Obesity potentiates development of fatty liver and insulin resistance, but not atherosclerosis, in high-fat diet-fed agouti LDLR-deficient mice

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Coenen KR, Hasty AH. Obesity potentiates development of fatty liver and insulin resistance, but not atherosclerosis, in high-fat diet-fed agouti LDLR-deficient mice. Am J Physiol Endocrinol Metab 293: E492–E499, 2007. First published June 12, 2007; doi:10.1152/ajpendo.00171.2007.—Obesity is increasing at an alarming rate, and its related disorders are placing a considerable strain on our healthcare system. Although they are not always coincident, obesity is often accompanied by hyperlipidemia. Both obesity and hyperlipidemia are independently associated with atherosclerosis, nonalcoholic fatty liver disease (NAFLD), and insulin resistance (IR). Thus, we sought to determine the relative contributions of obesity and hyperlipidemia to these associated pathologies. Obese agouti (A/a) mice and their littermate controls (a/a) were placed on an LDL receptor (LDLR)−/− background. At 4 mo of age, mice were either maintained on chow diet (CD) or placed on Western diet (WD) for 12 wk. These genetic and dietary manipulations yielded four experimental groups: 1) lean, a/a;LDLR−/−;CD; 2) genetic-induced obesity (GIO), A/a;LDLR−/−;CD; 3) diet-induced obesity (DIO), a/a;LDLR−/−;WD; and 4) genetic-plus diet-induced obesity (GIO/DIO), A/a;LDLR−/−;WD. Lipoprotein profiles revealed increased VLDL and LDL particles in WD-fed mice compared with CD-fed controls. The hyperlipidemia present in this mouse model was the result of both increased hepatic triglyceride production and delayed lipoprotein clearance from the plasma. Both WD-fed groups exhibited similar levels of atherosclerotic lesion area, with increased obesity in the GIO/DIO group having no impact on atherogenesis. However, the severe obesity in the GIO/DIO group did aggravate NAFLD and IR. These findings suggest that, although obesity and hyperlipidemia exert individual pathological effects, the combination of the two has the potential to exert an additive effect on NAFLD and IR but not atherosclerosis in this mouse model.

Obesity is a growing worldwide epidemic and is linked to development of hyperlipidemia, insulin resistance (IR), and hypertension (19, 21). When coincident, these disorders form what is referred to as the metabolic syndrome (19). The presence of the metabolic syndrome greatly increases the risk for development of diseases such as atherosclerosis, nonalcoholic fatty liver disease (NAFLD), and diabetes (5, 15, 19, 21, 29). Furthermore, obesity is independently associated with increased risk of these diseases (6, 16, 22). Thus, it is imperative to discern the relative contributions of obesity and hyperlipidemia to these diseases and, in the process, to develop models that encompass multiple aspects of the metabolic syndrome.

Traditionally, leptin-deficient (ob/ob) and leptin receptor-deficient (db/db) mice have been used for studies of obesity. Although the ob/ob and db/db mice have many advantages for obesity research, including studies of IR and NAFLD, their utility in studying atherosclerosis is limited. First, in contrast to what is often noted in human obesity, ob/ob and db/db mice have elevated HDL levels (9, 25, 26). In addition to the increased HDL, plasma VLDL and LDL levels of ob/ob and db/db mice do not increase upon high-fat diet feeding (our unpublished observations). Thus, ob/ob and db/db lipoprotein profiles, both on a chow and high-fat diet, are uncharacteristic of wild-type mice or of humans. Furthermore, the increased HDL levels and decreased VLDL and LDL levels cause ob/ob and db/db mice to be resistant to diet-induced atherosclerosis compared with lean controls (20).

In the current study, we sought to determine the relative contributions of obesity and hyperlipidemia to NAFLD, IR, and atherosclerotic lesion formation. To this end, we crossed yellow agouti (A/a) mice onto an LDL receptor (LDLR)−/− background (A/a;LDLR−/−). In A/a mice, the agouti protein is expressed ectopically and ubiquitously. In the brain, the agouti protein competes against the anorexigenic factor, α-MSH, for binding to the melanocortin-4 receptor, acting as an antagonist of this satiety signaling pathway (34). Thus, A/a mice display adult-onset obesity and IR (28), similar to what is seen in melanocortin-4 receptor-deficient mice (7, 12, 14). By crossing the A/a mice onto an LDLR−/− background, we developed a model that is susceptible to high-fat diet-induced obesity and hyperlipidemia. This model, therefore, provides a setting whereby the individual and combined effects of obesity and hyperlipidemia on atherosclerosis, NAFLD, and IR can be determined.

MATERIALS AND METHODS

Mice and diets. The A/a and LDLR−/− mice used in these studies were on the C57BL/6 background and were initially purchased from Jackson Laboratories (Bar Harbor, ME). The A/a;LDLR−/− mice were produced by intercrossing A/a and LDLR−/− mice. Four-month-old male and female mice were fed chow diet (CD; LabDiet, 12% of kcal from fat) or Western diet (WD; Harlan-Teklad, 42% of kcal from milk fat with 0.15% cholesterol added) ad libitum for 12 wk. Total body adipose tissue was determined by nuclear magnetic resonance using the Bruker Minispec (Woodlands, TX) at the Vanderbilt University Mouse Metabolic Phenotyping Center (MMPC). All animal care and experimental procedures were performed according to the regulations of and approved by the Institutional Animal Care and Usage Committee of Vanderbilt University.

Plasma lipid analyses. Mice were fasted for 5 h before being bled via the retroorbital venous plexus with the use of heparinized capillary collection tubes. Total plasma cholesterol (TC) and triglyceride (TG) levels were measured using enzymatic kits from Raichem (San Diego, CA), nonesterified fatty acids (NEFAs) were measured using the
NEFA C kit by Wako (Richmond, VA), and plasma glucose levels were determined using Lifescan’s OneTouch glucometer (Johnson & Johnson, Northridge, CA). Fast performance liquid chromatography was performed by separating 100 μl of plasma on a Superose 6 column (Amersham Biosciences) at a flow rate of 0.5 ml/min. Forty fractions of 0.5 ml were collected, and cholesterol and TG levels were measured in fractions 11–40. VLDL particles elute in fractions 15–18, LDL in fractions 19–24, and HDL in fractions 28–32.

**Hepatic TG production rate.** Tyloxapol (hereafter referred to as Triton), purchased from Sigma (St. Louis, MO), was diluted to 0.25 g/ml. Mice were fasted overnight and then bled via the retroorbital venous plexus for baseline TG analysis. The mice were then injected with Triton at a concentration of 500 mg/kg body wt. Subsequent blood collections occurred at 1 and 2 h after Triton injection, and plasma was isolated for TG analysis.

**Lipoprotein clearance.** VLDL (d < 1.019 g/l) was prepared by serial ultracentrifugation from plasma of fasted genetic-induced obesity (GIO)/diet-induced obesity (DIO) mice. The lipoproteins were labeled with 125I-NaI (IMS30; Amersham Biosciences), using iodination tubes according to the manufacturer’s instructions (Pierce, Rockford, IL). Labeled lipoproteins had a specific activity of 186 cpm/μg. Recipient lean, GIO, and DIO/GIO mice were injected with 12 μg of protein via the retroorbital plexus. Immediately after 125I-VLDL injection, and at 5 min, 15 min, 30 min, and 1 h postinjection, blood was collected and plasma isolated via centrifugation. Radioactive counts were measured from plasma samples, and clearance was determined as a percentage of the injected counts.

**Atherosclerotic lesion area.** Frozen hearts were sectioned at the aortic root, and oil red O (ORO) was used to stain neutral lipids. Lesion area was quantified using Kinetic Histometrix version 6 imaging and analysis software by Kinetic Imaging (Durham, NC). Images were captured using a Q-Imaging MicroPublisher camera mounted on an Olympus upright microscope. There were no differences in lesion area between male and female mice. Consequently, data for both sexes have been combined. En face analyses were performed as previously described (3, 27).

**Characterization of NAFLD.** Frozen liver sections were cut and stained with hematoxylin and orcein for 3 min, and images were captured at ×20 magnification using a Q-Imaging MicroPublisher camera mounted on an Olympus upright microscope. Fatty acid composition of livers was analyzed using gas chromatography (GC) as previously described (23).

**Hyperinsulinemic euglycemic clamps in conscious, unrestrained mice.** Clamps were performed in the MMPC at Vanderbilt University. Following anesthetization with pentobarbital, the right jugular vein and the left common carotid artery were catheterized for infusions and arterial blood sampling, respectively. The catheters were exteriorized and sealed with stainless steel plugs. The mice were housed individually following the surgery for a 5-day recovery period. Conscious, unrestrained mice were fasted for 5 h before the start of the insulin infusion. Two hours prior to the start of the experiment, the catheters were connected to tubing to allow connections to the infusion syringes as well as for access for arterial blood sampling. After this point, the mice were allowed to move freely and were not handled. A continuous infusion of d-[3-3H]glucose (0.05 μCi/min; NEN no. NET331C) was given during the 2-h acclimation period to estimate basal glucose turnover. After this 2-h period, a continuous infusion of 4 μU·kg⁻¹·min⁻¹ insulin (Humulin, Eli Lilly) was given for 2 h along with the aforementioned tracer at 0.1 μCi/min. The mice received continuous blood infusion from donor mice at 4 μl/min. Euglycemia (plasma glucose between 120 and 140 mg/dl) was maintained by a variable infusion of 50% glucose. Blood was collected 10 min before the start of the insulin infusion and was taken every 10 min after the start of the insulin infusion to analyze glucose (HemoCue, Mission Viejo, CA) as well as other plasma markers. Hematocrit was assessed before and after the 2-h insulin infusion. Rate of glucose disposal (Rg) was calculated by dividing the tracer infusion rate by the specific activity of the plasma glucose. The insulin sensitivity index was calculated by dividing Rg by steady-state clamp insulin levels.

**Statistical analyses.** Statistical analyses were performed using Graphpad PRISM (San Diego, CA). Data are presented as the mean ± SE and were considered significant at P < 0.05 upon ANOVA analysis with a Tukey post hoc test.

**RESULTS**

Gradation in obesity, adiposity, and plasma lipids in CD- and WD-fed A/a;LDLR⁻/⁻ mice. Hyperlipidemia-prone LDLR-deficient (LDLR⁻/⁻) mice were crossed with obesity-prone lethal yellow agouti (A/a) mice. Four-month-old A/a; LDRLR⁻/⁻ mice and their littermate controls (a/a;LDLR⁻/⁻) were fed either a low-fat CD or a high-fat WD for 12 wk. These combinations resulted in four experimental groups: 1) a/a;LDLR⁻/⁻ mice fed CD (lean); 2) A/a;LDLR⁻/⁻ mice fed CD (GIO); 3) a/a;LDLR⁻/⁻ mice fed WD (DIO); and 4) A/a;LDLR⁻/⁻ mice fed WD (GIO/DIO).

Irrespective of diet, A/a;LDLR⁻/⁻ mice weighed more than a/a;LDLR⁻/⁻ mice. The GIO and DIO groups exhibited 1.5- and 1.3-fold increases, respectively, in body weight compared with the lean group (P < 0.001; Table 1), whereas the combination of genetics and diet resulted in a significantly higher body weight in the GIO/DIO group (1.9-fold increase compared with the lean group, P < 0.001). Nuclear magnetic resonance analyses revealed that the weight gain seen in the mice could be defined by a significant gain in total fat mass, with dramatic five-, three-, and eightfold increases in the GIO, DIO, and GIO/DIO groups, respectively, compared with the lean group (P < 0.001; Table 1). Similar fold increases were seen in the abdominal fat pad masses when the mice were killed (P < 0.001; Table 1).

Plasma lipid levels were measured after 12 wk of diet treatment. WD feeding resulted in a significant increase in TC levels, as evidenced by five- and eightfold elevations in DIO and GIO/DIO groups, respectively, compared with the lean group (P < 0.001; Table 1). The WD feeding resulted in elevations in TG levels in the GIO/DIO group (6-fold higher increase compared with lean, P < 0.001; Table 1); however,

Table 1. Body composition and plasma parameters of agouti;LDLR-deficient mice

<table>
<thead>
<tr>
<th></th>
<th>Body Weight, g</th>
<th>Total Fat Mass, g</th>
<th>Fat Pad Mass, g</th>
<th>Cholesterol, mg/dl</th>
<th>Triglycerides, mg/dl</th>
<th>NEFA, mEq/l</th>
<th>Glucose, mg/dl</th>
<th>Insulin, ng/ml</th>
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<tbody>
<tr>
<td>Lean</td>
<td>23 ± 1</td>
<td>2.6 ± 0.1</td>
<td>0.31 ± 0.03</td>
<td>191 ± 5</td>
<td>58 ± 6</td>
<td>0.42 ± 0.03</td>
<td>131 ± 7</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>GIO</td>
<td>34 ± 1a</td>
<td>12.2 ± 0.7a</td>
<td>1.70 ± 0.09a</td>
<td>286 ± 17</td>
<td>154 ± 15</td>
<td>0.54 ± 0.03</td>
<td>150 ± 6</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>DIO</td>
<td>29 ± 1b</td>
<td>8.6 ± 0.8b</td>
<td>1.05 ± 0.12b</td>
<td>1,023 ± 67a</td>
<td>331 ± 36a</td>
<td>0.95 ± 0.10a</td>
<td>122 ± 5d</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>GIO/DIO</td>
<td>45 ± 1c</td>
<td>20.8 ± 0.3c</td>
<td>2.86 ± 0.10c</td>
<td>1,496 ± 29b</td>
<td>816 ± 42a</td>
<td>1.53 ± 0.06b</td>
<td>108 ± 6</td>
<td>7.7 ± 0.9</td>
</tr>
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Data are presented as means ± SE and are from 26 to 34 mice/group. LDLR, LDL receptor; NEFA, nonesterified fatty acids; GIO, genetic-induced obesity; DIO, diet-induced obesity. Body composition and plasma parameters of male and female mice were measured after 12 wk on chow diet or Western diet. a,b,c P < 0.001 vs. all other groups; *P < 0.01 vs. GIO; †P < 0.001 vs. lean and GIO.
this same diet resulted in a dramatic 14-fold elevation in TG levels in the GIO/DIO group compared with the lean group (P < 0.001; Table 1). NEFA levels were increased in WD-fed mice in a manner similar to TC levels (P < 0.001; Table 1). Thus, these combinations of diet and genetics produce a gradation of obesity and hyperlipidemia in which individual and combined effects of the two on atherosclerosis, NAFLD, and insulin resistance could be evaluated.

**Lipoprotein profiles in CD- and WD-fed mice.** Plasma samples collected 12 wk postdiet were used for fast performance liquid chromatography analysis. Cholesterol measured from the individual fractions revealed similar trends in WD-fed mice, with robust increases in VLDL and LDL particles compared with the CD-fed groups (Fig. 1A). There were no differences in HDL levels between the four experimental groups. TG levels were also measured in individual fractions and displayed a distinct difference in the amount of TGs present on VLDL particles from CD- and WD-fed mice (Fig. 1B). In addition, there was a significant increase in TGs present on VLDL particles from GIO/DIO mice compared with DIO mice.

**Hepatic TG production and lipoprotein clearance.** To determine whether increased lipoprotein production was one mechanism by which plasma lipid levels were elevated in the GIO/DIO mice, Triton studies were performed (Fig. 2A). Although there were no significant differences between the lean, GIO, and DIO groups (112 ± 20, 124 ± 14, and 110 ± 11 mmol TG/h, respectively), the GIO/DIO group displayed a significant increase in the rate at which hepatic TGs were produced (183 ± 16 mmol TG/h, P < 0.05, compared with lean and DIO), suggesting that increased hepatic TG production contributes to the disproportionate increase in plasma TG levels seen in the GIO/DIO group.

Reduced lipoprotein clearance is another possible mechanism by which GIO/DIO mice could sustain increases in their plasma lipid levels. To determine lipoprotein clearance rates, VLDL (d < 0.019) was collected from WD-fed A/2a;LDLR−/− mice and radiolabeled with 125I. This 125I-VLDL was injected into mice from each of the four groups, and radioactive counts remaining in plasma were assessed immediately as well as at 5, 15, 30, and 60 min postinjection. All three obese groups exhibited significantly higher amounts of radiolabel in their plasma at each time point compared with the lean group, suggesting delayed VLDL clearance by the liver (P < 0.05; Fig. 2B). In fact, the GIO/DIO group showed significantly delayed VLDL clearance from the plasma compared with both the lean and GIO groups at 5, 15, 30, and 60 min postinjection (P < 0.01) and a trend toward delayed lipoprotein clearance compared with the DIO group. Together, these data show that the hyperlipidemia present in the GIO/DIO group is the result of both increased lipoprotein production and delayed lipoprotein clearance.

**Atherosclerosis in agouti LDLR−/− mice.** To determine whether obesity potentiates the known atherogenic impact of hyperlipidemia, we quantified lesion area in the aortic root as well as en face preparations of the arch, thoracic, and abdominal aorta. ORO staining of the aortic root revealed that mice fed WD had significantly greater lesion area compared with the mice fed CD (P < 0.001). There were no significant differences in mean lesion area between DIO and GIO/DIO groups (Fig. 3). Furthermore, there were no significant differences in the lesion area found in the arch, thoracic, or abdominal segments of the aorta between DIO and GIO/DIO groups (Fig. 4).

**NAFLD in WD-fed mice.** To determine the relative effects of obesity and hyperlipidemia on NAFLD in GIO/DIO mice, hepatic TG levels were quantified (Fig. 5). ORO staining of neutral lipids revealed increased lipid accumulation in the groups fed WD compared with the groups fed CD (Fig. 5, C and D, compared with A and B). These observations were confirmed with quantitative lipid analyses of the liver using GC (Fig. 5E). There was no significant difference between the lean and GIO groups (both on CD); however, the WD-fed DIO and GIO/DIO mice had significantly greater amounts of total hepatic TGs compared with the CD-fed groups (P < 0.001). In addition, the GIO/DIO group displayed a significantly greater amount of total TGs in the liver compared with the DIO group (P < 0.01).

**Fatty acid composition of liver and adipose tissue TGs.** Further characterization of specific fatty acids present on TGs in the liver of WD-fed mice revealed a significant decrease in polyunsaturated fatty acids (PUFAs) and an increase in monounsaturated fatty acids (MUFAs) compared with CD-fed mice (Fig. 6, A and B). A similar composition of fatty acids was detected in TGs stored in white adipose tissue (WAT) between the four groups (Fig. 6, C and D). Longer-chain (n-3) PUFAs were detected in livers of CD- but not WD-fed mice in minor amounts (~5% of total fatty acids; data not shown).

**Effects of obesity and hyperlipidemia on insulin sensitivity.** Glucose levels were not elevated in any of the groups (Table 1), indicating that the mice were not overtly diabetic; however, the GIO/DIO group exhibited significant increases in fasting
plasma insulin levels, suggesting the presence of IR. To determine the degree of insulin sensitivity, hyperinsulinemic-euglycemic clamps were performed in conscious mice. When challenged with a constant infusion of insulin, the GIO/DIO group displayed a significantly decreased \( R_t \) compared with the lean group \((P < 0.05; \text{Fig. 7A})\). The GIO and DIO groups also exhibited a decrease in \( R_t \), but the difference was not significant. Although insulin infusion rates were identical between the four groups, the obese groups (GIO, DIO, and GIO/DIO) displayed increased steady-state clamp insulin levels, suggesting that insulin clearance was also impaired in these mice (Fig. 7B). To normalize the data for the prevailing insulin levels, the insulin sensitivity index was calculated by dividing \( R_t \) by steady-state clamp insulin levels (Fig. 7C). These data showed not only the dramatic peripheral IR seen in the severely obese and hyperlipidemic GIO/DIO group but also revealed the significant IR present in the GIO and DIO groups. Because of the increased plasma insulin levels seen in the three obese groups during the clamp, the impact on hepatic insulin resistance is difficult to discern.

**DISCUSSION**

We have previously reported that \( A^\mathrm{Y/a}\);LDLR\(^{-/-}\) mice develop mild obesity and hyperlipidemia on CD compared with \( a/a\);LDLR\(^{-/-}\) controls. However, when the mice are placed on WD, both obesity and hyperlipidemia are potentiated (4). In the current study, we utilized this model to determine the relative impact of obesity and hyperlipidemia on the development of atherosclerosis, NAFLD, and IR. Surprisingly, the GIO/DIO group did not have increased lesion area compared with the DIO mice despite their mild increase in plasma TC levels, their

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**Fig. 2.** A: lipoprotein production and clearance. Hepatic TG production rates were measured using the Triton assay as described in MATERIALS AND METHODS. Plasma TG levels at 0, 1, and 2 h are shown. Data are means ± SE from 4 to 6 mice/group. *\( P < 0.05 \) vs. lean and DIO. B: VLDL was collected from GIO/DIO mice, radiolabeled with \( ^{125}\)I, and used for clearance studies as described in MATERIALS AND METHODS. Blood was collected immediately after injection as well as 5, 15, 30, and 60 min postinjection. Counts remaining in plasma at each time point were divided by the originally injected dose. Data are means ± SE from 2 to 6 mice/group. &\( P < 0.05 \) vs. all; *\( P < 0.05 \) vs. lean; *\( P < 0.01 \) vs. lean; #\( P < 0.01 \) vs. lean and GIO. Symbols for groups are as follows: ○, lean; □, GIO; ●, DIO; ▲, GIO/DIO.

**Fig. 3.** Atherosclerotic lesion area in the aortic root. Hearts were collected from mice after 12 wk of CD or WD feeding, embedded in OCT, and frozen. Fifteen 10-μm sections were collected from the aortic root and stained with oil red O (ORO), and lesions were quantified using computer assisted densitometric analysis. Representative ORO-stained images are shown on left [lean (A), GIO (B), DIO (C), and GIO/DIO (D)]. E: mean lesion area ± SE for mice are shown for 19–24 mice/group. The DIO and GIO/DIO groups displayed significantly greater lesion areas \((P < 0.001 \) vs. lean and GIO groups). Symbols for groups are as follows: ○, lean; □, GIO; ●, DIO; ▲, GIO/DIO.
dramatic increase in plasma TG levels, and their increase in obesity. In contrast, the most obese GIO/DIO mice did exhibit the greatest degree of NAFLD and were the least insulin sensitive. Thus, in this mouse model, the combined presence of obesity and hyperlipidemia appeared to aggravate NAFLD and IR, but not atherogenesis; however, the significant increase in obesity in the GIO/DIO group cannot be ruled out as the major contributor to the perturbations seen in this group.

It is particularly interesting to compare the metabolic parameters of the GIO and DIO mice. Whereas body weight, total fat mass, and abdominal fat pad mass were relatively similar between these two groups, plasma and tissue lipids were dramatically increased in the DIO mice. This increase is likely due to the high fat content of the WD; yet, by comparing these two groups, the relative impact of obesity and hyperlipidemia on lipoprotein metabolism, atherosclerosis, NAFLD, and IR can be discussed. First, lipoprotein profiles in the DIO mice reflect a large increase in plasma VLDL and LDL compared with the GIO mice (Fig. 1). Second, hepatic TG production rates did not seem to be impacted by the hyperlipidemia, whereas VLDL clearance was delayed in the hyperlipidemic DIO mice (Fig. 2). Thus, prevailing hyperlipidemia appears to impact lipoprotein clearance to a greater extent than obesity in this model system. Third, obesity per se had no impact on atherosclerotic lesion formation, whereas hyperlipidemia was absolutely required (Fig. 3). Fourth, NAFLD was evident only in the WD-fed DIO mice (Fig. 5). Finally, IR appeared to be associated with the level of obesity, and the increased hyperlipidemia in the DIO group did not impact insulin sensitivity beyond what was seen in the GIO mice (Fig. 7). Taken together, a comparison of the GIO and DIO groups reveal that lipoprotein clearance defects, atherosclerosis, and NAFLD are aggravated by hyperlipidemia, whereas insulin sensitivity is impacted primarily by the extent of obesity.

There are many aspects of the GIO/DIO mice that make them a good model of the metabolic syndrome. They have delayed onset obesity, which is increased by high-fat diet feeding, and in contrast to ob/ob and db/db mice their obesity is associated with mild elevations in plasma leptin levels (4). These mice also develop hyperlipidemia characterized by dramatic increases in TG levels that are not seen in DIO mice. Finally, they exhibit IR, as evidenced by elevated plasma insulin levels and quantified by hyperinsulinemic-euglycemic clamps. Thus, these mice display most of the major features of the metabolic syndrome. In addition, they have increased adipose tissue-specific and systemic inflammation as well as decreased plasma adiponectin levels, as described in a previous report from our laboratory (4), all of which could be considered

Fig. 4. En face lesion area. Aortas were collected from the DIO and GIO/DIO groups and were pinned and stained with Sudan IV. Lesion area was quantified using computer-assisted densitometric analysis. Lesion area percentages for the arch (A), thoracic (B), and abdominal (C) sections of the aorta were measured separately (11–25 mice/group). Data are shown as mean %total lesion area ± SE. Symbols for groups are as follows: •, DIO; ●, GIO/DIO.

Fig. 5. Hepatic lipid content. Livers from lean (A), GIO (B), DIO (C), and GIO/DIO mice (D) were frozen, sectioned, and stained with ORO. Images are shown at ×20 magnification. E: gas chromatography (GC) analysis was performed to determine hepatic TG content. Data are means ± SE from 6 mice/group. *P < 0.001 vs. lean and GIO; ^P < 0.001 vs. lean and GIO, P < 0.01 vs. DIO. Study groups are indicated below graph.
novel markers of the metabolic syndrome. With regard to diseases that are associated with the metabolic syndrome, these mice develop atherosclerosis and NAFLD. Consequently, these mice will be useful for future studies of the metabolic syndrome.

The synergistic effect of genetically prone obesity and WD feeding on plasma TG levels (Table 1 and Fig. 1) is important to note. Our metabolic studies demonstrate that the hypertriglyceridemia is due to both increased hepatic TG synthesis and decreased hepatic VLDL clearance (Fig. 2). It is thought that one mechanism whereby obesity increases plasma TG levels is via incorporation of adipose tissue-derived fatty acids into newly synthesized lipoprotein particles in the liver (2). This hypothesis would suggest that the increase in hepatic TG production seen in the GIO/DIO mice is likely due to increased flux of NEFAs derived from lipolysis of adipose tissue TGs. It is also possible that the TG production is a reflection of insulin sensitivity in the animals. Indeed, both the GIO and DIO mice had similar plasma insulin levels, whereas the GIO/DIO mice had dramatic elevations in their plasma insulin levels, which could explain our observation that hepatic TG production was elevated only in the GIO/DIO mice. With regard to lipoprotein clearance, it is possible that the increased adipose tissue mass and increased TG accumulation in liver contribute to delayed hepatic VLDL clearance. Thus, the combination of obesity and hyperlipidemia creates a vicious cycle in which increased adipose tissue mass influences both hepatic lipoprotein production and clearance, resulting in increased plasma TG levels.

In addition to accumulation of TGs in plasma, high-fat diet consumption also influences the deposition of TGs in hepatocytes, leading to NAFLD. Because A/aj mice are known to be hyperphagic (18), the increased consumption of dietary fat most likely contributed to the elevated hepatic TG levels in the GIO/DIO mice (Fig. 5). Furthermore, dietary fatty acid composition can influence the specific type of fatty acids stored in the body (17). Hepatic fatty acid composition appears to play a pivotal role in the development of NAFLD and IR, with decreases in (n-3) PUFAs and increases in MUFAs found in

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**Fig. 7.** Hyperinsulinemic-euglycemic clamps. Hyperinsulinemic-euglycemic clamps were performed on conscious mice as described in MATERIALS AND METHODS. A: glucose disposal rate (Rg) was calculated by dividing the tracer infusion rate by the specific activity of the plasma. B: steady-state clamp plasma insulin levels were determined. C: insulin sensitivity index (M/I) was calculated by dividing Rg by steady-state clamp insulin levels. Data are expressed as means ± SE from 4 to 5 mice/group. #P < 0.001 vs. all; *P < 0.05 vs. lean; ^P < 0.001 vs. lean. Study groups are indicated below graphs.
hepatic TGs being the most detrimental (1, 15, 29, 30). Similar to these reports, the WD-fed mice in our study exhibited decreased (n-6) PUFA s and increased (n-3) MUFA s in hepatic TGs (Fig. 6 and data not shown). It is interesting to note that the fatty acid composition of hepatic TGs mirrors that found in adipose tissue (compare Fig. 6, A and B, with C and D). It has been shown that, in addition to the diet, NEFAs found in the liver can also originate from adipose tissue (15). Further studies are needed to determine the importance of WAT fatty acid composition to NAFLD and IR.

Although the increase in hepatic and plasma TG levels likely contributed to lipoprotein metabolism defects and to the decreased insulin sensitivity seen in GIO/DIO mice, they did not appear to influence atherosclerotic lesion formation. Despite the fact that, in humans, plasma TG levels correlate with risk for atherosclerotic disease (11), this association does not always hold true for mouse models (10). Although elevated plasma TC and TG are required to initiate lesion formation, as seen when comparing the normolipidemic lean and GIO mice to the DIO and GIO/DIO mice, further increases in plasma TGs do not seem to impact the progression of atherosclerotic lesion formation (compare DIO and GIO/DIO in Fig. 3) in this model system.

Macrophages are a key cell type in the development and progression of atherosclerosis (8, 13). Monocytes migrate through the endothelium into the intima of the arterial wall where they differentiate into macrophages and accumulate lipids, being transformed into macrophage foam cells. Their contribution to atherosclerosis involves both lipid accumulation and the release of inflammatory cytokines. Recently, a role of macrophages in IR has also been shown (33). Monocyte migration into WAT is increased in obesity and is temporally associated with both inflammation and IR (24, 31–33). Using the same model as in the current study, we previously demonstrated that obesity, but not hyperlipidemia, increases macrophage infiltration into WAT (4). Thus, taken together, our two studies show that diet-induced obesity is the driving force for macrophage infiltration into WAT, whereas hyperlipidemia is the driving force for macrophage infiltration of the artery wall. Future studies will be required to determine whether the population of macrophages found in the artery wall is physiologically different from the population of macrophages found in the adipose tissue and to characterize the stimuli that influence their migration to their respective locations.

In conclusion, our current studies show that, although obesity and hyperlipidemia can exert individual pathophysiological effects related to the metabolic syndrome, obesity has the capability to exert more potent effects on NAFLD and IR, but not atherosclerosis, in this mouse model. In addition, the GIO/DIO mouse model used in this study proves to be a useful model of the metabolic syndrome because it encompasses many disorders characteristic of the metabolic syndrome, such as obesity, hypertriglyceridemia, IR, NAFLD, and atherosclerosis.

GRANTS

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