Bile salt sequestration reduces plasma glucose levels in db/db mice by increasing its metabolic clearance rate
**ABSTRACT**

Bile salt sequestrants (BAS) reduce plasma glucose levels in type 2 diabetics and in murine models of diabetes but the mechanism herein are unknown. We hypothesized that sequestrant-induced changes in hepatic glucose metabolism would underlie reduced plasma glucose levels. Therefore, *in vivo* glucose metabolism was assessed in *db/db* mice on and off BAS using tracer methodology. Lean and diabetic *db/db* mice were treated with 2% (wt/wt in diet) colesevelam HCl (BAS) for 2 weeks. Parameters of *in vivo* glucose metabolism were assessed by infusing [U-13C]-glucose, [2-13C]-glycerol, [1-2H]-galactose and paracetamol for 6 hours, followed by mass isotopologue distribution analysis and related to metabolic parameters as well as gene expression patterns. Compared to lean mice, *db/db* mice displayed an almost 3-fold lower metabolic clearance rate of glucose (p=0.0001), a ~3-fold increased glucokinase flux (p=0.001) and a ~2-fold increased total hepatic glucose production rate (p=0.0002). BAS treatment increased glucose metabolic clearance rate by ~37% but had no effects on glucokinase flux nor total hepatic or endogenous glucose production. Strikingly, BAS-treated *db/db* mice displayed reduced long-chain acylcarnitine content in skeletal muscle (p=0.0317) but not in liver (p=0.189). Unexpectedly, BAS treatment increased hepatic Fgf21 mRNA expression 2-fold in lean mice (p=0.030) and 3-fold in *db/db* mice (p=0.002). BAS induced plasma glucose lowering in *db/db* mice by increasing metabolic clearance rate of glucose in peripheral tissues, which coincided with decreased skeletal muscle long-chain acylcarnitine content. BAS-mediated induction of hepatic Fgf21 might play a modulating role herein.
INTRODUCTION

Type 2 diabetes is a major health problem worldwide. The predominant features of type 2 diabetes entail increased fasting blood glucose levels, increased plasma triglycerides and LDL-cholesterol levels, as well as disturbed peripheral glucose utilization. The role of bile salt sequestrants (BAS) in treated LDL-cholesterol levels is well established. More recently, coleselam HCl a BAS has been indicated by the FDA to improve glycemic control in patients with type 2 diabetes. So far, however, the actual changes in hepatic and/or peripheral glucose metabolism upon BAS supplementation are not clearly understood and not extensively studied.

Bile salts are synthesized from cholesterol in the liver. Upon secretion into bile, bile salts function to emulsify fats in the small intestine. Most of the secreted biliary bile salts are reabsorbed in the ileum (enterohepatic circulation). Sequestrants interfere with the enterohepatic circulation of bile salts by binding them in the intestine, thereby inhibiting their reabsorption and promoting their fecal loss. As a consequence, the liver increases bile salt synthesis and subsequently cholesterol uptake from the circulation thereby reducing LDL-cholesterol levels.

To date, there is a lack of understanding how BAS reduce plasma glucose levels. However, a large body of research suggests that bile salts function as hormones and modulate hepatic glucose metabolism via signaling pathways mediated by the nuclear receptor FXR. FXR is expressed in the liver, intestine, adrenal gland and kidney, and it acts to inhibit de novo bile salt synthesis when activated by bile salts in the liver. Paradoxically, both agents that inhibit de novo bile salt synthesis, such as bile salts themselves and synthetic FXR ligands, as well as agents that increase de novo bile salt synthesis such as BAS, were shown to reduce plasma glucose levels in diabetic mice. Thus, regulation of bile salt-mediated changes in blood glucose levels is still not fully understood and remains elusive.

We therefore questioned whether BAS-induced changes in hepatic carbohydrate fluxes are responsible for the observed reduction in plasma glucose levels in db/db mice. To test this, we treated healthy lean mice and obese, diabetic db/db mice with the bile salt sequestinant coleselam HCl. Applying an in vivo infusion protocol of stable isotopes followed by mass isotopologue distribution analysis (MIDA), we first characterized specific disruptions of whole body glucose turnover and hepatic glucose metabolism in db/db mice. We then tested the hypothesis that BAS restores disrupted hepatic glucose fluxes thereby mediating the previously observed reduction in blood glucose levels in diabetic mice. In view of the strong interaction between glucose and fatty acid metabolism, we additionally tested the effect of BAS on levels of lipids and intermediates of fatty acid metabolism in liver and muscle.
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MATERIALS AND METHODS

Animals and diets

Ten week old male lean C57Bl/6J and obese, diabetic db/db mice on a C57Bl6/J background (B6.Cg-m +/+ Leprdb/J) were purchased from Charles River Laboratories (L’Arbresle, France and Brussels, Belgium, respectively). Mice were housed in a temperature controlled (21°C) room with a dark-light cycle of 12 h each. For all animal experiments the principles of laboratory animal care (NIH publication no. 85–23, revised 1985) were followed. The Ethics Committee for Animal Experiments of the University Groningen, the Netherlands, approved experimental procedures.

One week after arrival at the animal facility, 8 \(db/db\) and 6 lean (L) mice were put on a diet containing standard laboratory chow (RMH-B; Arie Blok, Woerden, The Netherlands) supplemented with 2% (wt/wt) colesevelam HCl (Daiichi Sankyo, Inc., Parsippany, NJ, USA) for 2 weeks. Another 8 \(db/db\) and 6 lean mice remained on standard laboratory chow. Body weights and food intake were recorded every other day. One week into the diets, mice were fitted with a permanent catheter in the right atrium via the jugular vein, as described previously. Mice were allowed to recover from surgery for 6 days.

Materials

The following isotopes were used: [2-\(^{13}\)C]-glycerol (99% \(^{13}\)C atom percent excess), [1-\(^2\)H]-galactose (98% \(^2\)H atom percent excess) (Isotec, Miamisburg, Ohio, USA), [U-\(^{13}\)C]-glucose (99% \(^{13}\)C atom percent) (Cambridge Isotope Laboratories, Andover, Mass., USA). All reagents and chemicals used were reagent pro analysis grade. Blood spots and urine were collected on Schleicher and Schuell No. 2992 filter paper (Schleicher and Schuells, ’s Hertogenbosch, The Netherlands). Infusates were freshly prepared and sterilized at the day before the experiment.

Animal Experiments

The infusion experiment was performed in conscious mice, as described previously. Mice were fasted for 4 hours (03:00-08:00 am) and then housed in individual cages to allow frequent collection of urine and blood spots on filter paper. Mice were infused with a sterile solution containing [U-\(^{13}\)C]-glucose (13.9 \(\mu\)mol/ml), [2-\(^{13}\)C]-glycerol (160 \(\mu\)mol/ml), [1-\(^2\)H]-galactose (33 \(\mu\)mol/ml) and paracetamol (1.0mg/ml) at a rate of 0.6 ml/h. Before and during the experiment, small blood samples were obtained via tail bleeding to allow for the determination of plasma glucose. Blood was immediately centrifuged and stored at -20°C until analysis. Blood spots were collected on filter paper before the start of the infusion and hourly afterwards until 6 h after the start of the infusion. Blood spots were air dried and stored at room temperature until analysis. Hourly urine samples were collected on filter paper, air
dried and stored at room temperature until analysis. At the end of the experiment, animals were anesthetized with isoflurane and a small blood sample was collected via orbital puncture for the determination of insulin.

Mice were allowed to recover and five days after the infusion experiment. Mice were then fasted for 7 hours (03:00-10:00h) and terminated by heart puncture under isoflurane anesthesia. A large blood sample was collected in heparin-containing tubes, immediately centrifuged and stored at -20 °C until analysis. Liver was excised, weighed, snap frozen and stored at -80 °C until further analysis. Skeletal muscle was collected, frozen in liquid N2 and stored at -80 °C until further analysis.

Measurement and Analysis of Mass Isotopologue Distribution Analysis by GC-MS

Analytical procedures for extraction of glucose in bloodspots and paracetamol-glucuronide from urine filter paper strips, and derivatization of the extracted compounds and GC-MS measurements of derivatives were all performed according to Van Dijk et al. The measured fractional distribution was corrected for natural abundance of 13C by multiple linear regression as described by Lee et al. to obtain the excess mole fraction of mass isotopologues due to incorporation and dilution of infused labeled compounds, i.e., [2-13C]-glycerol, [U-13C]-glucose and [1-2H]-galactose. This distribution was used in mass isotopologues distribution analysis (MIDA) algorithms of isotope incorporation and dilution according to Hellerstein et al. as described by Van Dijk et al.

Determination of metabolite concentrations

Commercially available kits were used to determine plasma levels of insulin (Merckodia, Uppsala, Sweden), triglycerides, total cholesterol, free cholesterol and NEFA (Wako Chemicals, Neuss, Germany). Hepatic glycogen and glucose-6-phosphate content were determined as previously described. Hepatic lipids were determined in liver homogenates by commercially available kits for triglycerides and total cholesterol (Wako Chemicals, Neuss, Germany) after lipid extraction as described by Bligh and Dyer. Plasma acylcarnitines were determined according to the method of Chase et al. as described by Derks et al. Profiles of long-chain acylcarnitines (C16:0, C16:1, C18:0, C18:1 and C18:2) in muscle and liver homogenates (15% (w/v) in PBS) were determined according to the method of Gates.

mRNA analysis

Total RNA was isolated from liver using TRI-reagent (Sigma, St. Louis, MO) according to the manufacturers’ protocol. cDNA was produced as described by Plösch and coworkers. Real-time PCR was performed on a 7900HT FAST real-time PCR system using FAST PCR master mix and MicroAmp FAST optical 96 well reaction
plates (Applied Biosystems Europe, Nieuwerkerk ad IJssel, The Netherlands). Primer and probe sequences have been published before (www.labpediatrics.nl). PCR results were normalized to 18S-rRNA abundance.

Statistics
All values are represented as mean ± standard deviation. Statistical significance was assessed using the Mann-Whitney-U-test (SPSS 12.0.1 for Windows). P-values were corrected for multiple comparison errors. Statistical significance was accepted for a p<0.05.

RESULTS

Bile salt sequestration reduced plasma glucose values in db/db mice
Previously, it has been observed that colesevelam HCl treatment lowers plasma glucose concentrations in db/db mice compared to untreated counterparts. To confirm these findings, basal parameters related to glucose metabolism were determined in lean and db/db mice treated with the bile salt sequestrant (BAS) for two weeks. As expected, BAS-treatment lowered blood glucose levels of diabetic mice (Table 1). No effects of BAS on body weight, liver weight or liver weight / body weight ratio were observed. Plasma insulin concentrations were decreased (~30%) and the HOMA-index was improved in sequestrant-treated diabetic mice compared to untreated counterparts. Moreover, we observed a more than 50% reduction in plasma 3-hydroxybutyrate levels and a non-significant decrease in plasma NEFA levels. Consistent with our previous observations, liver triglyceride contents were increased in sequestrant-treated lean and db/db mice compared to controls.

Bile salt sequestration increased metabolic clearance of glucose without affecting hepatic glucose production
BAS promotes specific changes in hepatic cholesterol and bile salt synthesis. To gain insight in hepatic glucose metabolism upon BAS-treatment, in vivo glucose metabolism was studied in lean and db/db mice after 2 weeks of BAS treatment. First, whole body and hepatic glucose metabolism in db/db mice was characterized. In db/db mice plasma glucose concentrations were almost 3 times higher than in lean mice (9.2 ± 0.5, 32.7 ± 3.9 mmol/l, lean vs db/db, Figure 1A). The rate of uptake of plasma glucose by peripheral tissue disposal was only slightly but significantly higher in db/db mice compared to lean mice (93.1 ± 13.1 vs. 117.0 ± 14.6 ml.kg⁻¹.min⁻¹, lean vs. db/db, Figure 1B). Consequently, the metabolic clearance rate of plasma glucose was lower in db/db mice (9.9 ± 1.3 vs. 3.6 ± 0.7 ml.kg⁻¹.min⁻¹, lean vs. db/db, Figure 1C). Diabetic mice had significantly higher hepatic glycogen contents compared to lean mice while hepatic glucose-6-phosphate contents did not differ between genotypes.
Figure 1. Effect of BAS on plasma glucose levels (A), rate of whole body glucose disposal (B) and metabolic glucose clearance rate (C) in lean mice (lean, n=5), lean mice supplemented with BAS (lean BAS, n=6), db/db mice (db/db, n=8) and db/db mice supplemented with BAS (db/db BAS, n=8). Each value represents the mean ± SD; *p<0.05 vs. same genotype untreated; #p<0.05 vs lean same condition.

Table 1. The effects of bile salt sequestration (BAS) on morphological, plasma and hepatic parameters in lean and diabetic mice. 7h fasted parameters (unless otherwise stated) in lean mice (Lean), lean mice supplemented with BAS (Lean BAS), db/db and db/db mice supplemented with BAS (db/db BAS). Data are shown as means ± SD; *p<0.05 vs. same genotype, #p<0.05 vs. lean same condition.
A strongly increased hepatic glucose cycling brought about by a massively increased glucokinase flux was observed in db/db compared to lean mice (46.7 ± 4.5 vs. 150.2 ± 58.3 μmol.kg⁻¹.min⁻¹, lean vs. db/db, Table 2). However, since hepatic clearance of glucose by glucokinase, as derived by division of blood glucose from the glucokinase flux, was not significantly different from hepatic glucose clearance in lean mice (5.1 ± 0.7 ml.kg⁻¹.min⁻¹ vs. 4.6 ± 0.7 ml.kg⁻¹.min⁻¹, lean vs. db/db mice), the observed increase in glucose cycling is most likely driven by the extreme hyperglycemia in db/db mice. In db/db mice de novo synthesis of glucose-6-phosphate was significantly decreased when compared to lean mice (86.0 ± 6.0 μmol.kg⁻¹.min⁻¹ vs. 73.7 ± 10.9 μmol.kg⁻¹.min⁻¹, lean vs. db/db, p<0.05, Table 2). Endogenous glucose production (excluding glucose cycling) was slightly higher (87.3 ± 13.0 vs. 115.0 ± 14.0 μmol.kg⁻¹.min⁻¹, lean vs. db/db, p<0.05, Table 2) whereas total glucose output (including glucose cycling) was massively higher in db/db compared to lean mice (118.0 ± 16.6 μmol.kg⁻¹.min⁻¹ vs. 243.5 ± 54.1 μmol.kg⁻¹.min⁻¹ lean and db/db mice, respectively (Table 2).

BAS treatment had differential effects on whole body glucose metabolism in lean and db/db mice. Irrespective of the decreased plasma glucose concentration, BAS treatment had no effect on the flux through glucokinase in livers of db/db mice. As a consequence, hepatic clearance of glucose by glucokinase was significantly increased
in db/db compared to lean mice, from 5.6 ± 0.4 ml.kg⁻¹.min⁻¹ in lean mice to 8.1 ± 0.7 ml.kg⁻¹.min⁻¹ in db/db mice (p<0.05). Since the glucokinase flux remained high, glucose cycling remained higher in treated db/db mice when compared to treated lean mice. In treated db/db mice the rate of endogenous glucose production did not change and the rate of total hepatic glucose output tended to increase, albeit non-significantly (Table 2). Although BAS was not effective to reduce rates of hepatic glucose consumption or production, we did observe a decrease of de novo glucose-6-phosphate synthesis in treated db/db mice (87.4 ± 9.4 μmol/kg⁻¹.min⁻¹ vs. 61.2 ± 7.4 μmol/kg⁻¹.min⁻¹, lean vs. db/db mice, p<0.05). This accounted for the decreased contribution of newly synthesized glucose-6-phosphate towards plasma glucose. Since the flux through glucokinase and glucose cycling remained invariably high, these decreases of de novo glucose-6-phosphate synthesis and newly synthesized glucose-6-phosphate partitioning towards glucose did not translate into a decreased total glucose output in db/db mice. Importantly, BAS treatment significantly increased peripheral metabolic clearance of glucose in db/db mice (~37%; Figure 1C) without affecting whole body glucose disposal (Figure 1B). Improved plasma glucose concentrations in BAS-treated db/db mice are therefore mainly attributed to increased peripheral glucose clearance.

To assess whether changes in hepatic glucose metabolism were in parallel with changes in gene expression patterns, expression levels of genes involved in hepatic glucose metabolism in untreated lean and db/db mice were compared. Compared to lean mice, in db/db mice expression levels of glucose transporter 2, glucokinase and glucose-6-phosphate hydrolase were increased, whereas no difference was observed in the expression of the gene encoding phospho-enol-pyruvate carboxykinase. (Figure 2A, B, C and D). Furthermore, expression of the glycolytic enzyme pyruvate kinase was not differentially expressed in lean and db/db mice (Figure 2E). Additionally, expression levels of these genes were measured in both models following BAS-treatment. BAS treatment had differential effects on expression of genes involved in hepatic glucose metabolism. In lean mice, expression of glucose transporter 2 and glucokinase were increased upon BAS treatment whereas expression levels remained high in db/db mice. Quite surprisingly, expression of the glycolytic enzyme pyruvate kinase was strongly increased upon treatment in db/db mice whereas expression levels remained unaffected in lean mice upon treatment. (Figure 2E). In addition, hepatic gene expression levels of fibroblast growth factor 21 (Fgf21), which has lately gained attention for its remarkable in vivo actions on glucose metabolism, were measured. Surprisingly, expression levels of Fgf21 were 2-fold increased in treated lean and 3-fold increased in treated db/db mice compared to untreated counterparts (Figure 2F).
Chapter 3

Bile salt sequestration reduced long-chain acylcarnitine content in muscle and plasma in db/db mice

Skeletal muscle is the major site of both glucose and fatty acid uptake and oxidation. It is known that under circumstances of high glucose concentrations muscle favors glucose uptake and oxidation over fatty acid uptake and oxidation, while accumulating excess fatty acids, in particular saturated free fatty acid species like stearate and palmitate. Recently, it has been shown that excessive fatty acid oxidation in diabetic mice, results in inefficient oxidation. Concomitantly, high intracellular concentrations of long-chain acylcarnitines, markers of inefficient mitochondrial fatty acid oxidation, were measured.

Figure 2. Relative hepatic mRNA expression levels of genes involved in hepatic glucose metabolism in lean mice (lean, n=5), lean mice supplemented with BAS (lean BAS, n=6), db/db mice (db/db n=8) and db/db mice supplemented with BAS (db/db BAS, n=8) for glucose transporter 2 (Glut 2, A), glucokinase (Gk, B), glucose-6-phosphate hydrolase (G6Pase, C), phospho-enol pyruvate carboxykinase (Pepck, D), pyruvate kinase (Pk, E) and fibroblast growth factor 21 (Fgf21, F). Expression levels were normalized to 18S. Data are mean ± SD, * p<0.05 vs. same genotype untreated; # p<0.05 vs lean same condition.
High concentrations of these intermediates can impair the switch to carbohydrate oxidation, a marker of "metabolic flexibility". To study whether BAS affected fatty acid metabolism, long-chain acylcarnitine contents in liver and skeletal muscle were measured. In db/db mice, both skeletal and liver long-chain acylcarnitines were strongly increased compared to lean mice (Figure 3). BAS treatment had no effect on hepatic long-chain acylcarnitine content in db/db mice (Figure 3A). In contrast, in skeletal muscle of db/db mice a strong reduction of long-chain acylcarnitine content was observed which almost reached the level of untreated lean mice (Figure 3B). Specifically saturated long-chain acylcarnitine species of palmitic (C16:0) and stearic acid (C18:0) were affected (Figure 3C). Apparently, increased metabolic clearance of glucose by peripheral tissue is paralleled by changes in long-chain acylcarnitine content of muscle indicative of increased efficiency of mitochondrial fatty acid oxidation.

Figure 3. Effect of BAS on hepatic long-chain acylcarnitine content (sum of C16:0, C16:1, C18:0, C18:1 and C18:2) (A), skeletal muscle long-chain acylcarnitine content (B), skeletal muscle saturated long-chain acylcarnitine content (C) and plasma acylcarnitine concentration (D) in lean mice (lean, n=5), lean mice supplemented with BAS (lean BAS, n=4), db/db mice (db/db, n=5) and db/db mice supplemented with BAS (db/db BAS, n=5). Data are represented as mean ± SD, * p<0.05 vs. same genotype untreated; # p<0.05 vs lean same condition.
DISCUSSION

The leptin receptor deficient \textit{db/db} mouse is a widely utilized mouse model of type 2 diabetes as it displays several of the features of human type 2 diabetes at 12 weeks of age \textsuperscript{39}. However, no characterization describing disturbances of \textit{in vivo} hepatic glucose metabolism in this mouse model is available. Here, we first show that leptin receptor deficiency results in a variety of alterations of \textit{in vivo} hepatic glucose metabolism, the most prominent perturbations being the massively increased glucokinase flux and glucose cycling in \textit{db/db} mice. Secondly, we tested whether the bile salt sequestrant colesvelam HCl (BAS) induces its reported blood glucose-lowering actions \textsuperscript{23,24} by specific alterations of \textit{in vivo} hepatic glucose metabolism. Our results, however, demonstrate that BAS treatment increased metabolic clearance of glucose by peripheral tissue without affecting hepatic glucose production. Unexpectedly, we observed a three-fold induction of hepatic \textit{Fgf21} gene expression and a decrease in skeletal muscle long-chain acylcarnitine content in treated \textit{db/db} mice. We speculate this lowering in skeletal muscle long-chain acylcarnitines to be indicative of an improved skeletal muscle insulin sensitivity and glucose uptake. Increased hepatic \textit{Fgf21} gene expression might provide a novel role for FGF21 as modulator of peripheral glucose metabolism upon BAS.

A major and novel observation of this study was the characterization of the disturbances in \textit{in vivo} hepatic glucose metabolism in \textit{db/db} mice. Most strikingly, glucokinase flux was drastically increased in \textit{db/db} mice compared to lean mice. Irrespective the dramatic increase of the HOMA index in \textit{db/db} compared to lean mice, the metabolic clearance of glucose by the liver was essentially not affected. Moreover, a similar ineffectiveness of insulin to modulate hepatic glucose metabolism was apparent from the data of \textit{de novo} synthesis of glucose-6-phosphate: biosynthesis of glucose-6-phosphate was only slightly decreased (~20%) in \textit{db/db} compared to lean mice. Moreover, fluxes through glucokinase and \textit{de novo} glucose-6-phosphate synthesis were insensitive towards changes in insulin concentration.

The metabolic clearance rate of glucose by peripheral organs was ~3-fold lower in \textit{db/db} mice at blood glucose concentrations more than 3 times higher and plasma insulin concentrations ~7.5 times higher than those of lean mice. It is of interest to compare the hepatic clearance of glucose by glucokinase with values obtained for peripheral glucose clearance in \textit{db/db} mice. In \textit{db/db} mice, peripheral clearance of glucose was strongly reduced, whereas hepatic clearance of glucose by glucokinase was hardly affected compared to lean mice. Furthermore, the rate of gluconeogenesis was hardly affected by the prevailing high glucose and insulin concentrations in \textit{db/db} mice. Similar observations were made previously by us in \textit{ob/ob} mice \textsuperscript{25}. It clearly indicates that the increase in blood glucose concentration in both \textit{db/db} and \textit{ob/ob} mice is driven by an impaired uptake and metabolism of glucose in peripheral organs rather than by increased hepatic glucose production.
Treatment with BAS has been shown to reduce plasma glucose levels in Type 2 diabetic humans \textsuperscript{40,41} and rodents \textsuperscript{23,24}. We tested whether the glucose-lowering actions of BAS in \textit{db/db} mice were due to improvement of disturbed hepatic glucose metabolism. Plasma glucose concentration and \textit{de novo} glucose-6-phosphate synthesis decreased upon BAS treatment in \textit{db/db} mice. Furthermore, insulin concentration tended to decrease, albeit not significantly, when compared to untreated \textit{db/db} mice. The glucokinase flux as well as the glucose cycling rate, however, remained invariantly high. Thus, changes in liver glucose metabolism do not mediate the glucose-lowering effect of BAS.

Yet, we found that BAS improved peripheral glucose clearance in \textit{db/db} mice. Interestingly, long-chain acylcarnitine content in \textit{db/db} skeletal muscle, specifically long-chain acylcarnitines of palmitic and stearic acid, decreased upon BAS treatment. Acylcarnitines are by-products of mitochondrial fatty acid oxidation and are formed upon acyl transfer from acyl-CoA to carnitine. Composition and content of acylcarnitines can reflect both high and low rates of mitochondrial fatty acid oxidation. In inborn errors of mitochondrial fatty acid oxidation, in which fatty acid oxidation is impaired, acylcarnitines typically accumulate in tissues and plasma. Additionally, it has been shown that excessive fatty acid oxidation in diabetic mice results in inefficient oxidation. Increased content of long-chain acylcarnitines in skeletal muscle of \textit{db/db} mice compared to lean mice could therefore be indicative of impaired oxidation of long-chain fatty acids in skeletal muscle mitochondria \textsuperscript{38}. This results in metabolic inflexibility, \textit{i.e.}, the switch to glucose oxidation cannot be made and insulin is unable to stimulate glucose oxidation \textsuperscript{42}. BAS treatment resulted in a clear-cut decrease of long-chain acylcarnitine content of skeletal muscle, nearly to the level that was observed in lean mice. This is suggestive of a more efficient mitochondrial fatty acid oxidation. Concomitantly, a higher metabolic clearance rate of glucose was observed, indicative of an increased ability of the mitochondria to switch to carbohydrate oxidation.

The question arises how BAS treatment, which interrupts the enterohepatic circulation of bile salts, can exert its beneficial effects in peripheral tissues. We observed that BAS induced a 2-fold increase in hepatic \textit{Fgf21} gene expression in lean and 3-fold increase in \textit{db/db} mice. Overexpression of \textit{Fgf21} in livers of \textit{db/db} or \textit{ob/ob} mice or administration of recombinant \textit{Fgf21} to \textit{db/db} or \textit{ob/ob} mice or to diabetic Zucker rats have been shown to have beneficial effects on insulin sensitivity and glucose clearance \textsuperscript{35,43,44}. In this respect, \textit{Fgf21} might provide a link to communicate changes in liver metabolism to peripheral tissues to allow for metabolic adaptation. How \textit{Fgf21} exactly brings about these changes remains, however, elusive at the moment.

In conclusion, this study is the first to characterize hepatic glucose fluxes in \textit{db/db} mice. Glucokinase flux and rates of hepatic glucose output were massively increased in these mice compared to lean mice. Additionally, \textit{db/db} mice had lower
metabolic clearance rates of glucose by peripheral tissues compared to lean mice. Decreased plasma glucose levels upon BAS treatment were mainly attributable to increased metabolic clearance of glucose by peripheral tissues: hepatic glucose output remained unaffected. Interestingly, skeletal muscle long-chain acylcarnitine content was decreased in BAS-treated db/db mice. Increased hepatic Fgf21 gene expression levels might play a crucial role in modulating peripheral glucose handling upon BAS-treatment. This hypothesis, however, requires further investigation.
Bile salt sequestration and hepatic glucose metabolism

REFERENCE LIST


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