Intestine-specific Deletion of Acyl CoA:monoacylglycerol Acyltransferase (MGAT) 2 Protects Mice from Diet-induced Obesity and Glucose Intolerance

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Background: Global MGAT2 knockout mice are protected from obesity.

Results: Intestine-specific MGAT2 knockout mice showed increased energy expenditure and were protected against excess weight gain and metabolic disorders induced by high-fat feeding.

Conclusion: Intestinal triacylglycerol metabolism is crucial in regulating systemic energy balance.

Significance: Intestinal MGAT2 may be a feasible intervention target for diseases associated with excess caloric intake.

ABSTRACT

The absorption of dietary fat involves the re-esterification of digested triacylglycerol in the enterocytes, a process catalyzed by acyl CoA:monoacylglycerol acyltransferase (MGAT) 2. Mice without a functional gene encoding MGAT2 (Mogat2<sup>-/-</sup>) are protected from diet-induced obesity. Surprisingly, these mice absorb normal amounts of dietary fat but increase their energy expenditure. MGAT2 is expressed in tissues besides intestine, including adipose in both mice and humans. To test the hypothesis that intestinal MGAT2 regulates systemic energy balance, we generated and characterized mice deficient in MGAT2 specifically in the small intestine (Mogat2<sup>IKO</sup>). We found that, like Mogat2<sup>-/-</sup> mice, Mogat2<sup>IKO</sup> mice also showed a delay in fat absorption, a decrease in food intake, and a propensity to use fatty acids as fuel when first exposed to a high-fat diet. Mogat2<sup>IKO</sup> mice increased energy expenditure, though to a lesser degree than Mogat2<sup>-/-</sup> mice, and were protected against diet-induced weight gain and associated comorbidities, including hepatic steatosis, hypercholesterolemia, and glucose intolerance. These findings illustrate that intestinal lipid metabolism plays a crucial role in the regulation of systemic energy balance and may be a feasible intervention target. In addition, they suggest that MGAT activity in extra-intestinal tissues may also modulate energy metabolism.

INTRODUCTION

Dietary fat is absorbed in the intestine, where its digested components trigger hormone release and neural output (1). These intestinal signals can in turn regulate food intake and coordinate metabolism, preparing the rest of the body for efficient use and storage of incoming nutrients (1-3). Acyl CoA:monoacylglycerol acyltransferase (MGAT) in the intestine is thought to be important for the absorption of dietary fat (4,5). The enzyme is involved in the re-synthesis of digested triacylglycerol (TAG), catalyzing the esterification of monoacylglycerol (MAG). Its product diacylglycerol (DAG) serves as substrate for the final step catalyzed by acyl CoA:diacylglycerol acyltransferase (DGAT) (6). This re-synthesis of TAG is crucial for the assembly and secretion of chylomicrons, which deliver absorbed fat to other...
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MGAT2 is one of the three known MGATs that share sequence homology (10-13) and is highly expressed in both mouse and human intestine. In contrast, MGAT1 is not expressed in the intestine, while MGAT3 is expressed in human but not mouse intestine.

Consistent with the concept that intestinal fat metabolism regulates energy balance, MGAT2 enhances systemic metabolic efficiency. Mice with a deletion of the gene encoding the enzyme (Mogat2–/–) are protected from obesity and associated metabolic disorders induced by high-fat feeding (14). Despite the putative role of MGAT2 in fat absorption, Mogat2–/– mice consume and absorb normal amounts of dietary fat. Interestingly, they exhibit an increase in energy expenditure. This difference is exacerbated with high-fat feeding, but the increase in energy expenditure persists even when the mice are fed a fat-free diet (15). Deficiency of MGAT2 protects mice from obesity caused by agouti mutation-induced hyperphagia as well as from excess weight gain induced by a diet rich in refined carbohydrate (15). These findings are consistent with the idea that MGAT2 normally enhances metabolic efficiency and mediates positive energy balance in response to a surplus of dietary calories.

MGAT2 is expressed in tissues besides intestine, including the kidney and adipose in both mice and humans (11,12,14). Recently, we found that re-introducing MGAT2 expression exclusively in the small intestine is not sufficient to restore metabolic efficiency in Mogat2−/− mice completely (16), implicating a role of extra-intestinal MGAT2 in modulating energy balance. To test the hypothesis that intestinal MGAT2 is necessary for maximizing metabolic efficiency, in this study, we examined if inactivating MGAT2 exclusively in the small intestine protects mice from excessive accretion of body fat and associated comorbidities induced by high-fat feeding.

EXPERIMENTAL PROCEDURES

Mice

To generate mice lacking Mogat2 specifically in the small intestine, embryonic stem cells bearing a targeted Mogat2 allele were obtained from the KOMP repository (EPD0093_1_A12, http://www.knockoutmouse.org/martsearch/project/26094), injected into blastocysts, and implanted into pseudopregnant mice. The offspring (C57BL/6N-Mogat2tm1a(KOMP)Wtsi) carry a trapping cassette “SA-geo-pA” flanked by FLP recombinase target sites (FRT) upstream from exon 2 of the Mogat2 allele (Fig. 1A; Mogat2 Targeted). After crossing with a mouse (B6.Cg-Tg(ActFLPe)9205Dym/J) expressing the FLP1 recombinase under the control of the human ACTB promoter (The Jackson Laboratory) to excise the trapping cassette, offspring carrying a “floxed” Mogat2 allele with exon 2 flanked by Cre recombinase target sites (loxP), were produced. Mice possessing the “floxed” Mogat2 allele without the FLP1 gene (Mogat2+/–) in the C57BL/6 genetic background were then produced through intercrossing. Next, Mogat2+/– mice were bred with a mouse expressing the transgene Cre recombinase under the control of the mouse villin 1 promoter (B6.SJL-Tg(Vil-cre)997Gum/J; The Jackson Laboratory), which drives expression in all four types of intestinal epithelia beginning by postcoital day 12.5 (17); through intercrossing, mice with an intestine specific deletion of Mogat2 (Mogat2IKO mice) were produced.

For experiments, Mogat2+/– mice were bred with Mogat2IKO mice to produce Mogat2IKO and Mogat2+/– littermate controls. To generate mice deficient in MGAT2 in all tissues, Mogat2+/– mice were also bred to produce Mogat2+/- and wildtype littermate controls as described (14). Differences in metabolic phenotypes were most pronounced in males; therefore, adult male mice were used for experiments. Female mice were used in a few key experiments as indicated to verify if the effects were sex dependent. Mice were housed at 22°C on a 12 h light, 12 h dark cycle. Weighing of mice and changes of diets and cages were performed between 3 and 6 p.m.. All animal procedures were approved by the University of Wisconsin–Madison Animal Care and Use Committee and were conducted in conformity with the Public Health Service Policy on Humane Care and Use of Laboratory Animals.
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Genotyping

Genotypes of mice were determined by PCR. To determine the presence of the Cre-recombinase transgene, the following four primers were used: Cre forward, 5’-CCCGGCAAAACAGGTAGTTA-3’; Cre reverse, 5’-TGCCAGGATCAGGGTTAAG-3’; Positive control forward, 5’-CCTTAGCCTGTCTAGGAGACGAG-3’; Positive control reverse, 5’-CAGCAAAGCCCCCTGCTGAATCTCTC-3’. This reaction produces a 194 bp amplicon and a 381 bp amplicon from the transgene and the internal control gene, respectively. To determine the presence of the wildtype or floxed Mogat2 allele, the following two primers were used: M2 forward, 5’-GTATGCCACCTGGTGGTAC-3’; M2 reverse, 5’-GCAGTCCTATACCAGTACAG-3’. This reaction produces a 478 bp amplicon and a 512 bp amplicon from the wildtype allele and the allele with the addition of a 34 bp loxP site, respectively.

Diets

Mice were fed a complete, fixed-formula chow (#8604, Teklad, Madison, WI). A series of semipurified (defined) diets containing 10, 45, or 60% calories from fat (D12450B, D12451, and D12492, Research Diets, New Brunswick, NJ) were used to examine the effect of dietary fat, replacing refined carbohydrate, on food intake and energy expenditure in metabolic chambers and during long-term feeding experiments. These defined diets contained 20% calories from protein (casein) and fixed amounts of micronutrients and fiber per calorie, but they varied in metabolizable energy (3.8, 4.7 or 5.2 kcal/g, corresponding to the fat content).

Real-time quantitative PCR analysis

To assess the levels of Mogat2 mRNA, tissues from age-matched mice fed a 10 or 60 kcal% fat diet for 12–16 weeks were homogenized, and total RNA was extracted with Aurum™ Total RNA Mini kit (Bio-Rad, Hercules, CA) and purified by on-column digestion of DNA with DNase I to eliminate residual genomic DNA. Total RNA was used for cDNA synthesis (iScript™cDNA synthesis kit, Bio-Rad). Real-time quantitative PCR was performed with iTaq™ SYBR Green Supermix (Bio-Rad) and analyzed with the ABI PRISM® 7000 Sequence Detection System (Applied Biosystems, Grand Island, NJ). Relative expression levels were calculated by the comparative C_T (cycle of threshold detection) method as outlined in the manufacturer’s technical bulletin. Cyclophilin B (Cyph) expression was used as an internal control. The primer sequences of the Cyph gene were 5’-TGCCGGAGTCGACAAATGAT-3’ (forward) and 5’-TGAAGAGCACCAAAGACACAGA-3’ (reverse). The primers to detect Mogat2 mRNAs were located on exon 1 and exon 2, respectively; the primer sequences were 5’-TGGGACGGCAGTTACAGA-3’ (forward) and 5’-CAGGTGGCATACAGGACAGA-3’ (reverse). The 2-ΔΔCt method was used to calculate the fold change in gene expression (18).

In vitro monoacylglycerol-O-acyltransferase assays

MGAT activity assays were performed with total tissue homogenates as previously described (11). Reactions were started by adding homogenates to the assay mixture and were stopped after 5 min by adding chloroform:methanol (2:1, v:v). The lipids were extracted, dried, and separated by thin-layer chromatography (TLC) on silica gel G-60 plates with the solvent system hexane:diethyl ether:acetic acid (80:20:1, v:v:v). Lipid bands were visualized with iodine vapor, and products were identified by comparison with the migration of lipid standards. The incorporation of radioactive substrates into lipid products was also visualized by an imaging scanner (Typhoon FLA 7000, GE Healthcare Life Sciences, Piscataway, NJ) followed by scraping and counting in a scintillation counter (Packard Tri-Carb 2200 CA Liquid Scintillation Counter Analyzer).

Monoacylglycerol uptake and processing in the small intestine

To examine the uptake and processing of MAG, micelles containing radiolabeled tracers were injected into ligated intestine pouches created in anesthetized mice. Taurocholate micelles were prepared with 2-monooleoyl-rac-glycerol [14C (U); ~15 Ci/mol] (American Radiolabeled Chemical, St.
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Louis, MO) and unlabeled oleate, and isolated pouches in both the proximal and distal intestine were created as previously described (16,19). Two minutes after the micelle preparation was injected, the proximal and distal small intestinal pouches were excised. The luminal content was washed out with 10 mM taurocholate in PBS and collected. Lipids from the proximal small intestine and the distal small intestine were extracted with chloroform:methanol (2:1, v:v) and separated by TLC using a two solvent system [first by chloroform:acetone:methanol:acetic acid:water (50:20:10:10:5, v:v:v:v:v) and then by hexane:diethyl ether:acetic acid (80:20:1, v:v:v)]. The bands corresponding to TAG, DAG, and MAG were scrapped after iodine staining and transferred into scintillation vials for counting of radioactivity.

Spatial and temporal distribution of fat absorption in the intestine

To examine the anatomical distribution of lipid absorption, we intragastrically challenged mice with a radiolabeled lipid bolus and assessed the levels of radioactivity along the length of the small intestine. Briefly, mice were acclimated to 60 kcal% fat diet for at least 1 week, fasted for 4 h, then gavaged with 2 µCi trioleoylglycerol [carboxyl-14C] (American Radiolabeled Chemical) in 200 µl olive oil. After 2 h, the entire gastrointestinal tract was excised. The small intestine was rinsed and cut into 2-cm sections. Each section was digested overnight in 500 µl 1N NaOH at 65°C. Radioactivity was counted after adding 4 ml of Optima Gold scintillation cocktail (Perkin Elmer, Santa Clara, CA) in a scintillation counter.

To assess the temporal distribution, the rate at which dietary fat entered circulation, mice acclimatized to high-fat feeding for one week were fasted for 6 h and injected with 500 mg/kg of the surfactant Pluronic F127 NF Prill Poloxamer 407 (a gift from BASF, Florham Park, NJ) to inhibit the clearance of plasma TAG. Ten minutes later, mice were intragastrically challenged with 200 µl of olive oil containing 2 µCi 14C-trioleoylglycerol. Blood was collected before (time 0) and at indicated times for TAG measurement and scintillation counting. Plasma TAG was measured by enzymatic assay (Infinity™ Triglycerides Lipid Stable Reagent; Thermo Fisher Scientific Inc., Middletown, VA).

Plasma GLP-1 and GIP levels

To determine postprandial GLP-1 and GIP levels in circulation, mice acclimatized to high-fat feeding for 1–2 weeks were fasted for 4 h and then challenged with an intragastric bolus of 300 µl mixed meal (Ensure® supplemented with olive oil) containing 0.65 kcal and 42 mg fat. Plasma was collected 2 h later in the presence of 100 µM DPP-IV inhibitor (EMD Millipore Corporation, Billerica, MA). GLP-1 and GIP levels in plasma were assessed using total GLP-1 and GIP ELISA kits (Millipore).

Metabolic phenotyping studies

To assess phenotypes related to energy balance, mice were housed in a metabolic phenotyping system with housing and wood chip bedding similar to the home cage environment (LabMaster modular animal monitoring system; TSE, Chesterfield, MO). Male mice (3 months old) were acclimated to individual housing and metabolic cages for 1 week before experiments and were fed indicated diets sequentially for three days each and high-fat diet for up to 2 additional weeks. Data collection and analysis were performed as previously reported (15,16). A subset of mice was housed continuously in the metabolic cages for 3 weeks to collect longitudinal food intake data in the metabolic chambers. Food intake after acclimation to high-fat feeding was also examined in an independent cohort of mice in standard cages.

Body weight response to chow or high-fat feeding

To examine the effects of the interaction between diet and MGAT2 on long-term energy balance, mice were fed a regular mixed meal chow at weaning (3 weeks) and switched from chow to a 60 or 10 kcal% fat diet at 12 weeks of age, and weighed weekly for 10 weeks. Female mice were switched from chow to high-fat diet at 10 months of age and weighed weekly for 10 weeks. Following high-fat feeding, tissues and
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Plasma were harvested for weighing and biochemical assays.

Biochemical assays

Hepatic and fecal lipids were extracted using the method of Folch et al. (20). Samples were homogenized in PBS and extracted in chloroform:methanol (2:1, v:v). After repeating the extraction, the organic phases were combined, dried and weighed in a new glass vial. To determine the amount of TAG, free fatty acids, and cholesterol in these extracts, samples were resuspended in 1 ml chloroform containing 1% Triton X-100, evaporated to dryness, resuspended in 1 ml H2O, and assayed using enzymatic kits (Infinity™ Triglycerides Lipid Stable Reagent, Thermo Fisher; Cholesterol E, Non Esterified Fatty Acid assay, Wako Diagnostics). Protein concentration was measured by the bicinchoninic acid assay (Thermo). Hepatic glycogen contents were determined as described (21). Fecal samples were collected from individually housed mice acclimatized to the 60 kcal% fat diet for 1 week.

Glucose metabolism

To examine glucose metabolism prior to high-fat feeding, chow-fed mice aged 3 months were bled after a 6 h fast for blood glucose and plasma insulin measurement. To examine glucose metabolism following high-fat feeding, mice were fed a 60 kcal% fat diet for 8–11 weeks, then a glucose tolerance test was performed. Briefly, male mice were fasted for 6 h beginning at 7 a.m., then injected with glucose (1 g/kg BW, IP, 10% glucose in PBS). Blood glucose was measured immediately before and at defined intervals after glucose injection using a hand-held glucometer (OneTouch Ultra, LifeScan, Inc., Milpitas, CA). Insulin was measured by ELISA (Crystal Chem, Inc., Downers Grove, IL).

Statistical analyses

All data are presented as mean ± SEM. P<0.05 was considered statistically significant. Each experiment was performed with independent samples at least twice to confirm reproducibility of the results. For comparisons between two groups, student’s t-tests were used. For comparisons between four groups, a one-way ANOVA followed by Tukey’s multiple comparison test was used. Differences measured over time were compared using repeated measures two-way ANOVA to determine main effects of, and interactions between, time and genotype. Analyses were conducted using GraphPad Prism statistical analysis software (version 5.01; GraphPad Software, La Jolla, CA).

RESULTS

Generation of intestine-specific MGAT2 deficient mice

To examine the effects of inactivating MGAT2 specifically in the intestine, we introduced a Cre-recombinase under the control of the intestine-specific villin promoter into Mogat2f/f mice, which carry two loxP sites flanking exon 2 of the Mogat2 gene (Fig. 1A), as described in the experimental procedures. All of the offspring were born with floxed Mogat2 alleles, and half expressed the tissue-specific Cre enzyme. To confirm the efficiency and tissue-specificity of MGAT2 ablation in the Cre expressing floxed mice (Mogat2IKO), we measured MGAT2 mRNA expression level and MGAT activity in several tissues, including the small intestine, kidney, and white and brown adipose tissue, where MGAT2 expression has been reported (11,12) as well as liver, where MGAT2 expression has been reported (11,12) as well as liver, where MGAT2 is not expressed in adult mice (11). We found that Mogat2IKO mice expressed MGAT2 mRNA at levels similar to that found in wildtype mice. In both groups, MGAT2 was expressed predominantly in the intestine when mice were fed either a high-fat (Fig. 1B) or a low-fat (data not shown) diet, and the intestinal expression levels were up-regulated more than five-fold upon high-fat feeding. Mogat2–/– mice had no detectable MGAT2 mRNA in any tissue examined. In contrast, Mogat2IKO mice showed similar MGAT2 expression levels as Mogat2+/+ littermates in examined tissues except in the small intestine, where MGAT2 expression remained absent even after the continuous renewal of intestinal epithelia. Mice in all four groups had no detectable MGAT2 mRNA in liver (data not shown), indicating that lacking MGAT2 specifically in the intestine does not induce hepatic MGAT2 expression in adult mice.
The levels of MGAT activity correlated with the levels of MGAT2 mRNA detected. With the highest levels found in the intestine, Mogat2<sup>+/+</sup> mice showed MGAT activity similar to that of wildtype mice. Ablation of MGAT2 message in the intestine of Mogat2<sup>KO</sup> mice reduced intestinal MGAT activity by approximately 70% as compared with Mogat2<sup>+/+</sup> mice (Fig. 1C). This difference in MGAT activity was similar to that seen between Mogat2<sup>+/+</sup> and wildtype mice. The levels of MGAT activity were low in all other tissues examined. In these tissues, the differences between wildtype and Mogat2<sup>+/+</sup> were statistically significant, whereas the differences between Mogat2<sup>+/+</sup> and Mogat2<sup>KO</sup> were not. These results indicated that Mogat2<sup>KO</sup> mice are deficient in MGAT2 specifically in the intestine.

**Deletion of MGAT2 in the intestine reduces MAG uptake/esterification and delays fat absorption**

Mogat2<sup>+/+</sup> mice exhibit reduced MAG uptake and esterification in the intestine (16). To confirm that deletion of MGAT2 in the intestine impairs MAG uptake and esterification, we infused taurocholate micelles containing fatty acids and <sup>14</sup>C-MAG directly into ligated pouches created in both the proximal and distal intestine. After a 2 min incubation, approximately 45% of label injected was taken up and accumulated in the proximal intestine segment of wildtype mice (Fig. 2A), while less than 1% of label appeared in the circulation with the rest still in the lumen (data not shown). A similar level of accumulation was found in Mogat2<sup>+/+</sup> mice. In contrast, less than 10% of MAG tracer was taken up in the proximal intestine of Mogat2<sup>–/–</sup> mice, and likewise in Mogat2<sup>KO</sup> mice. In wildtype and Mogat2<sup>+/+</sup> mice, uptake was greater in the proximal intestine than in the distal intestine. In Mogat2<sup>–/–</sup> and Mogat2<sup>KO</sup> mice, the uptakes were low compared with that in respective controls, and there was no difference between the proximal and the distal intestine. In general, the levels of MAG uptake were associated with the production of DAG and TAG. In wildtype and Mogat2<sup>+/+</sup> mice, 90% tracer was recovered as TAG in the proximal intestine. In Mogat2<sup>–/–</sup> and Mogat2<sup>KO</sup> mice, the proportion of MAG incorporated into DAG and TAG was greatly reduced compared to that in littermates, demonstrating the predominant role of intestinal MGAT2 in catalyzing the esterification process. Deletion of MGAT2 in the intestine inhibited both uptake and esterification of MAG.

To assess the spatial distribution of fat absorption, we challenged mice with an intragastric bolus of oil containing <sup>14</sup>C-TAG and measured radiolabeled lipid accumulated along the length of the small intestine. Consistent with the location of MAG uptake and esterification, a substantial amount of radiolabeled lipid accumulated in the proximal small intestine of wildtype and Mogat2<sup>+/+</sup> mice. In Mogat2<sup>–/–</sup> and Mogat2<sup>KO</sup> mice, there was significantly less accumulation of radiolabeled lipid in the proximal half of the intestine than in respective control mice (Fig. 2B). The pattern of radiolabeled lipids present in the small intestine of Mogat2<sup>KO</sup> mice resembled that of Mogat2<sup>–/–</sup> mice.

To determine the rate of fat absorption, we treated mice with a lipoprotein lipase inhibitor and an intragastric bolus of <sup>14</sup>C-TAG containing oil. In wildtype and Mogat2<sup>+/+</sup> mice, the labeled dietary and total fat increased in circulation at a similar rate; whereas, in Mogat2<sup>–/–</sup> and Mogat2<sup>KO</sup> mice, these increases were diminished to a similar degree (Fig. 2C and D). In all four genotypes, the majority of radioactivity in blood was found as triacylglycerol (data not shown). These findings suggest that deletion of MGAT2 in the intestine was sufficient to not only alter the kinetics of lipid processing in the intestine, but also modulate the delivery of dietary lipid into the circulation and other tissues.

**Deletion of intestinal MGAT2 increases postprandial plasma GLP-1**

To examine if the change in fat absorption leads to a change in gut hormone secretion, we measured the levels of glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) 2 h after an intragastric challenge of a high-fat meal in mice that had been acclimatized to high-fat feeding. Compared with wildtype mice, Mogat2<sup>–/–</sup> mice showed an increased level of plasma GLP-1 (Fig. 3A). Likewise, Mogat2<sup>KO</sup> mice had a higher level of GLP-1 than Mogat2<sup>+/+</sup> mice, linking the change in circulating GLP-1 level to the deficiency of MGAT2 in the intestine.
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In contrast, GIP levels were not significantly different across genotypes (Fig 3B).

**Deletion of intestinal MGAT2 protects mice against diet-induced obesity**

To determine if intestinal MGAT2 modulates long-term energy balance, we monitored body weight of mice over time. When mice were fed a standard mixed-meal chow low in fat (accounting for approximately 14 kcal%), deficiency of MGAT2 globally or specifically in the intestine did not alter long-term energy balance, as mice grew and gained weight similarly across genotypes (Fig. 4A). On the other hand, when mice were fed a high-fat diet, both wildtype and Mogat2<sup>f/f</sup> male mice exhibited positive energy balance and gained weight rapidly; whereas, Mogat2<sup>−/−</sup> and Mogat2<sup>KO</sup> mice gained weight at a significantly lower rate than their littermate controls (Fig. 4B). When switched to a high-fat diet at 3 months of age, wildtype and Mogat2<sup>f/f</sup> mice gained 17.7 and 17.1 g after 10 weeks, respectively. In contrast, Mogat2<sup>−/−</sup> and Mogat2<sup>KO</sup> mice gained only 6.0 and 10.7 g, resulting in body weights 28 and 15% lighter than their littermates, respectively. Thus, both Mogat2<sup>−/−</sup> and Mogat2<sup>KO</sup> mice gained less weight than their littermates, while Mogat2<sup>KO</sup> mice gained significantly more weight than Mogat2<sup>−/−</sup> mice.

The differences in body weight were largely due to differences in fat mass. White and brown adipose tissues were significantly smaller in Mogat2<sup>−/−</sup> mice (63 and 45%, respectively) compared with wildtype controls. In Mogat2<sup>KO</sup> mice, white and brown adipose tissues were also smaller (38 and 36%, respectively) when compared with Mogat2<sup>f/f</sup> controls (Fig. 4C). Lean mass, represented by calf and heart, was not decreased in Mogat2<sup>−/−</sup> or Mogat2<sup>KO</sup> mice. A small increase of heart mass in Mogat2<sup>−/−</sup> mice over controls reached statistical significance; whether the difference is biologically significant remains to be determined.

The effect of intestinal MGAT2 on weight gain was independent of age or sex, as mature female Mogat2<sup>KO</sup> mice were also protected from excess weight gain when they were switched to high-fat feeding at 10 months of age (Fig. 4D). After 10 weeks of high-fat feeding, female Mogat2<sup>KO</sup> mice gained only 59% as much as Mogat2<sup>f/f</sup> controls (12.9 v. 21.9 g, respectively). In addition, Mogat2<sup>KO</sup> mice gained less weight than Mogat2<sup>f/f</sup> controls (2.1 v. 3.8 g, respectively) after consuming a low-fat diet rich in refined carbohydrate (Fig. 4E), suggesting that the protective effect is not limited to high-fat feeding. Taken together, these data indicate that, like global deletion of MGAT2, deficiency of intestinal MGAT2 is sufficient to deter excess accumulation of body fat resulting from positive energy balance; however, the effect is to a lesser degree than deletion of MGAT2 in all tissues.

**Deletion of intestinal MGAT2 modulates short-term energy balance and substrate utilization**

To explore the physiological mechanisms by which intestinal MGAT2 modulates energy balance, we examined energy intake and expenditure using indirect calorimetry, when mice were fed the standard chow or a semi-purified diet containing 10, 45, or 60% of calories from fat consecutively for 3 days on each diet. Mogat2<sup>−/−</sup> mice exhibited increased energy expenditure compared with wildtype littermates, as indicated by increased oxygen consumption (Fig. 5A). The increases, ranging from 11 to 15%, were significant under all diet conditions and were more pronounced when dietary fat was high. Similar, but more moderate, elevations in oxygen consumption were observed when comparing Mogat2<sup>KO</sup> mice to their littermate controls (Fig. 5B). The increases ranged from 4 to 7% and were statistically significant, except when mice were exposed to the 45 kcal% diet. Together with the long-term weight gain, these data suggest that MGAT2 in the intestine contributes significantly to the regulation of energy expenditure, while MGAT2 in extra-intestinal tissues could also play a role.

Energy expenditure is fueled by energy-yielding nutrients from diet when intake is not limiting. In all four groups of mice, the respiratory exchange ratio (RER) of carbon dioxide production over oxygen consumption largely reflected the composition of nutrients in diets, with lower RERs when the levels of dietary fat increased (Fig. 5A and B, middle panels). Nonetheless, when fed diets rich in fat (45 or 60...
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Mogat2–/– mice oxidized higher proportions of fatty acids than wildtype controls did as indicated by 4% lower RERs. Mogat2IKO mice showed similar differences in RER when compared with Mogat2+/+ controls fed the corresponding diets.

Food intake was greater in Mogat2–/– mice when fed chow or a low-fat diet, compensating for increases in energy expenditure (Fig. 5A, lower panel) as previously reported (15). When they were switched onto high-fat diets, which contain more metabolizable energy, Mogat2+/+ mice decreased their food intake to a greater extent than wildtype littermates did (Fig. 5A). Interestingly, these contrasting effects of diet on food intake were recapitulated between Mogat2IKO and Mogat2+/+ mice, indicating an essential role of intestinal MGAT2 in modulating satiety or aversion induced by dietary fat (Fig. 5B).

During the 12-day experiment in metabolic chambers, wildtype mice gained weight when fed the calorie-dense, semi-purified diets (Fig. 5C). The weight gain was especially evident when fat content was high. In contrast, Mogat2–/– mice maintained their body weights throughout. These contrasting differences were also recapitulated between Mogat2+/+ and Mogat2IKO mice (Fig. 5C).

The effect on caloric intake caused by MGAT2 deficiency during high-fat feeding appears to be transient, while the effect on energy expenditure is persistent. Both Mogat2+/+ and Mogat2IKO mice showed acute decreases in caloric intake when they were first exposed to a high-fat diet (Fig. 5D, Arrow). After housing continuously in the metabolic cages for 3 weeks, all four groups of mice consumed a similar level of calories (Fig. 5D). Similar food intake following high-fat feeding was also confirmed in an independent experiment using regular cages (data not shown).

After acclimatizing to high-fat feeding, there was no difference in fat absorption quantitatively between Mogat2+/+ and wildtype mice or between Mogat2IKO and Mogat2+/+ mice, as indicated by total fecal output and residual lipids, free fatty acids and cholesterol in the excrement (Fig. 5E–H).

Deletion of intestinal MGAT2 protects mice from diet-induced metabolic disorders

Mogat2+/+ mice are protected from several comorbidities of obesity (14). We next investigated if intestine-specific ablation of MGAT2 can also protect mice from hepatic steatosis, hypercholesterolemia, and impaired glucose tolerance induced by high-fat feeding. After long-term high-fat feeding, wildtype mice developed hepatic steatosis, while Mogat2–/– mice were protected from increases in mass and TAG content of the liver (Fig. 6A and B). To a similar degree, Mogat2IKO mice had significantly smaller livers and lower hepatic TAG content than their littermate controls (Fig. 6A and B), while there was no difference in glycogen content between genotypes (Fig. 6C). In addition, Mogat2–/– and Mogat2IKO mice had lower plasma cholesterol than littermate controls (Fig. 6D). Their plasma TAG levels also trended lower than controls, but the differences did not reach statistical significance (Fig. 6E).

Prior to high-fat feeding, similar levels of fasting blood glucose and plasma insulin were found in mice across genotypes (Fig. 7A and B). After 8 weeks of high-fat feeding, levels of fasting blood glucose in Mogat2–/– and Mogat2IKO mice were 75% that of their littermates (Fig. 7C), while levels of fasting plasma insulin in these mice were approximately half that of their respective controls (Fig. 7D). A decrease in both fasting blood glucose and insulin suggests an enhancement in glucose tolerance. Indeed, when challenged with an intraperitoneal injection of glucose, both Mogat2–/– and Mogat2IKO mice dispose of glucose faster than control littermates do (Fig. 7E and F). Following a glucose challenge, Mogat2–/– and Mogat2IKO mice have significantly reduced net increases in blood glucose (38 and 46% lower area under the curve, respectively).

DISCUSSION

Mice without a functional MGAT2 enzyme absorb normal quantities of dietary fat but exhibit increased energy expenditure. As a result, they are protected from obesity and other metabolic disorders induced by the agouti mutation or by high-fat feeding (14,15). In this study, we examined if intestinal MGAT2 is responsible for these phenotypes. We found that deletion of MGAT2 specifically in the intestine of mice...
Inactivation of MGAT2 in the intestine altered intestinal TAG metabolism and delayed fat absorption. These Mogat2IKO mice had reduced weight gain and were protected against obesity-associated comorbidities induced by high-fat feeding. Although the protection against weight gain was not to the same extent as that in the global deficiency of MGAT2, the partial reduction in weight gain was sufficient to normalize hepatic steatosis and glucose intolerance as seen in Mogat2–/– mice. These findings support the concept that intestinal lipid metabolism plays a crucial role in the regulation of systemic energy balance and suggest that targeting MGAT2 in the small intestine may be a feasible approach to reduce weight gain and avoid associated metabolic disorders. Complementing findings from gain-of-function studies using intestine-specific transgenic mice (16), these findings also implicate extra-intestinal MGAT activity in the regulation of whole body energy balance.

MGAT2 is highly expressed in the small intestine, where the role of MGAT activity in fat absorption is well-established (4,5). MGAT2 appears to be a major intestinal MGAT, as Mogat2IKO mice, like Mogat2–/– mice, exhibited significantly reduced intestinal MGAT activity (Fig.1C). The residual activity could come from other enzymes. For example, acyl CoA:diacylglycerol acyltransferase 1 (DGAT1), one of the two known DGAT enzymes (6), also exhibits MGAT activity in vitro and is expressed in the intestine (22-24); however, it does not appear to account for the activity, as mice deficient in both MGAT2 and DGAT1 still exhibited similar levels of residual MGAT activity in the intestine (Yen, unpublished results). Lysophosphatidylglycerol acyltransferase1 (LPGAT1) also exhibits MGAT activity (25). As LPGAT1 is expressed in the intestine, it could contribute to the residual MGAT activity. Like that of DGAT1, the expression of LPGAT1 is not altered by MGAT2 deficiency (data not shown).

Despite the presence of alternative enzymes, the biochemical and physiological functions of MGAT2 cannot be fully compensated for; the uptake and esterification of MAG were greatly reduced and entry of dietary fat into circulation was delayed in Mogat2IKO mice (Fig. 2). Diacylglycerol, the product of MGAT, can also come from the glycerol-3-phosphate pathway, which is predominant in most tissues (26,27). The enzymes involved, glycerol-3-phosphate acyltransferase, 1-acylglycerol-3-phosphate acyltransferase, and phosphatidate phosphatase (lipin), are all expressed in the intestine, but the alternative pathway did not fully compensate for the MGAT pathway either. Consistent with our previous report (16), results from Mogat2IKO mice show that MGAT2 determines the levels of MAG uptake and esterification in the intestine, not because of its systemic effects, such as gastric emptying, but in a cell autonomous fashion. These findings support the model that lipid intermediates generated from different pathways have preferred destinations. MGAT2 likely mediates TAG synthesis coupled to chylomicron assembly, because lacking MGAT2 in the intestine altered spatial distribution and temporal kinetics of fat absorption.

We postulated that MGAT2 modulates systemic energy balance most likely through its role in the intestine, because MGAT2 is highly expressed only in the intestine in mice (11,12). Intestine initiates hormonal and neural signals in response to the presence of substances in the lumen (1-3). A change in the spatial distribution of fat absorption in Mogat2–/– mice is associated with altered levels of gut hormones, including glucagon-like peptide 1 (GLP-1) (14). In this study, we also found that Mogat2IKO mice had an increased GLP-1 level in response to a fatty meal (Fig. 3). In addition to its effects on glucose metabolism, GLP-1 is known to reduce food intake and contribute to weight loss (28). When exposed to a diet rich in fat (45 or 60 kcal%), Mogat2IKO mice exhibited a similar transient reduction in food intake as seen in Mogat2–/– mice (Fig. 5), indicating that changes in food intake are a result of altered intestinal lipid metabolism. Moreover, MGAT2 exhibits high activity toward MAGs with polyunsaturated fatty acyl groups (11), including sn-2 arachidonoylglycerol (2-AG). 2-AG is an endogenous ligand for the cannabinoid receptors. MGAT2 may thus modulate endocannabinoid signaling, which is known to regulate food intake and energy balance (29). It is not known if MGAT2 is expressed in the taste buds, enteroendocrine cells, or the enteric nerve system. Interestingly, overexpression of MAG
lipase, an enzyme competing with MGAT for MAG substrate, leads to decreases in 2-AG, increases in food intake, decreases in energy expenditure, and obesity in mice (30).

The reduction in food intake and the increases in energy expenditure may have both contributed to the decrease in weight gain of \( \text{Mogat}^{2\text{KO}} \) mice when fed a high-fat diet. However, the reduction in food intake induced by high-fat feeding was transient, whereas the increase in energy expenditure induced by the deficiency of intestinal MGAT2 was persistent. The differences in food intake disappeared after acclimatization to high-fat feeding mainly because wildtype and \( \text{Mogat}^{2\text{f/f}} \) mice also reduced their food intake. This adaptation could be due to long-term regulators, such as leptin secreted from their expanding adipose tissue (31). When \( \text{Mogat}^{2\text{KO}} \) mice first consumed a high-fat diet, the greater reduction in food intake would have decreased diet-induced thermogenesis and thus blunted their increases in energy expenditure. As the dietary fat content increased to 60 kcal%, differences in energy expenditure were pronounced, despite the reduced food intake. After acclimatization to the high-fat feeding for one week, all groups of mice ate the same and absorbed the same amount of fat. Yet, the weight gain in \( \text{Mogat}^{2\text{KO}} \) mice remained much lower than in controls (Fig. 4), consistent with an increase in energy expenditure. Compared with their respective littermate controls, \( \text{Mogat}^{2\text{f/f}} \) and \( \text{Mogat}^{2\text{KO}} \) mice both had significant decreases in RER, suggesting a higher proportion of fatty acid oxidation upon high-fat feeding. Throughout the 12-day metabolic chamber study, \( \text{Mogat}^{2\text{f/f}} \) and \( \text{Mogat}^{2\text{KO}} \) mice maintained energy balance, matching their energy intake with expenditure.

They likely oxidized most of the consumed dietary fat. In contrast, wildtype and \( \text{Mogat}^{2\text{f/f}} \) mice gained weight, likely storing some dietary fat. Moreover, when fed a diet low in fat (chow or 10 kcal% fat), \( \text{Mogat}^{2\text{f/f}} \) mice ate more to compensate for increased energy expenditure, and they lost weight when food intake was limited to the levels of wildtype littermates (15). \( \text{Mogat}^{2\text{KO}} \) mice also showed increases in food intake when fed chow or the low fat diet while gaining less weight than controls, indicating a persistent increase in energy expenditure regardless of dietary components.

The extent of energy expenditure increases in \( \text{Mogat}^{2\text{KO}} \) mice was not as great as that of \( \text{Mogat}^{2\text{f/f}} \) mice. These findings are complementary to studies performed in \( \text{Mogat}^{2\text{f/f}} \) mice with an intestine-specific MGAT2 transgene (16). Re-introducing MGAT2 in the intestine of \( \text{Mogat}^{2\text{f/f}} \) mice reduced energy expenditure, enhancing metabolic efficiency and propensity to gain weight upon high-fat feeding. However, the recovery is only partial, as these mice do not gain as much weight as wildtype mice. Taken together, these findings indicate that intestinal MGAT2 is necessary but not sufficient to maximize metabolic efficiency and suggest that MGAT2 in other tissues also regulates energy balance. The identity of these tissues remains to be determined. The low levels of MGAT2 expression and activity in the brown and white adipose tissues may have cell autonomous effects, modulating the partitioning of substrates between use and storage. Several lines of mice lacking other enzymes involved in TAG metabolism in the adipose tissues also exhibit alterations in systemic energy balance (32-36). In addition, the involvement of adipose tissues can be indirect. For example, the sympathetic nervous system is a major regulator of brown adipose tissue, which is crucial for energy expenditure in rodents and may also play a role in humans (37). The level of MGAT2 expression in brain was not above the detection limit (data not shown).

However, it is possible that MGAT2 may modulate the metabolism of the endogenous cannabinoid 2-AG in certain neurons in the central nervous system that regulate food intake and energy expenditure.

The phenotypes of \( \text{Mogat}^{2\text{KO}} \) mice recapitulated the protection against diet-induced metabolic disorders seen in \( \text{Mogat}^{2\text{f/f}} \) mice, suggesting that inhibition of intestinal MGAT2 alone is sufficient to protect against hepatic steatosis and hyperglycemia associated with obesity. These aspects of MGAT2 functions resemble those of DGAT1. Informed by data from genetically engineered mice, several DGAT1 inhibitors were developed and showed promising effects in delaying fat absorption, reducing weight gain, alleviating hepatic steatosis and enhancing insulin sensitivity in rodent models (38-40). However, for humans, their long-term efficacy is unknown and their safety could be a concern.
Unlike mice, humans do not express DGAT2 in the intestine (41). Inhibiting the only DGAT enzyme in human intestine may block TAG synthesis completely and lead to side effects with gastrointestinal distress and malabsorption, as observed in patients with a null mutation in the DGAT1 gene and in some clinical trials of DGAT1 inhibitors (42,43). Inhibiting human MGAT2, on the other hand, is less likely to block TAG synthesis to the same extent, as the alternative GPAT pathway is intact in humans. In addition, MGAT3, a homolog of MGAT2, is also expressed in human intestine. As MGAT3 is not expressed in mice, whether MGAT3 could compensate fully for MGAT2 in humans remains to be determined. MGAT3, as well as MGAT2, is highly expressed in human subjects with non-alcoholic fatty liver disease and its expression was reduced significantly after weight loss (44). Thus, inhibiting both MGAT2 and MGAT3 may present a therapeutic opportunity.

In summary, we generated and characterized mice lacking MGAT2 specifically in the intestine. Deficiency of intestinal MGAT2 was sufficient to deter excess fat accumulation resulting from high-fat feeding, suggesting that MGAT2 in the intestine normally coordinates nutrient absorption and substrate utilization in the periphery. Although the protection is not to the same extent as with global deletion of MGAT2, these findings suggest that inhibiting intestinal MGAT2 alone is sufficient to blunt excessive weight gain and that the moderate decrease in weight gain is sufficient to protect against fatty liver and glucose intolerance associated with obesity. Our findings support the idea that MGAT2 in the intestine could be a feasible target to decrease the propensity of storing excess dietary calories.
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**FOOTNOTES**

1. *Authors contributed equally to this work.*

2. The abbreviations used are 2-AG, sn-2 arachidonoylglycerol; DAG, diacylglycerol; DGAT, acyl CoA:diacylglycerol acyltransferase; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; LPGAT, lysophosphatidylglycerol acyltransferase; MAG, monoacylglycerol; MGAT, acyl CoA:monoacylglycerol acyltransferase; RER, respiratory exchange ratio; TAG, triacylglycerol
Inactivation of MGAT2 in the intestine

FIGURE LEGENDS

Figure 1. Generation of intestine-specific MGAT2 deficient mice. (A) Illustration of the Mogat2 targeted allele with a trapping cassette (flanked by the flp-recombinase target site, FRT) that introduces 2 Cre-recombinase target loxP sites flanking exon 2, the Mogat2 “floxed” allele after removal of the trapping cassette by flp recombination, and the Mogat2 deleted allele after removal of exon 2 by Cre-recombination. Efficiency and tissue-specificity of MGAT2 ablation were confirmed by measuring Mogat2 mRNA expression level (B) and MGAT activity (C) in jejunum (intestine), kidney, inguinal fat pad (WAT), and interscapular fat pad (BAT) of wildtype (WT), Mogat2f/f (KO), Mogat2f/f (M2f/f) and Mogat2f/f (M2f/f) mice. n=5–11/genotype. Within each tissue, differences between bars without the same letter are statistically significant. nd, not detected; ns, not statistically significant.

Figure 2. Intestine-specific inactivation of MGAT2 inhibits monoacylglycerol uptake and esterification, alters distribution of fat absorption, and delays appearance of TAG in the circulation. (A) Monoacylglycerol uptake and esterification in isolated intestinal pouches, after injection of a micelle preparation containing fatty acids and 2-monooeyl-rac-glycerol [14C (U)] tracer. Bars represent accumulation of MAG and incorporation of tracers into DAG and TAG in each intestinal segment of wildtype (WT), Mogat2f/f (KO), Mogat2f/f (M2f/f) and Mogat2f/f (M2f/f) mice. n=3–5/genotype. Differences between bars without the same letter are statistically significant. (B) Distribution of dietary TAG in 2-cm segments of the small intestine 2 h after an oral gavage of trioleoylglycerol [carboxyl-14C] in mice acclimatized to high-fat feeding. n=7–13/genotype. Blood radioactivity (C) and plasma TAG (D) in mice after injection of the lipase inhibitor P407 and gavage with olive oil containing 14C-trioleoylglycerol. n=5–8/genotype. Differences between curves without the same letter are statistically significant.

Figure 3. Inactivation of intestinal MGAT2 increases postprandial plasma GLP-1. Wildtype (WT), Mogat2f/f (KO), Mogat2f/f (M2f/f) and Mogat2f/f (M2f/f) mice were challenged with a liquid meal, and plasma samples were collected 2 h later. Plasma GLP-1 (A) and GIP (B) were measured by ELISA. n=7–10/genotype. Differences between bars without the same letter are statistically significant.

Figure 4. Intestine-specific inactivation of MGAT2 protects against excessive weight gain induced by diets. (A) Male mice fed chow from weaning through 23 weeks of age. n=7–13/genotype. (B) Male mice switched to high-fat diet at 12 weeks of age. n=17–28/genotype. (C) Tissue masses (WAT, inguinal fat; BAT, interscapular fat) of mice fed high-fat diet for approximately 3 months. n=13–15/genotype. (D) Females switched to high-fat feeding at 10 months of age. n=7–8/genotype. (E) Male mice switched to low-fat diet at 12 weeks of age. n=14/genotype. Wildtype (WT, black squares), Mogat2f/f (KO, white circles), Mogat2f/f (M2f/f, dark grey diamonds), and Mogat2f/f (M2f/f, light grey triangles). Error bars not shown are smaller than the symbols. * or #, p < 0.05 versus littermate controls by t-test or repeated measures ANOVA, respectively.

Figure 5. Inactivation of intestinal MGAT2 alters energy balance. (A, B) 3 month old male mice (Wildtype, black; Mogat2f/f, red; Mogat2f/f, blue; and Mogat2f/f, green) were sequentially fed chow, then fed semi-purified diets containing 10, 45, and 60% of calories from fat for 3 days each. Oxygen consumption adjusted for baseline body weight at the start of each diet (VO2), respiratory exchange ratio (RER), and cumulative food intake were measured continuously. Data from each mouse were pooled from the same time of the day of the same diet treatment. n=16–20/genotype. Graphs represent average days. Grey areas mark dark phase of the light cycle (6 pm to 6 am). (C) Short-term body weight of mice during the 12-day metabolic phenotyping experiment. Differences between curves without the same letters are statistically significant. (D) Food intake of mice housed continuously in the metabolic cages for 21 days (n=16–20/genotype, day 1 to 12; n=4–9/genotype, day 13 to 21). Arrow indicates the start of high-fat feeding. (E) Fecal output and (F) its lipid (G), free fatty acid (H) and cholesterol content during
the week following acclimatization to high-fat feeding. Wildtype (WT), $Mogat2^{-/-}$ (KO), $Mogat2^{ff}$ ($M2^{ff}$) and $Mogat2^{K0}$ ($M2^{K0}$). *ns*, not statistically significant.

**Figure 6. Intestine-specific inactivation of MGAT2 protects mice against hepatic steatosis induced by high-fat feeding.** Male wildtype (WT), $Mogat2^{-/-}$ (KO), $Mogat2^{ff}$ ($M2^{ff}$), and $Mogat2^{K0}$ ($M2^{K0}$) mice were 6-h fasted after approximately 3 months of high-fat feeding. Liver mass (A), hepatic triacylglycerol (B) and glycogen (C), plasma cholesterol (D) and triacylglycerol (E). n=6–9/genotype. Differences between bars without the same letter are statistically significant. *ns*, not statistically significant.

**Figure 7. Intestine-specific inactivation of MGAT2 protects mice from impaired glucose metabolism following high-fat feeding.** Blood glucose and plasma insulin from 6-h fasted wildtype (WT), $Mogat2^{-/-}$ (KO), $Mogat2^{ff}$ ($M2^{ff}$), and $Mogat2^{K0}$ ($M2^{K0}$) mice fed a regular chow (A and B) or high-fat diet (C and D). Differences between bars without the same letter are statistically significant. (E and F) Net blood glucose change following an IP injection of glucose (1 mg/g body weight, 10% glucose in PBS). n=6–14/genotype. #, $p<0.05$ versus area-under-the-curve of littermate controls.
Figure 1

A

*Mogat2 Targeted*

Exon 1

| Trapping Cassette |
|-------------------|
| FRT               |

*Exon 2*  

| loxP | loxP |

*Mogat2 Targeted X FLP*  

*Mogat2 Floxed*

Exon 1

| FRT |
|-----|

*Exon 2*  

| loxP | loxP |

*Mogat2 Floxed X Cre*  

*Mogat2 Deleted*

Exon 1

| FRT |

| loxP |

B

*Mogat2 mRNA*

| Intestine | Kidney | WAT | BAT |
|-----------|--------|-----|-----|

Relative Expression

| WT | KO | M2* | M2KO |
|----|----|-----|------|

C

*MGAT Activity*

| Intestine | Kidney | WAT | BAT |
|-----------|--------|-----|-----|

| nmol/mg/min |
|-------------|

| Intestine | Kidney | WAT | BAT |
|-----------|--------|-----|-----|

| 0 | 5 | 10 | 15 | 20 |

| nd | ns |

| a | b | a | b |

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Figure 2

A. Proximal Gut vs. Distal Gut

- **Labeled Lipid (pmol/mg protein)**
  - WT: a
  - KO: b
  - M2^{f/f}: a
  - M2^{KO}: ab

B. Intestine Radioactivity (X 10^3 CPM/2 cm)

- WT
- KO
- M2^{f/f}
- M2^{KO}

C. Blood Radioactivity (X 10^5 CPM/ml)

- Plotted against Time (h)
- WT: a
- KO: b

D. Plasma TG (mg/dL)

- Plotted against Time (h)
- WT: a
- KO: b

Intestine Radioactivity (X 10^3 CPM/2 cm)

Distal Gut

Labeled Lipid (pmol/mg protein)

WT KO M2^{f/f} M2^{KO}

Distal Gut

Blood Radioactivity (X 10^5 CPM/ml)

Plotted against Time (h)

Plasma TG (mg/dL)

Plotted against Time (h)
Figure 4

A  Chow

Body Weight (g)

Age (weeks)

KO  WT  M2IKO  M2f/f

Body Weight (g)

Age (weeks)

Chow

B  High-fat

Body Weight (g)

Weeks on diet

KO  WT  M2IKO  M2f/f

Body Weight (g)

Weeks on diet

High-fat

C  High-fat

Mass (g)

WT  KO

Mass (g)

WT  KO

High-fat

D  High-fat Female

Body Weight (g)

Weeks on diet

KO  WT  M2IKO  M2f/f

Body Weight (g)

Weeks on diet

High-fat Female

E  High-refined CHO

Body Weight (g)

Weeks on diet

KO  WT  M2IKO  M2f/f

Body Weight (g)

Weeks on diet

High-refined CHO
Figure 5

A

\begin{table}
\centering
\begin{tabular}{llll}
\hline
\textbf{Chow} & 10kcal\% & 45kcal\% & 60kcal\% \\
\hline
\hline
\textbf{VO}_2 (l/h/kg) & 4.5 & 3.5 & 2.5 \\
\textbf{RER} & 1.0 & 0.7 & 0.5 \\
\textbf{Food Intake (g)} & 4 & 2 & 0 \\
\hline
\end{tabular}
\end{table}

B

\begin{table}
\centering
\begin{tabular}{llll}
\hline
\textbf{Chow} & 10kcal\% & 45kcal\% & 60kcal\% \\
\hline
\hline
\textbf{VO}_2 (l/h/kg) & 4.5 & 3.5 & 2.5 \\
\textbf{RER} & 1.0 & 0.7 & 0.5 \\
\textbf{Food Intake (g)} & 4 & 2 & 0 \\
\hline
\end{tabular}
\end{table}

C

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5c.png}
\caption{Body Weight (g) over Days for WT, M2\textsuperscript{ff}, M2\textsuperscript{KO}, and KO mice on Chow 10%, 45%, and 60% diets.}
\end{figure}

D

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5d.png}
\caption{Food Intake (kcal/day) over Days for WT, M2\textsuperscript{ff}, M2\textsuperscript{KO}, and KO mice on Chow 10%, 45%, and 60% diets.}
\end{figure}

E

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5e.png}
\caption{Fecal Output (mg/day) for WT, M2\textsuperscript{ff}, KO, and M2\textsuperscript{KO} mice on Chow 10%, 45%, and 60% diets.}
\end{figure}

F

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5f.png}
\caption{Fecal Lipid (\% of dry weight) for WT, M2\textsuperscript{ff}, KO, and M2\textsuperscript{KO} mice on Chow 10%, 45%, and 60% diets.}
\end{figure}

G

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5g.png}
\caption{Fecal FFA (mmol/g feces) for WT, M2\textsuperscript{ff}, KO, and M2\textsuperscript{KO} mice on Chow 10%, 45%, and 60% diets.}
\end{figure}

H

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5h.png}
\caption{Fecal Cholesterol (mg/g feces) for WT, M2\textsuperscript{ff}, KO, and M2\textsuperscript{KO} mice on Chow 10%, 45%, and 60% diets.}
\end{figure}
Intestine-specific deletion of Acyl CoA:monoacylglycerol acyltransferase (MGAT)2 protects mice from diet-induced obesity and glucose intolerance
David W. Nelson, Yu Gao, Mei-I Yen and Chi-Liang Eric Yen

J. Biol. Chem. published online May 1, 2014

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