manuscript. A.V. designed the study, oversaw its conduct, researched data, contributed to the discussion and wrote the manuscript. A.V. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
References


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**Table 1:** Subject characteristics by genotype group. BMI = Body Mass Index; NGT = Normal Glucose Tolerance; IGT = Impaired Glucose Tolerance. Values reported are Means ± SEM. p-Value reports the result of an unpaired, two-tailed t-test or a two-tailed Wilcoxon test for values that are not normally distributed. *p-Value is the result of a Chi-Square test.
Table 2: Pulse and subject characteristics in subjects grouped by glucose tolerance status. NGT = Normal Glucose Tolerance; IGT = Impaired Glucose Tolerance; BMI = Body Mass Index. Values reported are Means ± SEM. *p-Value reports the result of an unpaired, two-tailed t-test or a two-tailed Wilcoxon test for values that are not normally distributed. *p-Value is the result of a Chi-Square test.
Figure 1: Glucose (Panel A), Insulin (Panel B), C-Peptide (Panel C) and Insulin Secretion Rate (Panel D) during the experiment in subjects with the CC (open circles) and TT (solid circles) genotype. Values plotted are Means ± SEMs.
Figure 2: Insulin secretion rate in a representative individual during the fasting (Panel A) and hyperglycemic phase of the experiment (Panel B). Individual basal (Panel C) and amplitude (Panel D) of insulin peaks in the fasting and hyperglycemic phase of the experiment are also shown for the CC (open circles) and TT (solid circles) genotype. Also shown are Means ± SEMs for each group. *p < 0.05 using a Wilcoxon matched-pairs signed rank test to examine within group changes (fasting vs. hyperglycemia).

Figure 3: Individual ApEn in the fasting (Panel A) and hyperglycemic phases of the experiment (Panel B) are shown for the CC (open circles) and TT (solid circles) genotype. Also shown are Means ± SEMs for each group. *p < 0.05 using a two-tailed Wilcoxon test.
Figure 4: Periodograms in a representative individual during the fasting (Panel A) and hyperglycemic phase of the experiment (Panel B). Individual pulse interval (Panel C) and frequency dispersion index (Panel D) in the fasting and hyperglycemic phase of the experiment are also shown for the CC (open circles) and TT (solid circles) genotype. Also shown are Means ± SEMs for each group. *p < 0.05 using a Wilcoxon matched-pairs signed rank test to examine within group changes (fasting vs. hyperglycemia).