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RESEARCH PAPER

Skeletal muscle mitochondrial uncoupling prevents diabetes but not obesity in NZO mice, a model for polygenic diabesity

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Abstract Induction of skeletal muscle (SM) mitochondrial stress by expression of uncoupling protein 1 (UCP1) in mice results in a healthy metabolic phenotype associated with increased secretion of FGF21 from SM. Here, we investigated whether SM mitochondrial uncoupling can compensate obesity and insulin resistance in the NZO mouse, a polygenic diabesity model. Male NZO mice were crossed with heterozygous UCP1 transgenic (tg) mice (mixed C57BL/6/CBA background) and further backcrossed to obtain F1 and N2 offspring with 50 and 75 % NZO background, respectively. Male F1 and N2 progeny were fed a high-fat diet ad libitum for 20 weeks from weaning. Blood glucose was reduced, and diabetes (severe hyperglycemia >300 mg/dl) was fully prevented in both F1- and N2-tg progeny compared to a diabetes prevalence of 15 % in F1 and 42 % in N2 wild type. In contrast, relative body fat content and plasma insulin were decreased, and glucose tolerance was improved, in F1-tg only. Both F1 and N2-tg showed decreased lean body mass. Accordingly, induction of SM stress response including FGF21 expression and secretion was similar in both F1 and N2-tg mice. In white adipose tissue, expression of FGF21 target genes was enhanced in F1 and N2-tg mice, whereas lipid metabolism genes were induced in F1-tg only. There was no evidence for induction of browning in either UCP1 backcross. We conclude that SM mitochondrial uncoupling induces FGF21 expression and prevents diabetes in mice with a 50–75 % NZO background independent of its effects on adipose tissue.

Keywords Polygenic obesity · Skeletal muscle · Uncoupling protein 1 · Fibroblast growth factor 21 · Diabetes · White adipose tissue · Body composition · Glucose tolerance test · Gene expression · Lipid metabolism · Glucose metabolism

Introduction

Ectopic expression of uncoupling protein 1 (UCP1) in skeletal muscle has been shown to result in a phenotype characterized by increased energy expenditure, reduced body weight, reduced fat mass, improved glucose tolerance, decreased muscle energy efficiency, and altered substrate oxidation (Couplan et al. 2002; Klaus et al. 2005; Li et al. 2000). UCP1-tg mice express the murine UCP1 gene in striated skeletal muscle, driven by the human skeletal actin promoter (Klaus et al. 2005). We have shown previously that UCP1-tg mice display a healthy metabolic phenotype and increased longevity. They are protected from the development of insulin resistance independent of the diet, displaying a dissociation of obesity and insulin resistance (Keipert et al. 2011; Neschen et al. 2008). Also, they show substantially improved in vivo insulin action due to an increased substrate flux through the glycolytic pathway paralleled by increased insulin-stimulated glucose uptake and increased substrate oxidation in SM (Keipert et al. 2013a; Neschen et al. 2008; Ost et al. 2014). Overall, UCP1 expression in skeletal muscle thus leads to beneficial effects on glucose homeostasis. This healthy metabolic phenotype of UCP1-tg mice is associated with the

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induction and secretion of fibroblast growth factor 21 (FGF21) from skeletal muscle (Keipert et al. 2014) which in turn linked to a metabolic remodeling program in skeletal muscle due to muscle mitohormesis (Ost et al. 2015). Overexpression and systemic administration of FGF21 has been shown to reduce obesity and improve glucose homeostatic in mice (Coskun et al. 2008; Inagaki et al. 2008; Kharitonenkov et al. 2005; Zhang et al. 2012). Thus, FGF21 is considered a potential therapeutic in obesity, diabetes and related disorders (Kharitonenkov and Adams 2014). Circulating FGF21 is largely increased In UCP1-tg mice and exerts endocrine effects mainly on white adipose tissue (WAT) such as a recruitment of inducible brown adipocytes (also termed "browning" of WAT) (Bartelt and Heeren 2014) characterized by increased mitochondrial content, UCP1 expression, and metabolic activity (Keipert et al. 2014). Interestingly, skeletal muscle expression of UCP1 delayed the development of diet-induced obesity and insulin resistance, thereby antagonizing the life-shortening effects of high-fat diets (Keipert et al. 2011). Expression of UCP1 in skeletal muscle was able to reduce body weight and improve glucose homeostasis in a monogenetic model of obesity, the lethal yellow (A^y/a) mice (Bernal-Mizrachi et al. 2002), but it is not known if it might also alleviate diabetes and obesity in a polygenic model such as the New Zealand obese (NZO) mice. These mice develop the metabolic syndrome and type 2 diabetes with beta-cell loss (Joost 2010). NZO mice originate from a colony of agouti mice selected for obesity which was subsequently fixed by inbreeding (Bielschowsky and Goodall 1970). NZO mice have been used in outcross studies aimed to locate genes responsible for obesity, insulin resistance and diabetes (Joost 2010; Joost and Schurmann 2014; Leiter et al. 1998). Quantitative trait loci (QTLs) affecting adiposity and blood glucose have been identified on a number of chromosomes and different loci (Plum et al. 2002; Taylor et al. 2001; Vogel et al. 2009, 2012, 2013) confirming the polygenic nature of obesity and diabetes development in this mouse model. On a high-fat diet NZO mice exhibit hypertension and hypercholesterolemia in addition to obesity, hyperinsulinemia and hyperglycemia (Joost and Schurmann 2014; Ortlepp et al. 2000). The development of obesity in NZO mice was found to be caused by hyperphagia combined with reduced energy expenditure and impaired voluntary physical activity (Jurgens et al. 2006). Development of diabetes and beta-cell failure in NZO mice is linked to the development of obesity and is also dependent on the presence of dietary carbohydrates (Jurgens et al. 2007; Kluth et al. 2011). In order to investigate if the disturbed glucose homeostasis and/or obesity of NZO mice can be rescued by skeletal muscle uncoupling, we crossed UCP1-tg mice with NZO mice and investigated body composition, glucose homeostasis, and gene expression in muscle and WAT in male offspring of the F1 progeny (50 % NZO background) and N2 progeny (75 % NZO background) challenged by a high-fat diet. The results show that the mitochondrial stress-induced remodeling of skeletal muscle including induction of FGF21 as a myokine is preserved in mice with increasing NZO background. These effects of the UCP1 transgene were associated with the prevention of diabetes and changes in body composition but not with a significant reduction of adiposity.

Materials and methods

Animal maintenance and backcross setup

All analyses were performed in male mice. Mice were maintained at 22 °C and a 12 h:12 h dark/light cycle with food and water provided ad libitum. Animal experiments were performed in compliance with the German animal protection law (TierSchG). The mice were housed and handled in accordance with good animal practice as defined by FELASA (www.felasa.eu/guidelines.php) and the national animal welfare body GV-SOLAS (www.gv-solas.de/index.html). The animal welfare committees of the DIfE as well as the local authorities (Landesamt für Umwelt, Gesundheit und Verbraucherschutz, Brandenburg) approved all animal experiments. To obtain F1 progeny, female heterogeneous UCP1-tg transgenic mice (on a mixed C57BL/6/CBA background, obtained as described (Klaus et al. 2005) were crossed with male NZO mice obtained from our own breeding colony (NZO/HlBom: Dr. R. Kluge, German Institute of Human Nutrition, Nuthetal, Germany). Female, transgenic F1-tg mice were backcrossed once more with male NZO mice to obtain the N2 progeny with 75 % NZO genetic background. Mice were genotyped for expression of the HSA-mUCP1 transgene as described (Klaus et al. 2005). Our backcross setup was chosen in a way to minimize possible prenatal maternal effects on the offspring since we used only male NZO mice for the backcross.

Experimental set up

Experiments were performed in male F1 and N2 offspring comparing wild-type (WT) and transgenic animals (UCP1). From weaning on at 4 weeks of age mice were fed ad libitum a commercial high-fat diet (Altromin C1057, Lage, Germany) containing 15 % fat (w/w) with an energy content of 18.7 kJ/g as determined by bomb calorimetry. In F1 wild type (F1-wt) and UCP1 transgenic mice (F1-tg) body

composition was determined weekly and mice were subjected to a glucose tolerance test at 20 weeks of age. Random, non-fasting blood glucose from tail blood was determined several times during the experiment between 8 and 9 a.m. Animals were killed at 23 weeks of age. N2 wild-type (N2-wt) and UCP1 transgenic mice (N2-tg) were treated similarly with the only difference that glucose tolerance tests were performed in week 16 and animals killed in week 20. Mice were killed in the morning under isoflurane anesthesia, 2 h after food withdrawal. Tissues and organs were snap frozen in liquid nitrogen after dissection and stored at −80 °C until further analyses.

Body composition and body length

Body composition was determined weekly using quantitative magnetic resonance (QMR) (Brukeŕs Minispec MQ10, Houston, TX, USA) as described (Klaus et al. 2005). Lean body mass was calculated by subtracting body fat values obtained by QMR from body weight obtained by weighing prior to QMR measurement. Body length was determined as nose to anus length in anaesthetized animals at time of killing.

Glucose tolerance test

Glucose tolerance tests were performed in week 20 (F1) or week 16 (N2) after 16 h of fasting. Glucose (2 mg/g body weight) was injected intraperitoneal, and tail blood samples were obtained before, as well as 15, 30, 60, 120, and 240 min after glucose injection for determination of blood glucose using a blood glucose sensor (Ascendia Elite, Bayer, Leverkusen). Approximately 20 µl blood per sample was obtained additionally, centrifuged and plasma frozen for subsequent analysis of insulin by ELISA as described (Neschen et al. 2008). Homeostasis model assessment index (HOMA) for insulin resistance was calculated as described (Lansang et al. 2001).

Plasma and biochemical analysis

Free fatty acids (Wako NEFA C Kit, Wako Chemicals, Neuss, Germany), total cholesterol (Wako CHOL-H L-Type R1/R2 Chemicals, Neuss, Germany), and triglycerides (triglyceride and free glycerol reagent, SIGMA, Germany) were analyzed in triplicates on 96-well plates as described (Noatsch et al. 2011). Insulin was analyzed by an ultrasensitive ELISA assay (Insulin mouse ultrasensitive ELISA, DRG Instruments GmbH, Germany). FGF21 (Mouse/Rat FGF-21 Quantikine ELISA Kit; R&D Systems) and IGF1 (Mouse/Rat IGF-I Quantikine ELISA Kit; R&D Systems) values were determined by ELISA. All measurements were taken according to the instructions of the manufacturers.

Gene expression analysis

RNA isolation and quantitative real-time PCR (qPCR) was performed as described (Keipert et al. 2013b). Quadriceps and gastrocnemius combined were used for SM, and epididymal WAT (eWAT) for adipose tissue gene expression analysis. Tissue-specific gene expression was calculated as $\Delta\Delta C_T$ using beta-actin or beta-2 microglobulin (B2M) for normalization and expressed relative to the F1-wt group which was normalized to a value of 1.

Statistical analysis

Data are expressed as mean ± SEM. Statistical analysis war performed using SPSS 14.0 or GraphPad Prism 5. Two-tailed Student's t tests were performed to compare WT and TG mice. Body composition development as well as glucose tolerance tests were analyzed by repeated measure factorial ANOVA. Differences in diabetes prevalence were assessed using Chi-squared test. Differences of $p < 0.05$ were considered significant.

Results

Body weight and composition

In the F1 progeny, transgenic mice (F1-tg) had a markedly reduced body weight compared to wild type (F1-wt) (Fig. 1a) which resulted from decreased lean body mass (Fig. 1b) as well as decreased absolute and relative body fat content (Fig. 1c; Table 1). Final body weight, lean mass and fat mass of F1-tg were reduced by 18, 7.3, and 10.6 g, respectively, compared to F1-wt. Because F1-tg mice also showed a reduced body length (Table 1), BMI was calculated to correct for body size. BMI was also significantly decreased in F1-tg compared to F1-wt mice (Table 1). Relative body fat content was similar at the start of high-fat feeding but increased to a higher extent in F1-wt mice (Fig. 1b) resulting in significantly higher percentage of body fat in F1-wt at 23 weeks of age (Table 1).

Mice of the N2 generation (75 % NZO genetic background) were overall heavier than F1 progeny and body weight as well as BMI of N2-wt mice was significantly higher than that of N2-tg mice throughout the experiment (Fig. 1d; Table 1). This was partly due to a consistently decreased lean body mass in N2-tg mice (Fig. 1e). Although numerical values of absolute and relative body fat mass content were also decreased in N2-tg mice, this did not reach statistical significance (Fig. 1f; Table 1). Because of the high individual variations, a larger number of animal would be required to clearly rule out a protective effect of SM UCP1 expression regarding obesity

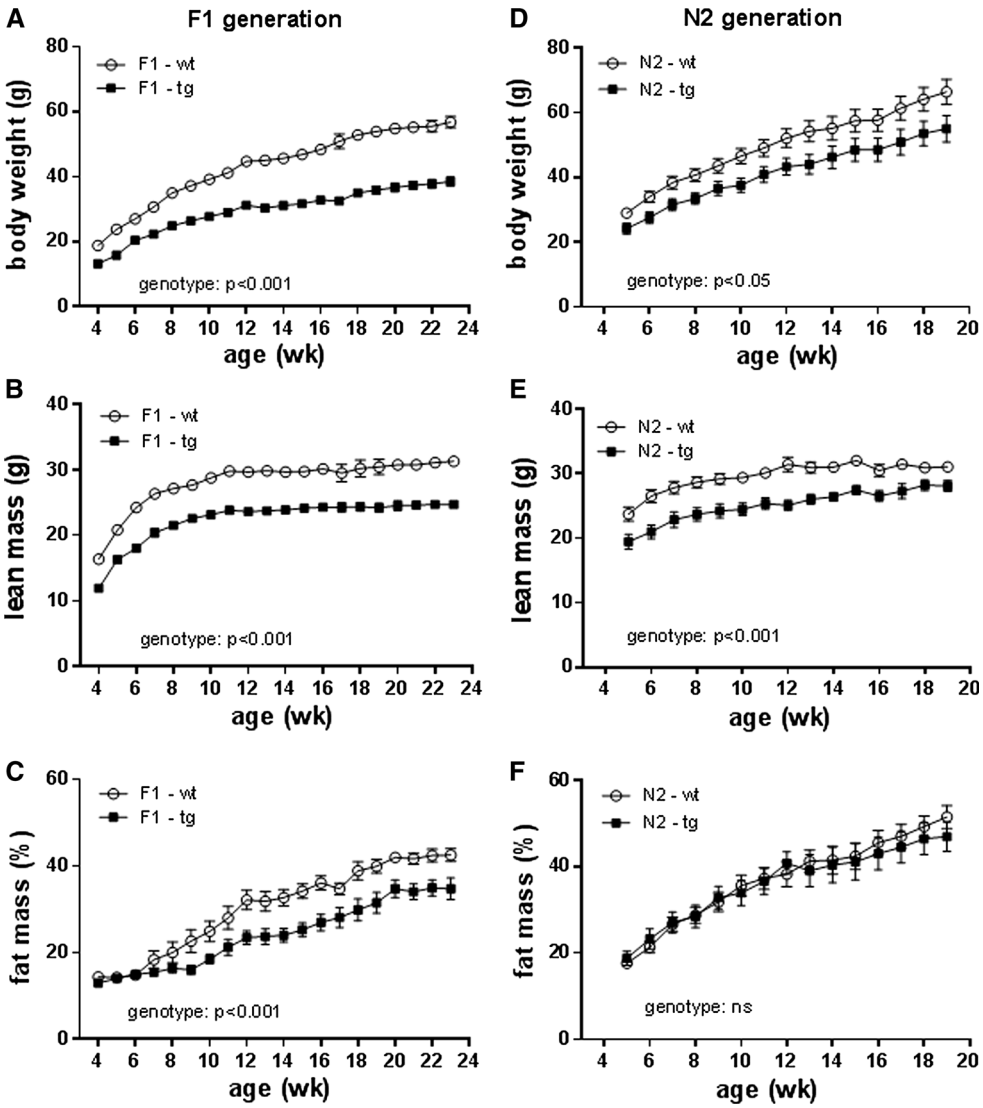

Fig. 1 Development of body composition in F1 (**a**–**c**) and N2 (**d**–**f**) NZO backcross wild type (wt, *open symbols*) and UCP1 transgenic (tg) mice (*filled symbols*) fed a high-fat diet from weaning on. Data are mean ± SE. $n = 20$–21 for F1 and 10–12 for N2. Differences between the groups were assessed by factorial ANOVA for repeated measurements

development. Body length differences disappeared in N2 mice consistent with similar levels of circulating IGF-1 (Table 1). Relative liver weights did not differ between wt and tg mice.

F1-tg mice showed a significantly improved glucose tolerance compared to F1-wt (Fig. 2a) with a lower and earlier glucose peak and a rapid subsequent drop in blood glucose. Area under the curve was also significantly lower [563 ± 31 arbitrary units (a.u.)] compared to F1-wt (856 ± 65 a.u., $p < 0.001$). Insulin levels during glucose tolerance test were significantly lower in F1-tg mice compared to F1-wt (Fig. 2b). F1-tg mice also showed significantly decreased blood glucose, plasma insulin and plasma free fatty acids compared to F1-wt. No significant differences were observed in plasma triglycerides (Table 1). Random blood glucose levels over 300 mg/dl (16.6 mM) were observed in wt mice only (Fig. 1e), and mice exceeding this threshold at any time were defined as diabetic. Diabetes prevalence was 15 % in F1-wt in line with a reduced HOMA index in F1-tg compared to F1-wt (Table 1).

In the N2 generation glucose response during GTT was very similar in wt and tg mice (Fig. 2c). Interestingly, although average plasma insulin levels during GTT were similar to those observed in F1, there were no more statistically significant differences due to high individual variability in both N2-wt and N2-tg mice. However, fed and fasted blood glucose concentrations were still significantly decreased in N2-tg mice compared to N2-wt (Fig. 2e; Table 1), resulting in a 50 % decrease of HOMA index that failed to reach statistical significance ($p = 0.067$) due to the large individual differences. However, despite these large individual variations, N2-tg mice still did not show any incidence of diabetes, whereas the prevalence in N2-wt mice was 42 % (Fig. 2e; Table 1). Curiously, N2 mice had overall lower plasma TG levels

Table 1 Body composition and plasma parameters of F1 and N2 NZO backcross wild type (wt) and UCP1 transgenic (tg) mice fed a high-fat diet from weaning

	F1-wt	F1-tg	N2-wt	N2-tg
Body weight (g)	56.0 ± 1.6 (20)	38.5 ± 1.2*** (21)	66.4 ± 3.9 (12)	55.1 ± 4.1 (10)
Lean mass (g)	31.8 ± 0.4 (20)	24.5 ± 0.3*** (21)	31.1 ± 0.5 (12)	28.1 ± 0.9** (10)
Lean mass (%)	56.9 ± 1.3 (20)	64.5 ± 1.7** (21)	48.5 ± 2.7 (12)	53.1 ± 3.4 (10)
Fat mass (g)	24.9 ± 1.5 (20)	14.3 ± 1.2*** (21)	35.3 ± 3.7 (12)	27.1 ± 3.5 (10)
Fat mass (%)	43.3 ± 1.3 (20)	35.7 ± 1.7** (21)	51.6 ± 2.7 (12)	47.0 ± 3.4 (10)
Body length (cm)	11.6 ± 0.05 (24)	11.1 ± 0.05*** (26)	11.7 ± 0.2 (12)	11.6 ± 0.1 (10)
BMI (kg/m^2)	4.18 ± 0.10 (19)	3.12 ± 0.08*** (21)	4.92 ± 0.21 (12)	4.22 ± 0.24* (10)
Liver weight (%)	4.03 ± 0.12 (20)	4.32 ± 0.11 (21)	4.07 ± 0.14 (12)	4.26 ± 0.22 (10)
Diabetes prevalence	15 % (22)	0 %* (20)	42 % (12)	0 %* (10)
Random blood glucose (mg/dl)	204.4 ± 30 (22)	116.6 ± 3** (20)	286 ± 35 (12)	172 ± 17** (10)
Random insulin (ng/ml)	21.9 ± 3.8 (11)	7.5 ± 1.9*** (20)	32.0 ± 11.9 (10)	21.7 ± 9.0 (8)
Fasting blood glucose (mg/dl)	89.2 ± 9.9 (9)	60.8 ± 3.3** (11)	122 ± 9.2 (10)	88 ± 9.0** (8)
Fasting insulin (ng/ml)	2.08 ± 0.18 (9)	1.16 ± 0.11*** (11)	2.34 ± 0.62 (10)	1.61 ± 0.40 (8)
HOMA index	11.7 ± 2.1 (9)	4.4 ± 0.6** (11)	20.5 ± 6.9 (10)	9.5 ± 3.1 (8)
Plasma TG (µg/ml)	830 ± 105 (25)	938 ± 85 (26)	586 ± 92 (12)	764 ± 92 (10)
Plasma FFA (mM)	1.21 ± 0.21 (25)	0.64 ± 0.09* (26)	0.93 ± 0.15 (12)	1.03 ± 0.23 (10)
Plasma IGF1 (ng/ml)	n.d.	n.d.	564 ± 32 (8)	509 ± 26 (8)

Data are from 23 weeks (F1) or 19 weeks (N2) old mice except fasted blood glucose, fasted insulin and HOMA index which were obtained in week 20 (F1) or 16 (N2). Diabetes was defined as random blood glucose levels >300 mg/dl. All data are mean ± SEM with *n*-number in parentheses

n.d. not determined

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to wt of the same generation

than F1 which could be due to the fact that they were 4 weeks younger. Individual variations were very high also in F1 generation, probably because mice were not fasted.

Circulating FGF21 levels were more than doubled in N2-tg mice compared to N2-wt (Fig. 1f), suggesting that effects of mitochondrial stress on skeletal muscle were not antagonized by the NZO genetic background. Gene expression analysis of skeletal muscle confirmed the induction of UCP1 and FGF21 in skeletal muscle of UCP1-tg mice which was even more pronounced in the N2 than in the F1 generation (Fig. 3). This was due to lower UCP1 gene expression in N2-wt [mean RQ-PCR cycle threshold (C_T) of 30.05] than in F1-wt (mean C_T of 28.12), whereas UCP1 expression levels were similar in both F1-tg and N2-tg (mean C_T of 22.45 and 22.39, respectively).

In SM of N2-tg mice there was a significantly increased expression of *activating transcription factor 4* (Atf4) and *C/EBP homologous protein* (Chop, also known as DNA-damage inducible transcript 3 (Ddit3)), two transcription factors which are part of the integrated stress response (ISR) (Marciniak and Ron 2006), as well as *glucose transporter 1* (Glut1, gene name Slc2a1). There were no expression changes of *glucose transporter 4* (Glut4, gene name Slc2a4), *cluster of differentiation 36* (Cd36) or *phosphoenolpyruvat-carboxykinase* (Pck1) in either F1-tg or N2-tg mice. Expression of *phosphoserine aminotransferase 1* (Psat1) and *methylenetetrahydrofolate dehydrogenase* (Mthfd2) was highly induced in both F1 and N2-tg mice (Fig. 3). These are two key genes of the serine, one-carbon, glycine (SOG) pathway which is induced by SM mitochondrial stress (Ost et al. 2015). UCP1 expression in SM has been shown to induce a fiber type switch from glycolytic type II fibers toward oxidative type I fibers (Couplan et al. 2002; Ost et al. 2014). In accordance, expression of *myosin heavy polypeptide 7, cardiac muscle, beta* (Myh7) encoding a myosin found in cardiac muscle and in type I skeletal muscle fibers was highly increased in increased in both F1 and N2-tg mice. Myh1 and Myh4, encoding skeletal muscle myosin heavy polypeptide 1 and 4 (markers of IIx and IIb fibers, respectively), showed no expression differences.

Analysis of gene expression in WAT as a major target of FGF21 is shown in Fig. 4. Overall gene expression changes induced by SM UCP1 expression were largely attenuated in the N2 generation. Expression of FGF receptor 1 (Fgfr1) and its co-receptor β-Klotho (Klb) was increased in F1-tg mice as well as genes of lipid metabolism such as *fatty acid synthase* (Fasn), *acetyl-CoA carboxylase alpha* (Acaca), *stearoyl-CoA desaturase 1* (Scd1), and *hormone sensitive lipase* (Lipe). In addition, expression of the adipokines

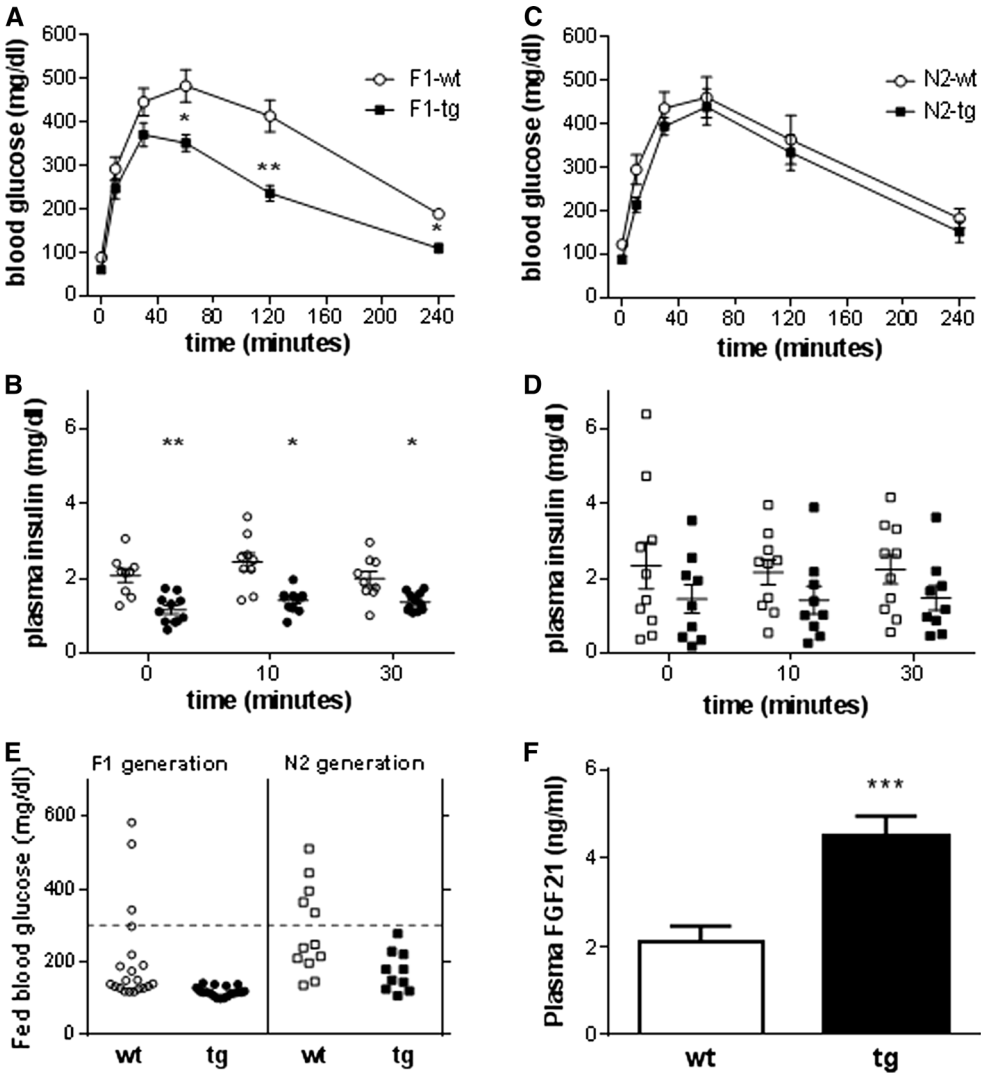

Fig. 2 Glucose homeostasis and plasma FGF21 in F1 and N2 NZO backcross wild type (wt) and UCP1 transgenic (tg) mice fed a high-fat diet from weaning until week 23 (F1-generation) or week 20 (N2-generation). Blood glucose levels and plasma insulin during glucose tolerance test performed in week 20 in F1 generation (**a**, **b**; $n = 7$–8) and week 16 in N2 generation (**c**, **d**; $n = 8$–10). **e** Highest individual random blood glucose levels achieved during HFD feeding period ($n = 10$–22). **f** FGF21 plasma levels of N2 wt and tg mice at 20 weeks of age ($n = 8$). Data are mean ± SE. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared to wt

adiponectin (Adipoq) and retinol-binding protein 4 (Rbp4) were induced. UCP1 and the transcriptional co-activator *peroxisome proliferator-activated receptor gamma coactivator 1-alpha* (PGC1α, gene name Ppargc1a) as markers for brown adipocyte were not induced in either F1 or N2-tg mice which is in contrast to findings in UCP1-tg mice on a Bl6 background (Keipert et al. 2014). Furthermore, no expression changes were observed in *leptin* (Lep) and *mesoderm specific transcript* (Mest) which have recently been established as predictive biomarkers of adipose tissue expansion (Voigt et al. 2015). With regard to glucose metabolism, there were no expression changes in *insulin receptor* (Insr), and Glut1, whereas Glut4 and Pck1 gene expression was induced in both F1 and N2-tg compared to wt. Overall, gene expression changes induced by SM UCP1 expression were considerably attenuated in the N2 generation. The remaining significant expression changes (Klb, Fasn, Rbp4, Glut4, and Pck1) were much less pronounced in N2-tg than in F1-tg mice (Fig. 4).

Discussion

Modifier genes can have a large impact on the phenotypical penetrance and expressivity of traits inherited in a simple Mendelian fashion (Nadeau 2001). Body size and thus body weight are highly heritable traits but possibly influenced by up to 6000 genes (Reed et al. 2008). It has also repeatedly been shown that the genetic background can influence the development of obesity and insulin resistance. Leptin deficiency in mice for example leads to multiple metabolic abnormalities including obesity, insulin resistance, reduced fertility and body temperature while the severity of these traits is strongly affected by the genetic background (Ewart-Toland et al. 1999; Haluzik et al. 2004; Qiu et al. 2001). The NZO mouse is a model for polygenic "diabesity" that shows obesity-associated insulin resistance, beta-cell failure, and ultimately chronic hyperglycemia leading to the development of type 2 diabetes thus closely resembling the human metabolic syndrome.

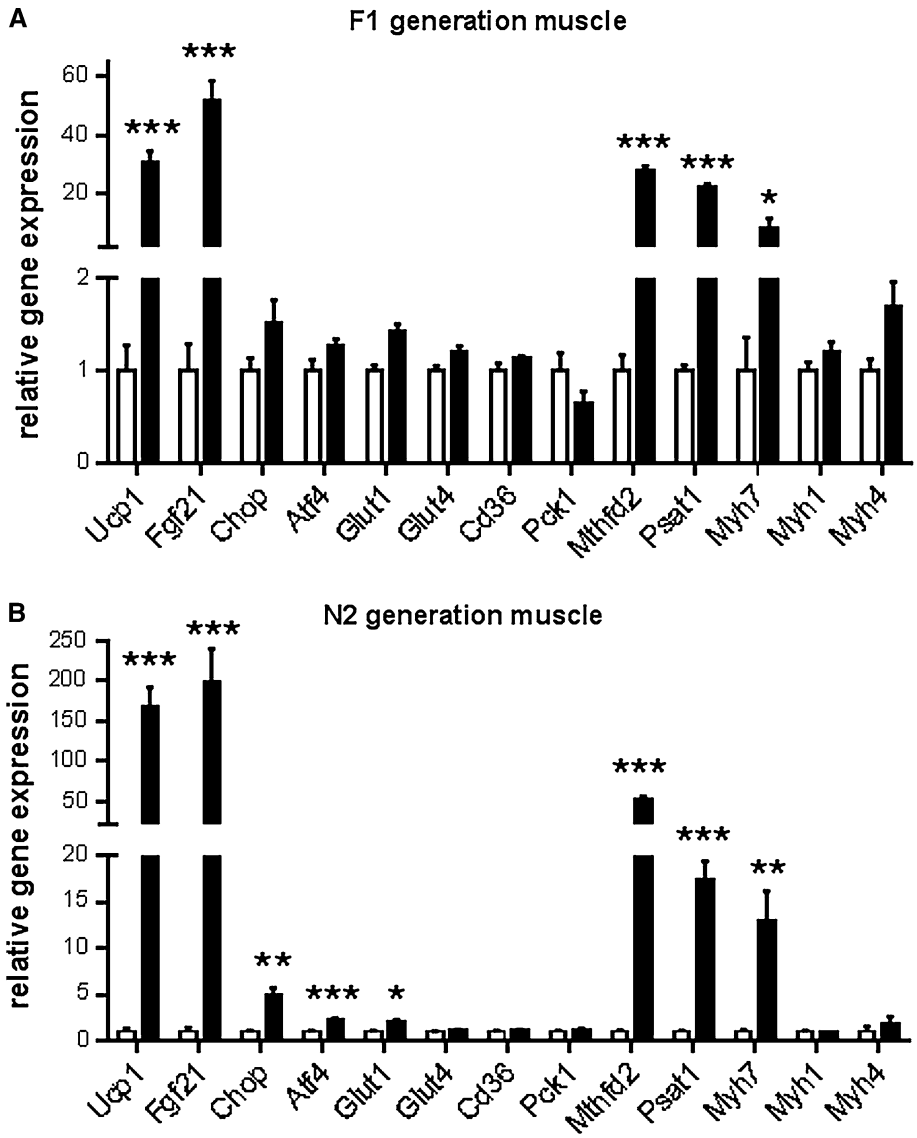

Fig. 3 Skeletal muscle gene expression in F1 (**a**) and N2 (**b**) NZO backcross wild type (wt, *open bars*) and UCP1 transgenic (tg, *black bars*) mice fed a high-fat diet from weaning until week 23 (F1-generation) or week 20 (N2-generation). $n = 8$–10. Data are mean ± SE. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared to wt which were set as 1

Investigations of the genetic basis of metabolic syndrome pathogenesis in this model lead to the identification of a number of adipogenic and diabetogenic gene variants which altogether suggest that fat oxidation and fat storage are crucial determinants of obesity and diabetes (Joost and Schurmann 2014). Skeletal muscle mitochondrial uncoupling profoundly affects substrate metabolism not only of muscle itself but also of other tissues such as WAT, leading to an overall improved glucose homoeostasis (Katterle et al. 2008; Keipert et al. 2011; Neschen et al. 2008). This is apparently still the case on the NZO polygenic diabetes and obesity background. NZO mice show a high incidence of obesity especially on high-fat diets, and it has been shown previously that the diabetes prevalence on a high-fat diet decreased from over 90 % in male mice of NZO background to 17 % a NZO × C57BL/6J intercross (Vogel et al. 2009). This is comparable with the diabetes prevalence of 15 % that we observed in the F1-wt and confirms that NZO-derived diabetes is markedly suppressed by the genetic background of lean, diabetes-resistant mice such as C57BL/6J. Diabetes prevalence was more than doubled in N2-wt with 75 % NZO background corroborating the strong effect of the NZO genome on high-fat-diet-induced hyperglycemia. Skeletal muscle UCP1 expression completely abolished the occurrence of severe hyperglycemia in both the F1 and N2 progeny which is supported by significantly lower fasted and random blood glucose levels in tg mice. Although HOMA index in the N2-tg was not significantly different from N2-wt due to high individual variations, this still suggests an increased insulin sensitivity in transgenic mice which is persistent even on a 75 % NZO genetic background.

The development of diabetes in N2-wt seems to be linked to an increased early adiposity in the affected mice. All five N2-wt mice developing diabetes showed over 21 % body fat already at 12 weeks of age. On the other

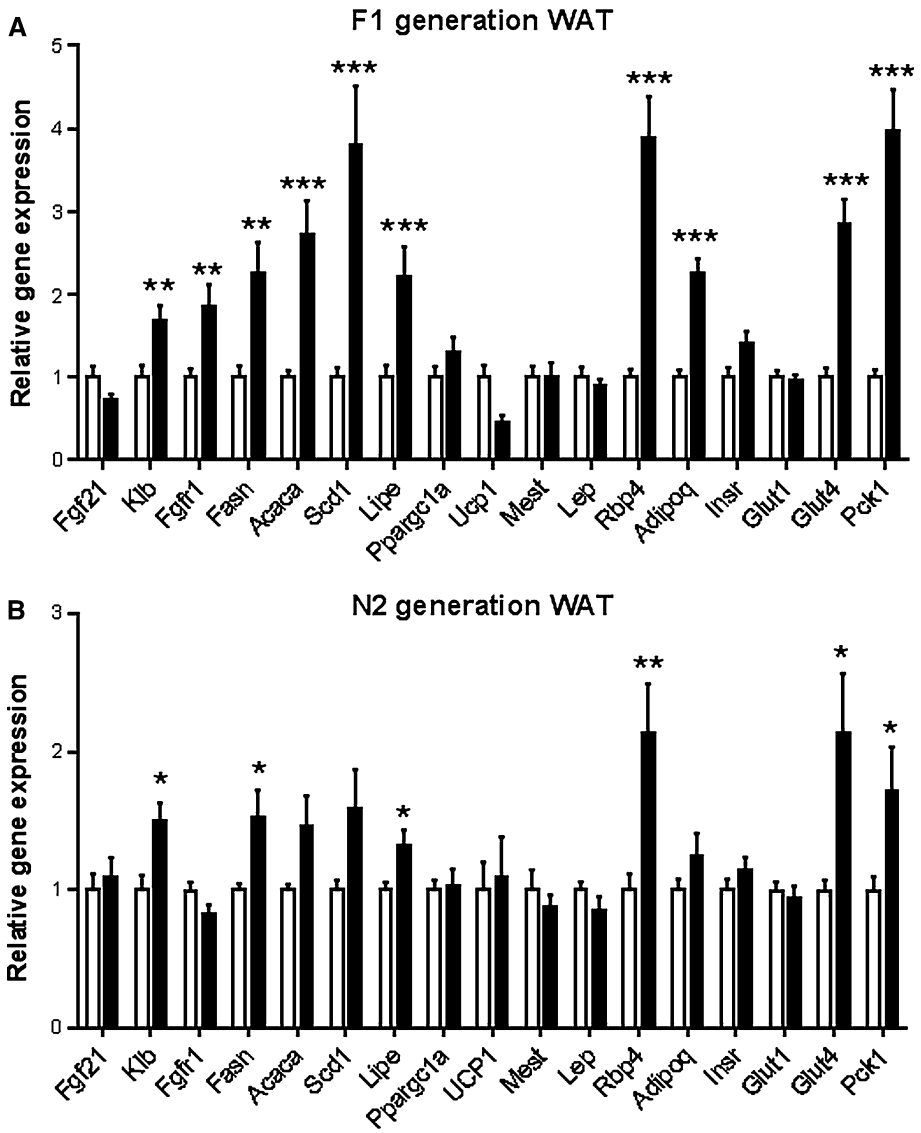

Fig. 4 White adipose tissue (eWAT) gene expression in F1 (**a**) and N2 (**b**) NZO backcross wild type (wt, *open bars*) and UCP1 transgenic mice (tg, *black bars*) fed a high-fat diet from weaning until week 23 (F1-generation) or week 20 (N2-generation). $n = 8$–11. Data are mean ± SE. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ compared to wt which were set as 1

hand, at this age 50 % of the N2-tg progeny also showed a relative body fat content above 21 % without developing diabetes. The prevention of diabetes development by skeletal muscle mitochondrial uncoupling is thus not due to the reduction of adiposity below the threshold necessary for diabetes development. This confirms that UCP1 expression in skeletal muscle leads to a dissociation of adiposity and impaired glucose homeostasis as shown before (Katterle et al. 2008; Keipert et al. 2013a) which is apparently independent of the genetic background.

Compared to C57BL/6J mice as reference strain, the genome of NZO mice contains over four million single nucleotide polymorphisms (SNPs) of which approximately 200,000 are specific to NZO when compared to other mouse strains (Keane et al. 2011). Using outcross populations of NZO mice, several variants have been identified in genes that are associated with different adipogenic and diabetogenic traits such as food intake, fat accumulation, and insulin levels (Joost and Schurmann 2014) in genes such as Pctp (Pan et al. 2006a, b), Tbc1d1 (Chadt et al. 2008), Zfp69 (Scherneck et al. 2009), and Ifi202b (Vogel et al. 2012). The activity of phosphatidylcholine transfer protein (Pctp) which is reduced in NZO mice is associated with serum insulin levels making this an interesting candidate to investigate in further studies.

Mitochondrial dysfunction has been implicated in the development of insulin resistance, but it is still unclear if there is a causal relationship (Montgomery and Turner 2015), and recently it has been suggested that rather an increase in mitochondrial efficiency precedes the development of insulin resistance as shown in a rat model of high-fat diet feeding (Crescenzo et al. 2014). It is interesting to note that an uncoupling induced decrease in mitochondrial efficiency (Neschen et al. 2008) in turn leads to a protection from diabetes development. Skeletal muscle mitochondrial uncoupling has profound effects on skeletal

muscle energy metabolism evident by a fiber type switch toward oxidative type 1 fibers (Couplan et al. 2002; Ost et al. 2014). This was preserved also with increasing NZO genetic background which could possibly account for the decreased lean body mass to which muscle contributes to a great extent. Furthermore, SM mitochondrial uncoupling induces a metabolic remodeling as a result of compensatory stress-signaling network that preserves cellular function as part of a muscle mitohormesis program (Keipert et al. 2013a; Ost et al. 2015). The induction of key genes linked to this remodeling was not attenuated but rather increased in N2-tg mice compared to F1-tg mice showing clearly that the normal response to skeletal muscle mitochondrial uncoupling is preserved in mice with increasing NZO genetic background. Part of the mitochondrial stress response induced in UCP1-tg mice is a massive induction of FGF21 expression and secretion from skeletal muscle leading to over fivefold increased circulating FGF21 levels (Keipert et al. 2014). Skeletal muscle FGF21 expression was completely preserved in F1 and N2-tg progeny resulting in elevated FGF21 plasma levels which in N2-tg mice were very similar to those previously reported in UCP1-tg mice (Keipert et al. 2014). FGF21 plasma levels are known to be increased in diet-induced obesity as shown in different mouse models as well as humans (Iglesias et al. 2012). FGF21 plasma levels of N2-tg mice on a high-fat diet were indeed similar to those observed in wt mice on a C57BL/6/CBA background fed a high-fat diet (Keipert et al. 2014). Induction of FGF21 in obesity is likely a compensatory response because FGF21 has been shown to have numerous beneficial metabolic effects. Systemic administration and overexpression of FGF21 was shown to reduce diet induced as well as genetic obesity, and FGF21 is now considered to have promising therapeutic potential (Ohta and Itoh 2014). By studying lipodystrophic mice (Veniant et al. 2012) or mice with fat-specific deletion of the FGF receptor FGFR1 (Adams et al. 2012), it was shown that adipose tissue is the key tissue for the majority of the FGF21-dependent metabolic effects by binding of FGF21 to the FGFR1-KLB complex (Luo and McKeehan 2013). The high-fat-diet-induced increase in FGF21 is thought to be linked to a FGF21 resistance as evident by an attenuation of FGF21 effects on liver and adipocytes (Fisher et al. 2010). It was suggested that this could be due to an inflammation induced repression of KLB expression in adipocytes (Diaz-Delfin et al. 2012). We found that KLB gene expression was rather increased than decreased in WAT of F1 and N2-tg mice which is not supportive of a FGF21 resistance at the level of WAT with increasing NZO genetic background. The expression of genes which are crucial for adipocyte function is regulated by numerous factors besides FGF21 and examination of downstream FGF21 signaling would be necessary to elucidate if impairment at this level could be causally linked to the attenuation of SM mitochondrial uncoupling induced WAT changes with increasing NZO background. Remarkable is the complete lack of browning in WAT even of F1-tg mice which is likely due to the NZO background which should be addressed in future studies. On a C57BL/6/CBA background we have observed the recruitment of brown adipocytes in epididymal WAT of UCP1-tg mice evidenced by an increased respiratory capacity and induction of brown fat marker genes such as UCP1 and PGC1α (Keipert et al. 2014). It is generally accepted that FGF21 induces this browning (Eckardt et al. 2014; Poher et al. 2015) which was also confirmed in UCP1-tg mice (Keipert et al. 2014). Recruitable brown adipocytes within typical WAT depots [also named “brite”, “beige” or “inducible brown adipocytes” (Harms and Seale 2013; Petrovic et al. 2010; Schulz et al. 2011)] were shown to be thermogenically functional (Shabalina et al. 2013) and associated with improved metabolic profiles (Bartelt and Heeren 2014). On the other hand, most metabolic effects of pharmacological FGF21 administration do not require the presence of UCP1 as recently reported in two studies using UCP1 knockout mice (Samms et al. 2015; Veniant et al. 2015) which is in line with our findings.

In conclusion, this study shows that the induction of mild mitochondrial stress in skeletal muscle and the subsequent increase in circulating FGF21 are not able to completely abolish the development of diet-induced obesity but fully prevent the development of diabetes in a polygenic diabesity model. WAT remodeling was attenuated with increased NZO genetic background which could possibly antagonize the FGF21 response at the level of WAT.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interests.

Animal rights All institutional and national guidelines for the care and use of laboratory animals were followed.

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