Meera Shah,[1] Ron T. Varghese,[1] John M. Miles,[1] Francesca Piccinini,[2] Chiara Dalla Man,[2] Claudio Cobelli,[2] Kent R. Bailey,[3] Robert A. Rizza,[1] and Adrian Vella[1]

TCF7L2 Genotype and α-Cell Function in Humans Without Diabetes

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The diabetes-associated allele in *TCF7L2* increases the rate of conversion to diabetes; however, the mechanism by which this occurs remains elusive. We hypothesized that the diabetes-associated allele in this locus (rs7903146) impairs insulin secretion and that this defect would be exacerbated by acute free fatty acid (FFA)–induced insulin resistance. We studied 120 individuals of whom one-half were homozygous for the diabetes-associated allele TT at rs7903146 and one-half were homozygous for the protective allele CC. After a screening examination during which glucose tolerance status was determined, subjects were studied on two occasions in random order while undergoing an oral challenge. During one study day, FFA was elevated by infusion of Intralipid plus heparin. On the other study day, subjects received the same amount of glycerol as present in the Intralipid infusion. β-Cell responsivity indices were estimated with the oral C-peptide minimal model. We report that β-cell responsivity was slightly impaired in the TT genotype group. Moreover, the hyperbolic relationship between insulin secretion and β-cell responsivity differed significantly between genotypes. Subjects also exhibited impaired suppression of glucagon after an oral challenge. These data imply that a genetic variant harbored within the *TCF7L2* locus impairs glucose tolerance through effects on glucagon as well as on insulin secretion.

Type 2 diabetes is characterized by inadequate insulin secretion for the prevailing level of insulin action and is caused by a complex interaction between genes and the environment. Although many genes have been associated with type 2 diabetes, the T allele at rs7903146 in the *TCF7L2* locus arguably has the greatest effect on disease predisposition (1). The diabetes-associated allele T raises postprandial glucose concentrations and decreases peripheral concentrations of insulin in response to an oral challenge (2,3). On the basis of these observations, *TCF7L2* has been assumed to impair β-cell function.

This conclusion may be somewhat premature because it is subject to several caveats. Most studies have used qualitative measures of insulin secretion and action that are based on changes in peripheral insulin concentrations rather than on directly measured β-cell function. This is problematic because changes in peripheral insulin concentrations are influenced by changes in insulin secretion and by changes in hepatic insulin clearance (4,5). Furthermore, most studies typically have used qualitative measures of insulin action (e.g., HOMA), creating uncertainty about the extent to which insulin secretion is appropriate for the prevailing level of insulin action (5,6) and perhaps explaining why some studies have reported that the T allele is associated with defects in insulin action (7,8) rather than in insulin secretion. This is important because an inability of the β-cell to compensate for the prevailing level of insulin action is an early step in the evolution of type 2 diabetes (9–11).

To determine the mechanism by which the TT genotype causes glucose intolerance, we hypothesized that the diabetes-associated allele in *TCF7L2* causes glucose intolerance by limiting the ability of β-cells to compensate for (acute or chronic) insulin resistance. We studied subjects matched for age, sex, weight, and fasting glucose level during a 75-g oral glucose tolerance test (OGTT) at the time of screening and then on two occasions in random order. On one occasion, acute insulin resistance was induced by means of an Intralipid and heparin infusion to raise circulating concentrations of free fatty acids (FFAs) (12). On the other occasion, an infusion of glycerol was

[1]Division of Endocrinology, Diabetes, Metabolism, and Nutrition Research, Mayo Clinic, Rochester, MN
[2]Department of Information Engineering, Università degli Studi di Padova, Padova, Italy
[3]Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN
Corresponding author: Adrian Vella, vella.adrian@mayo.edu.
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M.S. and R.T.V. contributed equally to this work.

administered at the same rate as that on the Intralipid study day. We report subtle differences in β-cell responsivity to oral glucose due to genotype and an altered relationship of this parameter to insulin sensitivity. A novel finding is that the diabetes-associated allele impaired suppression of glucagon in response to an oral glucose challenge, especially during an accompanying elevation in FFA. Taken together, these data imply that a genetic variant in the *TCF7L2* locus impairs glucose tolerance through effects on glucagon as well as on insulin secretion.

RESEARCH DESIGN AND METHODS

Subjects

After approval from the Mayo Clinic Institutional Review Board, we used the Mayo Clinic Biobank, a repository of 20,000 DNA samples collected from volunteers, to perform genotyping of 4,000 individuals at rs7903146. Subjects were randomly selected from the biobank cohort, their age spanned 20–70 years (thereby minimizing the potential confounding effects of age on glucose tolerance and insulin secretion), they had no history of diabetes, and they resided within a 100-mile radius of Mayo Clinic in Rochester, MN. Subjects homozygous for the disease-causing allele TT were matched for age, sex, fasting glucose level, and body weight with subjects homozygous for the disease-protective allele CC, and all were invited to participate in the study. After written informed consent, subjects underwent a 2-h 75-g OGTT to characterize their glucose tolerance status. All subjects were not taking medications that could affect glucose metabolism and had no history of chronic illness or upper gastrointestinal surgery. All were instructed to follow a weight-maintenance diet (approximately 55% carbohydrate, 30% fat, and 15% protein) for the duration of the study. Body composition was measured with DEXA (iDXA scanner; GE Healthcare, Wauwatosa, WI).

Experimental Design

Subjects were subsequently studied on two occasions in random order 2 weeks apart. On one occasion (FFA), subjects received an infusion of Intralipid and heparin to raise FFA concentrations, whereas on the other occasion (GLY), glycerol was infused at a rate of 5 μmol/kg/min to match the amount of Intralipid infused during the FFA study day. On each occasion, subjects were admitted to the clinical research unit at 1700 h on the day before the study. At 1800 h, they consumed a standard 10 kcal/kg meal (55% carbohydrate, 30% fat, 15% protein) followed by an overnight fast. At 0630 h (−210 min) the following morning, a forearm vein was cannulated for infusions. In addition, a cannula was inserted retrogradely into a vein of the contralateral dorsum of the hand, which was placed in a heated Plexiglas box maintained at 55°C to allow sampling of arterialized venous blood.

At 0700 h (−180 min), an infusion of Intralipid (20%, 0.011 mL/kg/min; Baxter Healthcare, Deerfield, IL) and heparin (200 units prime, 0.2 units/kg/min continuous) was commenced as previously described (13). The infusion was continued until the end of the study (1600 h [360 min]). At time 0 (1000 h), subjects ingested a glucose drink (1 g/kg body weight). Blood samples were obtained at periodic intervals for hormone and substrate measurement over the course of the experiment.

Analytical Techniques

Genotyping of the rs7903146 single nucleotide polymorphism was undertaken with TaqMan (Applied Biosystems, Foster City, CA). Plasma samples were placed on ice, centrifuged at 4°C, separated, and stored at −20°C until assayed. Samples for measurement of FFA were placed in tubes containing 50 μL Paroxon (diethyl *p*-nitrophenyl phosphate; Sigma-Aldrich, St. Louis, MO). FFA concentrations were measured by high-performance liquid chromatography (14,15). Glucose concentrations were measured by a glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was measured with a chemiluminescence assay (Access; Beckman Coulter, Chaska, MN). Plasma glucagon and C-peptide levels were measured by radioimmunoassay (Linco Research, St. Louis, MO).

Calculations

Data are presented as mean ± SEM. Values from –30 to 0 min were averaged and considered as basal. Area above basal was calculated using the trapezoidal rule.

Net insulin action (S_i) was measured using the oral minimal model (16). β-Cell responsivity indices were estimated by the oral C-peptide minimal model (17), incorporating age-associated changes in C-peptide kinetics (18). The model assumes that insulin secretion comprises static and dynamic components. The parameter ϕ_d defines the dynamic responsivity index and is proportional to the rate of increase of glucose concentrations. The parameter ϕ_s represents the provision of new insulin to the releasable pool. An index of total β-cell responsivity to glucose (Φ) was then derived from both indices (19).

A population-based approach was applied to each genotype group during the GLY and FFA study days by using nonlinear mixed-effects modeling as first described by

Table 1—Demographic characteristics of each genotype group

	CC	TT	*P* value
Sex			
Male	22	23	—
Female	38	37	—
Age (years)	41.1 ± 1.7	41.9 ± 2.0	0.75
Weight (kg)	82 ± 3	80 ± 2	0.40
BMI (kg/m^2)	27.4 ± 0.5	27.4 ± 0.6	0.95
Lean body mass (kg)	47.4 ± 1.2	48.7 ± 1.4	0.47

Data are mean ± SEM. *P* value reports the result of an unpaired, two-tailed *t* test.

Denti et al. (20) to obtain genotype-specific estimates of the power function law describing the disposition index (DI) = $\Phi \cdot S_i^{\alpha}$, which accommodates the relationship between secretion and action under different experimental conditions.

Statistics

Data presented in the text are (observed) mean ± SEM. The primary analyses compared differences in values and indices between genotype groups by unpaired, two-tailed *t* test. Secondarily, we compared the effect of FFA elevation between genotype groups using (symmetric) percent differences (21) calculated as 100 · Log_e (FFA value/GLY value). Within-group changes in fasting, peak, and integrated hormone concentrations or glucose flux (GLY vs. FFA) were assessed separately for each group by a paired *t* test or a signed rank test as warranted. $P < 0.05$ was considered statistically significant.

RESULTS

Volunteer Characteristics

Sixty subjects with the TT genotype and 60 with the CC genotype were studied (Table 1). The genotype groups were well matched, with no between-group differences in age, sex distribution, weight, and fasting glucose concentrations. Using a 120-min glucose value ≥7.8 mmol/L during the 75-g OGTT to classify subjects as glucose intolerant, 25 subjects in the CC group and 30 in the TT group had impaired glucose tolerance.

Plasma Glucose, Insulin, C-Peptide, and Glucagon Concentrations During a 75-g OGTT

Although fasting glucose concentrations did not differ, peak (10.1 ± 0.2 vs. 10.9 ± 0.2 mmol/L, $P = 0.009$) and integrated (356 ± 15 vs. 431 ± 19 mmol per 2 h, $P = 0.003$) glucose concentrations were lower in the CC group than in the TT group, respectively (Fig. 1*A*). In contrast, fasting, peak, and integrated concentrations of insulin (Fig. 1*B*) and C-peptide (Fig. 1*C*) did not differ between groups. Although fasting and nadir glucagon (Fig. 1*D*) did not differ significantly, postchallenge suppression of glucagon was greater in the CC group (−1,107 ± 159 vs. −1,610 ± 180 ng per 2 h, $P = 0.04$) (Supplementary Table 1).

Plasma Glucose, Insulin, C-Peptide, and Glucagon Concentrations During Challenge With 1 g/kg Glucose and Concomitant Glycerol Infusion

The differences in fasting, peak (10.1 ± 0.2 vs. 10.6 ± 0.2 mmol/L, $P = 0.06$), and integrated glucose concentrations between the CC and TT genotype groups, respectively, were not significant (Fig. 2*A*). Similarly, fasting, peak, and integrated concentrations of insulin (Fig. 2*B*) and C-peptide (Fig. 2*C*) did not differ between groups. Despite apparent, but nonsignificant, differences in the time taken to suppress to nadir values (102 ± 8 vs. 124 ± 10 min, $P = 0.08$), the main difference in integrated glucagon concentrations (Fig. 2*D*) was observed in the first hour after oral challenge when glucagon was suppressed less in the TT group (−398 ± 71 vs. −178 ± 71 ng per 1 h, $P = 0.04$).

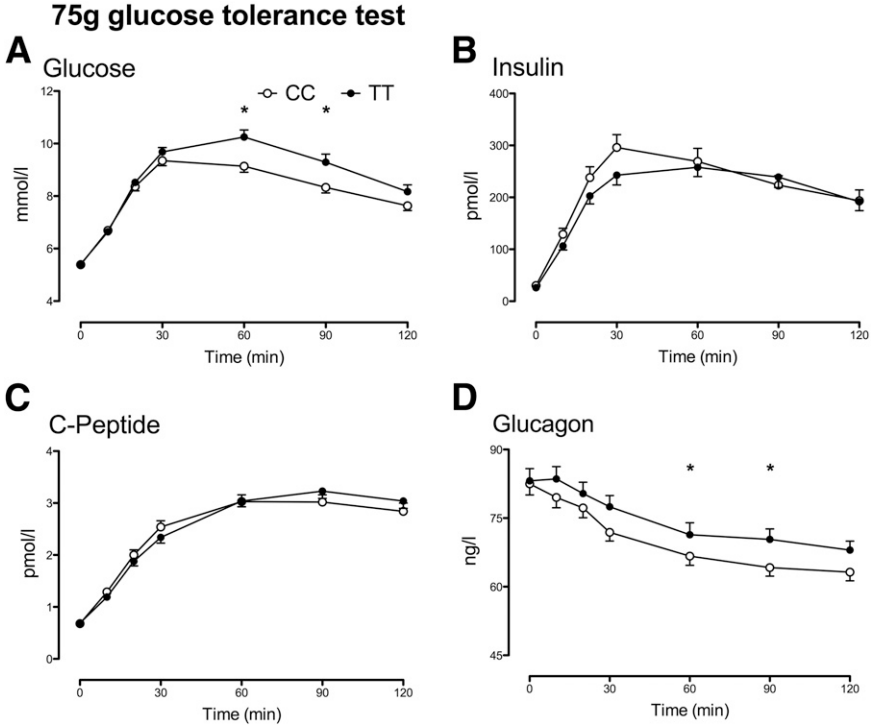

Figure 1—Glucose (*A*), insulin (*B*), C-peptide (*C*), and glucagon (*D*) concentrations in response to a 75-g oral glucose challenge in subjects with the CC and TT genotypes. Data are mean ± SEM. *$P < 0.05$ for a post hoc unpaired, two-tailed *t* test.

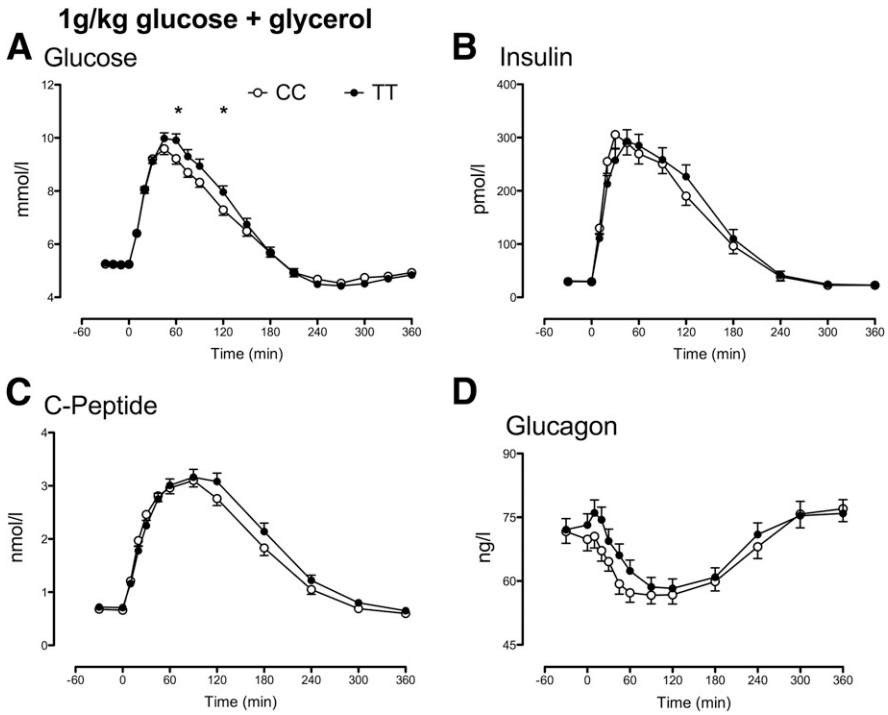

Figure 2—Glucose (*A*), insulin (*B*), C-peptide (*C*), and glucagon (*D*) concentrations in response to a 1 g/kg body weight glucose challenge with accompanying glycerol infusion in subjects with the CC and TT genotypes. Data are mean ± SEM. *$P < 0.05$ for a post hoc unpaired, two-tailed *t* test.

Plasma Glucose, Insulin, C-Peptide, and Glucagon Concentrations During Challenge With 1 g/kg Glucose and Elevated FFAs

By design, FFA concentrations were raised threefold in the FFA study compared with the GLY study (Supplementary Fig. 1). Although glucose ingestion (and subsequent insulin secretion) suppressed FFA in the postprandial state, differences in FFA concentrations compared with the GLY study persisted throughout the experiment.

FFA elevation resulted in higher postchallenge peak and integrated glucose concentrations ($P < 0.05$) (Supplementary Table 2) within both genotype groups than in the GLY study. Although there was a tendency to higher peak glucose concentrations in the TT group (Supplementary Tables 1 and 2), fasting, peak, and integrated glucose concentrations did not differ between genotype groups during FFA elevation (Fig. 3*A*).

Fasting and postprandial insulin and C-peptide concentrations were increased by FFA elevation ($P < 0.05$) (Supplementary Table 2) compared with the GLY study. However, insulin and C-peptide concentrations did not differ between genotype groups during FFA elevation (Fig. 3*B* and *C*).

FFA elevation raised fasting glucagon concentrations to a greater extent from the fasting concentrations observed during the GLY study in subjects with the TT genotype than in those with the CC genotype ($P < 0.05$) (Supplementary Table 2). Compared with subjects with the CC genotype, postprandial glucagon concentrations suppressed to a lesser degree, resulting in a higher nadir value of glucagon in the TT group (Supplementary Table 1). Indeed, although nadir glucagon was unchanged from the GLY study in the CC group (51 ± 2 vs. 52 ± 2 ng/L, $P = 0.47$), nadir glucagon in the TT group was increased by FFA elevation (53 ± 2 vs. 59 ± 2 ng/L, $P < 0.001$) (Supplementary Table 2).

Indices of Insulin Secretion and Insulin Action

S_i did not differ between genotype groups (Fig. 4*A*) during OGTT. However, Φ (Fig. 4*B*) was lower (55 ± 3 vs. 48 ± 2 $10^{-9}min^{-1}$, $P = 0.03$) in the TT group, a difference mainly explained by the dynamic component of β-cell responsivity (ϕ_d 46 ± 2 vs. 41 ± 2 10^{-9}, $P = 0.03$) (Supplementary Table 3).

S_i (Fig. 4*C*) and Φ (Fig. 4*D*) did not differ significantly between groups during the GLY study. As expected, elevation of FFA by infusion of Intralipid and heparin lowered S_i in both groups compared with the glycerol infusion. S_i did not differ between genotype groups during this experiment (Fig. 4*E*); however, Φ was lower (50 ± 3 vs. 42 ± 2 $10^{-9}min^{-1}$, $P = 0.02$) in the TT group (Fig. 4*F*). Differences were observed in both the dynamic (ϕ_d) and the static (ϕ_s) components of β-cell responsivity (Supplementary Table 3).

Relationship of β-Cell Responsivity to Oral Glucose and Insulin Action in Genotype Groups

To examine the relationship between S_i and Φ, individual values of S_i and Φ were used to calculate a DI for each

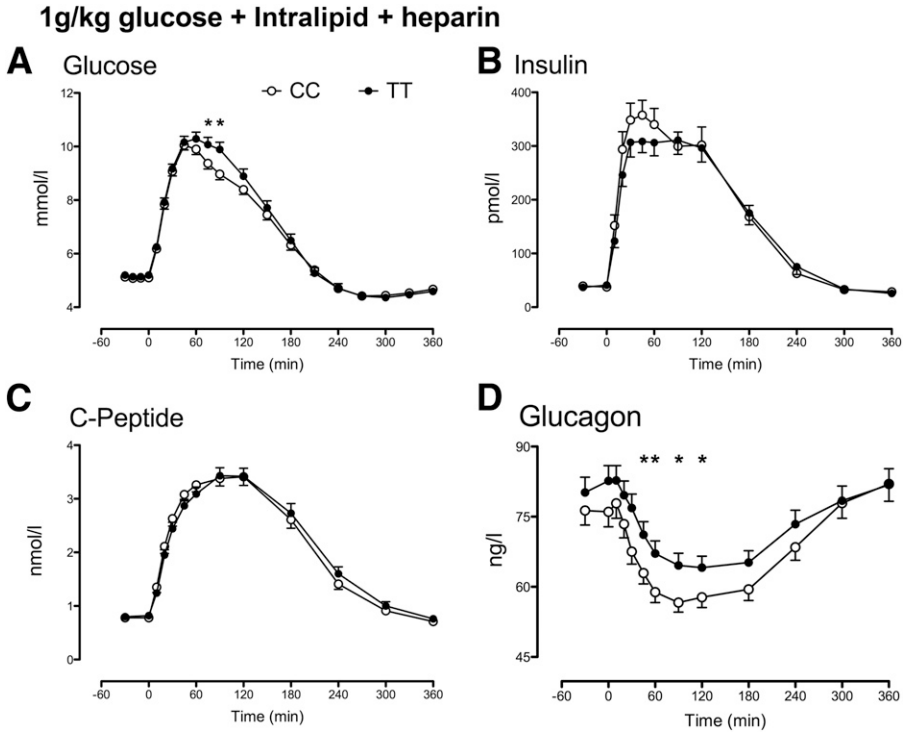

Figure 3—Glucose (*A*), insulin (*B*), C-peptide (*C*), and glucagon (*D*) concentrations in response to a 1 g/kg body weight glucose challenge with accompanying infusion of Intralipid and heparin in subjects with the CC and TT genotypes. Data are mean ± SEM. *$P < 0.05$ for a post hoc unpaired, two-tailed *t* test.

genotype group during the GLY and FFA studies by using a population instead of an individual approach as previously described (20) (Fig. 5). This approach includes an exponential parameter α to accommodate the (shape of the) relationship between insulin secretion and β-cell responsivity, where $DI = \Phi \cdot S_i^{\alpha}$. The DI during the GLY study was significantly decreased in the TT genotype group compared with the CC genotype group (931 ± 76 vs. $188 \pm 8\ 10^{-14}$ dL/kg/min^2 per pmol/L, $P < 0.001$). FFA elevation resulted in a significant decrease in DI in the CC group but in no change in the TT group so that DI did not differ between groups during the FFA study (209 ± 8 vs. $206 \pm 9\ 10^{-14}$ dL/kg/min^2 per pmol/L, *P* not significant). Differences in the exponent α (a parameter of the power function law describing insulin secretion and action [$DI = \Phi \cdot S_i^{\alpha}$]) were evident during the GLY study (1.16 ± 0.03 vs. 0.61 ± 0.01, $P < 0.001$) but to a lesser extent during the FFA study (0.72 ± 0.02 vs. 0.78 ± 0.02, $P = 0.04$) between CC and TT genotype groups, respectively (Supplementary Fig. 2).

Plasma Glucagon Concentrations in Subjects Classified by Glucose Tolerance Status

When classified by glucose tolerance status, fasting and nadir postprandial glucagon concentrations did not differ between subjects with CC and TT genotypes who had normal glucose tolerance during either the GLY (Fig. 6*A*) or the FFA (Fig. 6*C*) study. In contrast, in subjects with impaired glucose tolerance, glucose ingestion resulted in less suppression of glucagon in subjects with the TT genotype as represented by nadir values during either the GLY (51 ± 3 vs. 58 ± 3 ng/L, $P = 0.02$) (Fig. 6*A*) or the FFA (54 ± 3 vs. 64 ± 3 ng/L, $P = 0.05$) (Fig. 6*C*) study.

DISCUSSION

The *TCF7L2* T allele at rs7903146 is reproducibly associated with type 2 diabetes in various populations (22). The mechanism is uncertain, with some studies reporting that risk genotype is associated with defective insulin secretion (2,23–25). Some studies have reported an association with a decreased insulin action (8,26), and others have reported both (27). The present studies measured insulin secretion and insulin action in a large number of subjects with the TT genotype on three separate occasions and compared the results to those observed in carefully matched subjects with the CC genotype. On one occasion, subjects underwent a typical 2-h 75-g OGTT. On another occasion, they received a 1 g/kg oral glucose challenge during FFA-induced acute insulin resistance (by using an infusion of Intralipid and heparin). Finally, the subjects were studied after an identical challenge in the presence of glycerol infusion (to control for the glycerol infused with Intralipid). We reasoned that a modest effect of the TT phenotype on β-cell function only becomes evident when insulin secretory reserve is challenged by a decrease in insulin action.

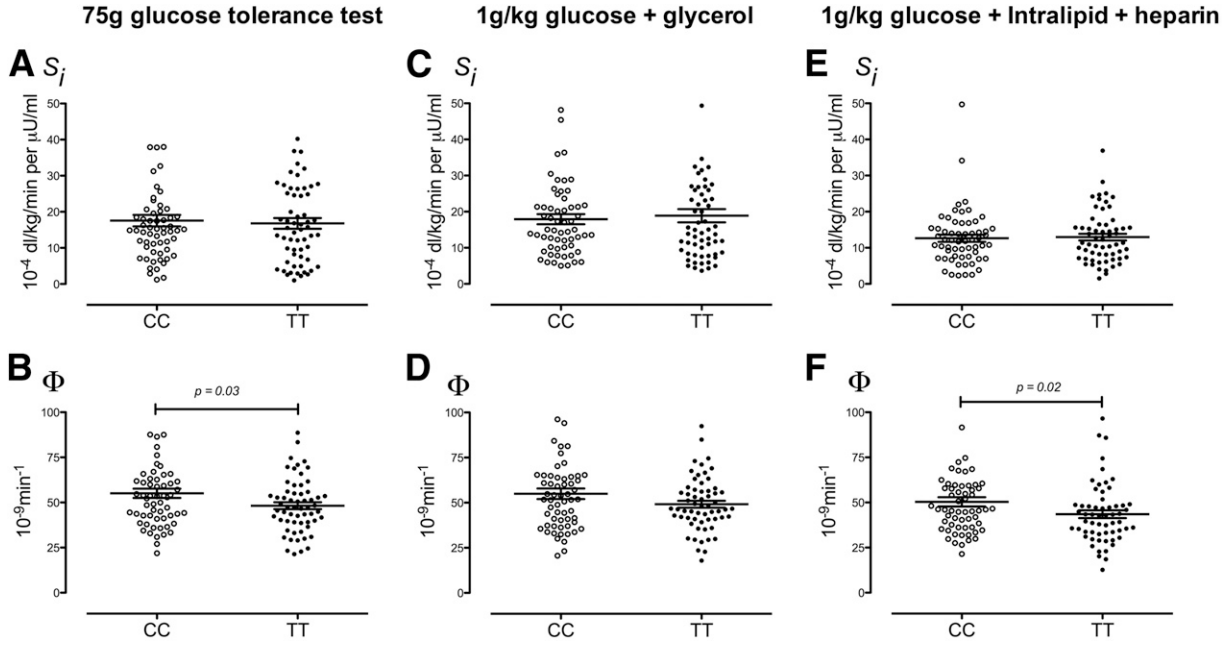

Figure 4—S_i (*A*, *C*, and *E*) and Φ (*B*, *D*, and *F*) in response to a 75-g oral glucose challenge (*A* and *B*), in response to a 1 g/kg body weight glucose challenge with accompanying glycerol infusion (*C* and *D*), and in response to a 1 g/kg body weight glucose challenge with accompanying infusion of Intralipid and heparin (*E* and *F*) in subjects with the CC and the TT genotypes.

In response to a 75-g OGTT, the glycemic excursion was greater in subjects with the TT genotype. This was accompanied by insulin concentrations that did not differ from those in subjects with the CC genotype, implying an inadequate secretory response for the prevailing glucose concentrations. Indeed, corrected insulin response, a commonly used surrogate of insulin secretion in genetic association studies, was lower in the TT group (Supplementary Table 3) in response to 75 g of glucose. The dynamic component of β-cell responsivity to glucose (believed to represent the secretion of preformed insulin secretory granules in response to rising glucose concentrations) as well as total β-cell responsivity (5) was slightly, but significantly decreased in the TT group (Fig. 3 and Supplementary Table 3). Insulin action was unaffected by genotype. Of note, there was an unexpected subtle impairment in glucagon suppression in subjects homozygous for the diabetes-associated allele of rs7903146.

An acute decrease in insulin action produced by FFA elevation worsened glucose tolerance comparably in both genotype groups. However, fasting and postprandial glucagon concentrations during these conditions were higher in the TT group (Supplementary Table 1). These differences in glucagon concentrations were accompanied by decreased β-cell responsivity to glucose during FFA elevation in the TT group (Fig. 3 and Supplementary Table 3). This was due to differences in both the static and the dynamic contribution to β-cell responsivity during the experimental conditions. Overall, these data suggest that impaired insulin secretion and impaired suppression of glucagon secretion function contribute to the diabetes predisposition conferred by *TCF7L2*. DI (which reflects the appropriateness of the β-cell response in light of the prevailing insulin action) differed significantly between genotype groups during the GLY study largely due to subtle differences in the hyperbolic relationship between insulin secretion and insulin action (Supplementary Fig. 3).

Net insulin action during all three studies did not differ in the TT and CC genotype groups. As anticipated, the Intralipid plus heparin infusion elevated plasma FFA and decreased insulin action. Of note, the increment in FFA and the accompanying decrement in insulin action did not differ in the TT and CC groups. Together, these observations imply that the TT genotype does not alter insulin action and does not exacerbate the fall in insulin action produced by an acute increase in FFA. These data argue against impaired insulin action as the mechanism by which the *TCF7L2* TT genotype predisposes to the development of type 2 diabetes.

Glucagon is not suppressed or may increase paradoxically after glucose ingestion in people with type 2 diabetes (28). In addition, although lack of glucagon suppression does not alter glucose tolerance when insulin secretion is intact (29,30), it can cause substantial postprandial hyperglycemia when insulin secretion is reduced or delayed (30,31). Therefore, the impaired suppression of glucagon in the TT relative to the CC genotype is particularly intriguing. This pattern is consistent with our previous observation that suppression of glucagon is lower in subjects with the TT genotype during a hyperglycemic clamp. Of particular interest, suppression of glucagon

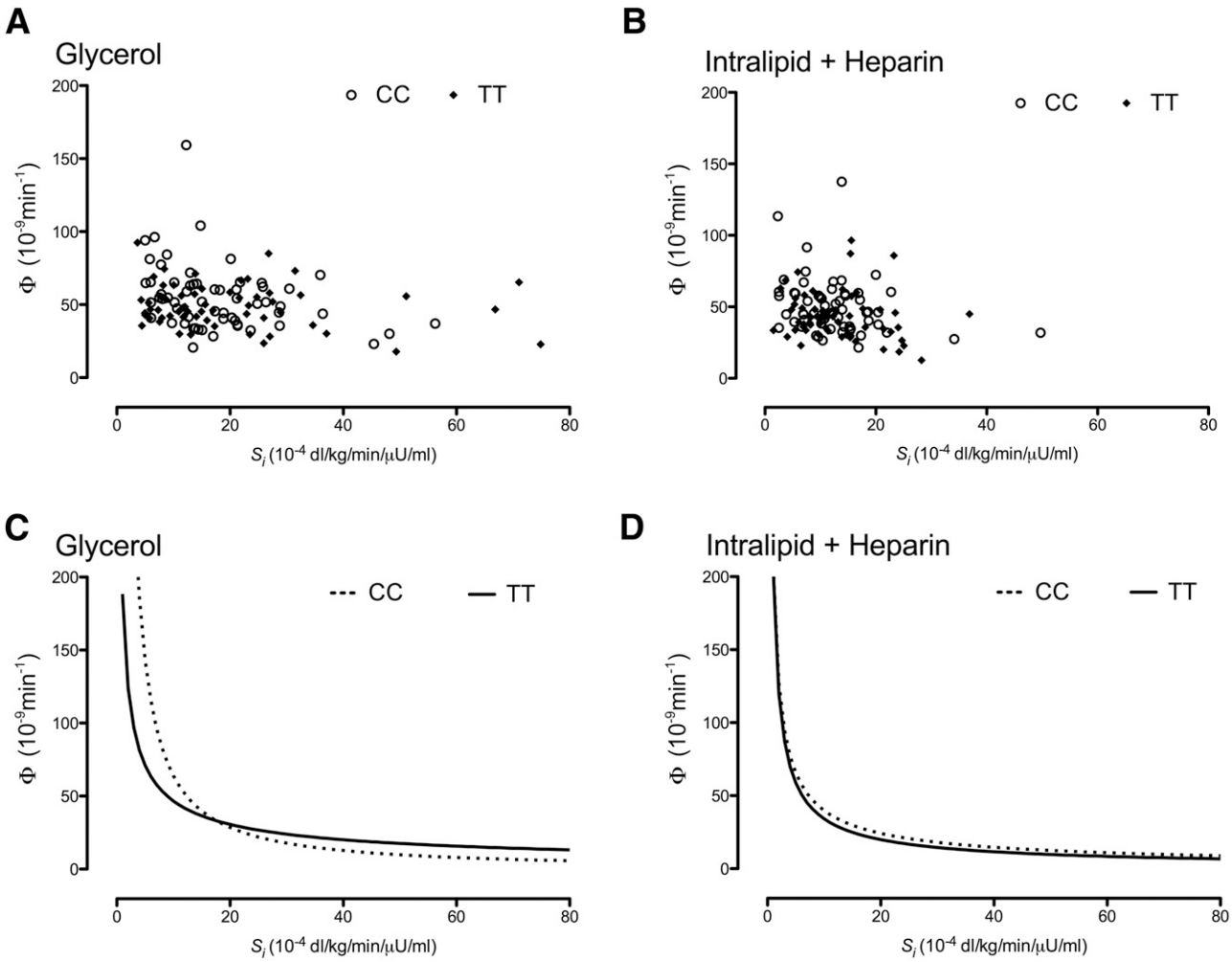

Figure 5—Relationship of Φ and S_i in subjects with the CC or TT genotypes during glycerol infusion (*A*) and during Intralipid and heparin infusion (*B*). The hyperbolic relationship for the two parameters during glycerol infusion (*C*) and Intralipid and heparin infusion (*D*) is shown.

during that experiment did not differ in subjects with the CT and CC genotypes (32). As in the current series of experiments, Lyssenko et al. (33) reported no effect of *TCF7L2* on fasting glucagon concentrations. Smushkin et al. (32) and Færch et al. (34) reported a trend suggesting impaired suppression of glucagon in subjects with the diabetes-associated allele. However, the small numbers of subjects mean that those studies may have been underpowered to detect a subtle abnormality in glucagon suppression. The current large cohorts used to study the effect of *TCF7L2* on diabetes predisposition did not report postprandial glucagon concentrations.

Defects in glucagon secretion are seen in prediabetes (35) and early in the course of type 1 diabetes, suggesting that subtle defects in insulin secretion also contribute to α-cell dysregulation (36). In the present cohort, post hoc examination of glucagon concentrations in subjects classified by glucose tolerance status at the time of screening suggests that genotype differences in postchallenge glucagon concentrations are most apparent in the group with impaired glucose tolerance, despite no apparent differences in insulin and C-peptide concentrations or β-cell responsivity (Supplementary Figs. 3 and 4) between genotypes of subjects with impaired glucose tolerance. Although impaired glucose tolerance is associated with impaired β-cell function (9), in the present cohort, groups discordant for genotype at rs7903146 differed in their ability to suppress glucagon, despite similar indices of β-cell function.

Of note, intravenous but not oral glucose suppresses glucagon in type 2 diabetes, leading to speculation that enteral signaling to the α-cells contributes to the pathogenesis of this disease (37). Glucagon arises from post-translational processing of proglucagon within the α-cell through the actions of prohormone convertase 2. On the other hand, prohormone convertase 1 processes proglucagon to produce GLP-1, an incretin hormone produced by enteroendocrine cells that produces glucose-dependent stimulation of insulin secretion and suppression of glucagon (38). Indeed, TCF7L2 acts as a regulator of proglucagon

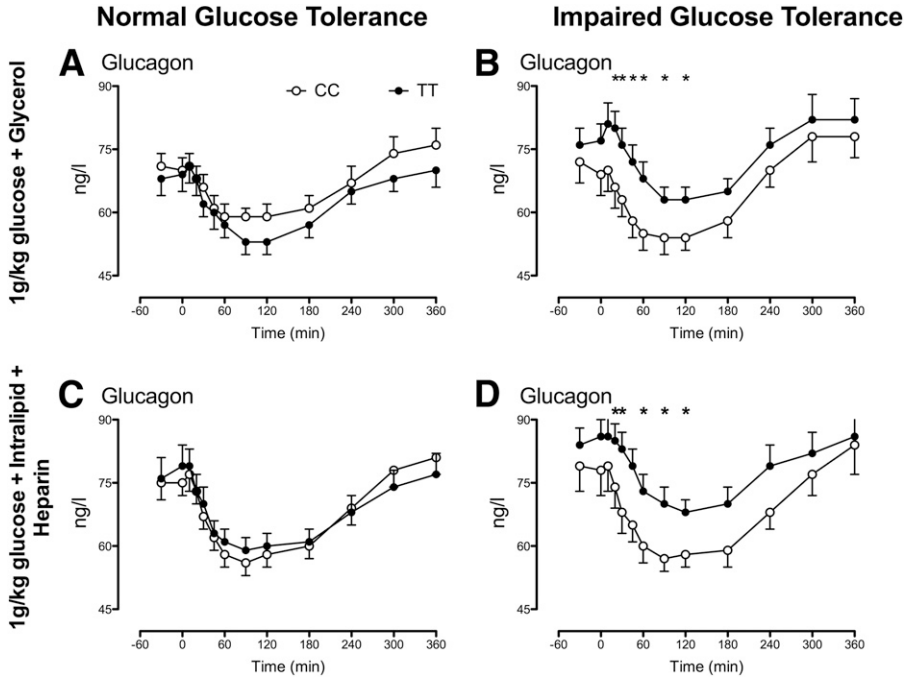

Figure 6—Glucagon concentrations in response to 1 g/kg oral glucose challenge during glycerol (*A* and *B*) and Intralipid and heparin (*C* and *D*) infusion in subjects with normal (*A* and *C*) and impaired (*B* and *C*) glucose tolerance at the time of screening OGTT. Data are mean ± SEM. *$P < 0.05$ for a post hoc unpaired, two-tailed *t* test.

expression in the gut, promoting synthesis and secretion of GLP-1 (39).

The possibility that *TCF7L2* predisposes to diabetes through effects on GLP-1 secretion was raised at the time of association of this locus with diabetes (40). Indeed, other investigators suggested that the diabetes-associated allele decreased responsivity to exogenous GLP-1 in humans without diabetes (7,41). However, an effect of *TCF7L2* on GLP-1 secretion (based on GLP-1 concentrations in the peripheral circulation) and action was not borne out in a larger study that used quantitative measures of insulin secretion in response to GLP-1 (32). Lyssenko et al. (33) reported that diabetes is associated with increased *TCF7L2* mRNA in islets, which is inversely correlated with glucose-stimulated insulin secretion. On the other hand, in a separate experiment, type 2 diabetes was associated with decreased islet TCF7L2 expression as well as with downregulation of islet incretin receptors (42). Islets obtained from humans with the TT genotype at rs7903146 exhibited a relative increase in α-cells and glucagon immunoreactivity (43), which supports the present findings.

By design, these experimental interventions were acute and may not replicate β-cell secretory response to a chronic decrease in insulin action produced by obesity or other chronic environmental influences. However, direct model-based measures of β-cell function during an intravenous glucose tolerance test (8,25,27,33,44) or insulin stimulation by arginine (33) have suggested an impairment of β-cell function attributable to the diabetes-associated allele of *TCF7L2*. The current study supports these conclusions to some extent. Diabetes-associated genetic variation in *TCF7L2* has been associated with elevated fasting glucose concentrations in subjects without diabetes (3). However, in the present cohort, genotype groups were matched for fasting glucose concentrations, which may have minimized genotype-attributable differences in β-cell function because subjects with higher fasting glucose concentrations (45) and subjects with both impaired fasting glucose and impaired glucose tolerance exhibit a decreased DI compared with subjects with normal fasting glucose and normal glucose tolerance (9).

Another limitation is that we only studied subjects homozygous for the diabetes-associated or diabetes-protective allele at rs7903146 to maximize *TCF7L2*-attributable differences in β-cell function (2). Whether the effects on α-cell function will be observed in subjects heterozygous for the T allele at rs7903146 remains to be determined.

FFA directly affects insulin secretion through the GPR40 receptor (46,47), likely explaining the small, but significant changes in fasting glucose, insulin, and C-peptide concentrations during FFA elevation (Supplementary Table 2). Theoretically, this could obscure differences in β-cell responsivity attributable to genotype. However, the experimental conditions clearly impaired insulin action, with evident differences in β-cell responsivity during FFA elevation. Of note, the between-group differences observed in response to a 75-g OGTT and during FFA elevation were not clearly demonstrated during the GLY experiment. We are unaware of evidence to suggest that glycerol might have salutary effects on β-cell function. Overnight admission

and standardization of dietary intake also may have obscured the effects of lifestyle in subjects with the TT genotype that may have been evident at the time of screening and during FFA elevation.

We conclude that the diabetes-associated allele of *TCF7L2* is associated with impaired postprandial suppression of glucagon, suggesting an additional mechanism by which *TCF7L2* predisposes to type 2 diabetes. Whether this can be explained by alterations in the synthesis of glucagon or other proglucagon derivatives and their signaling pathways remains to be ascertained (42,48). Determining the temporal relationship, if any, between *TCF7L2* genotype and defects in glucagon suppression and β-cell responsivity in a longitudinal study examining progression of glucose tolerance status in subjects without diabetes also will be important.

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Duality of Interest. A.V. is an investigator in multicenter studies sponsored by Novartis and GI Dynamics. He has consulted for XOMA, Sanofi, Novartis, and Bristol-Myers Squibb in the past 5 years. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. M.S. and R.T.V. researched data and conducted the studies. J.M.M. measured FFA concentrations. F.P. and C.D.M. contributed to the mathematical modeling of insulin secretion and action. C.C. reviewed and edited the manuscript. K.R.B. oversaw statistical analyses. R.A.R. contributed to discussion and reviewed and edited the manuscript. A.V. designed the study, oversaw its conduct, researched data, 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

1. Talmud PJ, Cooper JA, Morris RW, et al.; UCLEB Consortium. Sixty-five common genetic variants and prediction of type 2 diabetes. Diabetes 2015;64:1830–1840

2. Florez JC, Jablonski KA, Bayley N, et al.; Diabetes Prevention Program Research Group. TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. N Engl J Med 2006;355:241–250

3. Saxena R, Hivert MF, Langenberg C, et al.; GIANT Consortium; MAGIC Investigators. Genetic variation in GIPR influences the glucose and insulin responses to an oral glucose challenge. Nat Genet 2010;42:142–148

4. Waldhäusl W, Bratusch-Marrain P, Gasic S, Korn A, Nowotny P. Insulin production rate following glucose ingestion estimated by splanchnic C-peptide output in normal man. Diabetologia 1979;17:221–227

5. Cobelli C, Dalla Man C, Toffolo G, Basu R, Vella A, Rizza R. The oral minimal model method. Diabetes 2014;63:1203–1213

6. Xiang AH, Watanabe RM, Buchanan TA. HOMA and Matsuda indices of insulin sensitivity: poor correlation with minimal model-based estimates of insulin sensitivity in longitudinal settings. Diabetologia 2014;57:334–338

7. Pilgaard K, Jensen CB, Schou JH, et al. The T allele of rs7903146 TCF7L2 is associated with impaired insulinotropic action of incretin hormones, reduced 24 h profiles of plasma insulin and glucagon, and increased hepatic glucose production in young healthy men. Diabetologia 2009;52:1298–1307

8. Elbein SC, Chu WS, Das SK, et al. Transcription factor 7-like 2 polymorphisms and type 2 diabetes, glucose homeostasis traits and gene expression in US participants of European and African descent. Diabetologia 2007;50:1621–1630

9. Bock G, Dalla Man C, Campioni M, et al. Pathogenesis of pre-diabetes: mechanisms of fasting and postprandial hyperglycemia in people with impaired fasting glucose and/or impaired glucose tolerance. Diabetes 2006;55:3536–3549

10. Cali AM, Man CD, Cobelli C, et al. Primary defects in beta-cell function further exacerbated by worsening of insulin resistance mark the development of impaired glucose tolerance in obese adolescents. Diabetes Care 2009;32:456–461

11. Utzschneider KM, Prigeon RL, Faulenbach MV, et al. Oral disposition index predicts the development of future diabetes above and beyond fasting and 2-h glucose levels. Diabetes Care 2009;32:335–341

12. Boden G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 1997;46:3–10

13. Shah P, Vella A, Basu A, et al. Effects of free fatty acids and glycerol on splanchnic glucose metabolism and insulin extraction in nondiabetic humans. Diabetes 2002;51:301–310

14. Miles J, Glasscock R, Aikens J, Gerich J, Haymond M. A microfluorometric method for the determination of free fatty acids in plasma. J Lipid Res 1983;24:96–99

15. Miles JM, Ellman MG, McClean KL, Jensen MD. Validation of a new method for determination of free fatty acid turnover. Am J Physiol 1987;252:E431–E438

16. Dalla Man C, Caumo A, Basu R, Rizza R, Toffolo G, Cobelli C. Minimal model estimation of glucose absorption and insulin sensitivity from oral test: validation with a tracer method. Am J Physiol Endocrinol Metab 2004;287:E637–E643

17. Breda E, Cavaghan MK, Toffolo G, Polonsky KS, Cobelli C. Oral glucose tolerance test minimal model indexes of beta-cell function and insulin sensitivity. Diabetes 2001;50:150–158

18. Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes 1992;41:368–377

19. Cobelli C, Man CD, Sparacino G, Magni L, De Nicolao G, Kovatchev BP. Diabetes: models, signals, and control. IEEE Rev Biomed Eng 2009;2:54–96

20. Denti P, Toffolo GM, Cobelli C. The disposition index: from individual to population approach. Am J Physiol Endocrinol Metab 2012;303:E576–E586

21. Cole TJ. Sympercents: symmetric percentage differences on the 100 log(e) scale simplify the presentation of log transformed data. Stat Med 2000;19:3109–3125

22. Helgason A, Pálsson S, Thorleifsson G, et al. Refining the impact of TCF7L2 gene variants on type 2 diabetes and adaptive evolution. Nat Genet 2007;39:218–225

23. Wegner L, Hussain MS, Pilgaard K, et al. Impact of TCF7L2 rs7903146 on insulin secretion and action in young and elderly Danish twins. J Clin Endocrinol Metab 2008;93:4013–4019

24. Kirchhoff K, Machicao F, Haupt A, et al. Polymorphisms in the TCF7L2, CDKAL1 and SLC30A8 genes are associated with impaired proinsulin conversion. Diabetologia 2008;51:597–601

25. Ingelsson E, Langenberg C, Hivert MF, et al.; MAGIC Investigators. Detailed physiologic characterization reveals diverse mechanisms for novel genetic loci regulating glucose and insulin metabolism in humans. Diabetes 2010;59:1266–1275

26. Liu PH, Chang YC, Jiang YD, et al. Genetic variants of TCF7L2 are associated with insulin resistance and related metabolic phenotypes in Taiwanese adolescents and Caucasian young adults. J Clin Endocrinol Metab 2009;94:3575–3582

27. 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;55:2654–2659

28. Butler PC, Rizza RA. Contribution to postprandial hyperglycemia and effect on initial splanchnic glucose clearance of hepatic glucose cycling in glucose-intolerant or NIDDM patients. Diabetes 1991;40:73–81

29. Frank JW, Camilleri M, Thomforde GM, Dinneen SF, Rizza RA. Effects of glucagon on postprandial carbohydrate metabolism in nondiabetic humans. Metabolism 1998;47:7–12

30. Shah P, Basu A, Basu R, Rizza R. Impact of lack of suppression of glucagon on glucose tolerance in humans. Am J Physiol 1999;277:E283–E290

31. Shah P, Vella A, Basu A, Basu R, Schwenk WF, Rizza RA. Lack of suppression of glucagon contributes to postprandial hyperglycemia in subjects with type 2 diabetes mellitus. J Clin Endocrinol Metab 2000;85:4053–4059
32. Smushkin G, Sathananthan M, Sathananthan A, et al. Diabetes-associated common genetic variation and its association with GLP-1 concentrations and response to exogenous GLP-1. Diabetes 2012;61:1082–1089
33. Lyssenko V, Lupi R, Marchetti P, et al. Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. J Clin Invest 2007;117:2155–2163
34. Færch K, Pilgaard K, Knop FK, et al. Incretin and pancreatic hormone secretion in Caucasian non-diabetic carriers of the TCF7L2 rs7903146 risk T allele. Diabetes Obes Metab 2013;15:91–95
35. Vollmer K, Holst JJ, Baller B, et al. Predictors of incretin concentrations in subjects with normal, impaired, and diabetic glucose tolerance. Diabetes 2008;57:678–687
36. Greenbaum CJ, Prigeon RL, D'Alessio DA. Impaired beta-cell function, incretin effect, and glucagon suppression in patients with type 1 diabetes who have normal fasting glucose. Diabetes 2002;51:951–957
37. Bagger JI, Knop FK, Lund A, Holst JJ, Vilsbøll T. Glucagon responses to increasing oral loads of glucose and corresponding isoglycaemic intravenous glucose infusions in patients with type 2 diabetes and healthy individuals. Diabetologia 2014;57:1720–1725
38. Kieffer TJ, Habener JF. The glucagon-like peptides. Endocr Rev 1999;20:876–913
39. Yi F, Brubaker PL, 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
40. 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;38:320–323
41. Schäfer SA, Tschritter O, Machicao F, et al. Impaired glucagon-like peptide-1-induced insulin secretion in carriers of transcription factor 7-like 2 (TCF7L2) gene polymorphisms [published corrections appear in Diabetologia 2008;51:208 and Diabetologia 2009;52:557]. Diabetologia 2007;50:2443–2450
42. Shu L, Matveyenko AV, Kerr-Conte J, Cho JH, McIntosh CH, Maedler K. Decreased TCF7L2 protein levels in type 2 diabetes mellitus correlate with downregulation of GIP- and GLP-1 receptors and impaired beta-cell function. Hum Mol Genet 2009;18:2388–2399
43. Le Bacquer O, Kerr-Conte J, Gargani S, et al. TCF7L2 rs7903146 impairs islet function and morphology in non-diabetic individuals. Diabetologia 2012;55:2677–2681
44. Munoz J, Lok KH, Gower BA, et al. Polymorphism in the transcription factor 7-like 2 (TCF7L2) gene is associated with reduced insulin secretion in non-diabetic women. Diabetes 2006;55:3630–3634
45. Sathananthan A, Dalla Man C, Zinsmeister AR, et al. A concerted decline in insulin secretion and action occurs across the spectrum of fasting and post-challenge glucose concentrations. Clin Endocrinol (Oxf) 2012;76:212–219
46. Brownlie R, Mayers RM, Pierce JA, Marley AE, Smith DM. The long-chain fatty acid receptor, GPR40, and glucolipotoxicity: investigations using GPR40-knockout mice. Biochem Soc Trans 2008;36:950–954
47. Nolan CJ, Madiraju MSR, Delghingaro-Augusto V, Peyot M-L, Prentki M. Fatty acid signaling in the beta-cell and insulin secretion. Diabetes 2006;55(Suppl. 2):S16–S23
48. Smith EP, An Z, Wagner C, et al. The role of β cell glucagon-like peptide-1 signaling in glucose regulation and response to diabetes drugs. Cell Metab 2014;19:1050–1057