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ORIGINAL CONTRIBUTION

Opposing effects of dietary sugar and saturated fat on cardiovascular risk factors and glucose metabolism in mitochondrially impaired mice

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Abstract
Purpose Both dietary fat and dietary sucrose are major components of Western diets that may differentially affect the risk for body mass gain, diabetes mellitus, and cardiovascular disease.

Methods We have phenotypically analyzed mice with ubiquitously impaired expression of mitochondrial frataxin protein that were challenged with diets differing in macronutrient content, namely high-sucrose/low-fat and high-saturated fat/low-sugar diets.

Results We find here that a high-sucrose/low-fat diet has especially detrimental effects in mice with impaired mitochondrial metabolism promoting several independent cardiovascular risk factors, including impaired glucose metabolism, fasting hyperinsulinemia, reduced glucose-stimulated insulin secretion, increased serum triglycerides, and elevated cholesterol levels due to increased expression of HMG-CoA reductase. In contrast, a high-saturated fat/low-sugar diet protects mice with impaired mitochondrial metabolism from diet-induced obesity by increasing total energy expenditure and increasing expression of ACAA2, a rate-limiting enzyme of mitochondrial beta-oxidation, whereas no concomitant improvement of glucose metabolism was observed.

Conclusions Taken together, our results suggest that mitochondrial dysfunction may cause sucrose to become a multifunctional cardiovascular risk factor, whereas low-sugar diets high in saturated fat may prevent weight gain without improving glucose metabolism.

Keywords Mitochondria · Macronutrient metabolism · Diabetes · Obesity · Cardiovascular disease

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Introduction

In Western countries, both dietary sugars as well as saturated fatty acids represent increasingly important components of human diets [2, 3, 5, 6, 8, 10, 17, 18, 23, 31, 35]. Specifically, in US-Americans, sugars account for 25% of

daily calorie intake [11] and have been shown to negatively affect serum lipoprotein profiles promoting dyslipidemia in humans [20]. Accordingly, it has been recommended to reduce intake of nutritive sucrose in order to minimize cardiovascular disease risk [11, 16, 36]. Nevertheless, it has been repeatedly observed that individual response to dietary measures differs tremendously between subjects [33], suggesting an unknown systemic mechanism determining the fate of ingested sugars with regards to undesirable effects on risk for cardiovascular disease. Risk factors include four key features of the so-called metabolic syndrome, namely hyperglycemia, fasting hyperinsulinemia, hypertriglyceridemia, and hypercholesterolemia [25].

Regarding the underlying biochemical cause, we hypothesized that reduced efficiency of mitochondrial metabolism might prevent the key intermediate of cellular sucrose breakdown, acetyl-CoA, from being oxidized to carbon dioxide, thereby inducing *de novo* synthesis of lipids, including cholesterol, from acetyl-CoA. Unlike generation of carbon dioxide and of the concomitant proton gradient, this process requires only minimal mitochondrial activity. To test this hypothesis, we have used mice with ubiquitously reduced expression of the mitochondrial protein frataxin, which exhibit a limited impairment of mitochondrial capacity in a non-tissue-specific manner, as previously described [22]. As shown there, these frataxin knock-down mice develop obesity only when maintained on a so-called Western diet containing excessive amounts of both sucrose and saturated fat; while a less energy-dense, polysaccharide-rich rodent diet fully prevents this phenotype [22]. We now show that maintaining these knock-down animals on a sucrose-enriched/low-fat diet promotes several independent cardiovascular risk factors, including impaired glucose metabolism, fasting hyperinsulinemia, reduced glucose-stimulated insulin secretion, increased serum triglycerides, and elevated cholesterol levels, suggesting that impaired mitochondrial metabolism may convert sucrose into a significant health threat. In contrast, replacing sucrose by dietary fat in an isocaloric manner prevents the afore-mentioned alterations and rather protects from weight gain in this model of impaired mitochondrial metabolism.

Materials and methods

Generation of mice

For generation of mice with mitochondrial dysfunction, expression of the mitochondrial protein frataxin was downregulated by employing the Cre-loxP-recombinase-system. Namely, frataxin expression was impaired by loxP-targeting exon 4 of the corresponding gene as described [22, 24, 26], except that systemic expression of Cre recombinase was obtained by using a subline of mice carrying an aP2-promotor-driven Cre transgene [1, 22]. Expression of aP2-driven Cre recombinase was obtained with a single founder kindly provided by BIDMC (Boston, MA, USA) reported to be 90% C57BL/6. Frataxin knock-down mice were derived by intercrossing frataxin loxP heterozygous mice (frataxin$^{(+/lox)}$) with frataxin loxP heterozygous mice that also expressed Cre recombinase under the control of the aP2 promotor/enhancer (frataxin$^{(+/lox)}$ aP2Cre$^{(+/-)}$. Consequently, frataxin knock-down mice have the genotype Cre$^{(+/-)}$ frataxin$^{(lox/lox)}$. The remaining genotypes, frataxin$^{(+/+)}$, frataxin$^{(+/lox)}$, frataxin$^{(lox/lox)}$ (all without Cre), and frataxin$^{(+/+)}$ Cre$^{(+/-)}$, were used as controls. At an age of 30 weeks, frataxin knock-down and wild-type controls were matched for gender and body mass and placed on the three experimental diets. Mice at this age were chosen to avoid developmental effects of different diets. For most experiments, cre-positive and loxP-negative animals were additionally studied and showed no significant differences versus wild-type control animals. Low-caloric, low-fat, fiber-rich standard rodent chow (Altromin 1324) (convertible energy, 9 kJ/g; lipids, 40 g/kg; polysaccharides, 317 g/kg) [22]; high-caloric, high-sucrose-diet (Table 1) and high-caloric, high-fat diet (Table 1) were custom-made and obtained from Altromin GmbH, Lage, Germany. Animals were housed in air-conditioned rooms (temperature, 20 ± 2 °C; relative moisture, 50–60%) under a 12-h-light/dark schedule (lights on at 06:00 h) and had free access to chow and water. They were kept in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals, and all experiments were approved by the ethics committee of the Ministry of Agriculture, Nutrition, and Forestry (State of Brandenburg, Germany).

Table 1 Composition of diets

	'High-sugar'	'High-fat'
Proteins (casein, g/kg)	95	92
Polysaccharides (corn starch, g/kg)	362	338
Disaccharides (sucrose, g/kg)	364	13
Lipids (palm fat, g/kg)	51	231
Cholesterol (g/kg)	<0.001	<0.001
Convertible energy (kJ/g)	16	16

Genotype determinations

Genomic PCRs for detection of the loxP allele and the Cre transgene were performed as described [22, 26].

ATP content determinations

Liver tissue for ATP-determination was removed in the fed state, namely 2 h after beginning of the active dark phase at 20:00 h, and immediately clamp-frozen. Methods for ATP-measurements have been described [7, 22].

Body composition analysis

Body weight and body composition (including fat content) were measured as previously described, including use of quantitative nuclear magnetic resonance technique (Bruker Minispec mq10, Bruker Optics Inc., Billerica, MA) [22].

Food consumption quantification

Food intake was measured three times per week by weighing (BP 2100, Sartorius AG, Göttingen, Germany; detection limit 0.01 g) and normalized to 24 h and g of total body mass as previously described [22].

Respiratory quotient and total energy expenditure

Total energy expenditure and respiratory quotient were determined by indirect calorimetry of mice housed individually in metabolic cages, receiving food and water ad libitum as described [13]. Gas analysis was performed using the analyzing system Advanced Optima (ABB, Mannheim, Germany; former Hartmann & Braun GmbH & Co. KG, Frankfurt/Main, Germany) containing oxygen (Magnos 16) and a carbon dioxide (Uras 14) analyzer. Calorimetric measurements were performed for 48 h in parallel and every six min after an acclimation period of 24 h. Total energy expenditure was normalized to 24 h and metabolic body mass [13].

Glucose tolerance tests

Glucose tolerance tests were performed as described [26] by intraperitoneal (IP) glucose injection (D-(+)-glucose, Merck, Darmstadt, Germany) after mice were fasted 16 h overnight. Serum was collected before and 10, 30, and 120 min after glucose challenge and immediately frozen at −80 °C for measurement of glucose and insulin. If not indicated otherwise, GTTs were performed 6 weeks after initiation of diets.

Serum analyses

Blood samples for glucose measurements were obtained both in the fed state as well as after mice were fasted 16 h overnight. Determination of serum lipid concentrations was performed in samples collected after a 16-h overnight fasting period. Blood was collected by retro-orbital puncture. Serum samples were immediately prepared by centrifugation, and immediately frozen at −80 °C for further measurements as described [26].

Determination of glucose, non-esterified fatty acids (NEFA), triglycerides, and total cholesterol in serum was performed by using automated analyzer (Cobas Mira S, Hoffmann-La Roche, Basel, Switzerland) employing commercial kits (glucose HK125, triglycerides and total cholesterol from ABX, Montpellier, France, and NEFA C from Wako, Neuss, Germany). Mouse serum insulin levels were measured by ELISA for rat insulin using a mouse insulin standard (both from Crystal Chem Inc., Chicago, Illinois, USA) as described [26].

Immunoblots

Western blotting was performed on protein extracts obtained from clamp-frozen liver tissues collected in the fed state at 20:00 h. Liver tissue samples of four animals per diet and genotype were clamp-frozen, and protein was extracted by using Sörenson's phosphate buffer. After analyzing the protein content by using the Bradford assay, the protein extract was heated for 5 min at 95 °C with Laemmli buffer and DTT and finally stored at −20 °C until immunodetection. After SDS–PAGE, immunodetection was performed using primary antibodies against HMG-CoA-reductase (Anti-HMG-CoA Reductase, #07-457, Upstate, Lake Placid, NY, USA) and Acetyl-CoA-Acyltransferase 2 (ACAA2) (Anti-ACAA2, Clone 5C4, #H00010449-M01, Abnova Corporation, Taipei City, Taiwan) as well as alpha-Tubulin (Anti-alpha-Tubulin, Clone DM 1A, #T9026, Sigma, Taufkirchen, Germany) were used. Specific signals were detected (ECL Detection Reagents, Amersham Biosciences, Freiburg, Germany), quantified with luminescence imaging (LAS-1000, Fujifilm, Japan) and quantification software (AIDA, Raytest, Germany), and normalized to α-tubulin signals.

$^{13}CO_2$ breath tests for glucose oxidation experiments

$^{13}CO_2$ breath tests were applied as non-invasive methods to study the oxidation of injected or ingested ^{13}C-labeled glucose. The tests were performed in parallel between 0800 and 1700, in eight male mice of each genotype with free access to food and water. The ^{13}C-labeled substrate used in this study was D-glucose (U-$^{13}C_6$, 98%, M = 181.6 g/mol; Cambridge Isotope Lab., Inc., Woburn, MA, USA). Ten micromoles per kg ^{13}C-labeled glucose dissolved in sterilized 0.154 mol/l NaCl, and a volume of 50 μL/20 g body weight was injected intraperitoneally. Breath samples for determination of ^{13}C-glucose oxidation were obtained at baseline and at 10, 20, 30, 40, 50, 60, 80, 100, and

120 min in duplicate after injection, respectively. In order to collect $^{13}CO_2$ samples, mice were placed individually at each time point for 30 s into 140-mL syringes. This time interval was tested to be sufficient in CO_2 concentration and ^{13}C enrichment for mice (data not shown). The syringes were equipped with a wave to collect representative breath samples into evacuated 10-mL tubes (Exetainer, Labco Ltd., High Wycombe, U.K.) for storage and measurement.

Breath $^{13}CO_2$ enrichments were analyzed by isotope ratio mass spectrometry (BreathMAT. Thermo Scientific Corp., Bremen, Germany) and were expressed as $\delta^{13}C$ in the conventional delta per mill notation as described [21]. The delta over baseline values (DOB) were calculated by taking the difference between enrichments of each breath sample and the baseline breath sample. The baseline ^{13}C abundances in breath CO_2 (data not shown) were in accordance to a consumption of C_3 plant-based experimental diets with a relatively low natural ^{13}C abundance before the breath tests.

RNA extraction and RNA array analyses

RNA was extracted from tissue samples according to the TRIzol method (Invitrogen) according to the manufacturer's instructions. Crude RNA was subsequently loaded onto an RNA binding column (QIAGEN) to remove contaminating genomic DNA and proteins before further processing. Total RNA content was assayed, and equal amounts of RNA were used for cDNA synthesis and hybridization. For transcript-level analysis, the Affymetrix Mouse Genome 430 2.0 Array was used according to the manufacturer's instructions. Analysis of the presented data was performed with Affymetrix GCOS software by comparing means of signal intensities from RNA samples derived from control and knock-down animals. Ratios for transcript-level regulation as control versus knock-down signal intensities were obtained to illustrate the extent of regulation (Affymetrix GCOS software; threshold for regulation was set to 1.50). As individual transcripts are represented by a set of different probes on the chip, transcripts were only considered to be regulated when at least two-thirds of probes for the same transcript displayed similar regulation. Genes which were expressed at a fold change ratio greater than 1.5 in knock-down animals compared to controls were subjected to metabolic pathway analysis using the Gene Set Enrichment Analysis (GSEA) Software [19, 30].

Data analysis

Data are expressed as mean ± SEM. Equal distributions were tested by Kolmogorov–Smirnov test before applying *T*-tests. Unpaired Student's *T*-tests were used to compare knock-down and wild-type control animals of each dietary group as well as a two way ANOVA (diet, genotype) for analyzing the glucose clearance in the glucose tolerance test both by employing SPSS 15.0 (SPSS, Chicago, IL, USA).

Results and discussion

A high-sucrose diet reduces food uptake in mitochondrially impaired mice

Our previous study has shown that impaired frataxin expression in mice in an ubiquitous manner causes impaired mitochondrial metabolism and promotes weight gain following Western diet exposure due to impaired energy expenditure [22].

To further dissect the role of different macronutrients and specifically dietary sucrose in mammals, we have studied frataxin knock-down mice and their wild-type littermates at an age of 30 weeks. At this age, and comparable to young knock-down animals [22], they still show impaired mitochondrial function, as shown by an increased ratio of adenosine-diphosphate (ADP) to adenosine-triphosphate (ATP) in liver specimen obtained from these animals (Fig. 1a).

We have placed such mice and corresponding littermates on a diet containing high amounts of sucrose (Table 1) at an age of 30 weeks; the study was terminated at an age of 43 weeks. Unlike Western diets used for previous experiments [22], the high-sucrose diet used in this study did not contain cholesterol and was comparably low in total fat content, whereas convertible energy content was similar to that of previous experiments employing a Western diet [Table 1; 22]. When maintained on this particular diet, mitochondrially impaired knock-down mice showed body fat content (Fig. 1b) as well as body mass (Fig. 1c) similar to that of wild-type controls. We observed reduced daily food intake in knock-down mice (Fig. 1d, $P = 0.008$), whereas respiratory quotient (Fig. 1e) and total energy expenditure (TEE) (Fig. 1f) did not differ significantly between the two study groups. Therefore, it should be noted that all phenotypical alterations described below for these mice on a high-sugar diet are not related to obesity (Figs. 1b, c) and rather occur despite a significantly decreased energy intake (Fig. 1d) while food absorption has not been quantified. Furthermore, it should be noted that these animals, when being maintained on a Western diet, significantly gained body fat [22] whereas this seems no to be the case on the high-sucrose diet used in the present study (Table 1, Fig. 1b, c).

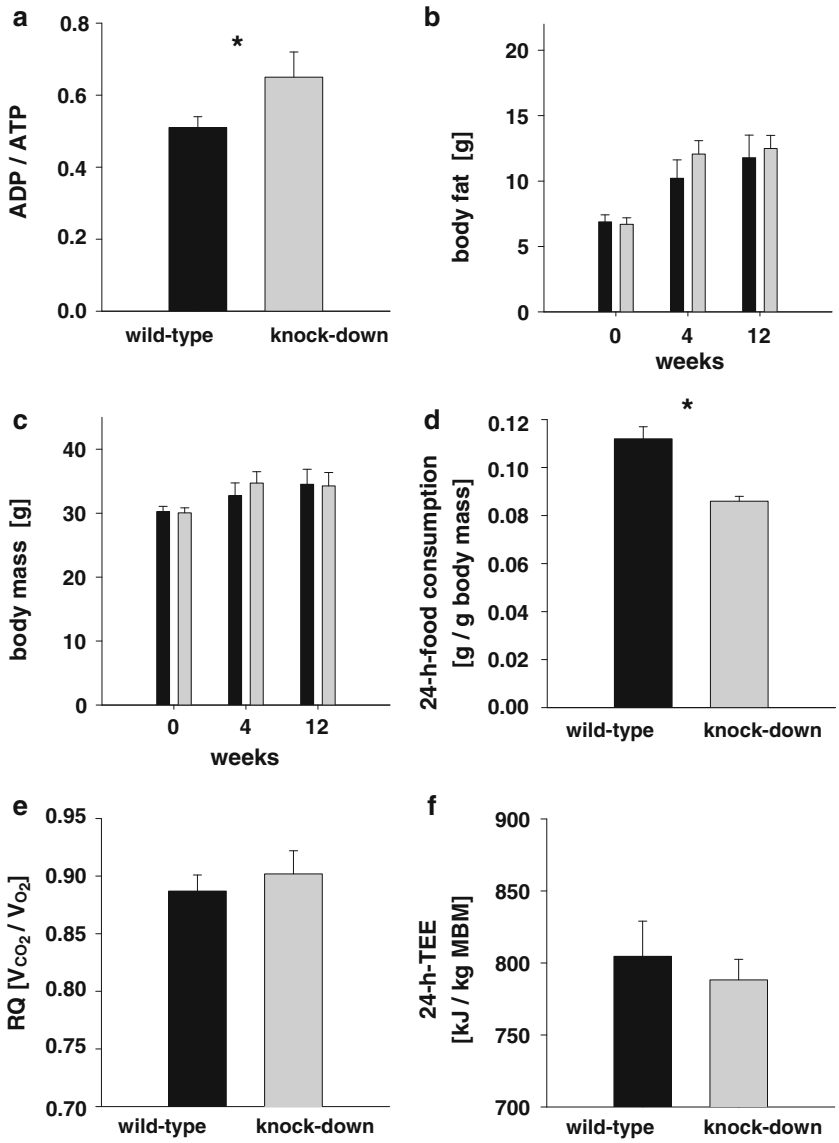

Fig. 1 Reduced mitochondrial metabolism does not affect body mass on a high-sucrose diet. **a** Ratio of hepatic ADP and ATP content in high-sucrose-fed mice; *black bars* correspond to control animals ('wild-type'), while *gray bars* reflect frataxin knock-down animals with impaired mitochondrial capacity (applies to all subsequent panels except for 3E and 4G) ($n = 5$ per genotype). *Error bars* reflect standard error of means, star indicates $P < 0.05$ (applies to all subsequent panels). **b** Body fat content in mice as in *Panel A* ($n = 10$ per genotype). **c** Body mass of mice as in *Panel A* ($n = 10$ per genotype). **d** Food uptake per individual mouse, depicted for mice as in *Panel A* ($n = 6$ per genotype). **e** Individual respiratory quotient of mice as in *Panel A* ($n = 6$ per genotype). **f** Total energy expenditure of mice as in *Panel A* ($n = 6$ per genotype)

Impaired glucose metabolism and reduced glucose-induced insulin secretion in sucrose-fed mitochondrially impaired mice

Next, we quantified glucose metabolism as previously described [26] and observed increased fasting serum glucose levels exclusively in mitochondrially impaired mice on a high-sucrose diet (Fig. 2a, $P = 0.047$). Accordingly, postprandial serum glucose levels were found to be elevated only in mitochondrially impaired mice on a high-sucrose diet (Fig. 2b, $P = 0.035$). While no differences in body mass were observed, as previously stated (Fig. 1c), intraperitoneal injection of D-glucose as described before [26] revealed a significant impairment of glucose disposal rates in mitochondrially impaired mice on a high-sucrose diet (Fig. 2c, $P = 0.024$), whereas no differences were observed in mice on standard rodent chow (data not shown). Similar differences were observed for the areas under the curves (AUCs) for glucose excursion after intraperitoneal injection of glucose (Fig. 2d, $P = 0.049$).

Quantification of serum insulin concentrations revealed increased levels during the fasting state only in mitochondrially impaired mice on high-sucrose diet (Fig. 2e, 0 min, $P = 0.024$ wild-type vs. knock-down genotype), while no differences were observed in mice on standard rodent diet (data not shown). This fasting hyperinsulinemia was accompanied by a complete loss of the so-called first-phase insulin secretion, which typically occurs a few minutes after injection of D-glucose in genetically unaltered animals. While control littermates still exhibited first-phase secretion on a high-sucrose diet (Fig. 2e, increases from 0 to 10 min after injection: $P = 0.034$ for wild-type, $P = 0.571$ for knock-down genotype), no differences were observed in mice on standard rodent diet (data not shown).

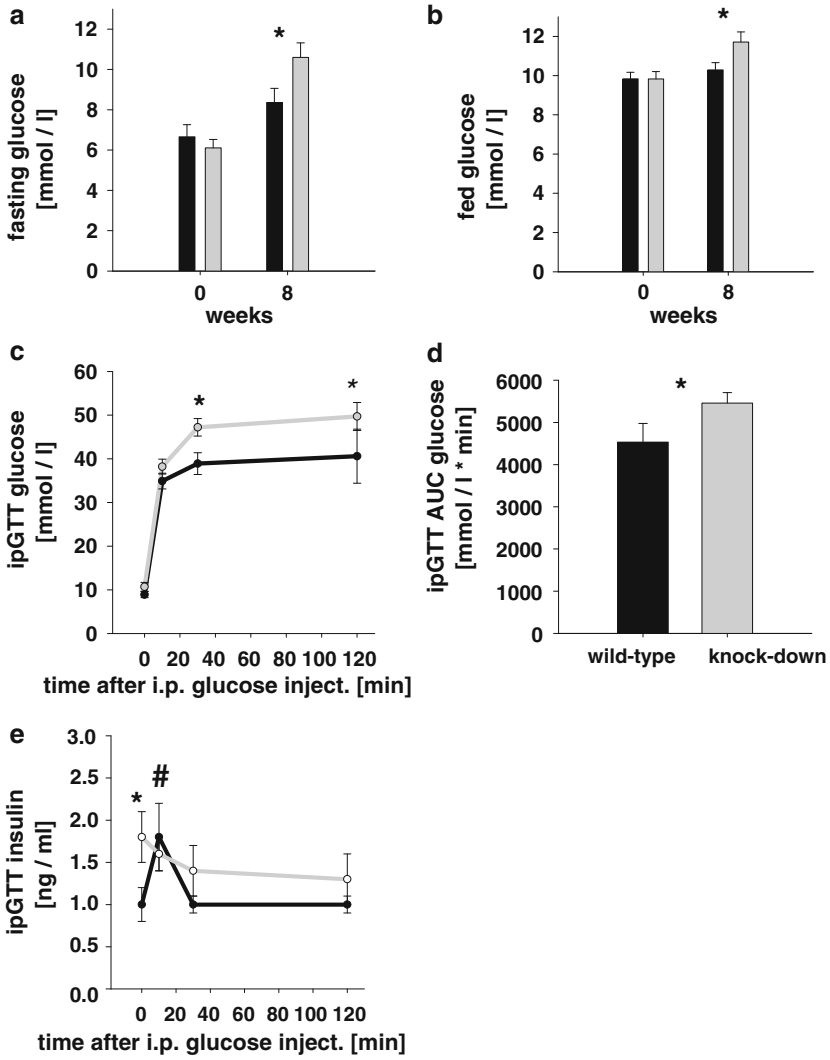

Fig. 2 Reduced mitochondrial metabolism causes impaired glucose metabolism in mice on a high-sucrose diet independent of body mass. **a** Fasting serum glucose levels of mice that were food deprived for 16 h ($n = 8$ per genotype). **b** Postprandial serum glucose levels of mice that had free access to sucrose-enriched diet ($n = 8$ per genotype). **c** Serum glucose excursions following intraperitoneal injection of D-glucose; *black line* corresponds to control animals, while *gray line* reflects frataxin knock-down animals with impaired mitochondrial capacity (also applies to panel E) ($n = 8$ per genotype); **d** Area under the curves (AUCs) for glucose excursions ($n = 8$ per genotype). **e** Serum insulin excursions following intraperitoneal injection of D-glucose ($n = 8$ per genotype); # indicates P-value < 0.05 for differences of insulin levels at 0 min compared with levels at 10 min for wild-type control animals

Dyslipidemia due to sucrose feeding in mitochondrially impaired mice

While both fasting and postprandial hyperglycemia, as well as fasting hyperinsulinemia and impaired glucose-stimulated first-phase insulin secretion are widely accepted to be indicative for type 2 diabetes mellitus and known to be independent risk factors for cardiovascular disease; we nevertheless questioned whether additional parameters contributing to the metabolic syndrome might be altered in response to impaired mitochondrial metabolism and sucrose feeding. We determined serum lipids as described before [13] in the above-mentioned state and observed no significant differences in serum concentrations for non-esterified fatty acids (NEFA) (Fig. 3a). In contrast, serum triglycerides (Fig. 3b, $P = 0.028$) and total cholesterol concentrations (Fig. 3c, $P = 0.037$) in serum from mitochondrially impaired mice on a high-sugar diet were found to be increased. Especially, since diets were depleted of cholesterol, we hypothesized that high-sucrose feeding induces hypercholesterolemia in mitochondrially impaired mice by increasing the rate of *de novo* synthesis of this lipid. Consistent with this mechanism, we observed increased protein expression of 3-hydroxy-3-methyl-glutaryl-Coenzyme A reductase (HMG-CoA reductase, EC 1.1.1.88) (Figs. 3d [$P = 0.043$] and e), the rate-limiting step for *de novo* synthesis of cholesterol from acetyl-CoA and a key target of cholesterol-lowering drugs.

A high-fat/low-sucrose diet protects from body mass gain in mitochondrially impaired mice

While the previous set of data suggests that mitochondrial dysfunction promotes dyslipidemia and glucose intolerance following increased disaccharide uptake, we also questioned whether replacing sucrose by saturated dietary fat in an isocaloric manner would exert differential effects on metabolism. In parallel to the before-mentioned

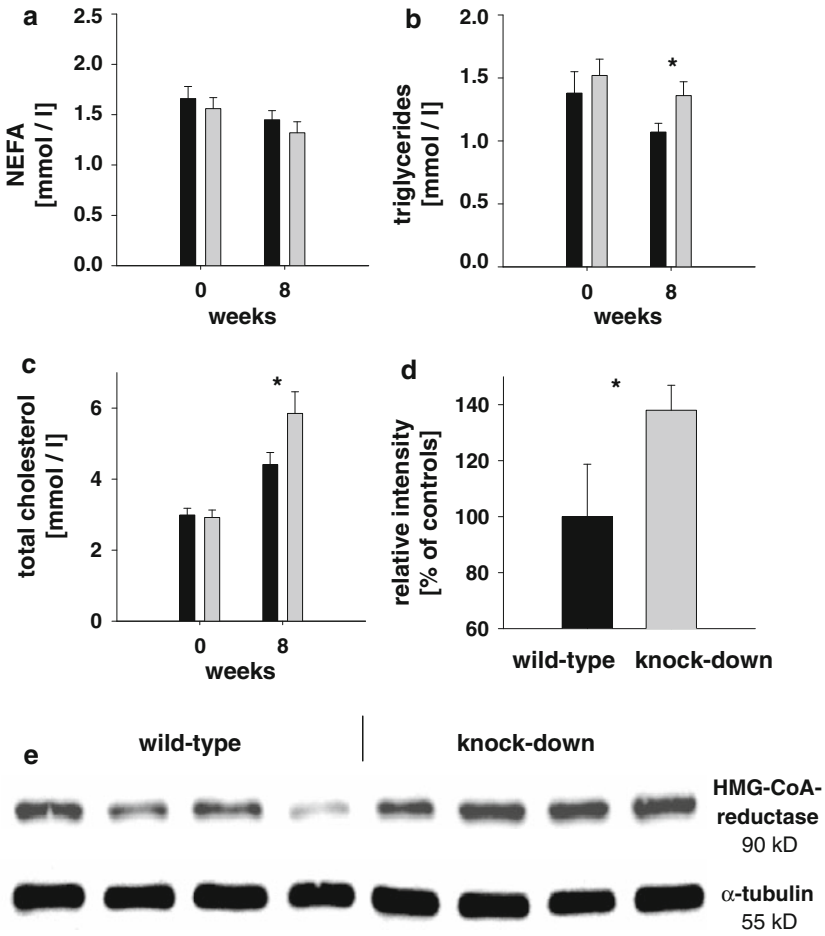

Fig. 3 Reduced mitochondrial metabolism promotes dyslipidemia in mice on a high-sucrose diet independent of body mass. **a** Serum concentrations of non-esterified fatty acids (NEFA) in fasted animals ($n = 8$ per genotype). **b** Serum concentrations of triglycerides in fasted animals as in *Panel A* ($n = 8$ per genotype). **c** Serum concentrations of cholesterol in fasted animals as in *Panel A* ($n = 8$ per genotype). **d**, **e** Western blot of hepatic tissues from random-fed animals as in *Panel A* using an primary antibodies raised against HMG-CoA reductase and α-tubulin. **d** Densitometric quantification of HMG-CoA-reductase signal intensities normalized to wild-type control signals on western blots of hepatic tissues from random-fed animals ($n = 4$ per genotype); **e** native blot

experiments, we placed both knock-down and control littermates of the above-mentioned sucrose-fed study group at the exact same age on a high-fat/low-sucrose diet (see Table 1 for details). Unexpectedly, applying this type of high-fat, Atkins-type regimen prevented body mass gain in mitochondrially impaired mice only (Fig. 4a), while control animals showed a significant increase in body mass over time (Fig. 4a, $P = 0.033$). This increase in body mass was preceded by a pronounced increase in body fat content in control mice only while mitochondrially impaired mice accumulated significantly less body fat (Fig. 4b, 4 weeks: $P = 0.021$, and 12 weeks: $P = 0.006$, respectively). Unlike for frataxin knock-down mice on a high-sucrose diet, food uptake was similar in both groups (Fig. 4c) while the respiratory quotient was similar in both groups as well (Fig. 4d) albeit lower than in the high-sucrose animals, as to be expected. However, it should be emphasized that ketones and/or glucose concentrations in urine samples were not determined; hence, ketonuria and/or glucosuria cannot be excluded to be a contributing factor affecting body mass and/or glucose metabolism.

Increased beta-oxidation paralleled by impaired substrate oxidation in high-fat fed mitochondrially impaired mice

Unaltered food uptake paralleled by differences in body fat accumulation and body mass suggest significant differences in energy conversion. To test this we first determined total energy expenditure in mitochondrially impaired mice on a high-fat diet (Fig. 4e, $P = 0.002$) and observed a striking induction of this parameter in the mitochondrially impaired animals protected from weight gain.

Next, we quantified oxidation of ^{13}C-labeled glucose in mitochondrially impaired mice fed a high-fat diet. The conversion of ^{13}C-glucose to $^{13}CO_2$ was found to be lower ($P = 0.017$ at 40 min) in frataxin knock-down genotype when compared to wild-type mice (Fig. 4f). The area under the curve (AUC) values were 1675 ± 84 delta over baseline values (DOB) and 1236 ± 90 DOB in wild-type and knock-down genotype, respectively. This effect was to be expected in frataxin knock-down mice based on reduced expression of a protein that affects Krebs cycle rate [27] by impairing activity of mitochondrial aconitase [28, 29, 32].

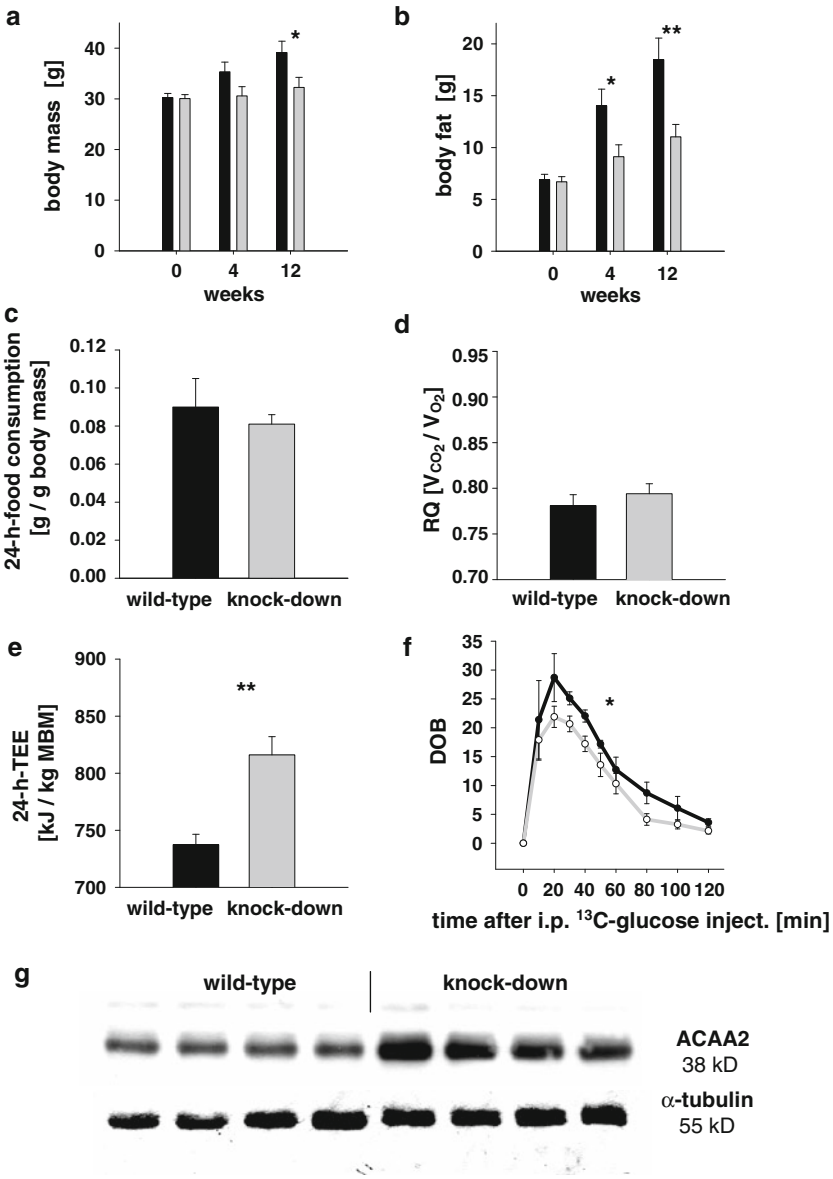

Fig. 4 Reduced mitochondrial metabolism protects from body mass gain by elevation of energy expenditure and beta-oxidation of fatty acids on a high-fat diet. **a** Body mass in mice ($n = 10$ per genotype); **b** body fat content in mice as in *Panel A* ($n = 10$ per genotype). **c** Food uptake per individual mouse, depicted for mice as in *Panel A* ($n = 6$ per genotype). **d** Individual respiratory quotient of mice ($n = 6$ per genotype). **e** Total energy expenditure of mice as in *Panel D* ($n = 6$ per genotype). **f** ^{13}C-enrichment in exhaled CO_2 expressed as DOB ($n = 5$ per genotype) after intraperitoneal injection of ^{13}C-labeled glucose. **g** Western blot of hepatic tissues from random-fed animals as in *Panel A* using primary antibodies raised against ACAA2 and α-tubulin

On the other hand, this reduction in glucose oxidation rate due to impaired Krebs cycle capacity cannot explain an increase in total energy expenditure in states of nutritive excess of saturated fatty acids. Therefore, we next quantified various enzymes of fatty acid metabolism, and most prominently found expression levels of acetyl-Coenzyme A acyltransferase 2 (ACAA2, also known as mitochondrial 3-oxoacyl-Coenzyme A thiolase) (EC:2.3.1.16) to be increased in mitochondrially impaired mice on a high-fat diet (Fig. 4g). Together with the before-mentioned decrease in glucose oxidation capacity (Fig. 4f), this latter finding suggests that mitochondrial dysfunction due to frataxin deficiency induces the rate-limiting step of beta-oxidation to generate C_2 intermediates, which cannot be further metabolized due to impaired Krebs cycle capacity [27, 29, 32]. Notably, almost identical metabolic sequelae have been recently proposed for the induction of insulin resistance by saturated fatty acids [15].

Relative impairment of glucose metabolism in high-fat fed mitochondrially impaired mice

Both decreased body mass as well as reduced body fat content are known to promote glucose metabolism in mice. We therefore assumed that mice on a high-fat diet might exhibit improved glucose metabolism in comparison with control mice, since the latter are significantly more obese (Fig. 4a, b). On the other hand, if a metabolic imbalance of increased fatty acid oxidation and reduced Krebs cycle capacity promotes or even causes impaired insulin action

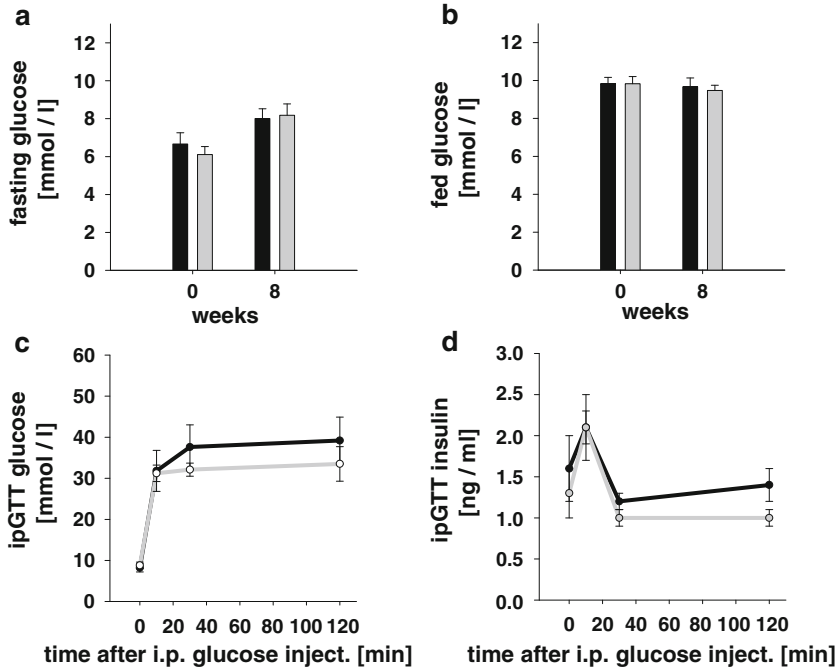

Fig. 5 Reduced mitochondrial metabolism does not alter glucose metabolism in mice on a high-fat diet. **a** Fasting serum glucose levels of mice that were food deprived for 16 h ($n = 8$ per genotype). **b** Postprandial serum glucose levels of mice that had free access to fat-enriched diet ($n = 8$ per genotype). **c** Serum glucose excursions following intraperitoneal injection of D-glucose; **d** Serum insulin excursions following intraperitoneal injection of D-glucose ($n = 8$ per genotype)

as previously suggested [15], the opposite might apply: Whereas the lack of increased body fat in our mitochondrially impaired mice (Fig. 4a, b) should promote glucose metabolism, this might be outweighed the metabolic imbalance within the mitochondria (Fig. 4f, g) culminating in unaltered glucose metabolism despite differences in body fat and body mass. Indeed, and despite reduced body mass and body fat content, we were unable to observe any differences in regards to serum glucose concentrations neither in the fasting (Fig. 5a) nor the postprandial, random-fed (Fig. 5b) state. Moreover, an intraperitoneal glucose challenge did not reveal any differences in glucose disappearance rates (Fig. 5c), and—unlike in sucrose-fed littermates (Fig. 2e)—insulin secretion was unaltered in both high-fat fed groups (Fig. 5d) suggesting a similar degree of insulin resistance [14] despite significant differences in body mass and body fat (Fig. 4a, b). These findings are consistent with alterations of mitochondrial lipid metabolism causing a relative impairment of glucose metabolism despite increased energy expenditure and decreased body mass at the same time, strongly supporting a recently proposed mechanism [15] where increased breakdown of fatty acids together with impaired Krebs cycle activity were shown to cause insulin resistance.

High-fat diet specifically affects expression of mitochondrial genes in mitochondrially impaired mice

To further validate our findings on increased mitochondrial lipid oxidation in mice with impaired energy metabolism and receiving a high-fat diet, we performed microarray gene expression analysis in liver and muscle samples from mice maintained on both diets.

In animals with impaired mitochondria receiving high-fat diet feeding, we observed an unexpected positive enrichment of pathways which are associated with lipid metabolism and mitochondrial energy metabolism in both muscle and liver (supplemental Tables 1, 2). We attribute this marked increase of mitochondrial gene expression levels to a counter regulatory mechanism that is activated due to dysfunctional maturation of iron-sulfur cluster-containing enzymes in tissues with reduced expression of frataxin. In this line, we also observe increased expression of frataxin mRNA in our microarray data, which is likely due to multiple probes which hybridize to different areas of the frataxin mRNA which are not affected by cre-mediated recombination and which hints toward autoregulatory mechanisms of frataxin expression.

Increased expression of mitochondrial genes could also be due to a surplus of metabolites coming from the initial steps of beta-oxidation. In fact, among those gene sets which were up-regulated in skeletal muscle of animals with mitochondrial impairment, we also found increased expression levels of several genes which are involved in mitochondrial fatty acid import and all subsequent steps of mitochondrial beta-oxidation, such as carnitine palmitoyltransferase and ACAA2, which is also consistent with the increased protein levels (Fig. 4g) observed in livers of high-fat fed animals with mitochondrial impairment (supplemental Table 3). In line with our previous observation, we observe a prevalence of negative enrichment, i.e.

reduced expression, of mitochondrial gene sets in liver samples from mice receiving the high-sucrose/low-fat diet (supplemental Tables 1, 2).

Choice of macronutrients determines metabolic consequences of mitochondrial dysfunction in mice

In line with the fact that physical exercise is an efficient inducer of mitochondrial metabolism known to extend life span by decreasing mortality from cardiovascular causes and/or impaired glucose metabolism [34] independently of body mass [12]; our findings indicate that impaired mitochondrial metabolism results in sucrose-induced changes to four independent risk factors for cardiovascular disease, including impaired glucose metabolism, fasting hyperinsulinemia, reduced glucose-stimulated insulin secretion, increased serum triglycerides, and elevated cholesterol levels due to increased expression of HMG-CoA reductase. Notably, these factors occur independent of increased body weight or obesity, since the sucrose-enriched diet in the current study did not cause significant differences in body mass between the different genotypes. While it is possible that sucrose promotes body weight gain in humans, our current findings suggest that additional risk factors may be induced by increased sucrose consumption, some of which are not readily detectable in epidemiological studies.

In contrast, replacing sucrose-contained calories by dietary fat, as suggested by the popular Atkins' diet regimen [4], prevents body weight gain only in states of impaired mitochondrial function when applied to our current murine model. This appears to be consistent with the fact that a high-fat, low-carbohydrate, ad libitum-available diet promotes weight loss more efficiently than conventional calorie restriction in humans [9]. Nevertheless, our findings suggest that this amelioration of body mass gain does not promote insulin sensitivity, indicating that body fat content may be of limited use in predicting overall metabolic health in states of mild mitochondrial dysfunction.

In summary, our results suggest that mitochondrial dysfunction may cause sucrose to become a multifunctional cardiovascular risk factor in mice, while high-fat/low-sugar diets may efficiently prevent weight gain without positively affecting glucose metabolism.

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Conflict of interest statement None.

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