

The Effect of Three Months of Resistance Training on TCF7L2 Expression in Pancreas Tissues of Type 2 Diabetic Rats

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Abstract

Background: Resistance exercise is recommended as a useful therapeutic tool for the treatment of type 2 diabetes (T2D); however, the frequency of studies is inadequate to establish the precise mechanisms of any association between them.

Objectives: In this study, we aimed to assess the effect of three months of resistance training on TCF7L2 expression in pancreatic tissues, serum insulin and glucose.

Materials and Methods: For this purpose, type 2 diabetes (T2D) was induced by intraperitoneal streptozotocin-nicotinamide in eighteen male Wistar rats aged 10 weeks (220 ± 30 g). Then, the rats were randomly divided into exercise and control groups. The exercise rats completed a three-month resistance training intervention that included climbing on a stepladder for 5 days weekly. The control group did not participate in exercise intervention. Fasting glucose and insulin were measured before and after injection (7 days) and after intervention. TCF7L2 gene expression of pancreatic tissues was measured in both groups after the exercise treatment, and the ratio between the two groups was calculated.

Results: Fasting glucose increased and serum insulin decreased significantly by T2D induction in the two groups at baseline. Resistance training resulted in a decrease in fasting glucose and an increase in insulin in exercise rats. Data also showed that TCF7L2 gene expression decreased after resistance training compared with the control group.

Conclusions: Based on these data, increased serum insulin can be attributed to a decrease in TCF7L2 gene expression of pancreatic cells by resistance training in T2D rats.

Keywords: Type II Diabetes, TCF7L2 Gene Expression, Resistance Training

1. Background

Diabetes mellitus type 2 (T2D) is the most common metabolic disorder of the present century (1). It is well known that in addition to reduced insulin sensitivity, impaired beta cell function has a key role in the pathogenesis of T2D, which is expected to affect more than 300 million people by 2025 (2). However, the exact mechanisms by which beta cell function is reduced in these patients are still not fully understood. Among the factors contributing to this disorder, the role of insulin receptors in the regulation of beta cell function (3) and beta cell mass (4) can be mentioned. Thus, longitudinal studies have always emphasized the importance of the damage progression of beta cell function in the prevalence and severity of T2D.

Although T2D is a multifactorial disease, obesity is one of the leading risk factors for its incidence. The crucial role of obesity in T2D is supported by many recent and past studies (5). On the other hand, the question arises why all obese people do not develop T2D or why some T2D patients have normal weight. Hence, it seems that apart

from obesity, other important factors play major roles in this disease, which has recently been the focus of many researchers. In this context, recent genetic studies, particularly from 2007 onward, on diabetics or pre-diabetics indicated that some recently discovered genes (CDKAL1, CDKN2A/B, IGF2BP2, HHEX, HNF1B, KCNJ11, PPARG, TCF7L2, SLC30A8, WFS1, ADAMTS9, CDC123, CAMK1D, JAZF1, NOTCH2, THADA, TSPAN8, and LGR5) set the stage for T2D, even in the absence of obesity (6). Interestingly, some of these genes do not affect body weight or obesity but alter beta cell function and insulin secretion (7-10).

In the meantime, it has been recently shown that Transcription Factor 7-Like 2 (TCF7L2) polymorphisms are associated with diabetes (11), and its increased expression increases the risk of developing T2D by 1.46 times (12). TCF7L2 is a T cell transcription factor that plays an important role in cellular signaling pathways of Wnt as the main components of the regulation of cell proliferation and differentiation (13). Although Kovacs et al. (2008) reported no significant difference in TCF7L2 gene expression in visceral and

subcutaneous adipose tissue between diabetics and non-diabetics as well as between obese and non-obese people (14), some recent studies reported a 5-fold increase in its expression in pancreatic cells of T2D patients compared with healthy people, which was also associated with decreased insulin secretion (9). Recent clinical studies have indicated that the decreased TCF7L2 expression in beta cells results not only in reduced blood glucose levels, but also in improved glucose tolerance (15).

Given the role of TCF7L2 and its variants on the prevalence of T2D reported in recent studies on the Iranian population (16, 17), it is of utmost importance to answer the question whether changes in the expression of this gene or its variants due to extrinsic interventions is associated with changes in insulin and blood glucose levels as the determinants of T2D. In this context, some studies have examined the combined effect of exercise training and diet on TCF7L2 expression. It was found in a recent study that in response to changes in lifestyle (exercise + diet), changes in rs7903146 of the TCF7L2 gene has a negative correlation with insulin secretion and insulin sensitivity (18). However, in the meantime, no study was found regarding the direct effect of resistance exercises on TCF7L2 expression in pancreatic cells of T2D.

2. Objectives

Hence, the present study was carried out to evaluate the effect of 12 weeks of resistance exercises on pancreas TCF7L2 expression and insulin and glucose fasting levels in male Wistar rats with T2D.

3. Materials and Methods

3.1. Experimental Animals

Eighteen 10-week-old male Wistar rats (220 ± 30 g), procured from the institutional animal house facility, were used for all the experiments. Animals were provided with a standard pellet diet and water ad libitum, and they were maintained under standardized conditions (12-hours light/dark cycle, $25 \pm 2^\circ\text{C}$ and humidity 45% - 55%). The rats were left for 1 week for acclimatization prior to the commencement of the experiment. The study was approved by the department of exercise physiology at Tehran university, Iran, and carried out in accordance with guidelines from the committee for the purpose of control and supervision of experiments on animals (CPCSEA).

3.2. Induction of Type 2 Diabetes

After 1 week of acclimation, the Wistar rats were randomized and divided into two groups: the exercise diabetes group (ED) and the control diabetes group (CD). T2D was induced by a single intraperitoneal (i.p.) injection of 60 mg/kg streptozotocin (dissolved in citrate buffer, pH 4.5), 15 min after the i.p. administration of 95 mg/kg of nicotinamide (dissolved in normal saline) (19). Hyperglycemia was confirmed by elevated blood glucose levels on day 7 after injection, and only animals with fasting blood glucose levels between 150 and 400 mg/dL were selected to serve as T2D rats and used in the study. The animal experimental protocols were approved by the animal ethics committee of Tehran university.

3.3. Training Protocol

At this phase, 7 days after the induction of diabetes, the rats in the exercise group climbed on a stepladder without any resistance for 6 times in 3 training sessions in order to learn how to exercise. Then, they participated in a 12-week course of resistance training for 5 days per week in the format of climbing a 26-step, 1 meter vertical ladder with a gradient of 80%. Each session of resistance training was performed in the form of 3 courses with 6 repetitions on each course, and resistance was increased through attaching a weight to each rat's tail. The attached weights were proportional to the body weight of the rats during exercise. Breaks between courses and between repetitions were 3 minutes and 45 seconds, respectively. The only method used to stimulate the rats to climb the ladder was touching and rubbing the tail. To warm up and cool down before and after the workout, the rats climbed and descended the ladder 2 times without any resistance.

The resistance was increased gradually during exercise as follows; repetitions with 10% of body weight in the first week, 20% of body weight in the second week, 40% of body weight in the fourth and fifth weeks, 60% of body weight in the sixth and seventh weeks, 80% of body weight in the eighth and ninth weeks, and 100% of body weight in the tenth, eleventh, and twelfth weeks ((20, 21): justified). Finally, all rats were dissected 48 hours after the last training session following 10 to 12 hours of overnight fasting. It should be noted that the diabetic control rats were not included in the training program during this period.

3.4. Sample Collection and Biochemical Assays

Finally, 48 hours after the last training session, the fasting rats in both groups (with 10 to 12 hours of no food overnight) were anesthetized through intraperitoneal injection of 10% ketamine at a dose of 50 mg/kg along with

2% xylosine at a dose of 10 mg/kg, after which they were underwent dissection. After the rats were anesthetized, blood samples were collected through cardiac puncture. Then, pancreatic tissue was removed and immersed in RNA until biochemical analysis was performed later to determine TCF7L2 expression. The blood samples were used to analyze blood glucose and serum insulin levels. The serum was separated by centrifugation (5 minutes, 3,000 rpm) and was analyzed for glucose using a Cobas 6000 analyzer (Roche, Germany). Glucose was determined by the oxidase method (Pars Azmoon kit, Tehran). The remaining serum samples were then stored at -20°C until the insulin determination was made by the ELISA method (Demeditec, Germany). The intra-assay and inter-assay coefficients of variation of the method were 2.6% and 2.88, respectively.

3.5. RNA Extraction/Real-Time PCR

To purify RNA, 20 milligrams of tissue were ground using a mortar and pestle, and extraction was then performed employing the RNeasy Protect Mini Kit (manufactured by Qiagen Inc. in Germany) according to the manufacturer's protocol.

In this stage, the One Step SYBR Prime Script RT-PCR Kit (manufactured by the Takara Bio, Inc., in Japan) was employed according to the manufacturer's protocol to prepare the reaction product. The thermal cycle program used for the Rotor-Gene Q instrument was as follows: 42°C for 20 minutes, 95°C for two minutes, and 40 cycles at 94°C for 10 seconds and 60°C for 40 seconds. Temperatures from 50 to 99°C were used for the melting curve after the PCR to study the characteristics of the primers. The comparative $\Delta\Delta\text{CT}$ method was used to quantify the TCF mRNA expression. We used RNA polymerase II as a normalizer.

3.6. Statistical Analysis

All the data are expressed as mean \pm SD. Data were analyzed by computer using the statistical package for social sciences (SPSS) for Windows, version 15.0. At baseline, comparisons of parameters between the two groups were made by unpaired student's t-test. Student's t-tests for paired samples were performed to determine whether there were significant within-group changes in the outcomes. Differences were considered to be statistically significant when $P < 0.05$.

4. Results

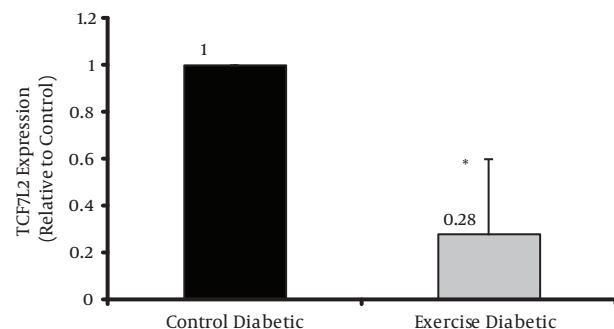
Based on statistical data, no significant differences were observed in fasting insulin between groups ($P > 0.05$). In control and exercise diabetes groups, fasting

glucose increased significantly after the streptozotocin-nicotinamide injection ($P < 0.05$). Fasting glucose decreased by resistance training in exercise diabetes subjects ($P = 0.000$) while no significant change was observed in the control diabetes group (Table 2).

Table 3 shows the changes in serum insulin in the 2 groups. Similar to fasting glucose, there were no statistically significant differences between exercise and control diabetic rats with regard to serum insulin at baseline ($P > 0.05$). Compared to baseline, fasting insulin concentration decreased significantly by streptozotocin-nicotinamide injection in both diabetes groups ($P < 0.05$). Insulin levels were significantly increased in exercise rats when compared with pre-training values ($P < 0.05$).

All changes in TCF7L2 expression in the exercise group with respect to the control group are standard. TCF7L2 expression decreased significantly with exercise training. On the other hand, 3 months of resistance training for 5 sessions per week led to a significant decrease in TCF7L2 expression in exercise diabetic rats compared to control diabetic rats. TCF7L2 expression in pancreas tissue decreased 72% in the resistance training group compared to the control group (Figure 1).

Figure 1. The Changes in TCF7L2 Expression of Pancreas Tissue by Resistance Training in T2D Rats



TCF7L2 expression decreased significantly (72%) by resistance training in exercise diabetic rats compared to control diabetic rats.* Significant difference compared to control diabetic group ($P < 0.01$).

5. Discussion

The major finding of the present study was the decreased TCF7L2 expression in response to 3 months of resistance training in T2D rats. The training course, on the other hand, decreased fasting blood glucose levels and increased serum insulin in diabetic rats with resistance training compared to those without resistance training. However, the answer to the question whether the increase in serum

Table 1. Genes

Genes	Primer Sequence	Product Size, bp	T m	Gene Bank
TCF7L2		159	60	NM_001191052.1
For:	CGTCCATGGTCCCTCCTC			
Rev:	ACTTCAATCAAGCAGGGGCAC			
RNA Polymerase II				
For:	ACTTTGATGACGTGGAGGAGGAC	164	60	XM_008759265.1
Rev:	GTTGGCCTGCGGTCGTTTC			

Table 2. Mean and Standard Deviation of Fasting Glucose in Studied Groups

Group	Baseline	After Injection ^a	After Intervention
Control diabetic	88 ± 13	358 ± 112	371 ± 112
Exercise diabetic	80 ± 11	363 ± 99	251 ± 71 ^b

^aSignificant difference compared to baseline level (P < 0.05).

^bSignificant difference compared to before intervention (P < 0.05).

Table 3. Mean and Standard Deviation of Serum Insulin in Studied Groups

Group	Baseline	After Injection ^a	After Intervention
Control diabetic	10.3 ± 4.3	6.3 ± 2.1	5.6 ± 2.4
Exercise diabetic	9.8 ± 2.7	5.9 ± 1.8	8.5 ± 2.1 ^b

^aSignificant difference compared to baseline level (P < 0.05).

^bSignificant difference compared to before intervention (P < 0.05).

insulin levels occurred in response to reduced TCF7L2 expression seems a little difficult. Type 2 diabetes is a result of complex interactions between genetic and environmental factors playing a role in the metabolism of fat and glucose, such as malfunctioning of hepatic and muscular insulin and defects in insulin secretion, adipose tissue metabolism, whole body lipolysis, and possibly metabolic defects in other organs of the body; nevertheless, the main cause of the disease, especially in its severe form, is the lack of sufficient insulin secretion from pancreatic beta cells to compensate for insulin resistance (21). In fact, although an increase in insulin resistance results in an increased mass of beta cells to secrete more insulin to compensate for insulin resistance (22-24), severe long-lasting insulin resistance is associated with reduced proliferation of beta cells. As a result, in response to long-term insulin resistance, levels of beta cell mass are not maintained for adequate secretion of insulin (25). If the capacity of insulin secretion is sufficient to compensate for insulin resistance, people with insulin resistance will not develop diabetes (22). Previous studies have noted that increased expression of TCF7L2 is associated with decreased insulin

secretion from pancreatic beta cells. Common genetic variations in TCF7L2 reveal the strongest association with type 2 diabetes known to date (26). The importance of genetic variations in TCF7L2 for type 2 diabetes has been reported in numerous studies in populations of diverse ethnic backgrounds, although the physiopathological mechanisms underlying these associations are largely unknown. On the other hand, the exact location of the genetic variations associated with the disease is different in cohorts of Asian descent (27).

Some studies on Asian populations have revealed that during increased insulin resistance after high-calorie or high-fat diets, secretion of insulin is insufficient to compensate for insulin resistance (28-30). In addition, a Korean study showed that a significant proportion of patients with type 2 diabetes are not obese and their insulin levels are normal or below normal (30). These studies point out in general that the non-obese Asians secrete insufficient amounts of insulin during insulin resistance, leading ultimately to type 2 diabetes (25, 28). In recent years, researchers have found 70 genetic variants with the risk for diabetes, but TCF7L2 is still identified as the most im-

portant diabetes susceptibility gene, so that the gene has carriers and variants with the greatest risk of type 2 diabetes (21, 29). TCF7L2 is a member of a transcription factors family and plays an important role in cellular signaling pathways of Wnt through regulation of cell proliferation and differentiation (30). The TCF7L2 gene is located on chromosome 10q25, where it has a strong linkage with diabetes type 2 (31, 32). It is known that Wnt signals affect secretion of glucagon-like peptide-1 (GLP-1) through nuclear receptors of TCF7L2 that trigger the release of insulin from beta cells of the small intestine (13).

Phenotypic changes in TCF7L2 indicate that type 2 diabetes exacerbates through or due to malfunctioning of beta cells (33-35). Changes in TCF7L2 expression and its variants are associated with impaired insulin secretion that reduces the capacity of insulin secretion in response to insulin sensitivity. Among variants of TCF7L2, rs7903146 T-allele is one of the most important genetic risk factors for T2D (36) and has been reported to have a strong direct relationship with T2D in different populations (12). This relationship is associated with defects in insulin production, and it is represented by reducing the release of insulin from pancreatic beta cells (37, 38). Other studies have reported the association between rs7903146 T-allele with impaired glucose tolerance (34, 38, 39), increased birth weight (40), and increased anthropometric indices which show the role of the TCF7L2 phenotype in several tissues (33). It seems that the diabetic effect of TCF7L2 rs7903146 or TCF7L2-related variants is presented as reduced insulin secretion or defects in insulin function, reduced effect of glucagon-like peptide-1 (GLP-1), and increased hepatic glucose production (37, 41, 42). Wegner et al. (2008) have recently shown that the TCF7L2 rs7903146 T-allele is associated with impaired insulin secretion in elderly twins (43). It is known that each TCF7L2 rs7903146 T-allele increases the risk of type 2 diabetes, even in the presence of a weight loss of 1.37-fold (11, 33, 34, 44). Several studies have also indicated that rs7903146 affects insulin production in type 2 diabetes through reducing the conversion of proinsulin to insulin. These studies in fact support the close relationship between rs7903146 and damaged conversion of proinsulin to insulin (37, 45). Genetic variations of rs7903146 are located in the non-coding region, and similar to other type 2 diabetes-related variants, it seems that their impact on the disease occurs through changes in gene expression.

Although one cannot be certain about the mechanisms of resistance training leading to reduced fasting blood glucose levels in diabetic rats in this study, according to previous evidence, it seems that reduced TCF7L2 expression following exercise is of great importance in improvement of blood glucose. Most previous studies in this area have introduced increased TCF7L2 expression in pancre-

atic cells as the most important genetic factor contributing to decreased insulin secretion. Thus, it appears that the mentioned resistance training protocol has led to increased secretion of insulin from beta cells through reducing the expression of TCF7L2 in the pancreas. In this context, some studies have noted that both diet and physical activity increase insulin secretion, while functional mechanisms are independent of each other. Diet increases beta cell mass through hypertrophy to overcome insulin resistance, while exercise training increases beta cell mass through hyperplasia, which is displayed as increased beta cell proliferation and reduced apoptosis (25). In addition, some studies have reported that exercise reduces symptoms of hepatic insulin through reducing hepatic glucose release in hyperinsulinemic conditions (46, 47). Nevertheless, some studies have shown that exercise training improves the mass and function of beta cells in type 2 diabetic patients.

The existing evidence supports increased expression of the TCF7L2 gene in the presence of type 2 diabetes, and the decline in insulin secretion in these patients has, in a way, been attributed to this higher gene expression. In this research, three months of resistance training significantly increased serum insulin levels accompanied by reduced fasting blood glucose levels in rats with type 2 diabetes that had been manipulated to develop defective insulin secretion. Moreover, this training program was accompanied by reduced levels of TCF7L2 expression in pancreatic tissue. Based on this evidence, higher insulin secretion in the studied rats probably can be attributed to lower TCF7L2 gene expression resulting from resistance training.

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Footnotes

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References

- Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004;**27**(5):1047-53. [PubMed: 15111519].
- Adeghate E, Schattner P, Dunn E. An update on the etiology and epidemiology of diabetes mellitus. *Ann NY Acad Sci*. 2006;**1084**:1-29. doi: 10.1196/annals.1372.029. [PubMed: 17151290].
- Da Silva Xavier G, Qian Q, Cullen PJ, Rutter GA. Distinct roles for insulin and insulin-like growth factor-1 receptors in pancreatic beta-cell glucose sensing revealed by RNA silencing. *Biochem J*. 2004;**377**(Pt 1):149-58. doi: 10.1042/BJ20031260. [PubMed: 14563207].
- Ogino J, Sakurai K, Yoshiwara K, Suzuki Y, Ishizuka N, Seki N, et al. Insulin resistance and increased pancreatic beta-cell proliferation in mice expressing a mutant insulin receptor (P1195L). *J Endocrinol*. 2006;**190**(3):739-47. doi: 10.1677/joe.1.06849. [PubMed: 17003275].
- Lazar MA. How obesity causes diabetes: not a tall tale. *Science*. 2005;**307**(5708):373-5. doi: 10.1126/science.1104342. [PubMed: 15662001].
- Ruchat SM, Rankinen T, Weisnagel SJ, Rice T, Rao DC, Bergman RN, et al. Improvements in glucose homeostasis in response to regular exercise are influenced by the PPARG Pro12Ala variant: results from the HERITAGE Family Study. *Diabetologia*. 2010;**53**(4):679-89. doi: 10.1007/s00125-009-1630-2. [PubMed: 20043145].
- Samson S. Role of Wnt signaling and TCF7L2 for beta cell function and regeneration in mouse models of diabetes. Houston, Texas: Baylor College of Medicine; 2011.
- Melzer D, Murray A, Hurst AJ, Weedon MN, Bandinelli S, Corsi AM, et al. Effects of the diabetes linked TCF7L2 polymorphism in a representative older population. *BMC Med*. 2006;**4**:34. doi: 10.1186/1741-7015-4-34. [PubMed: 17181866].
- Lyssenko V, Lupi R, Marchetti P, Del Guerra S, Orho-Melander M, Almgren P, et al. Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. *J Clin Invest*. 2007;**117**(8):2155-63. doi: 10.1172/JCI30706. [PubMed: 17671651].
- Villareal DT, Robertson H, Bell GI, Patterson BW, Tran H, Wice B, et al. TCF7L2 variant rs7903146 affects the risk of type 2 diabetes by modulating incretin action. *Diabetes*. 2010;**59**(2):479-85. doi: 10.2337/db09-1169. [PubMed: 19934000].
- Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, et al. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet*. 2006;**38**(3):320-3. doi: 10.1038/ng1732. [PubMed: 16415884].
- Cauchi S, El Achhab Y, Choquet H, Dina C, Krempler F, Weitgasser R, et al. TCF7L2 is reproducibly associated with type 2 diabetes in various ethnic groups: a global meta-analysis. *J Mol Med (Berl)*. 2007;**85**(7):777-82. doi: 10.1007/s00109-007-0203-4. [PubMed: 17476472].
- 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**(2):1457-64. doi: 10.1074/jbc.M411487200. [PubMed: 15525634].
- Kovacs P, Berndt J, Ruschke K, Kloting N, Schon MR, Korner A, et al. TCF7L2 gene expression in human visceral and subcutaneous adipose tissue is differentially regulated but not associated with type 2 diabetes mellitus. *Metabolism*. 2008;**57**(9):1227-31. doi: 10.1016/j.metabol.2008.04.016. [PubMed: 18702948].
- Hansson O, Zhou Y, Renstrom E, Osmark P. Molecular function of TCF7L2: Consequences of TCF7L2 splicing for molecular function and risk for type 2 diabetes. *Curr Diab Rep*. 2010;**10**(6):444-51. doi: 10.1007/s11892-010-0149-8. [PubMed: 20878273].
- Palizban A, Nikpour M, Salehi R, Maracy MR. Association of a common variant in TCF7L2 gene with type 2 diabetes mellitus in a Persian population. *Clin Exp Med*. 2012;**12**(2):115-9. doi: 10.1007/s10238-011-0144-7. [PubMed: 21678030].
- Amoli MM, Amiri P, Tavakkoly-Bazzaz J, Charmchi E, Hafeziyeh J, Keramatipour M, et al. Replication of TCF7L2 rs7903146 association with type 2 diabetes in an Iranian population. *Genet Mol Biol*. 2010;**33**(3):449-51. doi: 10.1590/S1415-4752010005000056. [PubMed: 21637413].
- McCaffery JM, Jablonski KA, Franks PW, Dagogo-Jack S, Wing RR, Knowler WC, et al. TCF7L2 polymorphism, weight loss and proinsulin:insulin ratio in the diabetes prevention program. *PLoS One*. 2011;**6**(7):ee21518. doi: 10.1371/journal.pone.0021518. [PubMed: 21814547].
- Shirwaikar A, Rajendran K, Dinesh Kumar C, Bodla R. Antidiabetic activity of aqueous leaf extract of *Annona squamosa* in streptozotocin-nicotinamide type 2 diabetic rats. *J Ethnopharmacol*. 2004;**91**(1):171-5. doi: 10.1016/j.jep.2003.12.017. [PubMed: 15036485].
- Molanouri Shamsi M, Hassanzadeh ZM, Mahdavi M, Gharakhanlou R, Azadmanesh K, Baghersad L, et al. Influence of Resistance Training on IL-15 mRNA Expression and the Protein Content in Slow and Fast Twitch Muscles of Diabetic Rats. *Iranian J Endocrinol Metab*. 2012;**14**(2):185-92.
- Alibegovic AC, Sonne MP, Hojbjerg L, Hansen T, Pedersen O, van Hall G, et al. The T-allele of TCF7L2 rs7903146 associates with a reduced compensation of insulin secretion for insulin resistance induced by 9 days of bed rest. *Diabetes*. 2010;**59**(4):836-43. doi: 10.2337/db09-0918. [PubMed: 20107109].
- Jetton TL, Lausier J, LaRock K, Trotman WE, Larmie B, Habibovic A, et al. Mechanisms of compensatory beta-cell growth in insulin-resistant rats: roles of Akt kinase. *Diabetes*. 2005;**54**(8):2294-304. [PubMed: 16046294].
- Rooman I, Lardon J, Bouwens L. Gastrin stimulates beta-cell neogenesis and increases islet mass from transdifferentiated but not from normal exocrine pancreas tissue. *Diabetes*. 2002;**51**(3):686-90. [PubMed: 11872667].
- Weir GC, Bonner-Weir S. Five stages of evolving beta-cell dysfunction during progression to diabetes. *Diabetes*. 2004;**53**(Supplement 3):S16-21. doi: 10.2337/diabetes.53.suppl_3.S16.
- Park S, Hong SM, Lee JE, Sung SR. Exercise improves glucose homeostasis that has been impaired by a high-fat diet by potentiating pancreatic beta-cell function and mass through IRS2 in diabetic rats. *J Appl Physiol (1985)*. 2007;**103**(5):1764-71. doi: 10.1152/japophysiol.00434.2007. [PubMed: 17761790].
- Renstrom E. Impact of transcription factor 7-like 2 (TCF7L2) on pancreatic islet function and morphology in mice and men. *Diabetologia*. 2012;**55**(10):2559-61. doi: 10.1007/s00125-012-2659-1. [PubMed: 22864463].
- Li WL, Zheng HC, Bukuru J, De Kimpe N. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. *J Ethnopharmacol*. 2004;**92**(1):1-21. doi: 10.1016/j.jep.2003.12.031. [PubMed: 15099842].
- Cockram CS. The epidemiology of diabetes mellitus in the Asia-Pacific region. *Hong Kong Med J*. 2000;**6**(1):43-52. [PubMed: 10793402].
- Anders R, Ola H. Mechanisms whereby genetic variation in the TCF7L2 gene causes diabetes: novel targets for anti-diabetic therapy? Sweden: Lund University Diabetes Centre; 2013.
- Ross SE, Hemati N, Longo KA, Bennett CN, Lucas PC, Erickson RL, et al. Inhibition of adipogenesis by Wnt signaling. *Science*. 2000;**289**(5481):950-3.
- Wiltshire S, Hattersley AT, Hitman GA, Walker M, Levy JC, Sampson M, et al. A genomewide scan for loci predisposing to type 2 diabetes in a U.K. population (the Diabetes UK Warren 2 Repository): analysis of 573 pedigrees provides independent replication of a susceptibility locus on chromosome 1q. *Am J Hum Genet*. 2001;**69**(3):553-69. [PubMed: 11484155].
- Reynisdottir I, Thorleifsson G, Benediktsson R, Sigurdsson G, Emilsson V, Einarsson AS, et al. Localization of a susceptibility gene for type 2 diabetes to chromosome 5q34-q35.2. *Am J Hum Genet*. 2003;**73**(2):323-35. doi: 10.1086/377139. [PubMed: 12851856].
- Cauchi S, Meyre D, Dina C, Choquet H, Samson C, Gallina S, et al. Transcription factor TCF7L2 genetic study in the French population:

- expression in human beta-cells and adipose tissue and strong association with type 2 diabetes. *Diabetes*. 2006;**55**(10):2903-8. doi: [10.2337/db06-0474](https://doi.org/10.2337/db06-0474). [PubMed: [17003360](https://pubmed.ncbi.nlm.nih.gov/17003360/)].
34. Cauchi S, Meyre D, Choquet H, Dina C, Born C, Marre M, et al. TCF7L2 variation predicts hyperglycemia incidence in a French general population: the data from an epidemiological study on the Insulin Resistance Syndrome (DESIR) study. *Diabetes*. 2006;**55**(11):3189-92. doi: [10.2337/db06-0692](https://doi.org/10.2337/db06-0692). [PubMed: [17065361](https://pubmed.ncbi.nlm.nih.gov/17065361/)].
 35. Saxena R, Gianniny L, Burt NP, Lyssenko V, Giuducchi C, Sjogren M, et al. Common single nucleotide polymorphisms in TCF7L2 are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in nondiabetic individuals. *Diabetes*. 2006;**55**(10):2890-5. doi: [10.2337/db06-0381](https://doi.org/10.2337/db06-0381). [PubMed: [17003358](https://pubmed.ncbi.nlm.nih.gov/17003358/)].
 36. Helgason A, Palsson S, Thorleifsson G, Grant SF, Emilsson V, Gunnarsdottir S, et al. Refining the impact of TCF7L2 gene variants on type 2 diabetes and adaptive evolution. *Nat Genet*. 2007;**39**(2):218-25. doi: [10.1038/ng1960](https://doi.org/10.1038/ng1960). [PubMed: [17206141](https://pubmed.ncbi.nlm.nih.gov/17206141/)].
 37. Kirchhoff K, Machicao F, Haupt A, Schafer SA, Tschritter O, Staiger H, et al. Polymorphisms in the TCF7L2, CDKAL1 and SLC30A8 genes are associated with impaired proinsulin conversion. *Diabetologia*. 2008;**51**(4):597-601. doi: [10.1007/s00125-008-0926-y](https://doi.org/10.1007/s00125-008-0926-y). [PubMed: [18264689](https://pubmed.ncbi.nlm.nih.gov/18264689/)].
 38. Raitakari OT, Ronnema T, Huupponen R, Viikari L, Fan M, Marniemi J, et al. Variation of the transcription factor 7-like 2 (TCF7L2) gene predicts impaired fasting glucose in healthy young adults: the Cardiovascular Risk in Young Finns Study. *Diabetes Care*. 2007;**30**(9):2299-301. doi: [10.2337/dc07-0539](https://doi.org/10.2337/dc07-0539). [PubMed: [17540954](https://pubmed.ncbi.nlm.nih.gov/17540954/)].
 39. Wang J, Kuusisto J, Vanttinen M, Kuulasmaa T, Lindstrom J, Tuomilehto J, et al. 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**(6):192-200. doi: [10.1007/s00125-007-0656-6](https://doi.org/10.1007/s00125-007-0656-6). [PubMed: [17437080](https://pubmed.ncbi.nlm.nih.gov/17437080/)].
 40. Freathy RM, Weedon MN, Bennett A, Hypponen E, Relton CL, Knight B, et al. Type 2 diabetes TCF7L2 risk genotypes alter birth weight: a study of 24,053 individuals. *Am J Hum Genet*. 2007;**80**(6):1150-61. doi: [10.1086/518517](https://doi.org/10.1086/518517). [PubMed: [17503332](https://pubmed.ncbi.nlm.nih.gov/17503332/)].
 41. Loos RJ, Franks PW, Francis RW, Barroso I, Gribble FM, Savage DB, et al. TCF7L2 polymorphisms modulate proinsulin levels and beta-cell function in a British European population. *Diabetes*. 2007;**56**(7):1943-7. doi: [10.2337/db07-0055](https://doi.org/10.2337/db07-0055). [PubMed: [17416797](https://pubmed.ncbi.nlm.nih.gov/17416797/)].
 42. Stolerman ES, Manning AK, McAteer JB, Fox CS, Dupuis J, Meigs JB, et al. TCF7L2 variants are associated with increased proinsulin/insulin ratios but not obesity traits in the Framingham Heart Study. *Diabetologia*. 2009;**52**(4):614-20. doi: [10.1007/s00125-009-1266-2](https://doi.org/10.1007/s00125-009-1266-2). [PubMed: [19183934](https://pubmed.ncbi.nlm.nih.gov/19183934/)].
 43. Wegner L, Hussain MS, Pilgaard K, Hansen T, Pedersen O, Vaag A, et al. Impact of TCF7L2 rs7903146 on insulin secretion and action in young and elderly Danish twins. *J Clin Endocrinol Metab*. 2008;**93**(10):4013-9. doi: [10.1210/jc.2008-0855](https://doi.org/10.1210/jc.2008-0855). [PubMed: [18611970](https://pubmed.ncbi.nlm.nih.gov/18611970/)].
 44. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature*. 2007;**445**(7130):881-5. doi: [10.1038/nature05616](https://doi.org/10.1038/nature05616). [PubMed: [17293876](https://pubmed.ncbi.nlm.nih.gov/17293876/)].
 45. Gonzalez-Sanchez JL, Martinez-Larrad MT, Zabena C, Perez-Barba M, Serrano-Rios M. Association of variants of the TCF7L2 gene with increases in the risk of type 2 diabetes and the proinsulin:insulin ratio in the Spanish population. *Diabetologia*. 2008;**51**(11):1993-7. doi: [10.1007/s00125-008-1129-2](https://doi.org/10.1007/s00125-008-1129-2). [PubMed: [18712344](https://pubmed.ncbi.nlm.nih.gov/18712344/)].
 46. Heled Y, Shapiro Y, Shani Y, Moran DS, Langzam I, Barash V, et al. Physical exercise enhances hepatic insulin signaling and inhibits phosphoenolpyruvate carboxykinase activity in diabetes-prone Psammomys obesus. *Metabolism*. 2004;**53**(7):836-41. [PubMed: [15254873](https://pubmed.ncbi.nlm.nih.gov/15254873/)].
 47. Perseghin G, Lattuada G, De Cobelli F, Ragogna F, Ntali G, Esposito A, et al. Habitual physical activity is associated with intrahepatic fat content in humans. *Diabetes Care*. 2007;**30**(3):683-8. doi: [10.2337/dc06-2032](https://doi.org/10.2337/dc06-2032). [PubMed: [17327341](https://pubmed.ncbi.nlm.nih.gov/17327341/)].