Loss of intestinal GATA4 prevents diet-induced obesity and promotes insulin sensitivity in mice

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The adult duodenal-ileal axis is maintained through spatiotemporal gradients of transcription factors, which persist throughout the adult lifespan. These include Gata4, Gata5, Gata6, Hnf1α, Cdx1, Cdx2, Pdx1, and the CCAAT displacement protein (CDP)/Cux (17, 42). Several of these factors regulate expression of genes leading to physiological diversity of the three parts of the small intestine, the duodenum, jejunum, and ileum. However, their study in adulthood has been impeded due to embryonic lethal phenotypes of their respective knockouts. So far, six members have been assigned to the GATA family of zinc finger domain transcription factors that bind to the conserved promoter motif “WGATAR,” identified as the GATA consensus site (31). GATA4 was identified as a gene encoding a zinc finger domain transcription factor involved in development and organogenesis. However, its mRNA and protein are also expressed in adult tissues, including heart, adrenal glands, and small intestine (11, 44).

Embryonic lethality in GATA4 knockout mice is known to arise mainly from defective heart tube formation and extraembryonic endoderm differentiation (33, 51). In the small intestine, GATA4 mRNA expression is restricted to the duodenum and jejunum, and the expression is undetectable in the terminal ileum (17). This expression pattern is conserved across several vertebrate species including humans (10). GATA4 is expressed throughout the length of the crypt-villus axis in epithelial cells (9, 10), where it regulates gene expression. It controls the expression of several genes that are important for carbohydrate, lipid, and steroid homeostasis and insulin secretion, including sucrase isomaltase, lactase (Lct), fatty acid-binding protein-1 (Fabp1), and glucose-dependent insulinotrophic polypeptide (Gip) (10, 48). Some of these genes are themselves expressed in distinct anteroposterior gradients with similar expression patterns to GATA4 (38, 41).

Even though GATA4 has been implicated in regulating physiologically relevant intestinal genes, few studies have described its role in metabolic diseases. Bosse et al. (10) generated viable intestine-specific inducible GATA4 knockout mice. They showed that, in the jejunum, expression of jejunal markers such as Fabp1 and Lct was downregulated, whereas ileal markers such as solute carrier family 10, member 2 (Slc10a2 also known as Asbt) and Fabp6 were upregulated, suggesting a jejunal-ileal transition (10). Using a villin-Cre approach, Battle et al. (5) generated intestine-specific GATA4 knockout (GATA4iKO) mice. The authors reported that 47% of ileal genes were ectopically expressed in jejunum of GATA4iKO mice and found a decrease (~20%) in jejunal lipid uptake and absorption. The observed jejunoileal transition closely resembles the bariatric surgical procedure of ileal interposition. Kohli et al. (26) recently reported that, in rats that had undergone ileal interposition surgery, the cycling of bile acids was increased, and these mice were protected from obesity associated comorbidities. GATA4iKO mice showed a decreased expression of genes involved in lipid uptake and transport, such as Cd36 antigen (Cd36) and scavenger receptor class B type 1 (Scarb1) (5). Both Cd36 and Scarb1 have been implicated in controlling triglyceride (TG) absorption in the small intestine (14, 29). Compared with controls, GATA4iKO mice showed decreased body weights during suckling but had similar body weights after weaning (5). Whether these effects

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on body weight persist throughout the adult lifespan has not been addressed so far. Therefore, we hypothesized that the lack of GATA4iKO might compromise dietary fat absorption due to changes in expression of epithelial genes implicated in absorption of dietary fat. Given the role of distal intestinal L-cells in producing glucagon-like peptide-1 (GLP-1) and regulating glucose homeostasis, we hypothesized that the observed posteriorization of the intestine might positively impact insulin sensitivity. In the present study, we challenged adult GATA4iKO and control littermates with a Western-type diet (WTD) for 20 wk. Our findings revealed that GATA4iKO mice had decreased plasma TG levels and were resistant to diet-induced obesity. Due to increased levels of GLP-1, GATA4iKO mice exhibited preserved insulin sensitivity even upon long-term WTD feeding.

**MATERIALS AND METHODS**

**Animals and diets.** Generation of viable Gata4loxPloxP (Gata4tm1Sad), Gata4null (Gata4tm1Eno), and VitCre [Tg(Vil-cre)997Gun] mice has been described previously (30, 33, 51). Homozygous GATA4loxPloxP mice were bred with the heterozygous GATA4nullVilCre mice to generate GATA4loxPnullVilCre intestine-specific knockout (GATA4iKO) and GATA4loxPloxP+VilCre controls. All experiments were performed on male mice. Mice had free access to food and water under a 12:12-h light-dark cycle in a temperature-controlled environment. Individually housed knockout and control littermates (n = 10/group) were switched to a Western type diet (WTD) or continued on a normal chow diet (11.9% caloric intake from fat, Sniff, Soest, Germany) at 6 wk of age. WTD contained 21% (wt/wt) fat content and 10% protein, and 43% from carbohydrate; Sniff). Mice were weighed weekly for a period of 20 wk.

**Biochemical analysis.** Blood was collected through the tail vein, and plasma was used for further determinations. Triglycerides (TG), total cholesterol (TC), and free fatty acids (FFA) were assayed in fasted plasma with enzymatic kits according to the manufacturers’ protocols (DiaSys, Holzheim, Germany; Wako Chemicals, Neuss, Germany). Plasma lipoproteins were separated using the Pharmacia protocols (DiaSys, Holzheim, Germany; Wako Chemicals, Neuss, Germany) at 65°C overnight, and feces were dried and pulverized. Radioactivity was measured by liquid scintillation counting and expressed as percentage of dose per gram of organ or feces. Additionally, the feces of both control diet- (n = 4/group) and WTD-fed mice (n = 7/group) collected daily over a 4-day period were dried, pulverized, and subjected to Folch extraction (19), with slight modifications. Briefly, tissues were weighed and homogenized, and lipids were extracted in chloroform-methanol (2:1) in a volume 20 times the weight of the sample. Lipid extracts were then solubilized in freshly prepared 0.2% Triton X-100 in chloroform, dried under nitrogen, and resuspended in water. FFA and TC were measured enzymatically.

**Fecal fat balance.** Seventy-two-hour fecal fat balance was measured as described previously (52), with slight modifications. WTD and fecal pellets from mice (n = 4/group) collected over 72 h were weighed, freeze-dried, and homogenized. Samples were hydrolyzed, derivatized, and measured using electron impact-gas chromatography–mass spectrometry (EI-GC-MS) for preparation of trimethylsilyl ether derivatives, preweighed lyophilized material was transferred to Pyrex tubes and extracted according to the Folch procedure in the presence of 100 µg of stearic-d35-acid (C18:0-d35, Isotec) as an internal standard to account for extraction efficiency. For quantification of FFA in mouse feces, 10 µl of 1,000-µl lipid extract was converted to the corresponding trimethylsilyl (Pierce, Rockford IL) ether derivatives in 100 µl of MSTFA solution (ABCr, Karlsruhe, Germany), [MSTFA, containing 1% TMCS, in pyridine (2:1, vol/vol) for 60 min at 25°C, and stored at −20°C until GC-MS analysis]. To quantify total amounts of fatty acids in the diet, esterified fatty acids were hydrolyzed in 0.5 M NaOH at 90°C for 60 min, neutralized with 0.5 M HCl, extracted according to the Folch procedure, and derivatized as mentioned above. For EI-GC-MS, a Thermo Scientific Trace GC coupled to a DSQII mass spectrometer was used. The GC was fitted with an SGE BPX5 capillary column (15 m, ID 0.25 mm, 0.25 µm methyl silicone film coating). The injector was operated in the splitless mode at 180°C. Helium was used as carrier gas at a flow rate of 1 ml/min. Initial column temperature was 80°C for 2 min, followed by an increase of 30°C/min to 170°C, an isothermal hold of 2 min, a second increase at 30°C/min to 230°C, an isothermal hold for 3 min, and increased by 20°C/min to 300°C with a hold for 3 min. The transfer line was kept at 310°C, and the ion source was 200°C. Electron impact spectra were recorded with electron energy of 70 eV and an emission current of 100 µA. All samples were monitored either in full scan mode or using selected ion recording (SIR). SIR was performed at m/z = 313 (C16:0; RT 8.97 min), m/z = 341 (C18:0; RT 10.01 min), m/z = 376 (C18:0:35; RT 9.83 min), m/z = 339 (C18:1;
retention time 9.84 min), and m/z = 337 (C18:2; RT 9.82 min). Quantification was performed by peak area comparison along with a standard calibration curve consisting of unlabeled primary fatty acid standards (Sigma, St. Louis, MO) mixed with the stable isotope-labeled standard. All solvents and reagents of analytical grade were from Sigma and Roth (Vienna, Austria). Absorption of four major dietary fatty acids (palmitate, stearate, oleate, linoleate) was determined as the difference between the amount of individual fatty acids ingested and excreted into feces over 72 h. The measurement was expressed as % total fat absorption = fat intake (g/day) − fecal fat output (g/day) × 100.

To measure fat intake (g/day), total fat absorption was quantified as the difference in fecal loss of the four major fatty acids from the total amount ingested.

RNA isolation and quantitative real-time PCR. Total RNA from tissues of mice fed WTD (n = 5/group) and/or Chow (n = 5/group) was extracted using TRIzol reagent according to the manufacturer’s protocol (Invitrogen, Carlsbad, CA). Two micrograms of total RNA was reverse transcribed with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA). Quantitative real-time PCR was performed on a Roche LightCycler 480 (Roche Diagnostics, Palo Alto, CA) using the QuantiFastTM SYBR Green PCR Kit (Qiagen, Valencia, CA). Samples were run in triplicates for each experiment with cyclophilin A as an internal control. For analyzing the expression profiles and associated statistical parameters, the public domain program Relative Expression Software Tool - REST 2008 (http://www.gene-quantification.com/download.html) was used (39). Primer sequences are available upon request.

In vivo MR imaging for body fat. Mice were anesthetized with isoflurane according to the guidelines of the local steering committee. MR images were acquired at a 3T MRI system (Siemens Tim-Trio, Erlangen, Germany) with an eight-channel multipurpose coil (Nora MRI products, Hoechenberg, Germany) to maximize signal-to-noise ratio. For fat measurements, two T1 weighted 2D Turbo Spin Echo (TSE) sequences, one with spectral selective fat suppression and one without, were carried out. The following imaging parameters were used: TR, 800 ms; TE, 37 ms; TF, 5 ms. A field of view 90 × 45 mm with a 384 × 192 matrix provided an in-plane resolution of 230 × 230 μm with a slice thickness of 1.2 mm. Twelve excitations were averaged. For postprocessing for the fat quantification, the image without fat saturation was divided by its fat-saturated counterpart in order to cancel signal variations coming from coil non-uniformities. The ratio between the images was then interpreted as (fat water)/(fat water) to get the fat volume. All experiments had been approved by the Committee for Genetic Engineering and Animal Experimentation at the Austrian Federal Ministry of Science and Research (Vienna, Austria) and by the Ethics Committee for Animal Experiments of the Medical University of Graz.

Oral glucose tolerance test. An oral glucose tolerance test was performed as described previously (4, 18). Briefly, blood was drawn through the tail vein from controls and GATA4iKO mice fasted for 6 h before gavage (0 min) and 15, 30, 60, and 120 min after an oral gavage of 2 g of n-glucose/kg body wt. Blood glucose levels were measured immediately using Glucometer Accu-check-active (Roche diagnostics, Palo Alto, CA).

Gastric emptying. Liquid gastric emptying was assessed in both genotypes as previously described (6, 32, 49). Briefly, mice fasted overnight were challenged with oral gavage of 200 μl (0.6 g/l) of phenol red in a glucose solution (6.5 mg/kg). Mice were killed by cervical dislocation after half an hour, and stomachs were collected in 0.1 N NaOH and homogenized. The homogenate was centrifuged at 8,500 rpm for 15 min. The supernatant (500 μl) was treated with 50 μl of TCA (20%) and centrifuged as above. Finally, supernatants were treated with 0.5 N NaOH (1:1 vol/vol) for maximum color development. Phenol red concentration in the mixture was then measured at 560 nM. Percent gastric emptying was calculated as %gastric emptying = 1 − (amount of phenol red in the stomach + average amount recovered from control stomach) × 100.

Statistical analysis. Plasma biochemical parameters in nonfasted and fasted states were compared independently. For body weights and plasma parameters, controls and GATA4iKO mice on Chow and WTD were compared using two-parameter analysis of variance (two-way ANOVA) followed by a Bonferroni posttest. For oral glucose tolerance tests, differences between control and GATA4iKO groups were compared using the two-way ANOVA with repeated measures followed by a Bonferroni posttest. For absorption studies, intergroup differences were calculated using two-tailed, unpaired Student’s t-test; P values < 0.05 were considered significant.

RESULTS

GATA4iKO mice exhibit decreased plasma lipid levels. GATA4iKO mice fed a Chow diet showed a slight but not significant decrease in plasma TG concentration in both fasted and nonfasted states (P = 0.0546). On WTD, GATA4iKO mice showed 23 and 60% decreases in plasma TG concentrations in the fasted and nonfasted states, respectively, compared with controls (Table 1). This effect was mainly due to decreased VLDL/Chylomicron-TG levels in GATA4iKO mice (Fig. 1A). Levels of plasma apoB-100 and apoB-48 were also lower in plasma of WTD-fed GATA4iKO compared with control mice (Fig. 1A, bottom inset). The food consumption in

| Table 1. Plasma levels of TG, TC, FFA, and glucose of Chow diet (n = 5) and WTD (n = 8) fed control and GATA4iKO mice in fasted and nonfasted states |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | Chow            | GATA4iKO        | WTD             |
|                                | Control         | GATA4iKO        | Control         | GATA4iKO        |
| **Fasted**                     |                 |                 |                 |
| TG, mg/dl                      | 55.78 ± 11.47   | 45.12 ± 8.01    | 110.06 ± 6.51   | 85.27 ± 12.60***|
| TC, mg/dl                      | 149.48 ± 13.60  | 75.25 ± 37.41*  | 232.76 ± 35.55  | 132.30 ± 24.52***|
| FFA, mmol/ml                   | 1.67 ± 0.40     | 1.38 ± 0.31     | 1.33 ± 0.05     | 1.19 ± 0.06     |
| Glucose, mg/dl                 | 97.5 ± 7.59     | 88.25 ± 8.66    | 114.5 ± 25.09   | 114.75 ± 12.58  |
| **Nonfasted**                  |                 |                 |                 |
| TG, mg/dl                      | 63.36 ± 11.86   | 41.68 ± 8.27    | 209.69 ± 41.23  | 84.36 ± 11.49***|
| TC, mg/dl                      | 178.00 ± 39.85  | 95.07 ± 22.28** | 391.75 ± 59.91  | 119.93 ± 28.53***|
| FFA, mmol/ml                   | 0.81 ± 0.18     | 0.64 ± 0.11*    | 0.83 ± 0.09     | 0.51 ± 0.12***  |
| Glucose, mg/dl                 | 98.43 ± 19.89   | 108.56 ± 22.34  | 158.45 ± 21.78  | 126.65 ± 22.24* |

Values are means ± SD. GATA4iKO, intestinal GATA4 knockout; WTD, Western-type diet; TG, triglycerides, TC, total cholesterol; FFA, free fatty acids. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control.
both groups was comparable (Fig. 1B). Plasma TC concentrations were significantly decreased on the chow diet in both fasted (49%) and nonfasted (57%) states (Table 1). On WTD, GATA4iKO mice showed 43 and 70% less plasma TC in fasted and nonfasted states, respectively, compared with controls. Nonfasted GATA4iKO mice had significantly lower plasma FFA concentrations in both chow diet- and WTD-fed mice compared with control mice (Table 1). Plasma glucose levels in nonfasted mice were significantly reduced only in GATA4iKO mice fed the WTD (Table 1). In the fasted state, plasma FFA and glucose levels were comparable in GATA4iKO and control mice fed either the chow diet or WTD (Table 1).

GATA4iKO mice exhibit impeded TG absorption. To investigate the underlying cause of reduced TG levels in plasma of GATA4iKO mice, we examined intestinal absorption and uptake of dietary fat. After oral administration of corn oil containing [3H]triolein, we observed delayed accumulation of lipids in plasma of GATA4iKO mice (Fig. 2, A and B). GATA4iKO mice also showed decreased amounts of [3H]counts in duodenum and jejunum but not in ileum, suggesting a compromised enterocytic lipid uptake into the proximal small intestine compared with controls (Fig. 2, A and B). The excretion of fecal [3H]counts in GATA4iKO mice fed the WTD (Table 1). In the fasted state, plasma FFA and glucose levels were comparable in GATA4iKO and control mice fed either the chow diet or WTD (Table 1).

Intestinal expression of key absorptive genes is misregulated in GATA4iKO mice. To study the impact of GATA4 deficiency on mRNA levels of key duodenal and jejunal transporters involved in lipid uptake and absorption, we performed quantitative real-time PCR analysis from small intestines obtained from GATA4iKO and control mice fed both chow diet and WTD. We found downregulation of Cd36, fatty acid transport protein-2 (Fatp2), and Fabp1 in duodenum and jejunum of GATA4iKO compared with control mice on both diets (Fig. 4, A and B). Expression levels of Scarb1 in the duodenum and jejunum of GATA4iKO mice were decreased on chow diet but comparable to control mice when fed a WTD. Although plasma TC levels of GATA4iKO mice were significantly reduced on both diets (Table 1), mRNA expression of the cholesterol transporter Niemann-Pick C1-like 1 (Npc1l1) in the duodenum and jejunum was comparable to that of controls fed either diet (Fig. 4, B and C). In accord with previous studies (47, 53), we also detected a significant increase in the expression levels of ATP-binding cassette transporter (ABC) G5 (Abcg5) and G8 (Abcg8) upon WTD in both genotypes (Fig. 4, B).
B and D). The mRNA expression level of hydroxymethylglutaryl (Hmg)-CoA reductase was upregulated 2.8-fold in the liver of GATA4iKO mice (Fig. 4F). To control for the lack of intestinal GATA4, we also measured the expression levels of small intestinal regional markers Fabp1 (proximal) and sodium/bile acid cotransporter (Asbt) (distal). Expression of Asbt was induced in the jejunum of GATA4iKO mice and was comparable to that of control ileum (Fig. 4E). The expression of jejunal Fabp-1 of GATA4iKO mice was decreased compared with control jejunum (Fig. 4E).

GATA4iKO mice are resistant to diet-induced obesity. Next, we examined the impact of intestinal GATA4 deficiency on body and adipose tissue mass in WTD-fed mice over a 20-wk period. We found that GATA4iKO mice weighed less from the beginning of the feeding study. This difference was even more pronounced during WTD feeding (Fig. 5A). The decrease in weight gain was attributed to lower adipose tissue mass in GATA4iKO mice (Table 2). Liver, heart, spleen, and brain showed comparable weights in both groups. To monitor body composition with regard to adipose tissue mass, GATA4iKO
mice and their control littermates were subjected to T1-weighted magnetic resonance imaging. Figure 5B shows representative coronal sections of epidermal, retroperitoneal, gonadal, and perirenal fat pads of control and GATA4iKO mice in the 18th wk of WTD feeding. GATA4iKO mice exhibited a 2.7-fold decrease in the fat volume compared with control (8.0 vs. 21.7 ml). In addition, adipocytes from the gonadal fat pads of GATA4iKO mice fed WTD were of a smaller size (Fig. 5C) and showed an intact regulation of two important adipokines, leptin and adiponectin. Fasting plasma leptin concentrations of WTD-fed GATA4iKO mice were as low as chow diet-fed mice (Fig. 5D). Levels of adiponectin were higher in GATA4iKO mice fed either diet compared with WTD-fed control mice (Fig. 5E).

**GATA4iKO mice are glucose tolerant and insulin sensitive upon WTD feeding.** To determine whether resistance to obesity was accompanied by increased insulin sensitivity, we measured plasma insulin levels and performed oral glucose tolerance test during the 18th wk of WTD feeding. GATA4iKO mice remained euinsulinemic in both nonfasted and fasted states (Fig. 6A) and showed increased glucose tolerance compared with controls (Fig. 6B). In chow diet-fed mice, insulin levels in both groups were comparable in fasted and nonfasted conditions (Fig. 6C). However, GATA4iKO mice showed improved glucose tolerance even upon chow diet feeding (Fig. 6D). Next, we assayed the release of GLP-1 in mice under both dietary conditions. GATA4iKO mice fed either diet had increased release of GLP-1 (Fig. 6, E and F). Furthermore, the release of Gip (a proximal intestinal peptide) in response to fat load was attenuated to 33% (30-min time point) in GATA4iKO mice (Fig. 6G), indicating that enteroendocrine factors are also subject to posteriorization, which might influence the malabsorption phenotype. An assay for glucose-stimulated insulin release upon fat load was performed. Although overall insulin levels were low due to overnight fasting, a 25% increase in the peak levels of plasma insulin in GATA4iKO mice was observed (Fig. 6H). Finally, we investigated liquid gastric emptying in GATA4iKO mice, which was reduced by 24% in GATA4iKO mice compared with controls (Fig. 6I).

**DISCUSSION**

Dietary lipids are predominantly absorbed by the proximal small intestine (duodenum and jejunum), whereas the terminal part (ileum) is mainly involved in bile acid reabsorption (35, 40). Here, we report that the posteriorization of the small intestine caused by the lack of intestinal GATA4 has a beneficial effect of lowering plasma TG by reducing intestinal absorption and promoting resistance to diet-induced obesity. Over a 72-h period, GATA4iKO mice excreted greater amounts of dietary fatty acids, implying that the underlying cause of obesity resistance is their inability to absorb dietary lipids. The lack of GATA4 had profound effects on the expression of several genes involved in intestinal lipid uptake and transport. This included the downregulation of Cd36, Fatp2, Fabp1, and Scarb1, genes involved in fatty acid and cholesterol uptake and/or transport (16, 21, 29, 34). The expression of all these transporters with the exception of Scarb1 was downregulated independently of whether mice were fed WTD or chow diet. However, Scarb1 expression was downregulated only in GATA4iKO mice fed chow diet. WTD is known to increase the levels of intestinal Scarb1 mRNA and protein in mice, and this contributes to the regulation of transintestinal cholesterol excretion (TICE) (47). Our finding implies that alternative pathways operating independently of GATA4 might regulate intestinal Scarb1 expression on WTD. Apart from the reduced plasma TG concentrations, GATA4iKO mice also have reduced plasma TC levels. However, the expression of the well-established cholesterol importer Npc1l1 was unchanged on both dietary regimens and genotypes. Based on our observations, there are several possible explanations for the decrease in plasma TC levels in GATA4iKO mice. 1) Downregulation of Cd36 might be extremely relevant, since a number of studies have proposed that Cd36 is a putative cholesterol importer (29, 40, 46). It was recently shown that Cd36 regulates cholesterol uptake into the proximal but not distal intestine (34). The ileal expansion observed in GATA4iKO mice lends further support for Cd36 as an important component for cholesterol uptake and or transport in the proximal intestine. 2) Our observation of increased fecal excretion of cholesterol in...
GATA4iKO mice fed WTD leads to the possibility of enhanced TICE as a probable cause of reduced plasma TC levels. Since GATA4 mice display defective patterning in the anteroposterior intestinal axis, it would be interesting to investigate the possible effects on TICE that have greater involvement of the proximal intestine.

Given the upregulated Hmg-CoA reductase mRNA in the livers of GATA4iKO mice, a lower cholesterol synthesis would be improbable to account for the drastic reduction in the plasma TC concentration. mRNA levels were normalized to cyclophilin A as a reference gene and expressed relative to gene expression in control mice on chow diet. Values represent means (n = 5/group) ± SE of 3 independent experiments for each gene on both dietary regimens. *P < 0.05, ***P < 0.001 vs. controls fed chow diet.

However, coadministration of [3H]triolein and [14C]oleate revealed that luminal lipolytic activities were comparable between GATA4iKO and control mice. Therefore, differences in activity of luminal lipases or formation of micelles do not account for the observed reduction in lipid absorption in GATA4iKO mice. In a recent study, GATA4iKO mice were shown to have a normal bile acid pool size, except for enhanced retention of tauro-β-muricholic acid (7). These findings support our observation that changes in luminal lipolysis do not account for the reduced lipid absorption seen in GATA4iKO mice. Reduced TG absorption of GATA4iKO mice effectively translated into a long-term obesity resistance.

Energy balance and food intake is regulated through a complex interaction of hormones produced in the periphery and central nervous system. Findings of the present study demonstrate that intestinal
GATA4 deficiency results in a shift of hormone production toward a metabolic situation efficiently counteracting obesity and hyperglycemia, as well as leptin and insulin resistance, major pathophysiological components of the metabolic syndrome. Plasma leptin is an indicator of body fat, and its circulating levels are suppressed upon fasting. Failure to respond to fasting by a decrease of plasma leptin levels is associated with obesity and is termed as peripheral leptin resistance (15, 22, 24). WTD-fed GATA4iKO mice failed to develop peripheral leptin resistance and exhibited lower levels of fasting plasma leptin, reflecting an intact physiological regulation of leptin in these mice.

A second adipose-derived hormone significantly impacting on energy balance is adiponectin. It exerts an anti-diabetic and anti-atherogenic effect and plays an important role in the regulation of lipid and glucose metabolism. Adiponectin levels are inversely proportional to body fat (2, 28). In line with lower fat volume and smaller adipocyte size, plasma adiponectin levels were higher in GATA4iKO mice compared with controls fed WTD. With regards to intestinal hormone production relevant to energy homeostasis, we observed increased GLP-1 and decreased GIP production. As described previously, a mechanism leading to a partial jejunoileal homeosis is oper-

Table 2. Organ weights of WTD-fed control and GATA4iKO mice

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control</th>
<th>GATA4iKO</th>
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<tbody>
<tr>
<td>Liver</td>
<td>4.87 ± 0.72</td>
<td>5.68 ± 1.28</td>
</tr>
<tr>
<td>WAT</td>
<td>4.19 ± 0.46</td>
<td>1.40 ± 0.42***</td>
</tr>
<tr>
<td>BAT</td>
<td>0.85 ± 0.20</td>
<td>0.57 ± 0.11*</td>
</tr>
<tr>
<td>Heart</td>
<td>0.66 ± 0.11</td>
<td>0.55 ± 0.08</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.32 ± 0.07</td>
<td>0.25 ± 0.09</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.55 ± 0.12</td>
<td>0.60 ± 0.16</td>
</tr>
<tr>
<td>Brain</td>
<td>1.37 ± 0.27</td>
<td>1.15 ± 0.12</td>
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<tr>
<td>Mean body weight</td>
<td>40.45 ± 3.28</td>
<td>32.7 ± 4.36</td>
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Values are means ± SD in grams. WAT, gonadal white adipose tissue; BAT, intrascapular brown adipose tissue. Results are expressed relative to total body weight of 9 mice per group. *P < 0.05, ***P < 0.001 vs. control.
This phenotype closely resembles ileal interposition, a form of bariatric surgery. In rats subjected to ileal interposition, obesity-related morbidities decrease (26). The interposed ileum adapts to surgical transposition but retains its ability to absorb bile acids and to release GLP-1, an ileal enteroendocrine peptide (12, 26). GLP-1 release is potentiated by the presence of dietary lipids (45). GLP-1 mainly enhances peripheral insulin sensitivity and stimulates glucose-dependent insulin release from the pancreatic \( \beta \)-cells (23, 37). We thus hypothesized that the ileal expansion in GATA4iKO mice would cause an increase in GLP-1. Concurrently, in GATA4iKO mice, GLP-1 release was elevated, which was reflected in a preserved glucose tolerance on both diets. Increased GLP-1 release in GATA4iKO mice was coupled with higher glucose-stimulated insulin release upon oral fat load. Insulin itself is known to inhibit the synthesis of TG-rich lipoproteins such as chylomicrons. Thus, the increased insulin release might be seen as an additional mechanism for decreased lipid absorption. Other physiological effects of GLP-1 include inhibition of gastric emptying. Consequently, GATA4iKO mice showed a slower liquid gastric emptying, which could explain, at least in part, the delayed appearance of triolein in plasma. Following ileal interposition surgery, the levels of Gip, a proximal intestinal enteroendocrine peptide, were shown to be decreased (12). Gip potentiates chylomicron production (43). Accordingly, we found that lack of intestinal GATA4 also led to decreased levels of circulating Gip. Gip release upon oral fat load was also lower in GATA4iKO mice.

Fig. 6. Glucose tolerance and insulin sensitivity in GATA4iKO mice after WTD feeding. Plasma insulin concentrations were determined in nonfasted and fasted states in control and GATA4iKO mice in the 18th wk of WTD feeding (\( n = 5/\)group) (A) and 11th wk of chow diet feeding (\( n = 4/\)group) (C). Glucose tolerance tests were performed in control and GATA4iKO mice fed either WTD (B), or chow diet (D) (\( n = 4/\)group). Glucagon-like peptide-1 (GLP-1) release in control and GATA4iKO mice fed WTD (E), or chow diet (F) (\( n = 4/\)group). Glucose-dependent insulinotropic polypeptide (Gip; G) and insulin (H) release upon oral fat load in control and GATA4iKO mice fed chow diet (\( n = 4/\)group). I: Percent liquid gastric emptying in control and GATA4iKO mice fed chow diet (\( n = 4/\)group). Values represent means ± SE. *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \) vs. controls.
These findings point to a possibility of decreased Gip release being an additional mechanism for the reduced TG absorption in GATA4iKO mice. In a canine model, Gip was shown to increase peripheral fat deposition by enhanced chylomicron clearance (50). In another study, it was found that Gip activates lipoprotein lipase on adipocytes and thus enhances lipid deposition (25). Therefore, lower Gip levels imply reduced adiposity as encountered in GATA4iKO mice. On the basis of our data, we conclude that intestinal lack of GATA4 could alleviate at least two components of the metabolic syndrome: diet-induced obesity and type 2 diabetes mellitus. Given the cholesterol-lowering effects of intestinal knockout of GATA4, it would be interesting to investigate its ability to reduce atherosclerosis susceptibility. Although direct clinical data are limited, an increasing number of studies present ileal interposition surgery as a novel and effective mode for treatment of type 2 diabetes mellitus (1, 3, 13, 27). In this respect, GATA4iKO mice are an interesting model to recapitulate the effects of ileal interpositioning, but they also provide a potential target for drug discovery.

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disclosures

No conflicts of interest are reported by the authors.

REFERENCES


