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Expression of the transcription factor 7-like 2 gene (*TCF7L2*) in human adipocytes is down regulated by insulin

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ABSTRACT

Variants in the TCF7L2 gene (transcription factor 7-like 2) have shown strong association with type 2 diabetes with two defined risk haplotypes, HapA and $HapB_{T2D}$. TCF7L2 may play a role in both glucose homeostasis and adipogenesis. Our aim was to characterize the *TCF7L2* mRNA expression and regulation in human adipose tissue.

We quantified *TCF7L2* mRNA levels in cultured human adipocytes and in biopsies from visceral (VAT) and subcutaneous (SAT) adipose tissue from 38 obese non-diabetic subjects, using real-time PCR. The influence of haplotype and clinical traits on *TCF7L2* mRNA levels were investigated.

In vitro, insulin decreased *TCF7L2* mRNA expression. This effect was attenuated in cells incubated with the free fatty acids palmitate or oleate. *In vivo*, we found significantly higher expression in SAT from more insulin resistant subjects. No correlations between *TCF7L2* mRNA expression and obesity measures were observed. *TCF7L2* expression was higher in VAT than in SAT and when stratifying for haplotype, this difference was seen in HapA carriers but not in non-HapA carriers.

In conclusion, *TCF7L2* mRNA levels in adipocytes are decreased by insulin and seem to increase in insulin resistant subjects and in HapA carriers.

Obesity is a rapidly increasing health problem especially in the western world. It is caused by a greater energy intake compared to the expenditure and is influenced by environmental, genetic and psychosocial factors. Obesity is a key risk factor for the metabolic syndrome and type 2 diabetes; two conditions characterized by elevated levels of circulating plasma free fatty acids and insulin resistance [1]. Type 2 diabetes develops when the insulin producing β-cells in the pancreas can no longer compensate for the insulin resistance by increased insulin secretion.

Recently, variants in *TCF7L2* were shown to have the strongest association to type 2 diabetes known so far [2]. TCF7L2 is a transcription factor regulated by the Wnt signalling pathway. Wnts are a family of secreted glycoprotein ligands involved in regulation of several transcription factors controlling cell fate. Wnts bind to cell surface receptors thereby initiating a signalling cascade resulting in the prevention of β-catenin degradation. β-Catenin translocates to the nucleus where it activates TCF7L2 [3]. In adipocytes Wnt signalling, via TCF7L2, negatively regulates adipogenesis [4]. The exact role of *TCF7L2* in pathogenesis of type 2 diabetes is not known, but recent data suggests a role in glucose homeostasis [5,6]. Several population studies have revealed an association between *TCF7L2* variants and impaired insulin secretion although an effect on insulin sensitivity has also been reported [5,7–12]. In particular, *TCF7L2* risk variants have been associated with a weaker insulin response to oral compared to intravenously administered glucose, suggesting a role in the enteroinsular axis [5]. Two risk haplotypes have been characterized [13]; $HapB_{T2D}$, which was associated with type 2 diabetes, decreased BMI and lower insulin secretion [9] and HapA, which in men was associated with increased BMI and increased insulin levels.

Considering the association with BMI and the regulatory effect on adipogenesis, we wanted to study the potential role of *TCF7L2* in adipose tissue. Our aim was to examine the regulation of *TCF7L2* expression *in vitro* and in adipose tissue from obese, insulin resistant subjects and the relationship between genotype and expression.

Research design and methods

Obese subjects. This study included 38 obese non-diabetic subjects undergoing bariatric surgery (4 men and 34 women; age 35.5 years [29.8–46.8]; BMI 41.6 kg/m^2 [37.6–45.2]; weight 112 kg [102–124.5]; waist 118 cm [106–129.6]; total cho-

Abbreviations: HOMA, homeostasis model assessment index of insulin resistance; SAT, subcutaneous adipose tissue; SGBS, Simpson Golabi–Behmel syndrome; SNP, single nucleotide polymorphism; TCF7L2, transcription factor 7-like 2; VAT, visceral adipose tissue.

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lesterol mmol/l 5.02 [4.25–5.57]; triglycerides 1.78 mmol/l [1.01–2.29]). Abdominal visceral and subcutaneous adipose tissue biopsies were obtained as previously described [14]. Data on glucose homeostasis were available from 25 of the subjects (insulin 14.4 mU/l [9.8–22.36]; glucose 4.8 mmol/l [4.5–5.25]) who were all insulin resistant according to their homeostasis model assessment for insulin resistance (HOMA 2.8 [2.1–4.1]) index. All patients gave their written consent, and the Local Ethics Committees approved the study.

Cell culture and treatment. Preadipocytes from the Simpson Golabi–Behmel syndrome (SGBS) cell strain of human origin was cultured and differentiated as previously described [14,15]. The adipocytes were incubated for 4 h with 2% human serum albumin medium supplemented with glucose (1, 5, 25 mmol/l), palmitate [C16:0] (0.2, 0.5, 1.6 and 5 mmol/l glucose) or oleate [C18:1] (0.2, 0.5, 1.6 and 5 mmol/l glucose) with or without 1 nmol/l insulin. This was replicated four times with duplicates.

Expression analysis. Total RNA was extracted as previously described [14,16]. cDNA was synthesized from 600 ng RNA with QuantiTect Reverse Transcription kit (QiaGen). Samples were analyzed with real-time PCR using the ABI 7900HT sequence detection system with 7.5 or 10 ng/μl cDNA in 10 μl reaction volumes and 2× TaqMan Universal PCR Master Mix (Assay on demand, *TCF7L2*: hs00181036_m1) according to the manufacturers recommendation (Applied Biosystems, USA). All samples were analyzed in duplicates and relative quantity was calculated using the standard curve method with either cyclophilin A (*PPIA*) or *HPRT* as endogenous control.

Genotyping. Two single nucleotide polymorphisms (SNPs), rs7903146 and rs10885406, were genotyped. rs7903146 T allele has been shown to represent $HapB_{T2D}$ and the rs10885406 A allele together with the rs7603146 C allele represent HapA [13]. The samples were genotyped using TaqMan allelic discrimination performed on ABI 7900HT. Genotyping success rate was >98%.

Statistical methods. Difference between groups was tested using Wilcoxon's signed rank test for paired comparison and Mann–Whitney *U* test for unpaired comparison. Kruskal–Wallis was used when testing for variance between more than two different treatments *in vitro* and when testing for variance between genotypes *in vivo*. Correlations between variables were tested using Spearman's test. Differences were considered significant at $p < 0.05$. Data are shown as mean ± SEM. Clinical characteristics are described as median with interquartile range (25–75th percentile). All statistics were calculated using Number Cruncher Statistical System 2004 software (NCSS, Kaysville, UT).

Results

The effect of glucose, insulin and fatty acids on TCF7L2 mRNA levels in cultured human SGBS adipocytes

Increasing glucose concentrations had no effect on *TCF7L2* mRNA levels in cultured adipocytes. However, *TCF7L2* mRNA levels were 55–66% lower ($p < 0.05$) in cells incubated in the presence of insulin for 4 h, irrespective of the level of glycemia (Fig. 1). The inhibitory effect of insulin was attenuated in cells incubated with increasing amounts of fatty acids at normoglycemic conditions (5 mM); 43% attenuated inhibitory effect ($p = 0.019$) at 0.5 mmol/l palmitate and 48% ($p = 0.012$) at 1.6 mmol/l oleate (Fig. 2A and B).

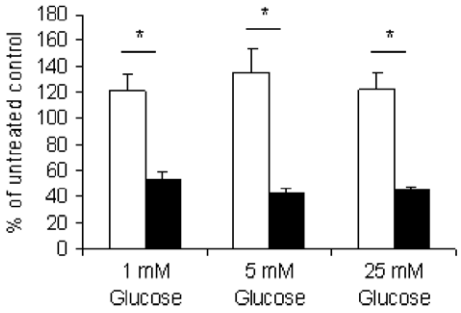

Fig. 1. *TCF7L2* mRNA levels in cultured human adipocytes treated without (white bars) or with (black bars) 1 nmol/l insulin and 1, 5 or 25 mmol/l glucose. *TCF7L2* mRNA levels were quantified by real-time PCR and normalized to *HPRT*. Data are presented as percentage of untreated control. $^{*}p < 0.05$, $n = 3–4$.

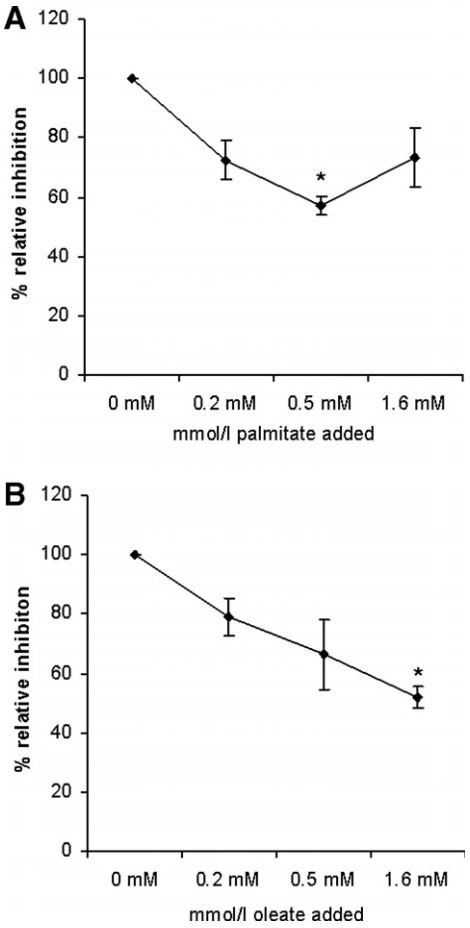

Fig. 2. Relative inhibition of *TCF7L2* mRNA levels when stimulated with insulin at normoglycemic conditions and (A) palmitate or (B) oleate in cultured human adipocytes. Data are shown as a percentage, where 100% is the decrease caused by insulin at normoglycemic conditions without free fatty acids. *TCF7L2* mRNA levels were quantified by real-time PCR and normalized to *HPRT*. $^{*}p < 0.05$, $n = 3–4$.

Genotype frequencies

The frequencies of the rs10885406 genotypes was AA 21.2% ($n = 7$), AG 63.6% ($n = 21$), GG 15.2% ($n = 5$) and of rs7903146 CC 58.8% ($n = 20$), CT 38.2% ($n = 13$) and TT 2.9% ($n = 1$). All rs10885406 A carriers also carried the rs7903146 C allele, thus representing HapA, whereas all rs7903146 T carriers represent HapB.

TCF7L2 mRNA levels in subcutaneous (SAT) and visceral (VAT) adipose tissue-TCF7L2

mRNA levels were measured in fat biopsies from SAT and VAT obtained from obese, non-diabetic subjects ($n = 35$). SAT and VAT *TCF7L2* mRNA levels were highly correlated to each other ($r = 0.53$, $p = 0.0011$), but VAT showed 26% higher levels of *TCF7L2* mRNA compared to SAT ($p = 0.00029$). The rs10885406 genotype influenced this difference; it was seen in carriers of the A allele but not in homozygous carriers of the G allele (Fig. 3A). In contrast, SNP rs7903146 had no effect on *TCF7L2* mRNA levels difference between SAT and VAT (Fig. 3B).

A significant difference was found between the mRNA levels in SAT in the tertile of the study subjects with lowest HOMA (1.9 [1.5–2.3]) compared to the mRNA levels in SAT in the tertile with highest HOMA (7.7 [4.5–7.0]) (0.76 [0.64–0.86] vs. 1.1 [0.79–1.4], $p = 0.043$). However, there was no correlation between either SAT

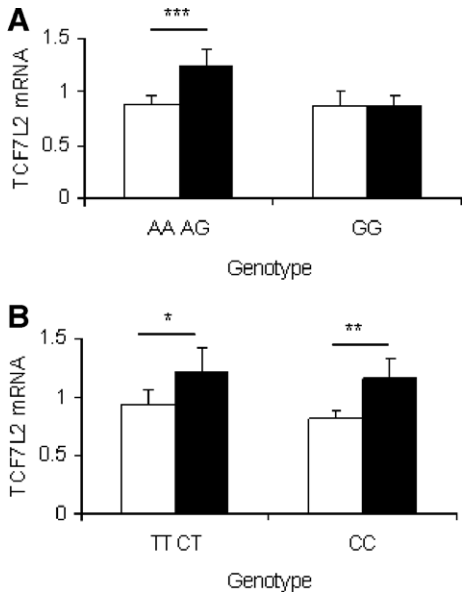

Fig. 3. *TCF7L2* mRNA levels in subjects carrying the at risk allele in (A) rs10885406 and (B) rs7903146 compared to wild type in subcutaneous (white bars) and visceral (black bars) adipose tissue. All rs10885406 A carriers also carried the rs7903146 C allele, thus representing HapA, whereas rs7903146 T carriers represents HapB. *TCF7L2* mRNA levels were quantified by real-time PCR and normalized to cyclophilin A. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

or VAT *TCF7L2* mRNA levels and measures related to obesity such as BMI, waist circumference, cholesterol, triglycerides (data not shown), except for a weak trend between SAT *TCF7L2* mRNA levels and HOMA ($r = 0.40$, $p = 0.058$).

Discussion

Recent data suggest that the strong association between *TCF7L2* and type 2 diabetes is explained by an effect on the β-cells and insulin secretion. However, TCF7L2 is a transcription factor known to have an inhibitory role of adipogenesis [4]. We therefore wanted to examine the expression of *TCF7L2* in adipocytes in relation to insulin and insulin resistance.

Firstly, we found that insulin inhibits or decreases *TCF7L2* mRNA expression in human adipocytes *in vitro*. Since one of the effects of insulin is to promote adipocyte differentiation, these findings may be potentially important. Experimental conditions were also chosen so as to mimic the elevated levels of circulating plasma free fatty acids that is often associated with human obesity and insulin resistance [1]. Interestingly, the inhibitory effect of insulin was attenuated in cells incubated with free fatty acids, possibly as a result of fatty acid induced insulin resistance. In order to investigate the physiological role of the *in vitro* findings, we studied *TCF7L2* mRNA expression in adipose tissue biopsies from obese non-diabetic subjects exhibiting varying degrees of insulin resistance. We found that *TCF7L2* mRNA levels in SAT were higher in more insulin resistant subjects, indicating that the regulation seen in response to insulin *in vitro* was lost in these insulin resistant subjects *in vivo*. It is feasible that this loss of regulation plays a role in adipogenesis and hence in the development of obesity and/or its consequences.

Secondly, we genotyped the obese study subjects to examine the possible influence of previously identified *TCF7L2* risk haplotypes HapA and $HapB_{T2D}$ on gene expression. HapA has been associated with increased BMI (1.4% per copy) and a suggestive increase in the fasting plasma concentration of insulin [13]. $HapB_{T2D}$ on the other hand has been associated with decreased BMI [13] and lower insulin secretion [9]. As previously hypothesized, the lower BMI is not necessarily a direct result of the *TCF7L2* variant, but could rather be due to the negative influence of $HapB_{T2D}$ on insulin secretion, which would lead to development of type 2 diabetes at a lower BMI [13]. The possibility that HapA and $HapB_{T2D}$ contribute to the increased risk for type 2 diabetes in different ways has also been discussed previously [13]. In light of these data we examined differences in *TCF7L2* mRNA expression between Hap A and $HapB_{T2D}$ haplotype carriers. Analyzing all subjects together we found greater *TCF7L2* mRNA expression in VAT compared to SAT in the obese subjects. However, this finding was only significant in carriers of the insulin resistance-associated HapA, whereas non-HapA (GG) carriers showed similar expression levels in both tissues. It is important to stress the limited statistical power due to the number of non-HapA carriers, but the results clearly indicate an attenuated suppression of the *TCF7L2* expression in VAT of HapA carriers, as one would expect from insulin resistance or a factor linked to insulin resistance being present. Because of its anatomical location VAT has a direct connection to the portal system and the liver and VAT is therefore assumed to be of greater importance as an endocrine organ regulating the metabolism compared to SAT [1,17]. There are many possible explanations for the observed difference between VAT and SAT mRNA expression. Regional differences in insulin sensitivity, being apparent only in the VAT of HapA carriers, may be one potential explanation. It remains to be determined weather this regional difference plays a role in the pathogenesis of obesity or if it is rather a consequence of obesity. Differences in expression levels between VAT and SAT were found for both $HapB_{T2D}$ and non-$HapB_{T2D}$ carriers confirming the lack of association between this haplotype and peripheral insulin resistance.

TCF7L2 expression in SAT and VAT from obese subjects has been investigated in one previous study by Cauchi et al. which, contrary to our findings, showed a non-significant but slightly higher expression in SAT compared to VAT [7]. This discrepancy could be explained by the fact that the previous study included only three subjects in each group of obese normoglycemic, glucose tolerant and type 2 diabetic patients and genotype. Insulin levels or resistance were not accounted for [7]. Similar to our study Cauchi et al. had an uneven distribution between genders (more female subjects). However, restricting the analysis did not significantly change our results (not shown). Previously, both a negative association [18] as well as lack of association [19] between *TCF7L2* risk genotypes and expression in adipocytes has been reported. In these studies however, they have not measured expression in both SAT and VAT and neither have they investigated SNP rs10885406.

In conclusion, we show that *TCF7L2* is an insulin sensitive gene that is inappropriately up regulated in insulin resistant subjects and in carriers of the HapA. These findings emphasize the need for further studies on the peripheral role of TCF7L2 in both adipogenesis and glucose homeostasis.

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