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ORIGINAL CONTRIBUTION

Improved glucose metabolism in mice lacking α-tocopherol transfer protein

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Abstract *Background* Conflicting evidence suggests a possible role for vitamin E in mammalian glucose metabolism and the protection from type 2 diabetes. The alpha-tocopherol transfer protein (α-TTP) mediates the transfer of α-tocopherol (α-TOH) from hepatocytes to very-low-density lipoproteins, thereby controlling plasma levels of α-TOH. *Aim of the study* The aim of this study was to investigate the putative impact of α-TTP knock-out on glucose metabolism in mice. *Methods* Mice deficient for α-TTP and wild-type control littermates were fed a diet containing 200 mg α-tocopheryl acetate per kg to ameliorate α-TOH deficiency in knock-out mice. We investigated fasting and postprandial plasma glucose, insulin and triglyceride levels of both groups of mice at different ages. All genotypes and age groups were further subjected to glucose and insulin tolerance tests, and number of insulin-producing islets of Langerhans were determined. *Results* Plasma α-TOH levels of knock-out mice were 34% the levels of wild-type controls: Any signs of α-TOH deficiency were absent at any age. Unexpectedly, serum glucose levels both in the fasted and in the fed state were lower in α-TTP-deficient mice at any age. Removal rates for intraperitoneally injected glucose were found to be significantly increased in young α-TTP-deficient mice. This improved glucose tolerance was caused by increased insulin secretion in response to an intraperitoneal glucose challenge due to an increased number of pancreatic islets, as well as by increased sensitivity to intraperitoneally injected insulin, both significantly promoting glucose metabolism in α-TTP-deficient mice. *Conclusions* Our findings suggest that α-TTP-deficiency in states of α-TOH supplementation unexpectedly promotes glucose tolerance in mice due to both increased insulin secretion and insulin action, suggesting differential roles of α-TTP and α-TOH in the pathogenesis of type 2 diabetes mellitus.

Key words alpha-tocopherol transfer protein – alpha tocopherol – glucose metabolism – diabetes mellitus type 2 – insulin secretion – insulin sensitivity

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Introduction

Since the discovery that vitamin E prevents fetus resorption in diet-restricted rats in 1922 [10] α-TOH-deficiency has been associated with several diseases including cancer, cardiovascular diseases and diabetes (reviewed in [4]). Natural vitamin E consists of a mixture of isomers which differ by methylation patterns of their chromanol ring and the saturation of the side-chain (α-, β-, γ-, δ-tocopherol and -tocotrienol). While its role as lipophilic antioxidant in vitro is widely accepted, the relevance in vivo is still a matter of debate. Non-antioxidant functions of individual members of the vitamin E family have also been demonstrated [2]. α-TOH has been reported to inhibit protein kinase C (PKC) by decreasing diacylglycerol (DAG) levels and the activation of protein phosphatases independent of its antioxidant capacity [24, 38]. Intraperitoneal injection of vitamin E prevented the increase of both DAG levels and PKC levels in different tissues such as heart, aorta and renal glomeruli of diabetic rats [23]. Many other examples for a role of vitamin E in cellular signaling and gene regulation have been reported (reviewed in [3]).

Vitamin C and vitamin E are believed to prevent oxidative events by scavenging of free radicals [8, 12]. Diabetes mellitus type 2 is a consequence of impaired glucose metabolism due to various reasons. Since hyperglycaemia is associated with the production of reactive oxygen species and causes oxidative stress in several model systems, a number of studies have been conducted investigating the possible role of α-tocopherol in diabetes and glucose homeostasis: Case–control as well as prospective studies have implicated a protective effect of plasma antioxidants with regard to type 2 diabetes mellitus [11, 22, 29, 36]. Intervention studies and a cohort study, however, did not find any correlation of vitamin E and the risk of type 2 diabetes [7, 27, 35].

Out of the eight vitamin E isomers, α-TOH is preferentially assorted by α-tocopherol transfer protein (α-TTP). The protein belongs to the SEC14 lipid ligand-binding protein family, all sharing a retinal binding motive sequence (so-called CRAL-TRIO domain) [42]. α-TTP mediates the transfer of α-TOH from liver into very-low-density lipoproteins (VLDL). As a result, α-TOH is preferentially retained in plasma over other forms of vitamin E, regardless of dietary intake levels. Humans with reduced α-TTP expression suffer from neuromuscular abnormalities characterized by spinocerebellar ataxia and myopathies [31]. This ataxia is associated with low systemic α-TOH levels and therefore called ataxia with vitamin E deficiency (AVED) [28]. Interestingly, clinical symptoms of AVED are extremely similar to Friedreich ataxia (FRDA), another inherited ataxia, which is known to be associated with increased incidence of diabetes mellitus [33]. Of note, and unlike in FRDA, to our knowledge AVED has never been associated with impaired glucose metabolism or diabetes mellitus [28], while the latter is a typical complication of FRDA [33] and accordingly found in animal models of FRDA [34].

Targeted disruption of the α-TTP (*Ttpa*) gene in mice results in low α-TOH levels in plasma as well as in several tissues, and typical signs of AVED [19, 39, 41]. Besides its predominant expression in liver [1] and Purkinje cells of the cerebellum [6], α-TTP is also located in the uterus and placenta [19, 20, 30]. High-density oligonucleotide arrays of *Ttpa* knock-out ($Ttpa^{-/-}$) mice furthermore indicated that the hepatic expression of mRNAs controlling hepatic glucose homeostasis is closely linked to the expression of α-TTP [13].

To investigate the possibility of an interaction of α-TTP with glucose metabolism, we subjected $Ttpa^{-/-}$ mice to a number of diabetes-relevant physiological tests. To dissect effects of α-TOH deficiency from α-TTP deficiency, animals were fed a diet containing high α-tocopherol levels (200 mg α-tocopheryl acetate/kg diet). Surprisingly, in comparison to wild-type mice, we found decreased fasting glucose levels, increased insulin secretion and increased insulin sensitivity in $Ttpa^{-/-}$ mice, altogether suggesting an improvement of glucose metabolism and possibly protection from diabetes mellitus caused by a lack of functional α-TTP.

Material and methods

Animals and diets

Mice were housed in standard barrier facilities according to the Federation of European Laboratory Animal Science Associations (FELASA) regulations. The study group consisted of $n = 6$ for both $Ttpa^{-/-}$ and age- and gender-matched wild-type control mice on a mixed (80% C57Bl6 and 20% 129SvJ) genetic background [39]. $Ttpa^{-/-}$ mice were obtained as a kind gift from Dr. R.V. Farese. All mice were fed a vitamin E-enriched diet based on Basis purified diets (Ssniff Spezialdiäten GmbH, Soest, Germany) supplemented with 200 mg/kg RRR-α-tocopheryl acetate (No. T3001 from Sigma-Aldrich, Deisenhofen, Germany). The basal diet was composed of the following components: casein, 240 g/kg; cornstarch, 500 g/kg; glucose, 110 g/kg; coconut oil, 30 g/kg; cellulose, 50 g/kg; vitamin premix without vitamin E, 10 g/kg; and mineral and trace element premix 60 g/kg. The α-tocopherol content of the major compounds was below 2 mg/kg. All tocopherol contents were analyzed

and confirmed by high performance liquid chromatography as previously described [21]. Diet was started directly after weaning and continued until the end of the study period. All animal experiments were approved by the governmental animal-ethics committee.

Genotype analyses and immunoblot analyses

Genotyping of mice was performed by polymerase chain reaction using primers A (5′-TGAGTGTGC GTGGGGCGGCGTCC-3′), B (5′-CTGTTTCCCAACCA ATGGCCCC-3′), and C (5′-CATTCAGGCTGCGCAAC TGTTGGG-3′) at 95°C for 10 min, followed by 30 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 1 min. Primers A and B were previously designed to amplify a 138 bp fragment from the wild-type allele, and primers A and C were previously designed to amplify a 266 bp fragment from the knock-out allele [39]. Immunoblots against (α-TTP were performed with our own (M.S.) polyclonal antiserum as described [30].

Determination of plasma and liver α-tocopherol concentration

Plasma of wild-type and *Ttpa*$^{-/-}$ mice was mixed with 1 ml methanol, 2.5 ml *n*-hexane and 4.3 nmol δ-tocopherol as internal standard. Tocopherols were extracted twice with 2.5 ml *n*-hexane and evaporated to dryness. Livers of wild-type and *Ttpa*$^{-/-}$ mice were minced with mortar and pistil under liquid nitrogen and weighed in a 15 ml tube. After addition of 1 ml pyrogallol (6% w/v in ethanol) and 4.3 nmol δ-tocopherol as internal standard the sample was saponified with 400 µl 40% potassium hydroxide for 30 min at 70°C. α-TOH was extracted twice with 2.5 ml *n*-hexane after addition of 1.6 ml 2% sodium chloride and evaporated to dryness.

Residues were dissolved in 95% methanol. Tocopherols were determined in a Summit HPLC-system with an ED 50 electrochemical detector (Dionex, Idstein, Germany) and a 250 × 4 mm, RP-18 end-capped column (Merck, Darmstadt, Germany) with a preceding guard column (4 × 4 mm) containing the same stationary phase. The mobile phase consisted of 95% methanol with 1.02 g/l lithium acetate and 0.05% v/v trifluoroacetic acid [A] and propanol [B]. The flow was set to 1.0 ml/min with a gradient program of 0% [B]: 0–35 min; 0–30% [B]: 35–38 min; 30% [B]: 38–105 min; 30–0% [B]: 105–108 min, and 0% [B]: 108–120 min. For colorimetric detection, the analytical cell was set to +0.85 V. α-TOH content was calculated using peak areas and concentration of the internal standard δ-tocopherol and a response factor of 0.4387.

Postprandial and fasting serum insulin, glucose and triglyceride levels

Blood samples of young (6 weeks) and old (36 weeks) mice were collected in the postprandial state as well as after an overnight fasting period for 14–16 h via retroorbital puncture under Isoflurane® inhalation anaesthesia. Samples were centrifuged at 8,000 rpm at 4°C, and serum was kept at −80°C until further analysis. Glucose and total triglyceride serum levels were analyzed by standard enzymatic assays (ABX, Montpellier, France) on an autoanalyzer (Cobas Mira S, Roche Diagnostics GmbH, Mannheim, Germany). Serum insulin levels were determined using an ELISA for rat insulin with a mouse insulin standard (both from CrystalChem Inc., Chicago, Illinois, USA) as previously described [32, 34].

Glucose and insulin tolerance test

Each assay was performed in young and old mice after overnight fasting. For glucose tolerance tests, glucose was injected intraperitoneally (2 g/kg body mass) and blood samples were collected before and 10, 30, 60, and 120 min after injection as previously described [17]. Serum glucose and serum insulin were determined as mentioned before. For insulin tolerance tests, we injected insulin (0.75 U/kg body weight) and measured blood glucose before and 15, 30, and 60 min after injection in tail vein blood using MediSense Precision Plus Electrodes (Abbott GmbH & Co. KG, Wiesbaden, Germany) as previously described [18].

Quantification of pancreatic islets of Langerhans

Quantification of islet number was performed as previously described [32, 34], briefly using pancreata from five *Ttpa*$^{-/-}$ mice and five wild-type littermates at 4 weeks of age, i.e. 10 animals in total. Three independent sections at least 200 µm apart of each animal were studied, i.e. 30 sections in total. Histology and immunohistochemistry were performed using standard procedures. The primary antibody used was anti-insulin (Camon, Wiesbaden, Germany). Islet morphometry was performed using a digital microscope (Eclipse E1000; Nikon Deutschland GmbH, Düsseldorf, Germany) and quantification software (Lucia G version 5.0; Nikon Deutschland GmbH) as previously described [32, 34].

Statistical analysis

Statistical calculations were performed with SPSS 12.0 (SPSS Inc., Chicago, Illinois, USA). All values are

given as mean ± SEM or SD, as indicated. Differences between two groups were assessed using unpaired student's *t* tests and two-tailed distribution with *P* below 0.05 considered statistically significant.

Results

Establishing supplementation of α-TOH

To dissect the differential effects of α-TOH deficiency from the effects of α-TTP-deficiency, we performed a series of experiments to establish the ideal supplementation of α-TOH (data not shown). Specifically we aimed to substitute α-TOH in an amount sufficient to obtain plasma levels of α-TOH known to prevent symptoms of α-TOH deficiency.

Dietary supplementation of knock-out animals with various concentrations of α-TOH resulted in reduced α-TOH plasma levels compared to wild type animals. Supplementing α-TTP-deficient animals with 200 mg/kg α-TOH was sufficient to avoid any obvious signs of a vitamin E deficiency, while plasma α-TOH levels were only 34% of control animals (additional data are shown below). According to previously published findings, neurological symptoms have not been observed in *Ttpa*$^{-/-}$ or vitamin E-depleted animals whenever plasma levels exceeded 10% of control animal α-TOH concentrations [15, 41]. Additionally, reduced fertility observed in vitamin E deficient *Ttpa* knock-out animals could be reversed by dietary vitamin E re-supplementation ([19] as well as in our hands, unpublished). Finally, both groups of animals tested in this study exhibited the same body mass in adolescent and aged stage, respectively (Fig. 1A).

Summarizing previously published and our own findings, all *Ttpa*$^{-/-}$ animals fed a vitamin E rich diet were accounted apparently healthy, and showed no signs gross of ataxia or growth retardation. Based on our experiments and previously published findings, we finally chose a supplementation of 200 mg α-tocopheryl acetate per kg diet.

Detection and efficacy of Ttpa-disruption in mice

To obtain *Ttpa*$^{-/-}$ mice both parental animals were kept in the heterozygous state. Following mendelian

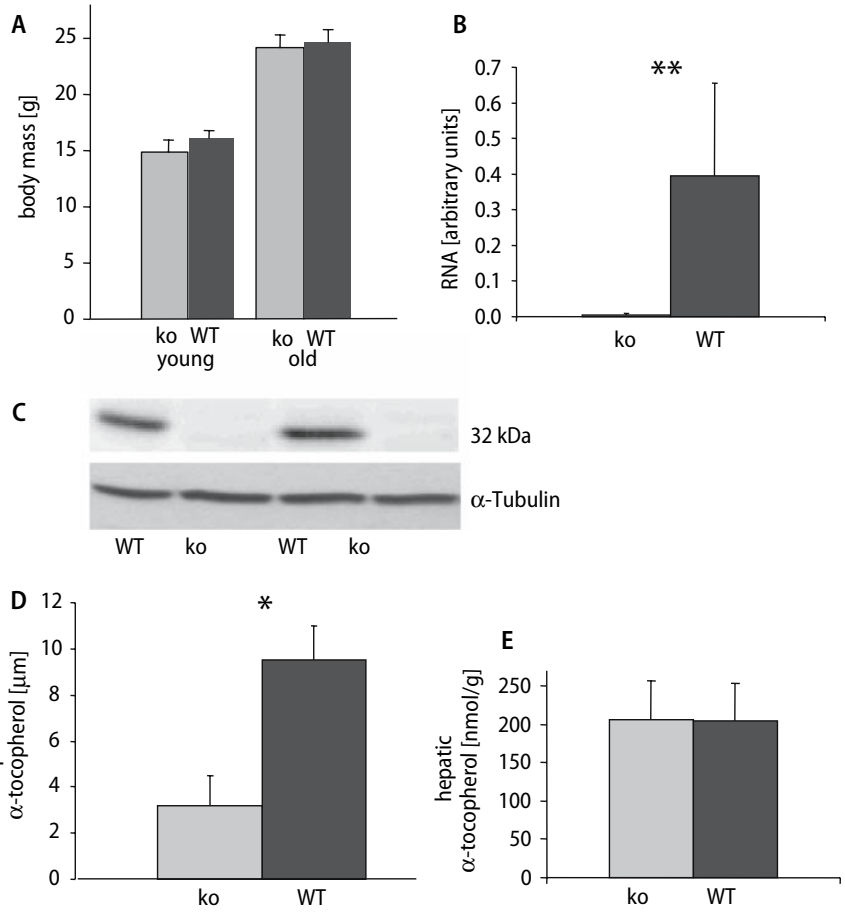

Fig. 1 Presence of *Ttpa* disruption and resulting alterations in α-TOH concentrations. Panel **A** depicts body mass values for animals subjected to experiments. Data are expressed as means ± SEM from at least six animals per genotype. Panel **B** depicts real-time PCR based quantification of *Ttpa*-mRNA prepared from homogenized liver tissue samples using SYBR®Green. The measurement of *Ttpa* mRNA was performed by quantitative real-time RT-PCR using SYBR®Green I. Panel **C** depicts a typical result of a Western blot analysis against α-TTP protein in liver homogenates derived from wild-type (WT) and *Ttpa*$^{-/-}$ animals (ko) (upper panel). A re-blot against α-tubulin is shown to indicate equal loading (lower panel). Panel **D** shows plasma α-TOH levels of wild-type (WT, black bars, applies to all subsequent panels) and *Ttpa* knock-out (ko, grey bars, applies to all subsequent panels) mice (36 weeks). Data are expressed as mean ± SD from four animals per genotype. Asterisk (*) indicates $P < 0.05$ (applies to all subsequent panels and figures). Panel **E** shows α-TOH content of liver homogenates from wild-type and *Ttpa* knock-out mice

distribution, offspring obtained from heterozygotes was approx. 25% knock-out and approx. 25% wild-type. The latter group was used as control. Analysis of *Ttpa*-mRNA levels in liver specimen using quantitative RT-PCR revealed complete absence of transcripts in *Ttpa*$^{-/-}$ mice while control mice had detectable levels of *Ttpa*-transcripts (Fig. 1B). Western blot analysis showed a complete lack of the 32 kDa α-TTP-protein in liver tissues, while this protein was abundantly expressed in controls livers (Fig. 1C).

Effects of Ttpa-disruption on α-TOH concentrations in plasma and liver specimen

To evaluate the direct effects of *Ttpa*-disruption on α-tocopherol compartmentalization in mice, plasma and hepatic α-TOH concentrations were determined. Plasma α-TOH concentration of control animals were in the range of 9.49 μM (±1.49) whereas knock-out animals exhibited plasma concentrations of 3.2 μM (±1.28) α-TOH (Fig. 1D). For comparison, *Ttpa*$^{-/-}$ animals receiving a vitamin E deficient diet exhibit markedly reduced α-TOH plasma levels of 200–500 nM [41]. α-TOH concentrations of liver extracts were in the range of 200 nmol/g tissue for both genotypes (205.5 ± 52.1 vs. 204.6 ± 48.8 nmol/g) (Fig. 1E). The ability of the liver to retain dietary α-TOH despite of a non-functional *Ttpa*-gene has been demonstrated before [26], and has been explained by the fact that lack of α-TTP is associated with the inability to secrete tocopherol in nascent VLDL and an accumulation of α-TOH in the liver.

Fasting and postprandial blood glucose levels

Serum glucose concentrations in *Ttpa*$^{-/-}$ animals as well as corresponding control littermates were determined in both the fasting and postprandial state of young and old mice. Mean fasting serum glucose levels of young (6 weeks) *Ttpa*$^{-/-}$ mice were found to be 38% lower than in their wild-type controls (4.4 vs. 7.1 mmol/l; $P < 0.01$). Similar observations were made with 36 week old mice (5.2 vs. 6.7 mmol/l; $P < 0.01$) (Fig. 2A). Mean postprandial serum glucose levels of young (6 weeks) *Ttpa*$^{-/-}$ mice were found to be 16% lower than in their wild-type controls (8.1 vs. 9.5 mmol/l; $P < 0.02$). Similar observations were made with 36 week old mice (8.8 vs. 11.0 mmol/l; $P < 0.01$) (Fig. 2B).

While not depicted in the figures, it should be noted that fasting as well as postprandial serum insulin levels were found to be unchanged in *Ttpa*$^{-/-}$ mice (6 weeks of age) compared to their wild-type controls (1.3 ± 0.3 vs. 1.2 ± 0.1 ng/ml; $n = 6$).

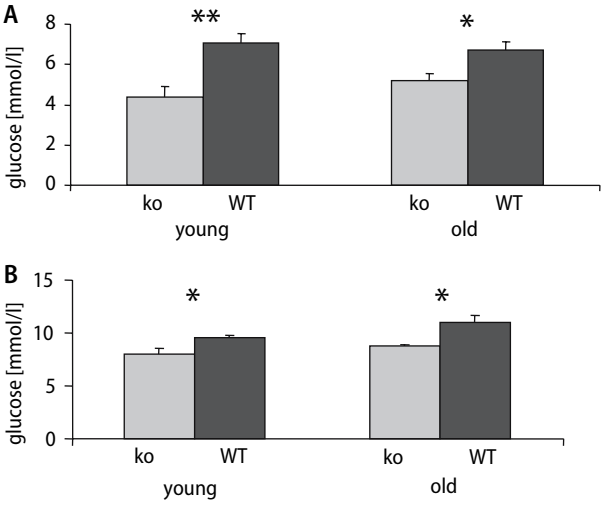

Fig. 2 Decreased fasting and postprandial serum glucose concentrations in mice lacking *Ttpa*. Panel **A** depicts fasting glucose levels of young (6 weeks) and old (36 weeks) mice. Data are expressed as means ± SEM from at least six animals per genotype. Asterisks (**) indicate $P < 0.01$. Panel **B** depicts postprandial glucose levels of young (6 weeks) and old (36 weeks) mice

Intraperitoneal glucose tolerance test

To gain further insight into the role of glucose metabolism and specifically glucose stimulated insulin secretion in *Ttpa*$^{-/-}$ mice, we performed intraperitoneal glucose tolerance tests. Both relative serum glucose levels and the area under the curve for serum glucose after glucose challenge were significantly lower in both young (10 weeks of age) and older (36 weeks of age) *Ttpa*$^{-/-}$ mice, respectively, in comparison to control animals. While a typical result is depicted in Fig. 3A, the area under the glucose response curves ($AUC_{glucose}$) for young mice was 2,323.7 mmol/l min (*Ttpa*$^{-/-}$) vs. 3,240.6 mmol/l min (wild-type controls) ($P < 0.02$, Fig. 3B), and $AUC_{glucose}$ for 36 week-old mice was 2,718.2 mmol/l min (*Ttpa*$^{-/-}$) vs. 3,548.5 mmol/l min (wild-type controls) ($P < 0.2$, Fig. 3B).

Accordingly, we determined serum insulin concentrations from the same samples used for glucose determinations (depicted in Fig. 3B): the area under the insulin curves ($AUC_{insulin}$) for young mice was 95.6 ng/ml min (*Ttpa*$^{-/-}$) vs. 69.03 ng/ml min (controls) ($P < 0.03$, Fig. 3C), and the ($AUC_{insulin}$) for 36 week-old mice was 111.14 ng/ml min (*Ttpa*$^{-/-}$) vs. 92.91 ng/ml min (wild-type controls) ($P < 0.14$, Fig. 3C).

Increased number of pancreatic islets of Langerhans

Increased insulin secretion as observed above may be caused by different reasons, most likely by an increase in secretory capacity of islets of Langerhans known to

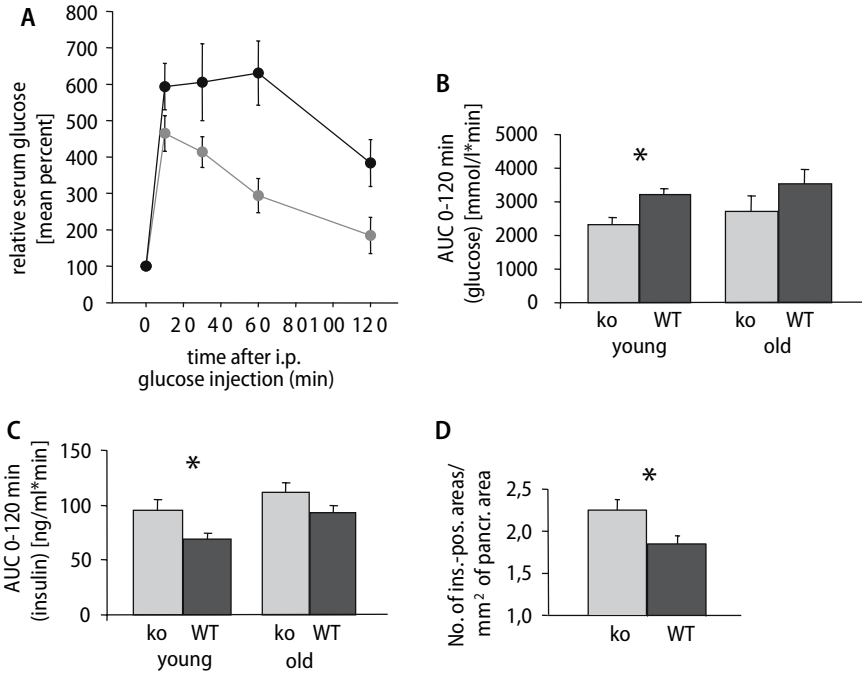

Fig. 3 Improved glucose tolerance and increased insulin secretion in mice lacking *Ttpa*. Panel **A** depicts relative blood glucose excursions following intraperitoneal injection of glucose (grey line corresponds to ko animals). Panel **B** depicts the corresponding areas under the curve (AUCs) for glucose excursions in experiments as shown in panel **A**, and Panel **C** depicts corresponding AUCs for glucose-stimulated insulin excursions following intraperitoneal injection of glucose. Panel **D** depicts the number of insulin positive areas per mm^2 of pancreatic tissue. Data are expressed as means ± SEM from at least six animals per genotype

contain pancreatic beta cells which are responsible for the production and secretion of insulin following stimulation by glucose. When quantifying pancreatic sections from *Ttpa*$^{-/-}$ mice and control littermates, we observed a significant increase in insulin-positive areas per pancreatic section ($P < 0.01$, Fig. 3D) known to reflect pancreatic islets of Langerhans, as previously established by others and ourselves [32, 34].

Intraperitoneal insulin sensitivity

To gain further insight into the role of glucose metabolism and specifically insulin action in *Ttpa*$^{-/-}$ mice, we performed intraperitoneal insulin tolerance tests. Following intraperitoneal injection of an amount of insulin adjusted to individual body mass, blood glucose levels typically decreased. Accordingly, the area under the glucose curves ($AUC_{glucose}$) for young mice was 195.4 mmol/l min (*Ttpa*$^{-/-}$) vs. 252.1 mmol/l min (controls) ($P < 0.01$, Fig. 4A), and $AUC_{glucose}$ for 36 week-old mice were 213.6 mmol/l min (*Ttpa*$^{-/-}$) vs. 238.3 mmol/l min (controls) ($P < 0.36$, Fig. 4A). Since insulin sensitivity is known to be dependent on individual body mass, we determined body mass in all study groups, and observed no significant differences in this regard (Fig. 1A), excluding the possibility that differences in insulin sensitivity was caused by differences in body mass. Lastly, since triglyceride levels might affect both insulin sensitivity and insulin secretion, we determined plasma triglyceride levels in wild-type and knock-out mice, and found no differences neither in young nor in old mice (Fig. 4B).

Discussion

Type 2 diabetes mellitus is a highly prevalent metabolic disorder affecting approx. 8% of the population in Westernized countries. The role of α-TOH in the development and prevention of type 2 diabetes mellitus is a matter of continuing debate. Alpha-TTP is required for the compartmentalization of α-TOH in specific tissues of mammals, and may influence putative effects of α-TOH on glucose metabolism, and hence development of type 2 diabetes mellitus.

We here unexpectedly find that ablation of *Ttpa*-gene in mice results in an improvement of glucose metabolism. This enhancement is caused by increased insulin secretion in response to glucose challenge, as well as increased insulin sensitivity, i.e., improved insulin action. Since impaired insulin secretion and impaired insulin action are both considered essential hallmarks of type 2 diabetes mellitus, lack of α-TTP may influence the development of this disease in mammals via a yet unknown mechanism. Notably, mice lacking expression of *Ttpa* develop an increased number of pancreatic islets known to be responsible for the production and secretion of insulin, providing an immediate mechanistic explanation for the phenotype observed in this study.

To dissect the possible effects of α-TOH-deficiency from the possible effects of α-TTP-deficiency, knock-out animals were fed a diet high in α-TOH (200 mg α-tocopheryl acetate per kg diet), resulting in somewhat reduced α-tocopherol levels in plasma, while no signs of vitamin E deficiency were observed. This approach

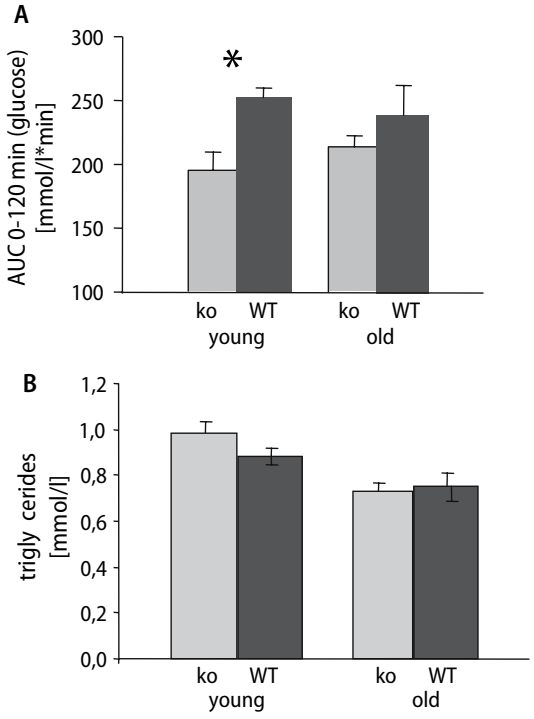

Fig. 4 Improved insulin sensitivity in mice lacking *Ttpa*. Panel **A** depicts AUCs for blood glucose levels following intraperitoneal injection of insulin. Panel **B** depicts fasting plasma triglyceride levels for young and adult mice. Data are expressed as means ± SEM from at least 10 animals per genotype

presumably enabled us to link the phenotype observed to the lack of α-TTP rather than to deficiency of α-TOH. While the plasma levels of α-tocopherol obtained in the present study have never been linked to any specific phenotype in rodents, we cannot fully exclude the possibility of some influence of reduced α-TOH availability on improved glucose metabolism observed in the present study.

Taken together, the unexpected improvement of glucose homeostasis of *Ttpa*$^{-/-}$ animals occurs presumably independent of vitamin E levels in these mice. This is supported by an earlier study showing that isolated adipocytes derived from vitamin E deficient rats exhibited no alterations of basal or insulin-stimulated glucose metabolism in comparison to fat cells isolated from control animals suggesting that α-tocopherol itself has no detectable influence on peripheral insulin sensitivity [25]. Hence our findings so far indicated that ablation of *Ttpa*-gene in mice results in an enhanced glucose metabolism. This enhancement is caused by increased insulin secretion as well as increased insulin sensitivity in mice lacking *Ttpa*-gene.

According to the analysis of Gohil et al. [13], several clusters of differentially expressed mRNAs could be associated with the lack of *Ttpa*$^{-/-}$. Apart from the down-regulation of genes for antioxidative response suggesting increased oxidative stress in *Ttpa*$^{-/-}$ livers, and repression of genes that determine neurological development in the cerebral cortex, a subset of regulated genes was related to glucose homeostasis. Out of 39 up-regulated genes in α-TTP-deficient liver, peroxisome proliferator-activated receptor-alpha (PPAR-alpha), a transcription factor that regulates lipid and glucose homeostasis, was found to be induced twofold. In several studies, activation of rodent PPAR-alpha by fibrates was associated with an improvement of insulin sensitivity by induction of insulin signaling [14, 40]. The use of PPAR-alpha agonists was accompanied by a reduction of triglyceride levels in liver and muscle. Another study reports an activation of PPAR-alpha by dietary oxidized fats, irrespective of the dietary vitamin E concentration [37]. In the present study, however, we found no differences in plasma triglyceride levels and therefore rule out PPAR-alpha as a regulator of glucose homeostasis in α-TTP-deficient mice.

Insulin-like growth factor binding protein-2 (IGFBP-2), a protein which recently has been suggested to be a marker for the metabolic syndrome [16], has also been found up-regulated 2-fold in α-TTP-deficient liver specimen [13]. Low circulating IGFBP-2 has been associated with elevated fasting glucose and insulin sensitivity [16]. Type I iodothyronine 5′-deiodinase was found to be up-regulated (2.5-fold) in *Ttpa* $^{-/-}$ mice [13]. Deiodinase type I increases active tyrosine T3 plasma levels that has been found to modulate glucose homeostasis and insulin sensitivity [5]. Finally, growth factor receptor-bound protein (Grb)10 interacts with the insulin (IR) and insulin-like growth factor-I (IGF-IR) receptor and may thus regulate IGF-I-stimulated growth [9]. The gene for Grb10 was up-regulated by a factor of 12 [13], further supporting the proposed causal relation of α-TTP-deficiency and improved glucose tolerance. Further studies are warranted to dissect the underlying molecular mechanisms how α-TTP-deficiency can improve glucose homeostasis.

Taken together, deficiency of α-TTP in states of α-tocopherol supplementation causes improved glucose tolerance due to an increased number of pancreatic islets followed by elevated insulin secretion, and improved insulin sensitivity in mice. These findings suggest differential roles of α-TTP and α-tocopherol in the pathogenesis of type 2 diabetes mellitus.

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References

1. Arita M, Sato Y, Miyata A, Tanabe T, Takahashi E, Kayden HJ, Arai H, Inoue K (1995) Human alpha-tocopherol transfer protein: cDNA cloning, expression and chromosomal localization. Biochem J 306:437–443
2. Azzi A, Ricciarelli R, Zingg JM (2002) Non-antioxidant molecular functions of alpha-tocopherol (vitamin E). FEBS Lett 519:8–10
3. Brigelius-Flohe R (2006) Bioactivity of vitamin E. Nutr Res Rev 19:174–186
4. Brigelius-Flohe R, Kelly FJ, Salonen JT, Neuzil J, Zingg JM, Azzi A (2002) The European perspective on vitamin E: current knowledge and future research. Am J Clin Nutr 76:703–716
5. Chidakel A, Mentuccia D, Celi FS (2005) Peripheral metabolism of thyroid hormone and glucose homeostasis. Thyroid 15:899–903
6. Copp RP, Wisniewski T, Hentati F, Larnaout A, Ben Hamida M, Kayden HJ (1999) Localization of alpha-tocopherol transfer protein in the brains of patients with ataxia with vitamin E deficiency and other oxidative stress related neurodegenerative disorders. Brain Res 822:80–87
7. Czernichow S, Couthouis A, Bertrais S, Vergnaud AC, Dauchet L, Galan P, Hercberg S (2006) Antioxidant supplementation does not affect fasting plasma glucose in the supplementation with antioxidant vitamins and minerals (SU.VI.MAX) study in France: association with dietary intake and plasma concentrations. Am J Clin Nutr 84:395–399
8. Dröge W (2002) Free radicals in the physiological control of cell function. Physiol Rev 82:47–95
9. Dufresne AM, Smith RJ (2005) The adapter protein GRB10 is an endogenous negative regulator of insulin-like growth factor signaling. Endocrinology 146:4399–4409
10. Evans HM, Bishop KS (1922) On the existence of a hithero unrecognized dietary factor essential for reproduction. Science 56:650–651
11. Feskens EJ, Virtanen SM, Rasanen L, Tuomilehto J, Stengard J, Pekkanen J, Nissinen A, Kromhout D (1995) Dietary factors determining diabetes and impaired glucose tolerance. A 20-year follow-up of the Finnish and Dutch cohorts of the seven countries study. Diabetes Care 18:1104–1112
12. Frei B (1994) Reactive oxygen species and antioxidant vitamins: mechanisms of action. Am J Med 97:5S–13S, Discussion 22S–28S
13. Gohil K, Schock BC, Chakraborty AA, Terasawa Y, Raber J, Farese RV Jr, Packer L, Cross CE, Traber MG (2003) Gene expression profile of oxidant stress and neurodegeneration in transgenic mice deficient in alpha-tocopherol transfer protein. Free Radic Biol Med 35:1343–1354
14. Haluzik MM, Haluzik M (2006) PPAR-alpha and insulin sensitivity. Physiol Res 55:115–122
15. Hayton SM, Kriss T, Wade A, Muller DP (2006) Effects on neural function of repleting vitamin E-deficient rats with alpha-tocopherol. J Neurophysiol 95:2553–2559
16. Heald AH, Kaushal K, Siddals KW, Rudenski AS, Anderson SG, Gibson JM (2006) Insulin-like growth factor binding protein-2 (IGFBP-2) is a marker for the metabolic syndrome. Exp Clin Endocrinol Diabetes 114:371–376
17. Isken F, Schulz TJ, Möhlig M, Pfeiffer AF, Ristow M (2006) Chemical inhibition of citrate metabolism alters glucose metabolism in mice. Horm Metab Res 38:543–545
18. Isken F, Schulz TJ, Weickert MO, Pfeiffer AF, Ristow M (2006) Chemical inhibition of citrate metabolism alters body fat content in mice. Horm Metab Res 38:134–136
19. Jishage K, Arita M, Igarashi K, Iwata T, Watanabe M, Ogawa M, Ueda O, Kamada N, Inoue K, Arai H, Suzuki H (2001) alpha-tocopherol transfer protein is important for the normal development of placental labyrinthine trophoblasts in mice. J Biol Chem 276:1669–1672
20. Kaempf-Rotzoll DE, Igarashi K, Aoki J, Jishage K, Suzuki H, Tamai H, Linderkamp O, Arai H (2002) Alpha-tocopherol transfer protein is specifically localized at the implantation site of pregnant mouse uterus. Biol Reprod 67:599–604
21. Kluth D, Landes N, Pfluger P, Muller-Schmehl K, Weiss K, Bumke-Vogt C, Ristow M, Brigelius-Flohe R (2005) Modulation of Cyp3a11 mRNA expression by alpha-tocopherol but not gamma-tocotrienol in mice. Free Radic Biol Med 38:507–514
22. Knekt P, Reunanen A, Marniemi J, Leino A, Aromaa A (1999) Low vitamin E status is a potential risk factor for insulin-dependent diabetes mellitus. J Intern Med 245:99–102
23. Koya D, Lee IK, Ishii H, Kanoh H, King GL (1997) Prevention of glomerular dysfunction in diabetic rats by treatment with d-alpha-tocopherol. J Am Soc Nephrol 8:426–435
24. Kunisaki M, Bursell SE, Clermont AC, Ishii H, Ballas LM, Jirousek MR, Umeda F, Nawata H, King GL (1995) Vitamin E prevents diabetes-induced abnormal retinal blood flow via the diacylglycerol-protein kinase C pathway. Am J Physiol 269:E239–E246
25. Lavis VR, Kitabchi AE, Williams RH (1969) Lipid peroxidation in vitro by isolated fat cells of rats. Correlation with total lipolysis, glucose utilization, and dietary tocopherol. J Biol Chem 244:4382–4386
26. Leonard SW, Terasawa Y, Farese RV Jr, Traber MG (2002) Incorporation of deuterated RRR- or all-rac-alpha-tocopherol in plasma and tissues of alpha-tocopherol transfer protein—null mice. Am J Clin Nutr 75:555–560
27. Lonn E, Yusuf S, Hoogwerf B, Pogue J, Yi Q, Zinman B, Bosch J, Dagenais G, Mann JF, Gerstein HC (2002) Effects of vitamin E on cardiovascular and microvascular outcomes in high-risk patients with diabetes: results of the HOPE study and MICRO-HOPE substudy. Diabetes Care 25:1919–1927
28. McKusick VA (2006) Familial isolated deficiency of vitamin E, http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?277460
29. Montonen J, Knekt P, Jarvinen R, Reunanen A (2004) Dietary antioxidant intake and risk of type 2 diabetes. Diabetes Care 27:362–366
30. Muller-Schmehl K, Beninde J, Finckh B, Florian S, Dudenhausen JW, Brigelius-Flohe R, Schuelke M (2004) Localization of alpha-tocopherol transfer protein in trophoblast, fetal capillaries' endothelium and amnion epithelium of human term placenta. Free Radic Res 38:413–420
31. Ouahchi K, Arita M, Kayden H, Hentati F, Ben Hamida M, Sokol R, Arai H, Inoue K, Mandel JL, Koenig M (1995) Ataxia with isolated vitamin E deficiency is caused by mutations in the alpha-tocopherol transfer protein. Nat Genet 9:141–145
32. Pomplun D, Florian S, Schulz T, Pfeiffer AF, Ristow M (2007) Alterations of pancreatic beta-cell mass and islet number due to Ins2-controlled expression of cre recombinase: RIP-cre revisited, part 2. Horm Metab Res 39:336–340
33. Ristow M (2004) Neurodegenerative disorders associated with diabetes mellitus. J Mol Med 82:510–529

34. Ristow M, Mulder H, Pomplun D, Schulz TJ, Müller-Schmehl K, Krause A, Fex M, Puccio H, Müller J, Isken F, Spranger J, Müller-Wieland D, Magnuson MA, Möhlig M, Koenig M, Pfeiffer AFH (2003) Frataxin-deficiency in pancreatic islets causes diabetes due to loss of beta-cell mass. J Clin Invest 112:527–534
35. Sacco M, Pellegrini F, Roncaglioni MC, Avanzini F, Tognoni G, Nicolucci A (2003) Primary prevention of cardiovascular events with low-dose aspirin and vitamin E in type 2 diabetic patients: results of the Primary Prevention Project (PPP) trial. Diabetes Care 26:3264–3272
36. Salonen JT, Nyyssonen K, Tuomainen TP, Maenpaa PH, Korpela H, Kaplan GA, Lynch J, Helmrich SP, Salonen R (1995) Increased risk of non-insulin dependent diabetes mellitus at low plasma vitamin E concentrations: a four year follow up study in men. Bmj 311:1124–1127
37. Sulzle A, Hirche F, Eder K (2004) Thermally oxidized dietary fat upregulates the expression of target genes of PPAR alpha in rat liver. J Nutr 134:1375–1383
38. Tasinato A, Boscoboinik D, Bartoli GM, Maroni P, Azzi A (1995) d-alpha-tocopherol inhibition of vascular smooth muscle cell proliferation occurs at physiological concentrations, correlates with protein kinase C inhibition, and is independent of its antioxidant properties. Proc Natl Acad Sci USA 92:12190–12194
39. Terasawa Y, Ladha Z, Leonard SW, Morrow JD, Newland D, Sanan D, Packer L, Traber MG, Farese RV (2000) Increased atherosclerosis in hyperlipidemic mice deficient in alpha-tocopherol transfer protein and vitamin E. Proc Natl Acad Sci USA 97:13830–13834
40. Tsuchida A, Yamauchi T, Takekawa S, Hada Y, Ito Y, Maki T, Kadowaki T (2005) Peroxisome proliferator-activated receptor (PPAR) alpha activation increases adiponectin receptors and reduces obesity-related inflammation in adipose tissue: comparison of activation of PPARalpha, PPARgamma, and their combination. Diabetes 54:3358–3370
41. Yokota T, Igarashi K, Uchihara T, Jishage K, Tomita H, Inaba A, Li Y, Arita M, Suzuki H, Mizusawa H, Arai H (2001) Delayed-onset ataxia in mice lacking alpha-tocopherol transfer protein: model for neuronal degeneration caused by chronic oxidative stress. Proc Natl Acad Sci USA 98:15185–15190
42. Zimmer S, Stocker A, Sarbolouki MN, Spycher SE, Sassoon J, Azzi A (2000) A novel human tocopherol-associated protein: cloning, in vitro expression, and characterization. J Biol Chem 275:25672–25680