Impact of *TCF7L2* rs7903146 on Insulin Secretion and Action in Young and Elderly Danish Twins

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Objective: We investigated the regulation and metabolic effects of *TCF7L2* gene expression in human sc fat and skeletal muscle and the impact of the *TCF7L2,* rs7903146, T-allele on gene expression and measures of glucose metabolism including insulin secretion and peripheral and hepatic insulin action.

Research Design and Methods: The rs7903146 was genotyped in 1) a population-based sample of 587 twins (55–64 yr) with glucose tolerance ranging from normal to type 2 diabetes and 2) a population of 196 nondiabetic young (22–31 yr) and elderly (57–66 yr) twins. All subjects underwent oral glucose tolerance tests, and population 2 was additionally examined with iv glucose tolerance tests and hyperinsulinemic, euglycemic clamps.

Results: Elderly T-allele carriers had decreased plasma insulin responses and lower disposition index, whereas insulinogenic index was similar between genotype groups. Elderly nondiabetic T-allele carriers had increased peripheral insulin sensitivity ($P = 0.03$). Young T-allele carriers had impaired hepatic insulin sensitivity ($P = 0.04$) independent of plasma insulin levels. *TCF7L2* gene expression in skeletal muscle and adipose tissue was not explained by genotype, sex, aerobic capacity, birth, or adult anthropometry and was not associated with *in vivo* glucose metabolism.

Conclusions: The rs7903146 T-allele associates with hepatic insulin resistance and diminished glucose-stimulated plasma insulin secretion. Our study does not provide evidence of a role of *TCF7L2* gene expression in sc fat tissue and muscle tissue in the regulation of glucose homeostasis. This suggests that the primary defect of rs7903146 T-allele carriers is impairment of insulin secretion rather than a defect in insulin action in peripheral tissues. **(*J Clin Endocrinol Metab* 93: 4013–4019, 2008)**

The transcription factor 7-like 2 (TCF7L2) is a member of the T-cell transcription factor family, which plays an important role in the WNT signaling pathway. This pathway is a major component in the regulation of cell proliferation and differentiation (1). It is also involved in the regulation of myogenesis and adipogenesis; furthermore, it is required for the development of pancreas and islets during embryonic growth (2, 3). Moreover, WNT signaling through the TCF7L2 nuclear receptor has been shown to influence glucagon-like peptide-1 (GLP-1) secretion (1).

The gene encoding TCF7L2 (*TCF7L2*) has been mapped to chromosome 10q25, a region with strong linkage to type 2 diabetes (T2D) (4–7). A microsatellite marker, DG10S478, within this region has shown an even stronger association with T2D (8). The rs7903146 T-allele was subsequently identified as either the risk variant itself or its known correlate (9), and its positive association with T2D has been extensively replicated across different populations (10). This association might, to some extent, be explained by decreased insulin secretion (11–13) probably through a defect in insulin processing (14, 15). Additional studies have reported associations between the rs7903146 T-allele and impaired glucose tolerance (11, 16, 17), increased birth weight (18), and adult anthropometry indicating the involvement of several organs in the *TCF7L2* phenotype (19). Genome-wide association studies have further established *TCF7L2* as a major

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Abbreviations: AUC, area under the curve; BMI, body mass index; CI, confidence interval; DZ, dizygotic; GLP-1, glucagon-like peptide 1; IGT, impaired glucose tolerance; incAUC, incremental AUC; IVGTT, iv glucose tolerance test; MAF, minor allele frequency; MZ, monozygotic; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; Rd, glucose disposal rate; TCF7L2, transcription factor 7-like 2; T2D, type 2 diabetes; VO_2 max, aerobic capacity; WHR, waist-to-hip ratio.

contributor to T2D (20–24). However, few studies have studied *TCF7L2* gene expression in skeletal muscle, liver, and adipose tissue (19, 25). Importantly, a previous study reported that *TCF7L2* gene expression in human pancreatic islets was increased 5-fold in T2D patients carrying the rs7903146 T-allele and was associated with reduced glucose-stimulated insulin secretion (12).

We aimed to explore the genetic *vs.* nongenetic regulation and metabolic effect of *TCF7L2* gene expression levels in human sc fat and skeletal muscle as well as the impact of the rs7903146 on gene expression and measures of glucose metabolism including glucose-stimulated insulin secretion and peripheral and hepatic insulin action in Danish monozygotic (MZ) and dizygotic (DZ) twins.

Subjects and Methods

Subjects

The *TCF7L2* rs7903146 was genotyped in two different populations of twins. 1) A population-based sample of 587 elderly Danish twins (67 ± 4.9 yr) included 66% (n = 389) who had normal glucose tolerance (NGT), 20% (n = 118) who had impaired fasting glucose tolerance (IGT), and 14% (n = 80) who had T2D (26, 27). Among the T2D subjects, 32 had known T2D and 48 had previously unknown T2D. The minor allele frequency (MAF) with a 95% confidence interval (CI) was 28.1% (25.5–30.7) and genotype frequencies for CC, CT, and TT were 52, 41, and 8%, respectively. For studies of quantitative traits, only treatment-naive subjects with genotypes available were included (n = 531). 2) A second population of 98 included MZ and DZ young (28 ± 1.9 yr) and elderly (62 ± 2.3 yr) twin pairs without known T2D (28, 29). The separation of this population into two distinct age groups was a prespecified classification. Among elderly twins, 74.5% had NGT, 22.0% had IGT, and 3.5% had previously unknown T2D. Among the young twins, 98.5% had NGT and 1.5% had IGT. The MAF of rs7903146 for the young twins with a 95% CI was 24.5% (18.7–30.3) with a genotype frequency for CC, CT, and TT of 55, 42, and 4%, respectively. For the elderly twins, the MAF with 95% CI was 38.1% (30.8–45.4) with a genotype frequency for CC, CT, and TT of 33, 38, and 19%, respectively.

The present study was approved by the regional Scientific Ethical Committees and conducted according to the Helsinki Declaration.

Methods

Both populations underwent anthropometric measurements of body mass index (BMI) and waist-to-hip ratio (WHR) and an oral glucose tolerance test (OGTT). In addition, subjects from population 2 underwent a dual-energy x-ray absorptiometry scan with measures of total and regional body fat percentage, a bicycle test to determine aerobic capacity, a 30-min iv glucose tolerance test (IVGTT), and a 2-h hyperinsulinemic-euglycemic clamp (40 mU/m^2/min) using tritriated glucose as previously described (30).

Surrogate measures of insulin sensitivity and secretion based upon the OGTT were performed in both population 1 and 2 and included the insulin sensitivity index (10,000/glucose$_{fasting}$× insulin$_{fasting}$× glucose$_{meanOGTT}$× insulin$_{meanOGTT}$) and insulinogenic index (insulin$_{30min}$− insulin$_{0min}$/glucose$_{30min}$− glucose$_{0min}$), respectively. Furthermore, disposition index during OGTT was calculated as the product of the insulin sensitivity index and insulinogenic index.

In population 2, calculations of basal and insulin-stimulated metabolic rates including peripheral insulin sensitivity [glucose disposal rate, (Rd)] and hepatic glucose production have previously been described in detail (29). The hepatic insulin sensitivity index was calculated as hepatic glucose production × fasting insulin. Disposition index during IVGTT was calculated as the product of the insulin secretion capacity [area under the curve (AUC)$_{0–10min\ insulin}$/AUC$_{0–10min\ glucose}$] and insulin sensitivity.

Tissue biopsies

Basal sc adipose tissue biopsies from the abdomen were obtained in a subgroup (n = 235) of population 1, and basal and insulin-stimulated skeletal muscle biopsies from the vastus lateralis muscle were obtained from subjects of population 2. The tissue specimens were taken under local anesthesia (lidocaine) using a Bergström needle with suction applied and were quickly blotted on filter paper and frozen in liquid nitrogen. The tissues were stored at −80 C until further processed.

Analysis of *TCF7L2* mRNA levels in skeletal muscle and adipose tissue

Total RNA was extracted from frozen skeletal muscle specimens using the Tri Reagent kit according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO). The cDNA was synthesized using RevertAid H Minus First Strand cDNA Synthesis Kit with random hexamer primers (Fermentas, Ontario, Canada). *TCF7L2* mRNA levels were quantified using TaqMan Real-Time PCR with an ABI 7900 system [Applied Biosystems (ABI), Foster City, CA]. Gene-specific probes and primer pairs for *TCF7L2* were obtained from ABI (Assays-on-Demand, Hs00181036_m1). The transcript quantity was normalized to the mRNA level of cyclophilin A (Hs99999904_m1; ABI).

Genotyping

Genomic DNA was extracted from blood using conventional methods. The *TCF7L2* rs7903146 was genotyped using allelic discrimination performed with an ABI 7900 system (KBioscience, Herts, UK). The overall genotyping success rate was more than 96%.

Statistical methods

The extent to which MZ twins are more alike than DZ twins is presumed to reflect a genetic influence on the phenotype in question. Heritability (expressed as h^2) gives the proportion of the total variation of a trait attributable to genetic variation and is expressed as twice the difference of the intraclass correlation of MZ and DZ twins [$h^2 = 2(r_{MZ-}r_{DZ})$] (31). Intraclass correlation expresses similarity within twin pairs: r = covariance (twins A, twins B/$\sqrt{}$variance (twins A) × variance (twins B). Statistical comparisons of intraclass correlations were made after transformation using the Fisher z transformation. Phenotypes were compared using SAS (version 8.2; SAS Institute, Cary, NC) proc mixed model. Using twin samples in genetic studies reduces the variability from several environmental sources; however, twins cannot be considered as independent observations. Therefore, we included a random-effects term for twin pair membership and a fixed-effects term for zygosity acknowledging the fact that twins cannot be considered as independent observations. Multiple regression analyses were performed to identify determinants of tissue *TCF7L2* gene expression including fat percentage, aerobic capacity (VO_2 max), birth weight, zygosity, and sex and to determine the impact of *TCF7L2* gene expression on *in vivo* metabolism. Adjustments were made for sex and age (bimodal variable) in each model. The regression analyses were performed with a stepwise elimination of insignificant covariables until obtaining the final reduced models in the SAS systems for Windows. The significance level for variable elimination was $P < 0.05$.

Results

Influence of rs7903146 genotype on anthropometry and body composition

In population 1 (n = 531), no differences in BMI or WHR between genotype groups were observed (Table 1). In addition, in population 2, the T-allele was not associated with BMI, WHR, total or regional (leg and trunk) body fat percentage, or VO_2 max in either young or elderly subjects (Table 1).

TABLE 1. Anthropometry/body composition of treatment-naive twins from population 1 (n = 531) and population 2 (n = 190)

rs7903146	CC	CT	TT	*P*
Population 1				
n (men/women)	278 (131/147)	211 (202/109)	42 (24/18)	
Age (yr)	67 ± 5	66 ± 5	66 ± 6	0.6
BMI (kg/m^2)	26.2 ± 4.8	25.4 ± 3.6	25.4 ± 3.0	0.1
WHR	0.87 ± 0.09	0.87 ± 0.09	0.88 ± 0.09	0.9
Population 2 (young twins)				
n (men/women)	58 (39/19)	44 (19/25)	4 (2/2)	
Age (yr)	28 ± 2	28 ± 0	27 ± 0	0.4
BMI (kg/m^2)	24.5 ± 3.3	23.6 ± 3.0	24.2 ± 2.0	0.7
Total fat (%)	21.1 ± 7.4	22.6 ± 5.9	26.1 ± 12.7	0.4
Trunk fat (%)	18.0 ± 6.8	18.3 ± 5.6	24.4 ± 12.5	0.5
Leg fat (%)	24.5 ± 10.1	27.6 ± 8.4	28.4 ± 14.5	0.4
VO_2 max (ml/kg FFM·min)	39.8 ± 6.7	39.0 ± 8.4	43.3 ± 16.5	0.7
Population 2 (elderly twins)				
n (men/women)	36 (15/21)	32 (16/16)	16 (6/10)	
Age (yr)	62 ± 3	62 ± 2	62 ± 2	0.3
BMI (kg/m^2)	25.6 ± 3.8	26.9 ± 4.8	25.5 ± 4.8	0.6
Total fat (%)	28.2 ± 8.8	27.4 ± 8.5	27.8 ± 12.5	0.3
Trunk fat (%)	25.2 ± 9.9	25.0 ± 8.7	24.3 ± 13.3	0.3
Leg fat (%)	32.2 ± 9.7	30.6 ± 11.0	31.9 ± 13.7	0.5
VO_2 max (ml/kg FFM·min)	25.8 ± 7.0	26.6 ± 6.2	27.1 ± 8.2	0.6

Anthropometry and body composition of population 1 and 2. *P* values were calculated using SAS (version 8.2; SAS Institute) proc mixed model. The full model includes a random-effects term for twin pair membership and a fixed-effects term for zygosity. Values are mean ± SD. FFM, Fat-free mass.

Influence of rs7903146 genotype on glucose tolerance and insulin secretion during an OGTT

In population 1, T-allele carriers had significantly increased fasting 30- and 120-min post-OGTT plasma glucose levels and a larger 120-min incremental AUC (incAUC$_{glucose0-120}$) for glucose (Table 2). Furthermore, carriers of the minor T-allele had significantly lower 30-min insulin post-OGTT levels and a tendency toward a smaller 30-min (incAUC$_{insulin0-30}$) and 120-min (incAUC$_{insulin0-120}$) incremental AUC for insulin. The disposition index, in which insulin secretion is adjusted for the degree of insulin sensitivity, was significantly lower among T-allele carriers ($P = 0.03$). However, when adjusting for the degree of glycemia, the plasma insulin response expressed by the insulinogenic index did not reach the level of significance ($P = 0.1$) (Table 2).

In both young and elderly nondiabetic subjects from population 2, the plasma glucose and plasma insulin profiles during an OGTT were similar in the three genotype groups, and insulinogenic and disposition indices did not differ with genotype (data not shown).

Influence of rs7903146 genotype on insulin secretion during an IVGTT

An IVGTT was performed in subjects from population 2. In the young twins, no effect of rs7903146 genotype was demonstrated on plasma levels of insulin and glucose or disposition index (data not shown). In elderly twins, homozygous carriers of the T-allele had significantly lower plasma insulin levels at 6 ($P = 0.049$), 8 ($P = 0.03$), and 15 min ($P = 0.03$) during an IVGTT. Conversely, plasma glucose levels were similar (Fig. 1), and

TABLE 2. Plasma glucose and insulin profiles of treatment-naive twins from population 1

rs7903146	CC	CT	TT	*P*
n (men/women)	278 (131/147)	211 (202/109)	42 (24/18)	
Plasma glucose (mmol/liter)				
Fasting	5.7 ± 0.7	5.9 ± 1.0	6.1 ± 1.3	0.005
30-min	9.2 ± 1.6	9.4 ± 1.9	10.0 ± 2.4	0.01
120-min	6.8 ± 2.5	7.4 ± 2.6	7.9 ± 4.2	0.0009
incAUC$_{30\text{-min}}$	52.5 ± 18.9	51.8 ± 21.6	58.9 ± 24.2	0.2
incAUC$_{120\text{-min}}$	258.7 ± 133.4	274.5 ± 144.1	316.6 ± 199.9	0.006
Plasma insulin (pmol/liter)				
Fasting	44 ± 27	45 ± 26	45 ± 19	1.0
30-min	335 ± 248	282 ± 196	282 ± 163	0.05
120-min	296 ± 295	313 ± 285	252 ± 209	0.5
incAUC$_{30\text{-min}}$	4338 ± 3474	3560 ± 2710	3562 ± 2310	0.06
incAUC$_{120\text{-min}}$	28701 ± 22283	26308 ± 19924	23293 ± 14028	0.08
Insulinogenic index	89 ± 68	87 ± 116	69 ± 72	0.1
Disposition index	1642 ± 101	1591 ± 2136	1024 ± 2256	0.03

OGTT in relation to *TCF7L2* rs7903146 genotype (n = 531). Insulin and glucose profiles for population 1 are shown. *P* values were calculated using SAS (version 8.2; SAS Institute) proc mixed model. The full model includes a random-effects term for twin pair membership and a fixed-effects term for zygosity. Values are mean ± SD.

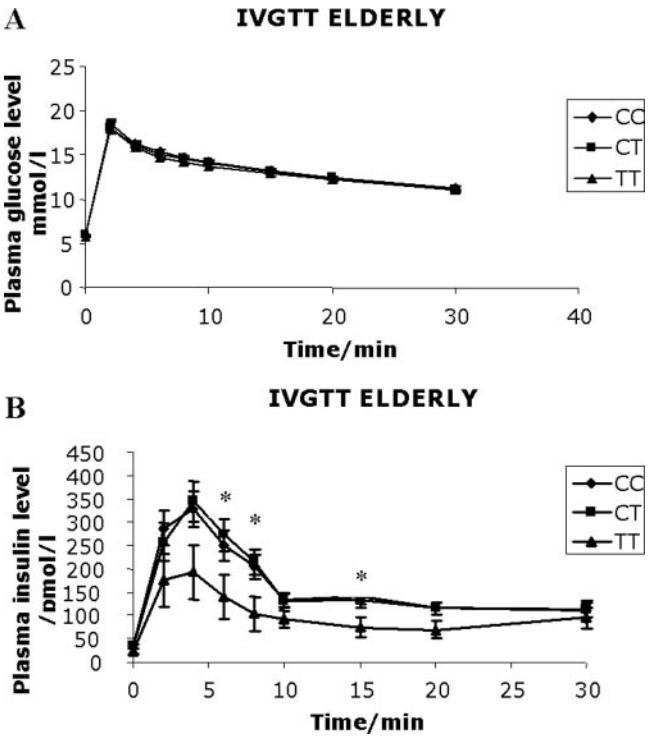

FIG. 1. Plasma glucose concentrations (A) and insulin concentrations (B) during an IVGTT. Values are mean ± SE for each genotype group. *, $P < 0.05$, assuming a recessive model.

no effect of genotype on disposition index was observed (CC, $1.1^{-7} \pm 8.1^{-8}$; CT, $1.2^{-7} \pm 8.7^{-8}$; TT, $1.4^{-7} \pm 8.6^{-8}$; $P = 0.5$).

Influence of genotype on hepatic and peripheral insulin sensitivity

Hepatic and peripheral insulin sensitivity was determined in subjects from population 2 and compared between genotype groups. In the elderly, homozygous rs7903146 T-allele carriers had a significantly higher Rd as compared with C-allele carriers ($P = 0.03$) with a tendency toward both a higher oxidative and nonoxidative glucose metabolism (Table 3). In the young subjects, the association between the T-allele and increased hepatic insulin resistance as determined by hepatic insulin sensitivity index was significant ($P = 0.04$), whereas no relationship between genotype and peripheral insulin sensitivity was seen (Table 3).

TCF7L2 gene expression in skeletal muscle

The level and determinants of *TCF7L2* expression in skeletal muscle in young and elderly nondiabetic twins from population 2 were investigated as were the metabolic effects of *TCF7L2* gene expression. Heredity of basal and insulin-stimulated *TCF7L2* gene expression in skeletal muscle was determined by heritability estimates (h^2) in both age groups (young: $h^2_{basal} = 0.76$, $P = 0.14$; $h^2_{insulin\text{-}stimulated} = 0.46$, $P = 0.20$; elderly: $h^2_{basal} = 0.74$, $P = 0.21$, $h^2_{insulin\text{-}stimulated} = 0.98$, $P = 0.047$). Despite some indication of a genetic component there, was no effect of rs7903146 on *TCF7L2* gene expression levels either in young for (basal state: CC, 0.16 ± 0.014; CT, 0.16 ± 0.016; TT, 0.14 ± 0.053, $P = 0.9$; insulin-stimulated state: CC, 0.16 ± 0.013; CT, 0.16 ± 0.017; TT, 0.16 ± 0.050, $P = 1.0$) or elderly subjects (basal state: CC, 0.06 ± 0.006; CT, 0.07 ± 0.006; TT, 0.06 ± 0.009, $P = 0.4$; insulin-stimulated state: CC, 0.07 ± 0.014; CT, 0.12 ± 0.014; TT, 0.08 ± 0.018, $P = 0.06$).

No change in *TCF7L2* gene expression levels in skeletal muscle was observed upon insulin stimulation in either young or elderly subjects; however, *TCF7L2* gene expression levels during both steady-state periods were significantly lower in elderly compared with younger subjects (Fig. 2). To identify additional nongenetic determinants of skeletal muscle *TCF7L2* gene expression, we performed multiple regression analyses with the

TABLE 3. Hepatic and peripheral insulin action in young (n = 106) and elderly (n = 84) twins

rs7903146	CC	CT	TT	*P*
Population 2 (young twins)				
n (men/women)	58 (39/19)	44 (19/25)	4 (2/2)	
Age (yr)	28 ± 0.2	28 ± 0.3	27 ± 0.4	0.4
Basal (mg/kg·min)				
Hepatic glucose production	3.0 ± 0.6	3.0 ± 0.3	3.6 ± 0.7	0.2
Hepatic insulin resistance index	112 ± 49	111 ± 45	181 ± 23	0.04
Clamp (mg/kg·min)				
Glucose disposal rate (Rd)	11.6 ± 3.5	11.7 ± 2.8	12.4 ± 4.2	1.0
Glucose oxidation	4.6 ± 1.4	4.6 ± 1.6	5.6 ± 2.5	0.1
Nonoxidative glucose metabolism	7.1 ± 3.1	7.1 ± 2.6	6.8 ± 2.4	0.9
Population 2 (elderly twins)				
n (men/women)	36 (15/21)	32 (16/16)	16 (6/10)	
Age (yr)	62 ± 0.4	62 ± 0.4	62 ± 0.5	0.3
Basal (mg/kg·min)				
Hepatic glucose production	3.0 ± 0.3	3.1 ± 0.4	3.1 ± 0.5	0.2
Hepatic insulin resistance index	128 ± 117	118 ± 76	111 ± 65	0.9
Clamp (mg/kg·min)				
Glucose disposal rate (Rd)	9.5 ± 2.7	9.4 ± 3.7	11.9 ± 3.5	0.03
Glucose oxidation	4.1 ± 1.2	3.7 ± 1.1	4.6 ± 1.7	0.1
Nonoxidative glucose metabolism	5.4 ± 2.5	5.8 ± 3.8	7.3 ± 2.9	0.1

IVGTT in relation to *TCF7L2* rs7903146 genotype. Metabolic rates during basal and insulin-stimulated conditions are shown. *P* values were calculated using SAS (version 8.2; SAS Institute) proc mixed model. The full model includes a random-effects term for twin pair membership and a fixed-effects term for zygosity. Data are mean values± SD.

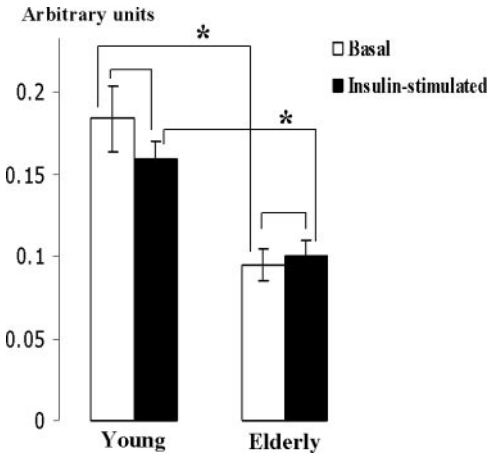

FIG. 2. *TCF7L2* gene expression levels in nondiabetic young and elderly twins. *TCF7L2* mRNA levels were normalized to cyclophilin (*PPIA*). Differences in gene expression between basal and insulin-stimulated states were calculated using a two-sided paired *t* test. Differences in gene expression between young and elderly were calculated using a two-sample *t* test. *White bars* represent *TCF7L2* mRNA level in the basal state. *Black bars* represent mRNA level during insulin stimulation. *, $P < 0.0001$. Values are mean ± SE.

following explanatory variables: sex, birth weight, zygosity (prenatal environment), VO_2 max (aerobic capacity), and fat percentage (body composition). None of these variables were associated with *TCF7L2* gene expression, and skeletal muscle *TCF7L2* gene expression was not related to any quantitative metabolic traits including peripheral and hepatic insulin sensitivity (data not shown).

TCF7L2 gene expression in adipose tissue

The level and determinants of *TCF7L2* gene expression in sc adipose tissue were investigated in a subgroup (n = 235) of elderly twins from population 1. The similarity regarding *TCF7L2* gene expression in sc adipose tissue was higher within DZ twins ($r_{DZ} = 0.59$; 95% CI = 0.32–0.75) than MZ twins ($r_{MZ} = 0.51$; 95% CI = 0.13–0.72), so further determination of heredity was not appropriate. In accordance, there was no difference in *TCF7L2* gene expression levels between genotype groups (CC, 0.25 ± 0.042; CT, 0.22 ± 0.044 TT, 0.32 ± 0.092; $P = 0.6$). Furthermore, the gene expression levels were similar in subjects with T2D (n = 39) and NGT (n = 130) (0.24 ± 0.07 and 0.28 ± 0.04, $P = 0.6$).

Multiple regression analyses including sex, birth weight, zygosity, BMI, and WHR were performed to identify nongenetic determinants of adipose tissue *TCF7L2* gene expression. Among these, no significant explanatory variables were demonstrated. Finally, adipose tissue *TCF7L2* gene expression was not related to any quantitative metabolic traits including glucose tolerance (data not shown).

Discussion

In accordance with numerous studies in different populations, we demonstrated an influence of the rs7903146 T-allele on plasma insulin profiles during oral glucose testing in a population-based sample of elderly twins and in response to iv glucose administration in a population of elderly nondiabetic twins. Few studies have adjusted insulin secretion for the degree of insulin sensitivity. When doing so, the insulin secretion capacity during the oral glucose challenge, expressed as disposition index, was significantly decreased in elderly carriers of rs7903146 T-alleles in the population-based cohort. In contrast, disposition indices during both the oral and iv glucose tolerance tests were similar between the genotype groups in nondiabetic elderly subjects, suggesting an intact insulin secretion relative to the degree of insulin resistance. The reason for the normal insulin secretion relative to insulin action in elderly nondiabetic carriers of the T-allele was a surprisingly increased peripheral insulin sensitivity. Of importance, the elderly subjects of population 2 were selected by age and no diagnosis of T2D, which is why patients with T2D as a consequence of the rs7903146 variant were not included in the present study population. Apparently, these subjects either possess a unique physiological flexibility to compensate for the reduced insulin secretion associated with the T-allele or have cosegregating protective genetic variants causing increased insulin sensitivity in the periphery.

The conventional view is that *in vivo* insulin secretion should be corrected for the ambient degree of insulin action (calculating the disposition index) to take into account the ability of the normal pancreatic β-cell to increase insulin secretion to compensate for the degree of insulin resistance, thereby defining the well known hyperbolic relationship between these two parameters. Nevertheless, it is most often forgotten that insulin-sensitive tissue also, to some extent, is capable of adjusting the degree of *in vivo* insulin action to the ambient degree of insulin secretion, *i.e.* the reverse scenario. The idea that genetically determined reduced insulin secretion in carriers of rs7903146 T-alleles may be responsible for the relatively increased insulin action, at least in nondiabetic elderly carriers, is supported by the previous report of a relatively increased *in vivo* insulin action in carriers of HNF-1α gene variants associated with reduced insulin secretion and the development of maturity-onset diabetes of the young type 3 (MODY3) (32). Altogether, the present as well as previous results of the relationship between insulin secretion and insulin action in carriers of gene polymorphisms with known reduced insulin secretion capacity are consistent with the idea that the compensatory interactions between peripheral insulin action and pancreatic insulin secretion work both ways in a highly biologically plausible and coordinated way. Subsequently, this should also be taken into account when evaluating the biological importance of absolute *vs.* relative (to insulin action) insulin secretion disposition indices.

Noteworthy, the young rs7903146 T-allele carriers exhibited decreased hepatic insulin sensitivity. The finding is in accordance with a few other studies (12) (Pilgaard, K., C. B. Jensen, J. H. Schou, L. Wegner, C. Brøns, T. Vilsbøll, T. Hansen, S. Madsbad, J. J. Holst, A. Vølund, P. Poulsen, and A. Vaag, submitted) and may either be a primary phenotypic characteristic of the T-allele of *TCF7L2* rs7903146 or be explained by lower fasting and glucose-stimulated levels of circulating insulin.

Several studies suggest that defects in the enteroinsular axis mediate the diabetogenic effects of TCF7L2 (8, 33). Taken together, studies have implied a defective incretin effect in

rs7903146 T-allele carriers rather than a secretion defect (12, 34). In the present study, we demonstrated an absolute defective insulin secretion during both the OGTT and IVGTT. We have not measured the secretion or action of the incretin hormones gastric inhibitory peptide and GLP-1. However, although highly hypothetical, it is noteworthy and of potential interest that the phenotypic traits characterizing the T-allele carriers in the present study including an impaired glucose-stimulated insulin secretion, increased peripheral insulin sensitivity, and decreased hepatic insulin sensitivity are similar to the metabolic features seen in the GLP-1 receptor knockout mouse and during brain infusion of GLP-1 receptor antagonist (35). According to these findings, GLP-1 has distinct extrapancreatic metabolic effects in several tissues involved in the pathogenesis of T2D including liver, fat, and skeletal muscle, and the GLP-1 regulation of glucose homeostasis, to some extent, may be central. In theory, the phenotype of the *TCF7L2* rs7903146 T-allele carriers may be due to a global or perhaps central defect in GLP-1 action.

Due to the association between the rs7903146 variant and peripheral insulin sensitivity and the role of the WNT pathway in myogenesis and adipogenesis, we investigated the gene expression levels of *TCF7L2* in skeletal muscle and sc fat specimens. Although the heritability estimates for *TCF7L2* gene expression levels in skeletal muscle gave some indication of genetic control, the expression levels were not influenced by genotype. Similarly, the expression level in fat tissue was independent of genotype. In addition, the expression levels in both skeletal muscle and fat tissue were not influenced by sex, body composition, aerobic capacity, or prenatal environmental factors including birth weight and zygosity status. However, a statistically significant decrease in *TCF7L2* gene expression with age was observed in the elderly subjects compared with the young subjects. Importantly, the *TCF7L2* expression level in neither fat nor skeletal muscle was associated with measures of glucose homeostasis including glucose tolerance and insulin sensitivity. Moreover, skeletal muscle *TCF7L2* expression levels did not change upon insulin stimulation. These findings are in accordance with a previous smaller study of 138 subjects of European and African descent (25). Although a previous study demonstrated a 5-fold increase in *TCF7L2* gene expression in pancreatic islets from T2D patients compared with nondiabetic donors (12), our present results taken together do not provide evidence of a role of *TCF7L2* gene expression in sc fat tissue and muscle tissue in the regulation of glucose homeostasis. The lack of association between expression of *TCF7L2* in adipose tissue and skeletal muscle, two key organs involved in the pathogenesis of T2D, and *in vivo* measures of glucose metabolism supports our hypothesis that the primary defect of rs7903146 T-allele carriers is an impairment of insulin secretion rather than a defect in insulin action in peripheral tissues.

In conclusion, the diabetogenic rs7903146 T-allele of *TCF7L2* is associated with an absolute and relative insulin secretion defect. In healthy nondiabetic subjects, the T-allele is associated with increased peripheral insulin sensitivity and decreased hepatic sensitivity and is not explained by altered *TCF7L2* gene expression levels in skeletal muscle or sc fat tissue.

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