Distinct metabolic and vascular effects of dietary triglycerides and cholesterol in atherosclerotic and diabetic mouse models

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Submitted 7 March 2013; accepted in final form 26 June 2013

THE WESTERN-STYLE DIET rich in saturated triglycerides and cholesterol is commonly used as a reference of poor dietary habits leading to the development of obesity, type 2 diabetes (T2D), and vascular diseases. This diet has been adapted for mouse studies on obesity and is used to worsen the development of atherosclerosis in genetically altered murine models such as the low-density lipoprotein (LDLr) receptor-deficient mouse (14, 15).

While dietary triglycerides or dietary cholesterol are often used in combination, each one has its own distinctive effects on vascular and metabolic disorders. Current evidence suggests that dietary cholesterol is more important than triglycerides for plaque formation. Indeed, cholesterol at >0.15% in a low-fat diet could worsen lesion size (27). Dietary triglycerides, despite promoting insulin resistance, also made little difference in aortic root lesion size and only in very old mice (31). On the other hand, the diabetogenic potential of dietary cholesterol is less clear. A cholesterol-rich diet did not affect glucose tolerance in a LDLr deficient mouse (11). However, studies in pancreatic cell lines and isolated pancreatic islets showed that hypercholesterolemia may impair β-cell function and insulin response to glucose (2, 5, 10). In humans, the link between atherosclerosis and metabolic disorders is of particular interest. T2D morbidity and mortality strongly correlates with coronary artery disease. However, the development of atherosclerosis associated with familial and nonfamilial hypercholesterolemia has been suggested to be independent from insulin resistance (1, 7, 21).

The rationale of the present study was to clarify the metabolic effects of both dietary triglycerides and cholesterol and assess whether or not diabetes could worsen atherosclerosis in three different murine models: wild-type C57BL6 (C57), atherosclerotic LDLr–/– ApoB100/100 (LRKOB100) and atherosclerotic/diabetic IGF-II × LDLr–/– ApoB100/100 (LRKOB100/IGF) mice. Each group was fed either a standard chow diet, a 0.2% cholesterol diet, a high-fat diet (HFD), or a high-fat 0.2% cholesterol diet for 6 mo. The triglyceride-rich HFD increased body weight, glucose intolerance, and insulin resistance but did not alter endothelial function or atherosclerotic plaque formation. Dietary cholesterol, however, increased plaque formation in LRKOB100 and LRKOB100/IGF animals and decreased endothelial function regardless of genotype. However, cholesterol was not associated with an increase of insulin resistance in LRKOB100 and LRKOB100/IGF mice and, unexpectedly, was even found to reduce insulin-resistant effects of a high-fat diet in all mouse models. Strikingly, we found that dietary cholesterol reduces plaque formation in the LRKOB100 and LRKOB100/IGF models. Strikingly, we found that dietary cholesterol reduces the insulin-resistant effects of a high-fat diet in all mouse models. Our results also demonstrate that insulin resistance and...
T2D per se are not primary factors causing atherosclerosis and endothelial dysfunction in obese hypercholesterolemic mice.

MATERIALS AND METHODS

Animals. Animal handling was conducted according to the Laval University Animal Care Committee guidelines. Studies were performed in accordance with the Canadian Guide for the Care and Use of Laboratory Animals and were approved by the Laval University Animal Care Committee. Male mice were housed in a pathogen-free, temperature-controlled environment under a 12:12-h light-dark cycle and fed ad libitum either a standard chow diet (SD; Harlan Teklad T-2018), a cholesterol diet (CD; 0.2% cholesterol; Harlan Teklad TD-07798), a high-fat diet (HFD, 55% kcal from fat; Harlan Teklad TD-93075), or a high-fat 0.2% cholesterol diet (HFCD; Harlan Teklad TD-07799) starting at 6 wk of age. Both LRKO100 and LRKO100/IGF-1 mice were generated from original founders, kindly provided by Drs. Seppo Ylä-Herttuala and Markku Laakso (Kuopio University, Kuopio, Finland) and backcrossed on a C57B1/6J background from Jackson Laboratories (Bar Harbor, ME). Age-matched C57B1/6J mice were used as a control. The addition of cholesterol did not substantially change the caloric content of the diets (see Supplemental Tables 1 and 2 for diet compositions. Supplemental data for this article may be found at http://andremarette.com/projects/ccd-diets-for-ajp-paper-2013-laplante-et-al).

Lipid and lipoprotein measurement. Plasma lipids were measured following a 6-h fast with a 2-h refeeding period between 4:00 A.M. and 6:00 A.M. Enzymatic kits were used for measuring triglyceride, cholesterol (Randox Laboratories, Kearneysville, WV), and nonesterified fatty acid (Wako Diagnostics, Richmond, VA). Apolipoprotein B and apolipoprotein AI were quantified from fasting plasma samples by immunoblot (antibodies from Midland Bioproducts, Boone, IA).

Plasma lipoprotein fast protein liquid chromatography. Equal volumes of plasma from three to four mice of the same genotype and diet were filtered through a 0.45-μm filter and 200 μl aliquots were injected on a Superose 6 100/300 GL column. Lipoproteins were eluted using Tris buffer each 15 min. Three different cumulative dose-response curves were tested: phenylephrine, phenylephrine precontraction at 10 μM, and set on two vessel holders (Harvard Apparatus, Montreal, QC) in an oxygenated bath at 37°C on a force transducer with an initial tension of 1.5 g. A 1-h resting period was applied with changes of buffer every 15 min. Three different cumulative dose-response curves were tested: phenylephrine, phenylephrine precontraction at 10−6 M with carbachol, and phenylephrine precontraction with sodium nitroprusside (SNP).

En face lesion analysis. En face preparation was performed following the protocol published by the Animal Models of Diabetic Complications Consortium (www.diacom.org). Images of vessels were taken, and the percentage atherosclerotic lesions area positive for Sudan IV was quantified using ImagePro software (Media Cybernetics, Bethesda, MD).

Data and statistical analysis. Hepatic glucose production and Rf during the clamp were determined using Mari’s non-steady-state equations for a two-compartmental model (4). Data are presented as means ± SE. A P < 0.05 was considered significant. Two-way ANOVA followed by Tukey-Kramer post hoc test was performed.

Table 1. Metabolic phenotype of C57 mice following 6 mo on different diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>CD</th>
<th>HFD</th>
<th>HFCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>34.0 ± 0.6a</td>
<td>38.6 ± 1.0b</td>
<td>42.9 ± 1.2b</td>
</tr>
<tr>
<td>Body wt change, g</td>
<td>15.9 ± 0.9c</td>
<td>20.4 ± 1.1c</td>
<td>24.6 ± 1.2c</td>
</tr>
<tr>
<td>Energy intake, kcal/day</td>
<td>11.70 ± 0.1c</td>
<td>11.50 ± 0.26b</td>
<td>14.79 ± 0.22b</td>
</tr>
<tr>
<td>Liver wt, g</td>
<td>1.30 ± 0.05b</td>
<td>1.85 ± 0.10c</td>
<td>2.40 ± 0.23b</td>
</tr>
<tr>
<td>eWAT wt, g</td>
<td>1.24 ± 0.15b</td>
<td>1.87 ± 0.22b</td>
<td>3.25 ± 0.27a</td>
</tr>
<tr>
<td>Gastrocnemius wt, mg</td>
<td>188 ± 9g</td>
<td>200 ± 6i</td>
<td>181 ± 5b</td>
</tr>
<tr>
<td>Pancreas wt, mg</td>
<td>184 ± 9h</td>
<td>197 ± 11h</td>
<td>215 ± 9g</td>
</tr>
<tr>
<td>Fasting glycaemia, mEq/l</td>
<td>9.65 ± 0.47b</td>
<td>11.12 ± 0.38b</td>
<td>11.28 ± 0.37h</td>
</tr>
<tr>
<td>Fasting insulin, mg/ml</td>
<td>0.84 ± 0.13b</td>
<td>1.34 ± 0.32b</td>
<td>3.00 ± 0.58a</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = 10–22 mice/group. SD, standard chow diet; CD, 0.2% cholesterol diet; HFD, high-fat diet; HFCD, high-fat 0.2% cholesterol diet; eWAT, epididymal adipose tissue. Body weights were recorded before death. Plasma for glucose or insulin measurement was isolated from blood drawn after a 5-h fast. Organ weights were taken after death following a 5-h fast. Food intake represents an average of kcal/day measured over the course of 6 mo. Groups not sharing the same letter are considered significantly different when comparing effects of both diet × genotype among the different groups. Symbols represent differences between diet groups (*), differences between genotypes (†), and differences between diet × genotype (¶).
using JMP 9.0 software (SAS Institute, Cary, NC). Groups not sharing the same letter are considered statistically different.

**RESULTS**

**Metabolic phenotypes of LRKOB100 and LRKOB100/IGF mice.** To better understand the effect of obesity-linked insulin resistance and diabetes on the development of cardiovascular pathologies, 6-wk-old LRKOB100 and LRKOB100/IGF mice were fed either low-fat SD, atherogenic CD, obesogenic HFD, or atherogenic/obesogenic HFCD for 24 wk after which their phenotypes were assessed (Tables 1–3). Regardless of genotype, mice fed the HFD or HFCD consumed more calories and gained more weight compared with mice of the same genotype fed a SD diet. Within each genotype, liver mass was also increased by CD, HFD, or HFCD, whereas there were no relevant changes in gastrocnemius muscle or pancreas mass. C57 mice also gained a significant amount of weight on the CD compared with C57 mice on the SD despite these two diets being isocaloric. Even though weight gain was seen in all groups, both LRKOB100 and LRKOB100/IGF groups fed CD, HFD, or HFCD weighed slightly less compared with the C57 group fed the same diet.

In C57 mice, fasting insulinemia was increased in response to HFD and HFCD. These dietary interventions had a lesser impact on the glycemia and insulinemia of LRKOB100 animals, which is coherent with the lessened weight gain observed in these groups. LRKOB100/IGF animals were hyperinsulinemic regardless of diet, and a HFD resulted in a significant increase in fasting glycemia. This hyperglycemic state failed to promote further insulin production, suggesting a lack of β-cell pancreatic response, an expected outcome of this model of T2D (6). Interestingly, fasting hyperglycemia was not observed in HFCD, suggesting that adding cholesterol in the diet prevented the development of diabetes independent from changes in insulinemia.

As expected, deletion of the LDL receptor increased both plasma cholesterol and apoB100 in LRKOB100 and LRKOB100/IGF mice compared with C57 controls, an effect that was observed even in animals fed the SD (Fig. 1, B and E). In addition to the genotype effect, there was an additive and significant diet effect on plasma cholesterol. High-fat feeding (HFD or HFCD) increased circulating cholesterol in both LRKOB100 and LRKOB100/IGF mice. We were able to confirm by fast protein liquid chromatography that the majority of circulating cholesterol in LRKOB100 and LRKOB100/IGF mice was found in the LDL fraction while the majority of cholesterol in C57 mice was found in the HDL fraction (Fig. 1F). On the other hand, there were no significant differences in circulating apoA1 levels between any of the groups (Fig. 1D). HFD and HFCD increased plasma triglycerides significantly in the LRKOB100 and LRKOB100/IGF mice (Fig. 1C). HFD and HFCD also induced an accumulation of triglyceride in the LDL fraction that was also shifted over a lower-density range for both LRKOB100 and LRKOB100/IGF mice (Fig. 1G).

**Impact of diets on glucose intolerance in genetic mouse models.** We performed IPGTT to assess the impact of the genotype and diets on glucose homeostasis (Fig. 2A). As expected, C57 mice on CD, HFD, or HFCD were more glucose intolerant than

### Table 2. Metabolic phenotype of LRKOB100 mice following 6 mo on different diets

<table>
<thead>
<tr>
<th></th>
<th>LRKOB100</th>
<th>SD</th>
<th>CD</th>
<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g*n,†</td>
<td>33.6 ± 0.8c</td>
<td>35.6 ± 0.6de</td>
<td>39.3 ± 0.9c</td>
<td>39.2 ± 0.8c</td>
</tr>
<tr>
<td>Liver wt, g*n,†</td>
<td>15.3 ± 0.7a</td>
<td>17.0 ± 0.9b</td>
<td>21.0 ± 0.8c</td>
<td>21.0 ± 0.7c</td>
</tr>
<tr>
<td>Fasting glycemia, mM*</td>
<td>11.54 ± 0.7c</td>
<td>11.58 ± 0.7c</td>
<td>14.83 ± 0.10b</td>
<td>14.88 ± 0.14b</td>
</tr>
<tr>
<td>Pancreas wt, mg*</td>
<td>1.21 ± 0.03d</td>
<td>1.65 ± 0.09e</td>
<td>1.82 ± 0.10b</td>
<td>2.45 ± 0.15b</td>
</tr>
<tr>
<td>Gastrocnemius wt, mg*</td>
<td>9.48 ± 0.38b</td>
<td>10.38 ± 0.37b</td>
<td>10.41 ± 0.49b</td>
<td>10.34 ± 0.38b</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = 10–22 mice/group. Body weights were recorded before death. Plasma for glucose or insulin measurement was isolated from blood drawn following a 5-h fast. Organ weights were taken at death following a 5-h fast. Food intake represents an average of kcal/day measured over the course of 6 mo. Groups not sharing the same letter are considered significantly different when comparing effects of both diet × genotype among the different groups. Symbols represent differences between diet groups (*), differences between genotypes (†), and differences between diet × genotype (¶).

### Table 3. Metabolic phenotype of LRKOB100/IGF mice following 6 mo on different diets

<table>
<thead>
<tr>
<th></th>
<th>LRKOB100/IGF</th>
<th>SD</th>
<th>CD</th>
<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g*n,†</td>
<td>33.1 ± 0.9e</td>
<td>37.3 ± 1.01de</td>
<td>40.0 ± 1.6c</td>
<td>38.8 ± 0.7b</td>
</tr>
<tr>
<td>Liver wt, g*n,†</td>
<td>15.0 ± 0.9e</td>
<td>19.2 ± 1.1c</td>
<td>21.8 ± 1.5b</td>
<td>20.4 ± 0.8c</td>
</tr>
<tr>
<td>Fasting glycemia, mM*</td>
<td>11.46 ± 0.1c</td>
<td>11.38 ± 0.2c</td>
<td>14.86 ± 0.04b</td>
<td>14.69 ± 0.12b</td>
</tr>
<tr>
<td>Pancreas wt, mg*</td>
<td>1.32 ± 0.05c</td>
<td>1.89 ± 0.13c</td>
<td>1.90 ± 0.14c</td>
<td>2.44 ± 0.17c</td>
</tr>
<tr>
<td>Gastrocnemius wt, mg*</td>
<td>0.76 ± 0.09e</td>
<td>1.21 ± 0.12c</td>
<td>1.65 ± 0.19c</td>
<td>1.62 ± 0.08c</td>
</tr>
<tr>
<td>Fasting insulin, ng/mL</td>
<td>178 ± 3.8e</td>
<td>167 ± 6.8b</td>
<td>170 ± 6.8b</td>
<td>168 ± 6.8b</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = 10–22 mice/group. Body weights were recorded before death. Plasma for glucose or insulin measurement was isolated from blood drawn following a 5-h fast. Organ weights were taken at death following a 5-h fast. Food intake represents an average of kcal/day measured over the course of 6 mo. Groups not sharing the same letter are considered significantly different when comparing effects of both diet × genotype among the different groups. Symbols represent differences between diet groups (*), differences between genotypes (†), and differences between diet × genotype (¶).
their SD-fed counterparts. The diets did not exacerbate glucose intolerance to the same extent in LRKOB100 mice as the C57 genotype. The relative protection from glucose intolerance in the LRKOB100 mice may be explained by the lesser weight gain in this genotype (Table 1). However, LRKOB100/IGF mice under HFD displayed glucose intolerance (Fig. 2A).

Worthy of note, the addition of cholesterol to the HFD (HFCD) reduced, to a great extent, glucose intolerance (Fig. 2A and B), which is consistent with the prevention of fasting hyperglycemia in this group (Table 1).

**HFD but not CD or HFCD decreases insulin sensitivity in LRKOB100 and LRKOB100/IGF mice.** To exclude the influence of defective pancreatic responses to glucose challenge, we evaluated whole body insulin sensitivity by performing HIIC studies. In C57 mice, CD decreased the glucose infusion rate (GIR) compared with SD, whereas HFD severely impaired insulin sensitivity as seen by a fourfold decrease in GIR relative to SD (Fig. 3A). Comparing across genotypes fed SD, the LRKOB100 and LRKOB100/IGF mice had a lower GIR compared with C57 mice, suggesting that dyslipidemic mice already have compromised insulin sensitivity even in the absence of dietary intervention. Surprisingly, the CD and the HFCD improved insulin sensitivity in both LRKOB100 and LRKOB100/IGF mice. In C57 mice, the insulin resistance caused by HFD was also partly prevented by a concomitant cholesterol input in the HFCD, but the mice remained insulin-resistant compared with SD-fed controls. This effect of dietary cholesterol on insulin sensitivity was explained by a lower
basal (Fig. 3B) and insulin-mediated suppression of hepatic glucose production (Fig. 3, C and D) and an increase in insulin-stimulated glucose uptake by peripheral tissues (Fig. 3, F and G). Conversely, the HFD was found to reduce GIR (Fig. 3A), increase hepatic glucose production (Fig. 3C), and decrease the rate of peripheral glucose uptake (Fig. 3F) compared with the CD and HFCD in all genotypes. Overall, these data indicate that dietary cholesterol can exert some protective effects on hepatic and peripheral insulin resistance but only when administered in mice with compromised insulin sensitivity.

Adipose tissue inflammation. Mice from all genotypes fed HFD or HFCD showed increased macrophage infiltration in their adipose tissues compared with mice fed SD or CD, as shown by the presence of crown-like structures (CLS) (Fig. 4, A and B). Feeding CD, HFD, or HFCD increased several chemokines/cytokines [monocyte chemoattractant protein-1, regulated on activation, normal T cell expressed and secreted (RANTES), IL-1β, IL-6, interferon-γ inducible protein 10 (IP-10)] in eWAT of C57 mice, suggesting an effect on adipose tissue inflammation (Fig. 4, C–G). We also observed a further increase in the diabetic LRKOB100/IGF mouse model independent of obesity or CLS number. In LRKOB100/IGF mice on CD and HFCD, there was a tendency toward a decrease in RANTES, IL-1β, and IP-10 compared with the HFD group of the same genotype; however, this failed to attain statistical significance. This may be related to the improvement of insulin resistance previously observed (Fig. 3).

Dietary cholesterol exacerbates aortic plaque formation and impairs endothelial function. We then assessed atherosclerosis to examine the influence of the insulin-resistant/diabetic state on vascular complications. As expected, C57 mice failed to develop atherosclerosis regardless of the diet (Fig. 5, A and C). The LRKOB100 and LRKOB100/IGF groups developed atherosclerotic plaques on SD, and there was a trend of increased lesion area with the CD and HFCD in LRKOB100 mice. In contrast, relative to SD, the HFD did not exacerbate plaque area in either LRKOB100 or LRKOB100/IGF mice despite their overt glucose intolerance. Therefore, a dyslipidemic genotype and dietary cholesterol additively promoted atherogenesis in these mouse models, whereas a triglyceride-rich diet led to glucose intolerance but did not alone or in combination with cholesterol exacerbate the development of atherosclerotic plaques.

With regard to vascular reactivity, following an initial contraction with phenylephrine, there was a lessened maximal vasodilatory effect of carbacholine on aortic rings from mice fed the CD or HFCD compared with HFD or SD. Genotype had no significant influence on endothelial function. C57 mice fed CD and HFCD developed endothelial dysfunction but without concomitant plaque formation, suggesting that the two phenomena are not necessarily linked together (compare Fig. 5, C and D). Indeed, endothelial dysfunction is often described as an early event that can develop in the absence of structural vascular or microvascular modification (3). In our models, endothelial dysfunction was associated with an increased plaque size only when combined with dyslipidemia in the LRKOB100 genetic background. SNP responses were not significantly different among groups, showing that the response to nitric oxide or the mechanical properties of the vessel wall were not altered by the genotypes or diets (Fig. 6).

**DISCUSSION**

We have explored the contributions of dietary cholesterol, triglycerides, and of insulin resistance/diabetes on the development of atherosclerosis using genetic and dietary approaches. Our work discriminated between the effects of either triglycerides or cholesterol and is the first to establish a detailed glucose homeostasis and insulin sensitivity profile in atherogenic mouse models fed these diets. Of interest, we documented that, whereas a HFD is accompanied by insulin resistance/diabetes, it does not exacerbate the level of plaque area in atherogenic mouse models. Conversely, a cholesterol-rich diet reduced insulin resistance in HFD-fed animals and exacerbated atherosclerosis. Hence, these findings highlight a differential effect of the cholesterol vs. the triglyceride-rich diet on insulin resistance and atherosclerosis. Furthermore, this study has brought to light a novel and intriguing finding. The development of lipid-rich atherosclerotic plaques within the aorta is not related to the insulin-resistant state in atherosclerosis-prone mouse models.

The LDLr<sup>−/−</sup> mouse presents a resistance to diet-induced metabolic alterations. In this regard, CD, HFD, and HFCD caused glucose intolerance in C57 and LRKOB100/IGF, but this was less pronounced in the LRKOB100 mice. These mice also gained less weight in response to dietary intervention. Metabolic differences such as lower epididymal adipose tissue weight, lower body weight, and improved glucose tolerance under a HFD were previously reported in LDLr<sup>−/−</sup> mice and could at least be partially explained by an enhanced thermogenesis, but this observation on weight gain was not reproduced in one study with diets richer in carbohydrates, suggesting complex genetic-dietary interactions (20, 24). Despite the lack of increased plasma glucose in LDLr<sup>−/−</sup> mice following prolonged high-cholesterol feeding, a study showed increased plasma insulin hinting at the existence of insulin resistance in these animals independent of weight gain (11). Genetic deletions of the LDL receptor and ApoB48 therefore appear to have an overall metabolic impact, preventing weight gain but also increasing insulin resistance, an effect that was observed through our clamp studies.

To the best of our knowledge, this is the first study to document insulin resistance in the LRKOB100 model relative to C57 mice. This insulin resistance could be a consequence of elevated circulating triglycerides since we previously have shown a strong correlation between elevated levels of circulating lipids and insulin resistance (4). Previous studies also found that circulating cholesterol had deleterious hepatic effects in LDLr<sup>−/−</sup> mice and may increase endoplasmic reticulum stress that could explain the occurrence of insulin resis-
 spite even in the face of a low-fat diet (9, 26). This is especially important considering that the LDLr−/− is a model often used in metabolic studies.

Our clamp studies further documented that dietary cholesterol promotes insulin resistance in C57 mice. However, we also made the surprising observation that insulin sensitivity was improved by dietary cholesterol in both LRKOB100 and LRKOB100/IGF mice even upon feeding a HFD. The molecular mechanisms underlying this effect of dietary cholesterol in mice lacking the LDL receptor remains to be clarified, but our clamp studies indicate that both a decreased hepatic glucose output and an increased peripheral tissue glucose uptake contribute to this effect. Cholesterol derivatives have important signaling properties in the liver through the liver X receptor and may be involved in this unexpected improvement of insulin sensitivity (13). The suppression of the lecithin cholesterol acyltransferase gene in LDLr−/− animals led to increased circulating free cholesterol and improved hepatic insulin signaling (18). A lack of functional LDL receptors has previously been reported to protect peripheral tissues against ectopic accumulation of cholesterol and the lipotoxicity resulting from cholesterol accumulation (17). A similar peripheral tissue protection may have occurred in LRKOB100 and LRKOB100/IGF groups. However, some insulin-sensitizing effect of dietary cholesterol was also seen in high-fat-fed C57 mice, suggesting that this protective effect is not simply related to the lack of LDL receptor in the atherogenic models. In this regard, our finding of reduced adipose tissue inflammation in LRKOB100/IGF mice fed CD and HFCD suggests that dietary cholesterol may also improve insulin sensitivity by dampening obesity-linked inflammation.

Previous studies have reported that human familial hypercholesterolemia is not associated with significant alterations in whole body glucose uptake, glucose oxidation, or lipid oxidation, arguing against a deleterious effect of cholesterol on glucose metabolism (7, 16). Our observations should also be considered in the context of recent evidence from the JUPITER trial that showed that statins increased the occurrence of T2D despite improvements in cholesterolemia and atherosclerosis (23). In fine, if dietary cholesterol is known to promote arterial lesions in mice, its metabolic effects are likely to be more complex and even perhaps beneficial in certain circumstances of already compromised insulin resistance.

Another interesting finding of our study is that we could clearly dissociate the level of insulin resistance (as measured by clamp studies) from the development of atherosclerosis in an atherosclerosis-prone model. Indeed, dietary cholesterol clearly impaired endothelial function and promoted plaque formation despite its metabolic benefits in LRKOB100 and LRKOB100/IGF mice. Moreover, LRKOB100/IGF mice showed no alteration in plaque formation or endothelial function above that of LRKOB100 regardless of the impact of diet on glucose intolerance and insulin resistance. These data are in line with Merat et al. (19) who showed that a normoinsulinemic fructose-fed LDLr−/− mouse developed significantly greater atherosclerosis than a hyperinsulinemic and glucose-intolerant Western diet-fed LDLr−/− mouse. They are also consistent with the report that dietary fat cannot alter plaque formation unless the lipoprotein profile was made more atherogenic (31).

A recent study has also shown that hyperinsulinemia did not affect plaque formation in ApoE−/− mice, another genetic model of atherosclerosis (22). However, previous studies could not exclude the possibility that hyperglycemia and T2D were actually required to further increase plaque formation, vascular function, and atherosclerosis pathology. Our data using diabetic LRKOB100/IGF mice address this limitation of previous studies and argue against the role of insulin resistance and diabetes in promoting the development of lipid-rich atherosclerotic plaques. However, we should be cautious in inferring that diabetes does not impact the development of atherosclerosis. The present study investigated the presence of lipid-rich atherosclerotic plaques by the en face technique and did not investigate plaque biology or stability. In this regard, other effects of diabetes on plaque composition were not measured and cannot be excluded. For instance, increased mineralization of blood vessels is a hallmark of insulin resistance/diabetes in both humans and animal models (12).

One intriguing observation is that, even though circulating cholesterol levels were barely affected by dietary cholesterol, increased endothelial dysfunction was still evident in mice fed cholesterol regardless of genotype. Surprisingly, this was true even in C57 mice in the absence of plaque formation. Zilversmit (32) extensively discussed the possibility that chylomicrons loaded with cholesterol from the diet may have important atherogenic properties. Chylomicrons are the main carrier of cholesterol postprandially, and the first stage of their catabolism takes place on endothelial cells where uptake by the arterial wall can occur (25). The interactions between oxidized chylomicron remnants and endothelial cells were reported to be deleterious to endothelial cell function measured by carbachol-mediated vasorelaxation (8). At least one other recent study confirmed that addition of cholesterol to the diet only contributes marginally to the total circulating amount of cholesterol but has important effects for atherosclerotic plaque formation and that blocking intestinal cholesterol absorption could alleviate these effects (11, 28). This could explain why cholesterol derived specifically from dietary origin can have such important vascular consequences, even before plaque formation is visible. Finally, it cannot be ruled out that the distinct metabolic impact of the HFD and HFCD are somewhat linked to the overwhelming genetic effects on the lipid profiles in the LDLRKO100 models. Future work is also needed to determine whether the metabolic effect of cholesterol in those models can be linked to regulation at the level of the central nervous system.

In summary, dietary triglycerides promote insulin resistance and glucose tolerance but have marginal effect on atheroscler-
Fig. 5. Assessment of vascular pathologies in C57, LRKO100, and LRKO100/IGF mice following 24 wk on SD, CD, HFD, or HFCD. A: quantification of atherosclerotic plaque by the en face method. Representative aortas for atherosclerotic plaque formation were stained with Sudan IV, n = 5–7. B and C: carbacholine vasorelaxation for aortic rings (B) and quantification of atherosclerotic plaques in percent surface area (C). D: the maximal vasorelaxations are displayed in a separate graphic for comparison, n = 7 animals/group. Groups not sharing the same letter are considered statistically different when comparing effects of both diet × genotype among the different groups.
rosis. Dietary cholesterol also induces metabolic impairments in C57 mice but remarkably improves insulin resistance in high-fat-fed insulin-resistant animals. This dual action of cholesterol on metabolism may be linked to adipose tissue inflammation, but further studies will be required to determine whether such inflammation promotes endothelial dysfunction before the onset of measurable atherosclerosis or whether these two events proceed concurrently. We conclude that dietary triglycerides and cholesterol have distinct metabolic and vascular consequences in obese atherogenic mouse models. We also found a clear dissociation between the impairment of glucose homeostasis and the development of atherosclerosis and endothelial function in these mouse models, suggesting that diabetes per se does not play a dominant role in the development of lipid-rich atherosclerotic plaques.

ACKNOWLEDGMENTS

We thank Christine Dion and Kim Denault (Centre de Recherche de l’Institut Universitaire de Cardiologie et Pneumologie de Québec) for help with animal procedures and Dr. Dominic Ng (St. Michael’s Hospital/Research Institute, University of Toronto) for critical reading of this manuscript.

GRANTS

This work was supported by a grant (no. 161971 to A. Marette) from the Canadian Institutes of Health Research (CIHR) and a CIHR/Pfizer Research Chair to A. Marette in the pathogenesis of insulin resistance and cardiovascular diseases. M.-A. Laplante was supported by the Heart and Stroke Foundation of Canada.

DISCLOSURES

André Marette is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS


Fig. 6. A: effect of a nitric oxide donor (SNP) on aortic vasorelaxation. B: the maximal vasorelaxations are displayed in a separate graphic for comparison, n = 7 animals/group. Groups not sharing the same letter are considered statistically different when comparing effects of both diet x genotype among the different groups.
REFERENCES