The T-Allele of TCF7L2 rs7903146 Associates With a Reduced Compensation of Insulin Secretion for Insulin Resistance Induced by 9 Days of Bed Rest

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OBJECTIVE—The aim of this study was to determine whether the type 2 diabetes–associated T-allele of transcription factor 7-like 2 (TCF7L2) rs7903146 associates with impaired insulin secretion to compensate for insulin resistance induced by bed rest.

RESEARCH DESIGN AND METHODS—A total of 38 healthy young Caucasian men were studied before and after bed rest using the hyperinsulinemic-euglycemic clamp technique combined with indirect calorimetry preceded by an intravenous glucose tolerance test. The TCF7L2 rs7903146 was genotyped using allelic discrimination performed with an ABI 7900 system. The genetic analyses were done assuming a dominant model of inheritance.

RESULTS—The first-phase insulin response (FPIR) was significantly lower in carriers of the T-allele compared with carriers of the CC genotype before bed rest, with and without correction for insulin resistance. The incremental rise of FPIR in response to insulin resistance induced by bed rest was lower in carriers of the T-allele compared with carriers of the CC genotype after bed rest. While carriers of the CC genotype developed increased hepatic insulin resistance, the TCF7L2 rs7903146 did not influence peripheral insulin action or the rate of lipolysis before or after bed rest.

CONCLUSIONS—Healthy carriers of the T-allele of TCF7L2 rs7903146 exhibit a diminished increase of insulin secretion in response to intravenous glucose to compensate for insulin resistance as induced by bed rest. Reduced paracrine glucagon stimulation may contribute to the impairment of β-cell function in the carriers TCF7L2 rs7903146 T-allele associated with increased risk of type 2 diabetes. Diabetes 59:836–843, 2010

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RESEARCH DESIGN AND METHODS—A total of 38 healthy young Caucasian men completed the study. Subjects were recruited from a cohort of young men with low birth weight and normal birth weight via the Danish National Birth Registry. Low birth weight was defined as the lowest 10 percentile, and the control subjects were recruited from the 50–75 birth weight percentiles. Twenty-one subjects were carriers of the T-allele of TCF7L2 rs7903146 (combined TT and CT alleles), and 17 subjects...
FIG. 1. Schematic presentation of the experimental day(s). Whole-body glucose metabolism was measured by a hyperinsulinemic-euglycemic clamp technique. Steady-state measurements of plasma glucose and plasma glycerol enrichments were performed during the basal period (before the insulin stimulation) to determine hepatic glucose production and whole-body lipolysis rate. Arrows show points for collecting of blood samples for basal-state determination of stable isotope kinetics.

The disposition index expressing the inverse hyperbolic relationship between insulin secretion and insulin action is an estimate of the “true” in vivo pancreatic β-cell insulin secretion capacity. The peripheral disposition index ($D_{\text{peripheral}}$) was calculated as (FPIR × M) and the hepatic disposition index ($D_{\text{hepatic}}$) as (FPIR/hepatic insulin resistance [HHR]). The HIR was calculated as the product of fasting plasma insulin concentration and the basal hepatic glucose production as previously described (25).

Stable isotope tracer calculations. Tracer-to-tracee ratios for both glucose and glycerol were calculated as previously described (20).

Statistics. Statistical analysis was performed with the SAS statistical analysis package (version 9.1, SAS Institute, Cary, NC). Due to the low number of subjects, TT and CT genotypes were pooled and a dominant model was applied. The selection of the dominant model for this locus is arbitrary and one of convenience, as most of the evidence published on rs7903146 indicates an additive model of risk transmission. One-way ANOVA analyses were performed to test for differences between groups before and after bed rest. The paired-sample t test was used to test statistically significant differences within groups in response to bed rest. The Kolmogorov-Smirnov test was used to test whether data were normally distributed and whether logarithmic transformation of non–normally distributed data rendered them normally distributed. P values of <0.05 were considered as significant, and data are presented as means ± SD. In a post hoc power calculation with the first-phase insulin secretion data as the end point, we had an 80% chance of detecting differences between carriers of the T-allele and carriers of the CC genotype of ~66% after bed rest with a group 1 size of 21 subjects and a group 2 size of 17 subjects.

RESULTS

Clinical characteristics of study participants. As presented in Table 1, we found no significant differences in age, weight, height, systolic and diastolic blood pressure, maximal oxygen uptake ($V_{O_2,max}$), total fat mass, fat percentage, trunk fat mass—total fat mass ratio, leg fat mass—total fat mass ratio, plasma triglycerides, plasma cholesterol, and plasma concentration of other lipoproteins between TT/CT carriers and CC carriers prior to bed rest. We demonstrated a significant decrease in $V_{O_2,max}$, plasma total cholesterol, and plasma HDL concentrations in carriers of the CC but not the TT/CT genotypes following 9 days of bed rest. However, we observed a similar BMI in carriers of the T-allele compared with carriers of the CC genotype before and after bed rest.

No differences were observed between TT/CT and CC carriers with regard to age, weight, height, systolic and diastolic blood pressure, $V_{O_2,max}$, total fat mass, fat percentage, trunk fat mass—total fat mass ratio, leg fat
mass–to–total fat mass or plasma concentrations of triglycerides, total cholesterol, LDL, and VLDL after bed rest. **Impact of the T-allele of rs7903146 on insulin secretion during IVGTT.** As presented in Table 2, first-phase insulin secretion (FPIR) was significantly diminished in carriers of the T-allele compared with carriers of the CC genotype before as well as after 9 days of bed rest. The decreased FPIR in carriers of the T-allele remained significant after correction for BMI before \( (P = 0.01) \) and after \( (P = 0.0001) \) bed rest. We showed a significantly lower insulin secretion during the time period from 10 to 30 min after glucose infusion \( (i.e., \text{AUC}_{10-30 \text{ min}}) \), to some extent reflecting a reduced late or second-phase insulin secretion in carriers of the T-allele compared with carriers of the CC genotype after bed rest \( (P < 0.001) \). The estimates of second-phase insulin secretion data are included also in Table 2. The AUC during IVGTT, \( \text{AUC}_{0-10 \text{ min}} \), for plasma insulin and C-peptide were similar in TT/CT and CC genotype carriers before bed rest \( (\text{Fig. 2}) \). However, we unmasked a significantly lower the AUC during IVGTT, \( \text{AUC}_{0-10 \text{ min}} \), for plasma insulin and C-peptide in carriers of the T-allele compared with carriers of the CC genotype after bed rest \( (\text{Fig. 3}) \).

Fasting plasma insulin and C-peptide levels were significantly decreased in the TT/CT genotype as compared with the CC genotype carriers on day 9 during the bed rest experiments \( (\text{Fig. 4}) \). Furthermore, carriers of the T-allele had significantly reduced fasting plasma glucagon concentrations in the basal state before and after bed rest compared with carriers of the CC allele, as presented in Table 2. FPIR increased significantly in response to bed rest in both groups, but the increment in FPIR was significantly lower in carriers of the T-allele compared with carriers of the CC genotype \( (P < 0.001) \).

The total AUC \( (\text{AUC}_{\text{total}}) \) was significantly lower in carriers of the T-allele compared with carriers of the CC genotype after bed rest. Furthermore, in response to bed rest we demonstrated a significant increase in \( \text{AUC}_{\text{total}} \) in CC carriers but not in carriers of the risk T-allele (Table 2).

### TABLE 1
Clinical characteristics of male study participants according to the TCF7L2 rs7903146 genotype before and after bed rest

| rs7903146 | Before bed rest | After bed rest |
|-----------|----------------|---------------|
|           | TT/CT          | CC            | TT/CT          | CC            |
| n         | 21             | 17            | 21             | 17            |
| Age (years) | 25.6 ± 2.0    | 25.2 ± 2.1    | 25.6 ± 2.0    | 25.2 ± 2.1    |
| Weight (kg) | 75.6 ± 11.7   | 82.4 ± 11.8   | 76.8 ± 10.4   | 81.5 ± 12.2   |
| Height (m)  | 1.81 ± 0.06    | 1.82 ± 0.06   | 1.81 ± 0.06   | 1.82 ± 0.06   |
| BMI (kg/m²) | 23.0 ± 2.7     | 24.4 ± 3.1    | 23.2 ± 2.5    | 24.5 ± 3.0    |
| Vo₂_max (ml·min⁻¹·kg⁻¹) | 43.0 ± 6.8     | 43.7 ± 7.8    | 42.9 ± 7.1    | 40.7 ± 7.8*   |
| Systolic blood pressure (mmHg) | 125 ± 10     | 129 ± 14      | 124 ± 11      | 127 ± 10      |
| Diastolic blood pressure (mmHg) | 69 ± 8       | 70 ± 10       | 70 ± 5        | 70 ± 8        |
| Waist-to-hip ratio | 0.85 ± 0.05    | 0.86 ± 0.05   | 0.85 ± 0.06   | 0.87 ± 0.06   |
| Total fat mass (kg) | 12.6 ± 6.6     | 16.7 ± 8.3    | 12.9 ± 6.9    | 17.2 ± 8.8    |
| Whole-body fat percentage (%) | 16.5 ± 6.5     | 19.8 ± 7.5    | 16.2 ± 6.6    | 20.3 ± 7.8    |
| Trunk fat mass-to-total fat mass ratio | 0.50 ± 0.04    | 0.51 ± 0.04   | 0.50 ± 0.05   | 0.52 ± 0.05   |
| Leg fat mass-to-total fat mass ratio† | 0.36 ± 0.04    | 0.35 ± 0.03   | 0.35 ± 0.04   | 0.35 ± 0.04   |
| Percent trunk fat mass-to-leg fat mass ratio | 1.41 ± 0.29    | 1.48 ± 0.30   | 1.46 ± 0.35   | 1.53 ± 0.33   |
| Triglycerides (mmol/l) | 0.9 ± 0.3      | 1.1 ± 0.9     | 0.9 ± 0.3     | 1.2 ± 0.6     |
| Cholesterol (mmol/l) | 3.8 ± 0.6      | 4.2 ± 1.0     | 3.8 ± 1.0     | 3.8 ± 1.0*    |
| HDL (mmol/l) | 1.2 ± 0.2      | 1.3 ± 0.5     | 1.2 ± 0.2     | 1.1 ± 0.4*    |
| LDL (mmol/l) | 2.1 ± 0.5      | 2.3 ± 1.0     | 2.2 ± 0.6     | 2.0 ± 0.6*    |
| VLDL (mmol/l) | 0.4 ± 0.2      | 0.5 ± 0.4     | 0.4 ± 0.2     | 0.5 ± 0.3     |

Data are means ± SD. *Significant difference before versus after bed rest; \( P < 0.05 \). †Log-transformed data.

Impact of the T-allele of rs7903146 on hepatic and peripheral insulin sensitivity. As seen in Table 2, fasting plasma insulin and glucose concentrations were similar between genotypes before as well as after bed rest. The peripheral (muscle) insulin action, measured by the hyperinsulinemic-euglycemic clamp, was significantly decreased in response to bed rest without differences between the genotype groups. Despite decreased peripheral insulin action in all subjects in response to bed rest, carriers of the T-allele tended to be more insulin sensitive after bed rest as determined by the homeostasis model assessment (HOMA) index. Furthermore, carriers of the T-allele demonstrated a similar decrease in the \( M \) value, as measured by the hyperinsulinemic-euglycemic clamp technique, compared with carriers of the CC genotype.

Estimates of insulin secretion adjusted for peripheral insulin sensitivity \( (i.e., \text{peripheral and hepatic disposition index}) \) were lower in carriers of the T-allele before bed rest. Notably, the peripheral disposition index, but not the hepatic disposition index, was also significantly decreased in carriers of the T-allele compared with carriers of the CC genotype after bed rest.

As presented in Table 3, carriers of the CC genotype, but not carriers of the T-allele, developed a significant increase in the HIR index \( (P < 0.01) \) in response to bed rest. However, we found no significant differences in rate of appearance of glucose and glycerol between genotype groups neither before nor after bed rest. **Impact of the T-allele of rs7903146 on gaseous exchange measurements.** Basal glucose oxidation increased and basal fat oxidation decreased significantly in response to bed rest in the both genotype groups as presented in Table 2. The glucose and fat oxidation rates during insulin infusion were not significantly affected by bed rest in any of the genotype groups. The insulin-stimulated nonoxidative glucose metabolism decreased significantly in carriers of the CC genotype as well as in carriers of the T-allele \( (P < 0.01) \), with no differences between the groups before and after bed rest.
DISCUSSION

Previous studies (27–29) have demonstrated that the mechanisms by which the T-allele of TCF7L2 rs7903146 is a marker for increased risk of type 2 diabetes most probably involve impairment at various steps of insulin biosynthesis and release. The most important finding from the present study is that young healthy carriers of the risk T-allele exhibit a diminished compensatory increase in glucose-stimulated plasma insulin and plasma C-peptide secretion during 9 days of bed rest, indicating a greater vulnerability to bed rest compared with carriers of the low-risk CC genotype.

Previous results from the Diabetes Prevention Program as well as from the Finnish Diabetes Prevention Study suggested an increased risk of type 2 diabetes in less physically active carriers of the TCF7L2 rs7903146 (28,30). In this study, we document that this significant adverse gene-environment interaction mechanistically may be caused by an inability of the carriers of the T-allele of TCF7L2 rs7903146 to increase insulin secretion to compensate for insulin resistance when exposed to physical inactivity. The extent to which other lifestyle factors, including diet, sleep deprivation, stress, etc., may interact with distinct susceptibility genotypes increasing the risk of developing overt type 2 diabetes remain to be documented.

It is generally recognized that in vivo insulin secretion should be corrected for the ambient degree of whole-body insulin sensitivity and expressed as a disposition index, taking into account the ability of the normal pancreatic β-cell to adapt insulin secretion to the level of insulin sensitivity (31). Lyssenko et al. (29) demonstrated reduced insulin sensitivity and expressed as a disposition index, in particular, after bed rest (Table 2). Our finding provides direct evidence for an impaired pancreatic insulin secretion relative to the level of peripheral insulin resistance in

### TABLE 2

Data on IVGTT, hyperinsulinemic-euglycemic clamp, and indirect calorimetry in male study participants according to TCF7L2 rs7903146 genotype before and after bed rest

| rs7903146 | Before bed rest | After bed rest |
|-----------|----------------|---------------|
|           | TT/CT          | CC            | TT/CT         | CC            |
| n         | 21             | 17            | 21            | 17            |
| Plasma glucose (mmol/l) |  |
|   Basal | 4.6 ± 0.4 | 4.6 ± 0.5 | 4.6 ± 0.4 | 4.6 ± 0.4 |
|   Insulin-stimulated state | 5.0 ± 0.2 | 5.0 ± 0.2 | 5.0 ± 0.4 | 5.1 ± 0.4 |
| Plasma insulin (pmol/l)* |  |
|   Basal | 26 ± 14 | 33 ± 23 | 30 ± 11 | 47 ± 27†‡ |
|   Insulin-stimulated state | 745 ± 151 | 848 ± 258 | 821 ± 186 | 837 ± 192 |
| Plasma glucagon (pmol/l) |  |
|   Basal | 6.7 ± 2.9 | 10.5 ± 4.3§ | 6.7 ± 3.6 | 9.4 ± 4.0† |
| HOMA-IR (10^{-6} \times \text{mmol}^{-1} \cdot \text{l}^{-1} \cdot \text{mmol}^{-1} \cdot \text{l}^{-1})* |  |
|   Basal | 5.3 ± 3.2 | 6.9 ± 5.2 | 6.1 ± 2.5 | 9.7 ± 5.8†‡ |
|   Insulin-stimulated state | 14.0 ± 1.9 | 14.0 ± 1.8 | 11.0 ± 1.7‡ | 10.0 ± 2.5‡ |
| Glucose oxidation rate (mg \cdot \text{min}^{-1} \cdot \text{kg} \cdot \text{fat-free mass}^{-1}) |  |
|   Basal | 1.6 ± 0.4 | 1.4 ± 0.4 | 2.3 ± 0.9‡ | 2.8 ± 0.7‡ |
|   Insulin-stimulated state | 4.2 ± 0.6 | 4.3 ± 0.6 | 4.5 ± 0.6 | 4.2 ± 0.7 |
| Fat oxidation rate (mg \cdot \text{min}^{-1} \cdot \text{kg} \cdot \text{fat-free mass}^{-1}) |  |
|   Basal | 1.0 ± 0.3 | 1.1 ± 0.4 | 0.7 ± 0.4‡ | 0.5 ± 0.3‡ |
|   Insulin-stimulated state | 0.1 ± 0.2 | 0.2 ± 0.4 | -0.1 ± 0.3 | 0.1 ± 0.3 |
| Nonoxidative glucose oxidation rate (mg \cdot \text{min}^{-1} \cdot \text{kg} \cdot \text{fat-free mass}) |  |
|   Insulin-stimulated state | 9.9 ± 1.7 | 9.7 ± 2.0 | 6.5 ± 1.5‡ | 6.4 ± 1.8‡ |
| FPPIR (pmol^{-1} \cdot \text{l}^{-1} \cdot \text{min}^{-1})* |  |
|   Basal | 1,559 ± 1,330 | 2,122 ± 1,464§ | 2,108 ± 1,339‡ | 3,503 ± 1,670†‡ |
|   Insulin-stimulated state | 2,671 ± 2,954 | 2,618 ± 602 | 2,632 ± 1,012 | 4,960 ± 2,778‡†‡ |
| Second-phase insulin response (pmol^{-1} \cdot \text{l}^{-1} \cdot \text{min}^{-1})* |  |
|   Basal | 4,469 ± 3,905 | 5,032 ± 2,985 | 5,026 ± 2,190 | 8,946 ± 4,063‡†‡ |
| AUC_total (pmol^{-1} \cdot \text{l}^{-1} \cdot \text{min}^{-1})* |  |
|   Basal | 21.9 ± 20.4 | 30.4 ± 21.5§ | 24.0 ± 16.2 | 34.3 ± 17.7† |
|   Insulin-stimulated state | 6.6 ± 6.4 | 14.2 ± 18.1‖ | 7.8 ± 5.6 | 9.4 ± 6.0 |

Data are means ± SD. *Log-transformed data. †Significant difference between the TT/CT and CC groups after bed rest, P < 0.05. ‡Significant difference before versus after bed rest; P < 0.05. Significant difference between the TT/CT and CC groups before bed rest, $P < 0.05 and $P = 0.05.
young healthy carriers of the risk T-allele. Importantly, we show that carriers of the T-allele of TCF7L2 rs7903146 were unable to increase pancreatic insulin secretion to compensate appropriately for peripheral insulin resistance as induced by physical inactivity, providing proof of concept for an adverse interaction between genotype and an important lifestyle determinant such as physical inactivity on risk of developing type 2 diabetes. Furthermore, we confirmed our previous finding of significantly lower fasting plasma glucagon levels in carriers of the T-allele of TCF7L2 rs7903146 prior to and after bed rest (19). As discussed in this report by Pilgaard et al. (19), this may be explained by reduced expression of proglucagon in the \( \beta \)-cells or by altered posttranslational processing of proglucagon to glucagon. Importantly, our confirmation of reduced plasma glucagon in the context of a documented reduced insulin secretion in response to intravenous glucose supports the idea of impaired paracrine glucagon stimulation playing a significant role in the development of impaired \( \beta \)-cell function in carriers of the T-allele of TCF7L2 rs7903146.

Carriers of the T-allele of TCF7L2 have an increased risk of nonalcoholic liver steatosis and fibrosis (32), and other studies (18,19,29) report that the T-allele of TCF7L2 is associated with an increased rate of endogenous glucose production in the basal state and during insulin infusion. In this study, the slightly elevated rate of hepatic glucose production in carriers of the T-allele (\( P = 0.10 \)) at baseline did not reach statistical significance. Furthermore, carriers of the T-allele exhibited a significantly, and paradoxically, decreased HIR index compared with carriers of the CC genotype after bed rest (Table 3). This interesting reversal of phenotype according to hepatic glucose production and hepatic insulin sensitivity is most likely to be explained by the clear separation of the fasting plasma insulin and C-peptide levels in the two study groups with increasing duration of bed rest (Fig. 4). These curves illustrate the unmasking effects of bed rest on a significant type 2 diabetes abnormality of reduced insulin secretion, even in the fasting state, in carriers of the T-allele. In that situation, the apparently increased hepatic insulin sensitivity in the carriers of the T-allele is due only to reduced fasting plasma insulin levels and the question may therefore be raised to which extent this calculated sensitivity index is a true biological phenomenon or a result of an inaccurate estimate of hepatic insulin sensitivity calculated from peripheral and not portal plasma insulin levels. However, the parallel differences of circulating plasma insulin as well as plasma C-peptide levels suggest that this is not only a result of altered hepatic insulin extraction. Although the lower fasting plasma glucagon levels may contribute to the lower HIR in carriers of the T-allele after bed rest, this is unlikely to be the full explanation. Thus, in agreement with the data from Pilgaard et al. (19), fasting plasma glucagon levels were reduced to the same extent in carriers of the T-allele prior to bed rest when HIR was not reduced in these subjects.

Wegner et al. (18) demonstrated a reduced insulin secretion in response to intravenous glucose in the abso-
lute sense as well as an increased peripheral insulin sensitivity with no difference in the peripheral disposition index in elderly carriers of the T-allele of TCF7L2 rs7903146 in a twin study. We speculated that the relatively increased peripheral insulin action in the elderly nondiabetic twins carrying the T-allele might be the result of an increase of peripheral (muscle) insulin action to compensate for a primary and long-lasting genuine impairment of insulin secretion (18). Along with this line of thinking, the relatively decreased HOMA, as well as pentameter of insulin secretion (18). Along with this line of thinking, the relatively decreased HOMA, as well as pentameter of insulin secretion (18). Along with this line of thinking, the relatively decreased HOMA, as well as pentameter of insulin secretion (18).

Data are means ± SD. *Log-transformed data. †Significant difference between TT/CT and CC group after bed rest, P < 0.05. ‡Significant difference before versus after bed rest; P < 0.05. R_a glucose; Glucose rate of appearance; R_a glycerol; glycerol rate of appearance of glycerol.

In conclusion, young healthy men who are carriers of the type 2 diabetes–associated T-allele of TCF7L2 rs7903146 and who are exposed to 9 days of physical inactivity develop an insulin response to intravenous glucose that is insufficient to compensate for the induced insulin resistance.
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The data presented in this manuscript are a part of a larger study on the influence of physical inactivity in young healthy subjects with and without risk of later development of type 2 diabetes, including subjects with low birth weight and first-degree relatives of patients with type 2 diabetes. Parts of the study have been published elsewhere (20). This work is initiated and funded by the European Union Framework VI, EXGENESIS project.

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REFERENCES

1. Vaag A. On the pathophysiology of late onset non-insulin dependent diabetes mellitus: current controversies and new insights. Danish Med Bull 1999;46:197–234
2. Ross SE, Hennat N, Longo KA, Bennett CN, Lucas PC, Erickson RL, MacDougald OA. Inhibition of adipogenesis by Wnt signaling. Science 2000;289:950–953
3. Papadopoulou S, Edlund H. Attenuated Wnt signaling perturbs pancreatic growth but not pancreatic function. Diabetes 2005;54:2344–2351
4. Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadottir A, Styrkarsdottir U, Magnusson KP, Walters GB, Palsdottir E, Jonsdottir T, Gudmundsdottir T, Gylfason A, Saemundsdottir J, Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Gudnason V, Sigurdsson G, Thorsteinsdottir U, Gulcher JR, Kong A, Stefansson K. Variation of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet 2006;38:320–323
5. McCarthy MI. A genomewide scan for loci predisposing to type 2 diabetes. Nat Genet 2007;39:218–225
6. Helgason A, Palsson S, Thorleifsson G, Grant SF, Emilsson V, Gunnarsdottir S, Adeyemo A, Chen Y, Chen G, Reynoldsottir I, Benediktsson R, Hinney A, Hansen T, Andersen G, Borgh-Johnsen K, Jorgensen T, Schafer H, Faruque M, Donny M, Zhou J, Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Sigurdsson G, Hebebrand J, Pedersen O, Thorsteinsdottir U, Gulcher JR, Kong A, Rotimi C, Stefansson K. Refining the impact of TCF7L2 gene variants on type 2 diabetes and adaptive evolution. Nat Genet 2007;39:218–225
7. Loos RJF, Franks PW, Francis RW, Barroso I, Griﬃble FM, Savage DB, Ong KK, O’Rahilly S, Wareham NJ. TCF7L2 polymorphisms modulate proteinulin levels and β-cell function in a British europid population. Diabetes 2007;56:1943–1947
8. Kirchhoﬀ K, Machicao F, Haupt A, Schafer SA, Tschritter O, Staiger H, Stefan N, Haring HU, Fritsche A. Polymorphisms in the TCF7L2, CDKAL1 and SLC30A8 genes are associated with impaired proinsulin conversion. Diabetologia 2008;51:597–601
9. Stolerman ES, Manning AK, McAteer JB, Fox CS, Dupuis J, Meigs JB, Florez JC. TCF7L2 variants are associated with increased proinsulin/insulin ratios but not obesity traits in the Framingham Heart Study. Diabetes 2009;58:614–620
10. Legner L, Hussain MS, Pilgaard K, Hansen T, Pedersen O, Vaag A, Poulsen P. Impact of TCF7L2 rs7903146 on Insulin Secretion and Action in Young and Elderly Danish Twins. J Clin Endocrinol Metab 2008;93:4013–4019
11. Pilgaard K, Jensen CB, Schou JH, Lysenkov V, Legner L, Brons C, Viiblsv T, Hansen T, Madbsdol S, Holst JJ, Volund A, Poulsen P, Groop L, Pedersen O, Vaag AA. 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;52:1298–307
12. Albigevic AC, Hojbjerg L, Sonne MP, Van HG, Stalkechts B, Dela F, Vaag A. Impact of nine days of bed rest on hepatic and peripheral insulin action, insulin secretion and whole body lipolysis in healthy young male offspring of patients with type 2 diabetes. Diabetes 2009;58:2749–2756
13. Holst JJ. Evidence that enteroglucon (II) is identical with the C-terminal sequence of glucagon. Biochem J 1982;207:381–388
14. Johnson R, McKunn P, MacMahon S, Rosbon R. Use of the Friedewald formula to estimate LDL-cholesterol in patients with chronic renal failure on dialysis. Clin Chem 1997;43:2183–2184
15. Plomgaard P, Bouzakri K, Krogh-Madsen R, Mittendorfer B, Zierath JR, Pedersen BK. Tumor necrosis factor-alpha induces skeletal muscle insulin resistance in healthy insulin sensitive male subjects via inhibition of Akt substrate 190 phosphorylation. Diabetes 2005;54:2093–2095
16. van Hall G, Sacchetti M, Radegran G, Saltin B. Human skeletal muscle fatty acid and glycerol metabolism during rest, exercise and recovery. J Physiol 2002;543:1047–1058
17. Bajaj M, Suraamornkul S, Romanelli A, Cline GW, Mandarino LJ, Shulman GI, DeFronzo RA. Effect of a sustained reduction in plasma free fatty acid concentration on intramuscular long-chain fatty Acyl-CoAs and insulin action in type 2 diabetic patients. Diabetes 2005;54:3148–3153
18. Prayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol 1983;55:628–634
19. Damcott CM, Pollin TI, Reinhart LJ, Ott SH, Shen H, Silver KD, Mitchell BD, Shulidder AR. Polymorphisms in the transcription factor 7-like 2 (TCF7L2) gene are associated with type 2 diabetes in the Amish: replication evidence for a role in both insulin secretion and insulin resistance. Diabetes 2006;55:2654–2659
20. Florez JC, Jabonson KS, Bayley N, Pollin TI, de Bakker PW, Shulidder AR, Knowler WC, Nathan DM, Alshuter D. TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. N Engl J Med 2006;355:241–250
21. Lyssenko V, Lum Y, Marchetti P, Del GS, Orho-Melander M, Almgren P, Stefan N, Haring HU, Fritsche A. Polymorphisms in the TCF7L2 gene increase risk of type 2 diabetes. J Clin Invest 2007;117:2155–2163
22. Wang J, Kuusisto J, Yannitine M, Kuulasmaa T, Lindstrom J. Eurotiolo J,
Uusitupa M, Laakso M. Variants of transcription factor 7-like 2 (TCF7L2) gene predict conversion to type 2 diabetes in the Finnish Diabetes Prevention Study and are associated with impaired glucose regulation and impaired insulin secretion. Diabetologia 2007;50:1192-1200

31. Bergman RN, Finegood DT, Kahn SE. The evolution of beta-cell dysfunction and insulin resistance in type 2 diabetes. Eur J Clin Invest 2002;32(Suppl. 3):35-45

32. Musso G, Gambino R, Pacini G, Pagano G, Durazzo M, Cassader M. Transcription factor 7-like 2 polymorphism modulates glucose and lipid homeostasis, adipokine profile, and hepatocyte apoptosis in NASH. Hepatology 2009;49:426-435

33. Brons C, Jensen CB, Storgaard H, Hiscock N, White A, Appel J, Jacobsen S, Nilsson E, Larsen C, Astrup A, Quistorff B, Vaag A. Impact of short-term high-fat feeding on glucose and insulin metabolism in young healthy men. J Physiol 2009;587:2387-97

34. Nauck MA, Meier JJ. The enteroinsular axis may mediate the diabetogenic effects of TCF7L2 polymorphisms. Diabetologia 2007;50:2413-2416

35. Schafer SA, Tschritter O, Machicao F, Thamer C, Stefan N, Gallwitz B, Holst JJ, Dekker JM, 't Hart LM, Nijpels G, van Haeften TW, Haring HU, Fritsche A. Impaired glucagon-like peptide-1-induced insulin secretion in carriers of transcription factor 7-like 2 (TCF7L2) gene polymorphisms. Diabetologia 2007;50:2443-2450