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Overexpression of apolipoprotein A5 in mice is not protective against body weight gain and aberrant glucose homeostasis

Nathalie Pamir[a], Timothy S. McMillen[a], Yu-I Li[a], Ching-Mei Lai[b], Howard Wong[b], Renée C. LeBoeuf[a,*]

[a]*Department of Medicine, Division of Metabolism, Endocrinology and Nutrition, University of Washington, Seattle, WA, USA*
[b]*Department of Medicine, University of California at Los Angeles and The Lipid Research Laboratory, Veterans Administration Greater Los Angeles Healthcare System, Los Angeles, CA, USA*

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Abstract

Apolipoprotein A5 (APOA5) is expressed primarily in the liver and modulates plasma triglyceride levels in mice and humans. Mice overexpressing APOA5 exhibit reduced plasma triglyceride levels. Because there is a tight association between plasma triglyceride concentration and traits of the metabolic syndrome, we used transgenic mice overexpressing human APOA5 to test the concept that these mice would be protected from diet-induced obesity and insulin resistance. Male and female transgenic and wild-type mice on the FVB/N genetic background were fed standard rodent chow or a diet rich in fat and sucrose for 18 weeks, during which time clinical phenotypes associated with obesity and glucose homeostasis were measured. We found that APOA5 transgenic (A5tg) mice were resistant to diet-induced changes in plasma triglyceride but not total cholesterol levels. Body weights were similar between the genotypes for females and males, although male A5tg mice showed a modest but significant increase in the relative size of inguinal fat pads. Although male A5tg mice showed a significantly increased ratio of plasma glucose to insulin, profiles of glucose clearance as evaluated after injections of glucose or insulin failed to reveal any differences between genotypes. Overall, our data showed that there was no advantage to responses to diet-induced obesity with chronic reduction of plasma triglyceride levels as mediated by overexpression of APOA5.

1. Introduction

Hypertriglyceridemia is a feature of the metabolic syndrome [1,2] and is frequently associated with obesity, insulin resistance [2-4], and atherosclerosis [5]. The transition from insulin sensitivity to insulin resistance is progressive and is thought to be due in large part to increased fatty acid uptake and accumulation into specific tissues such as muscle, adipose, and liver that result in aberrant whole-body glucose homeostasis. Furthermore, elevations in circulating triglyceride (TG) levels have been directly related to leptin resistance in brain, suggesting that TG levels have a direct bearing on appetite control [6]. Thus, modulating circulating TG levels is a potential therapeutic avenue that could be used to ameliorate features of the metabolic syndrome.

Apolipoprotein A5 (APOA5) is secreted primarily from the liver as a 366–amino acid protein associated with high-density lipoprotein (HDL). Apolipoprotein A5 was originally identified as a new apolipoprotein [7] and as an important factor required for liver regeneration [8]. Postprandially, APOA5 also associates with very low-density lipoprotein (VLDL) [9]. Evidence establishing that APOA5 is an important determinant of plasma TG levels is 2-fold: (1) epidemiologic studies show a strong association between alleles of *APOA5* and plasma TG levels [10-15], and (2) altering the levels of APOA5 protein using genetic engineering or adenoviral expression leads to significant changes in plasma lipid levels [7-9,16-18]. Mice and humans [19] deficient in APOA5 have markedly elevated TG levels, and overexpression of APOA5 leads to marked decreases in plasma TG concentrations. Apolipoprotein A5 can reduce TG levels by decreasing hepatic production of VLDL TG [9,17,18,20], increasing lipoprotein lipase lipolysis of lipoprotein TG [16-18], and increasing lipoprotein uptake

* Corresponding author. Tel.: +206 543 5208; fax: +206 685 3662.
E-mail address: leboeuf@u.washington.edu (R.C. LeBoeuf).

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by the liver [16]. Thus, APOA5 contributes to whole-body flux of free fatty acids.

In humans, specific alleles of *APOA5* not only alter plasma lipid levels but exhibit significant interactions with diet that are related to body mass index (BMI) and the risk for obesity [4]. When participants of the Framingham Offspring Study were studied, a strong gene-diet interaction was observed between the *APOA5* −1131T>C polymorphism and dietary fat that was predictive of the extent of obesity. Data were significant even in a multivariate model that included adjustments for sex and plasma TG levels. These data suggest that APOA5 plays an important role in modulating body composition, further pointing to the importance of continued studies of APOA5 in humans and model systems such as the mouse.

Overall, APOA5 represents a potential therapeutic target for modulation of plasma TG levels and obesity. Of particular interest to us is whether chronic reduction of circulating TG levels protects against the development of adiposity and insulin resistance. We used mice overexpressing human APOA5 [7] to test the hypothesis that chronically reduced plasma TG levels predispose to reduced adiposity and improved insulin sensitivity after a high-fat–diet challenge. We report, for the first time, responses to high-fat and sucrose feeding for APOA5 transgenic (A5tg) mice and show that reduced TG levels do not protect these mice from obesity and changes in glucose homeostasis associated with body weight gain.

2. Materials and methods

2.1. Mice

Apolipoprotein A5 transgenic mice on the FVB/NJ background (The Jackson Laboratories, Bar Harbor, ME) were obtained as a generous gift from Drs Len A Pennacchio and Edward M Rubin (Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA), and their development using a human bacteria artificial chromosomes and singular expression in the liver has been described [7]. Isogenic FVB/NJ mice were purchased from The Jackson Laboratory as described [7]. All animals were maintained in a specific pathogen-free animal facility at the University of Washington in a temperature-controlled room (25°C) with a fixed 12-hour light/dark cycle. Mice had free access to food and water.

2.2. Experimental design

Male and female mice were initially fed pelleted rodent chow (Wayne Rodent BLOX 8604; Teklad, Madison, WI) and randomly assigned to 1 of 2 diet groups: rodent chow or high-fat/high-sucrose (HFHS) diet. Rodent chow contained 4% fat (wt/wt), 24% protein, and 4.5% crude fiber. The HFHS diet (Bioserve No. F1850, Frenchtown, NJ) contained 35.5% fat (primarily lard), 20% protein, and 36.6% carbohydrate (primarily sucrose) as described previously [21-23]. At 8 to 10 weeks of age, diet groups were initiated; and diets were fed for 18 weeks. During weeks 14 to 16, mice were subjected to glucose tolerance and insulin sensitivity tests. At the end of the study, mice were fasted for 4 hours in the morning, bled from the retroorbital sinus into tubes containing 1 mmol/L EDTA, and killed by cervical dislocation; and tissues were collected for analyses. Plasma and tissues were stored at −80°C until analyses. All procedures were done in accordance with current National Institutes of Health guidelines and approved by the Animal Care and Use Committee of the University of Washington.

2.3. Analytical procedures

Blood glucose levels were measured with a portable glucose-measuring device (Accu-Chek Advantage, Nutley, NJ). Plasma insulin levels were determined using a commercial enzyme-linked immunosorbent assay kit (catalog no. EZRMI-13K; Linco Research, St Charles, MO) using rat insulin as a standard. Plasma total cholesterol (TC) levels were determined using a colorimetric kit (Diagnostic Chemicals, Oxford, CT) with cholesterol standards (catalog no. c0532; Sigma Chemical, St Louis, MO). Plasma TG levels were determined colorimetrically after the removal of free glycerol (Trig/GB Kit 450032; Roche Diagnostics, Indianapolis, IN). Plasma lipoproteins were separated by fast-performance liquid chromatography gel filtration using a Superose 6 column (GE Healthcare, Piscataway, NJ). A 150-μL aliquot of plasma from pools of 4 mice for each sex and sex group was analyzed at a flow rate of 0.2 mL/min using phosphate-buffered saline (PBS). Afterward, 100-μL aliquots from each 0.5-mL fraction were used for total lipid determinations.

2.4. Glucose tolerance test

Mice were fasted overnight (18 hours), and glucose was injected intraperitoneally at a dose of 1 g glucose per kilogram body weight. Blood glucose was monitored before glucose injection and at 15, 30, 60, and 120 minutes after injection [24].

2.5. Insulin sensitivity assay

Mice were fasted overnight and injected intraperitoneally with 0.1 U/mL Humulin R insulin (Eli Lilly, Indianapolis, IN) in sterile PBS at a dose of 0.5 U insulin per kilogram body weight. Blood glucose was monitored before and at 5, 15, 30, 60, and 120 minutes after insulin injection as described [24].

2.6. Lipoprotein lipase

Adipose tissue samples from abdominal and inguinal depots were weighed and made 20% (wt/vol) in ice-cold buffer (10 mmol/L Tris-HCL [pH 8.0], containing 0.1% Triton X-100, 10% glycerol, and 10 U/mL heparin). Tissues were homogenized using a Polytron (Glen Mills, Clifton, NJ) (output setting, 40) for 1 minute on ice and then centrifuged for 10 minutes at 20 000*g*. The infranatant region above the

cell debris and below the floating fat layer was removed and stored at −80°C for further analyses. Neutral lipase activity was determined using radiolabeled triolein as outlined previously [25]. Briefly, phosphatidylcholine and triolein were sonicated together and then mixed with buffer containing albumin. Rat serum was added as a source of apolipoprotein C-II to maximize lipoprotein lipase activity. Infranatant samples were assayed in duplicate, were found to have equivalent activity levels compared with samples not previously frozen, and were linear with added protein.

2.7. Statistics

Values are reported as means ± SEM. Statistical differences were assessed using the SPSS program (SPSS, Chicago, IL). Groups were compared using analysis of variance, and Tukey post hoc tests were applied to determine differences between means. In some cases, the Student *t* test was used to compare independent means. *P* less than .05 was accepted as statistically significant.

3. Results

3.1. Plasma lipid and lipoprotein profiles for wild-type and A5tg mice

In rodents, insulin resistance is frequently induced by feeding high-fat diets. Furthermore, fructose feeding also leads to a stimulation of VLDL secretion and to obesity and insulin resistance [21,26-28]. Thus, it was important to establish that, for the human A5tg mice, plasma TG levels were maintained when mice were challenged with dietary fat and sucrose.

As seen previously [7,9,29], A5tg mice fed rodent chow had nearly 2-fold lower plasma TG levels than wild-type mice; and importantly for our study, levels did not change for the A5tg mice upon feeding the HFHS diet (Fig. 1A). In contrast to these results, plasma TC levels for A5tg mice were comparable with those of wild-type mice; and values increased significantly upon feeding the HFHS diet (Fig. 1B). Overall, A5tg mice were resistant to diet-induced changes in plasma TG but not TC levels.

There were several significant interactions between diet and sex ($P < .05$) or genotype ($P < .0003$). Among wild-type mice, male but not female mice showed a significant increase in plasma TG levels with HFHS diet feeding (Fig. 1A). Because A5tg showed no such change for plasma TG levels, there was also a significant genotype by diet interaction for plasma TG levels. For both A5tg and wild-type mice, plasma TC levels (Fig. 1B) increased significantly with feeding of the HFHS diet. However, male A5tg mice had higher TC values than females under both rodent chow and HFHS diet conditions. Overall, both sex and genotype interacted with diet changes that resulted in differential responses in plasma lipid levels.

To investigate the nature of the lipoprotein particles seen for A5tg mice, we compared the lipoprotein profiles for A5tg and wild-type mice fed the HFHS diet. Equal plasma aliquots were pooled from 4 mice in each group to create the profiles shown in Fig. 2. Cholesterol was mainly carried by particles within the HDL fraction, and levels were modestly reduced for A5tg mice (Fig. 2B). Triglyceride was dispersed among VLDL, intermediate-density lipoprotein, and low-density lipoprotein (LDL) fractions for wild-type mice (Fig. 2A). For A5tg mice, TG particles consisted primarily of VLDL and LDL; and the VLDL fraction was markedly reduced compared with wild-type mice. Overall, lipoprotein profiles showed several quantitative differences in lipoprotein fractions between A5tg and wild-type animals.

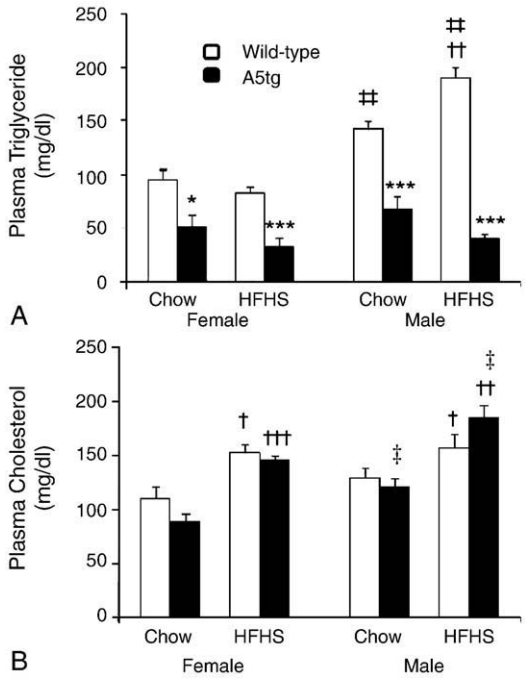

Fig. 1. Plasma total TG (A) and cholesterol (B) levels for mice fed rodent chow or the HFHS diet for 18 weeks. Wild-type (open bars) and A5tg (filled bars) mice were fasted for 4 hours in the morning before bleeding via the retroorbital sinus, and plasma lipids were quantified as described in "Materials and methods" using colorimetric kits purchased from commercial vendors. Plasma TG levels for A5tg mice were significantly lower than wild-type values for both sexes and diets. Male wild-type but not female mice showed increased TG levels with the HFHS diet. Data are presented as mean ± SE for 6 to 10 mice per group. Significance between genotypes: $^{*}P < .05$, $^{***}P < .0001$; between diets: $^{\dagger}P < .05$, $^{\dagger\dagger}P < .005$, $^{\dagger\dagger\dagger}P < .0001$; and between sexes: $^{\ddagger}P < .03$, $^{\ddagger\ddagger}P < .002$.

3.2. Body weight, fat pad size, and tissue lipase levels

Initial body weights were comparable between genotypes, ranging between 19 and 23 g for males and females. Final weights for mice fed rodent chow were not significantly different between the A5tg and wild-type genotypes (data not shown). For mice fed the HFHS diet, no differences were seen among females (29 ± 0.9 g and 30 ± 0.6 g for wild-type and A5tg mice, respectively). For the males fed HFHS, A5tg mice were 3 g heavier than wild-type mice, although differences were not significant (35 ± 1 g vs 38 ± 1 g for

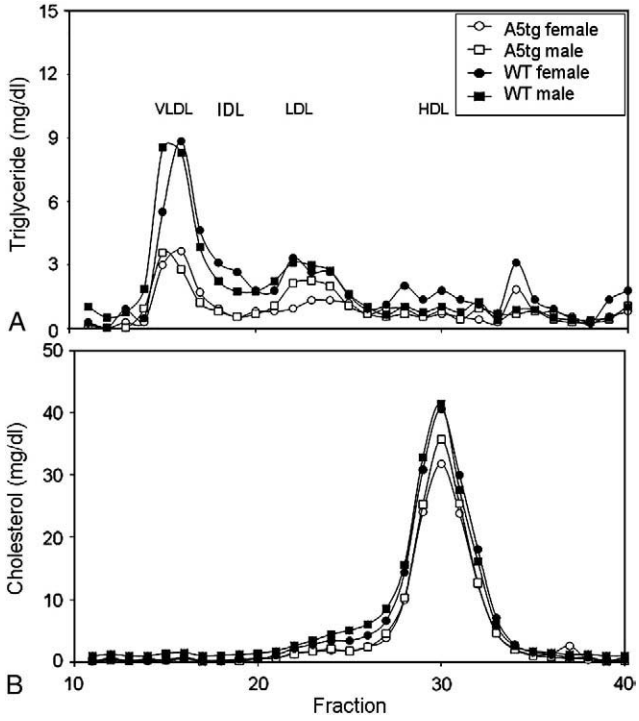

Fig. 2. Lipoprotein profiles for wild-type and A5tg mice fed the HFHS diet for 18 weeks. Equal plasma aliquots were pooled from 4 mice in each sex and sex group, and lipoproteins were separated using fast-performance liquid chromatography as described in "Materials and methods." Triglyceride (A) and cholesterol (B) contents are presented for male (square) and female (circle) mice of strains A5tg (open symbols) and FVB (filled symbols). The VLDL, intermediate-density lipoprotein, LDL, and HDL elution profiles are indicated. WT indicates wild-type mice.

wild-type and A5tg mice, respectively; $P = .06$; n = 11-12). The greater body weight gain for A5tg males was reflected in part by significant increases in relative inguinal (~20%, $P < .05$) (Fig. 3A) and brown adipose tissue (BAT) ($P < .05$, not shown) fat pad weights. The A5tg mice also showed a small but significant decrease in relative abdominal fat pad weight ($P < .05$). No differences in renal fat pad weights were seen between genotypes (data not shown).

Adipose tissues are the major storage sites for TG, primarily because of the rapid hydrolysis of circulating TG by lipoprotein lipase. Because APOA5 stimulates the activity of lipoprotein lipase [9], we tested whether changes in adipose tissue lipase activity contributed to the selective changes in size of fat pad as seen for male A5tg mice. Lipase activity was not different between genotypes but increased 2-fold with HFHS diet feeding in abdominal fat tissue (Fig. 3B). Thus, lipase activity did not mediate differences in adipose depot sizes between genotypes.

3.3. Assessment of glucose homeostasis

A role for APOA5 in glucose homeostasis has been indicated by others because polymorphisms in the human APOA5 locus are associated with altered glucose tolerance in humans [30]. In addition, mice deficient in APOA5 are hypertriglyceridemic and develop insulin resistance when fed high-fat diets [16]. Thus, at the beginning of our study, we hypothesized that A5tg mice fed an HFHS diet would show improved glucose homeostasis as compared with wild-type mice.

Glucose homeostasis was examined in several ways. We first compared plasma glucose and insulin levels taken after 4 hours of fasting and examined the ratio of glucose to insulin (G/I), which would be reduced for insulin-resistant mice. Table 1 shows that, overall, plasma glucose and insulin levels increased and the G/I decreased for mice fed the HFHS diet as compared with rodent chow. An exception to these changes was female A5tg mice that showed comparable insulin and G/I values for both diets. The most important observation is that male A5tg mice showed the highest levels of circulating glucose and insulin, and the G/I value reflected a requirement for higher insulin to maintain their glucose levels. These data suggest that no benefit to glucose homeostasis is seen with overexpression of APOA5, and this was actually detrimental in male mice.

To examine glucose homeostasis in more detail, we measured plasma glucose levels over time in response to injections of glucose (glucose tolerance) or insulin (insulin

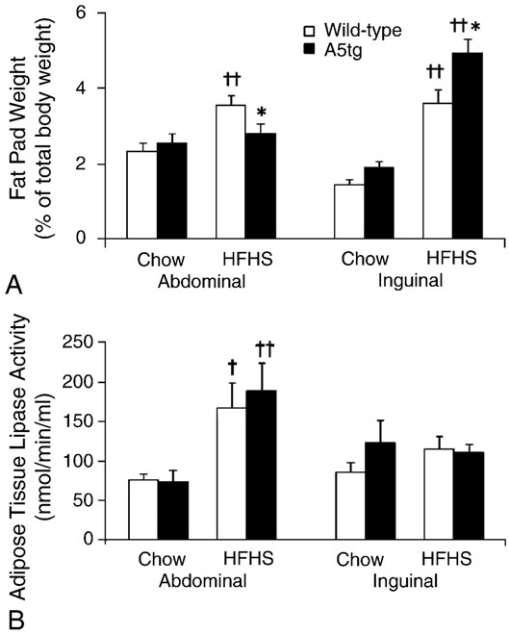

Fig. 3. Fat depot weights as a percentage of body weight (A) and lipase activity values (B) for male mice fed diets for 18 weeks. Wild-type (open bars) and A5tg (filled bars) mice were killed after 4 hours of fasting, and individual fat depots were removed and weighed. Adipose tissue fat pads are given as a percentage of total body weight. Adipose tissue lipase activity measurements were performed as described in "Materials and methods." Briefly, tissues were homogenized; and neutral lipase activity was determined using radiolabeled triolein and using rat serum as a source of apolipoprotein C-II as described in the text. Activity units are given as nanomoles free fatty acid hydrolyzed per minute per milliliter of lysate. Data are presented as mean ± SE for 6 to 13 mice per group. Significance between genotypes: $^{*}P < .05$; between diets: $^{\dagger}P < .025$, $^{\dagger\dagger}P < .002$.

Table 1
Plasma glucose and insulin levels for wild-type and A5tg mice fed rodent chow or HFHS diet for 18 weeks

	Genotype	Glucose (mg/mL)	Insulin (pg/mL)	Ratio (G/I)
Female				
Chow	WT	133 ± 10	0.64 ± 0.24	388 ± 95
Chow	A5tg	100 ± 12*	0.57 ± 0.10	186 ± 22*
HFHS	WT	151 ± 6	1.51 ± 0.33††	144 ± 30†
HFHS	A5tg	133 ± 7*,††	0.82 ± 0.18	194 ± 28
Male				
Chow	WT	138 ± 3	0.51 ± 0.05	280 ± 19
Chow	A5tg	155 ± 7*	0.56 ± 0.03	282 ± 23
HFHS	WT	158 ± 12	1.31 ± 0.33††	164 ± 30†
HFHS	A5tg	213 ± 10**,††	3.89 ± 1.06*,††	64 ± 13*,††

Data are presented as mean ± SE. WT indicates wild-type mice.
Significance between genotypes: *$P < .05$, **$P < .005$; between diets: $^{\dagger}P < .03$, $^{\dagger\dagger}P < .005$.

sensitivity). As shown in Fig. 4, except for a significant difference at 60 minutes between females, responses for these measures of glucose homeostasis were the same between A5tg and wild-type mice.

Overall, our data do not support a beneficial effect on body weight or glucose homeostasis after overexpression of APOA5 in mice.

4. Discussion

Metabolic syndrome is a risk factor for type 2 diabetes mellitus and cardiovascular diseases [31]. It is characterized by a collection of factors including hypertriglyceridemia, reduced HDL levels, obesity, and insulin resistance. The onset is progressive; and mechanisms for aberrant changes in lipoprotein profiles include increased rates of production of TG-rich lipoproteins from the intestine and liver, decreased hydrolysis of circulating TGs, alterations in specific concentrations of circulating apolipoproteins, and changes in gene expression in tissues responsible for the maintenance of lipid homeostasis [32-34]. We used the hypotriglyceridemic A5tg mouse strain to test the hypothesis that chronic reduction in circulating TGs would protect animals from diet-induced obesity and insulin resistance. Our data did not support this idea, as there was no improvement in body weight or glucose homeostasis accompanying sustained reductions in plasma TG levels. Sexual dimorphism was evident, as male A5tg mice showed worsened metabolic traits as compared with the wild-type mice.

When fed the HFHS diet, male A5tg showed a modest increase in body weight as compared with wild-type mice attributed to increases in inguinal and BAT fat pad weights. Furthermore, an assessment of G/I suggested that male A5tg mice were more glucose intolerant than wild-type mice. Of note is that all A5tg mice retained markedly reduced plasma TG levels with feeding of the HFHS diet, suggesting that plasma TG levels were not predictive of metabolic clinical outcomes among these mice. The results involving the males were surprising and demonstrate that an interaction exists between sex-based factors and expression of the *APOA5* transgene.

In humans, interactions between sex and *APOA5* polymorphisms have been difficult to discern. One report found an association between the *APOA5* promoter allele 1131C and postprandial TG levels that was more significant for males than females among a cohort of healthy participants in the United Kingdom [35]. Among males and females selected from a Czech population, another *APOA5* polymorphism (Val153>Met) was associated with plasma HDL cholesterol levels in females but not males [36]. In concert with apolipoprotein E alleles, the *APOA5* −1131C genotype was related to plasma lipid levels in females but not males [37]. In contrast to these reports, Corella et al [38] found no sex by genotype differences in outcome measures of BMI among individuals participating in the Framingham Heart Study with *APOA5* alleles (−1131T>C and 56C>G). Additional epidemiologic studies are still needed to investigate whether *APOA5* alleles, especially those affecting APOA5 plasma levels, combine with sex to influence outcomes of obesity and risk factors for heart disease.

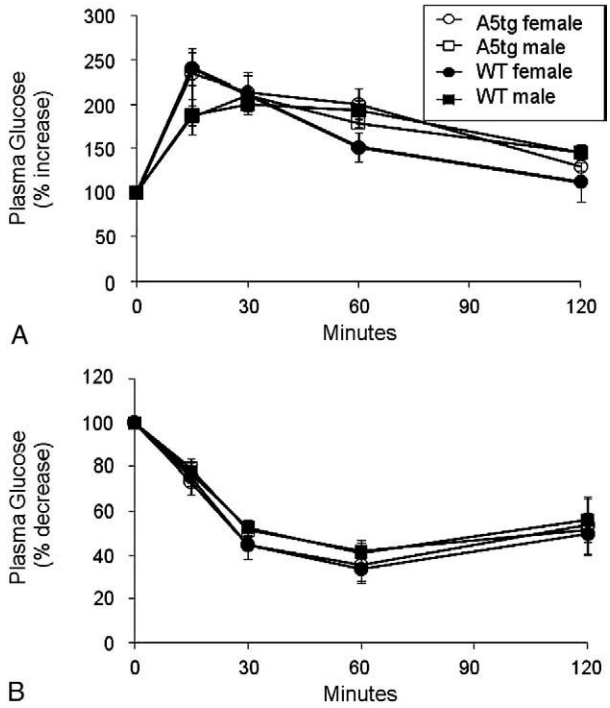

Fig. 4. Glucose tolerance (A) and insulin sensitivity (B) curves for mice fed the HFHS diet for 16 or 14 weeks, respectively. For glucose tolerance, mice were fasted overnight and gavaged with a 25% glucose solution (in sterile PBS) at a dose of 2 g glucose per kilogram body weight; and blood glucose was monitored over 120 minutes. For insulin sensitivity, mice were fasted overnight and injected intraperitoneally with 0.1 U/mL Humulin R insulin in sterile PBS at a dose of 0.5 U insulin per kilogram body weight. Wild-type (open symbols) and A5tg (filled symbols) mice show comparable responses between genotypes for both male (square) and female (circle) mice. Values are presented as percentage changes with respect to starting plasma glucose levels.

We also found interactions between APOA5 genotype (wild type vs transgenic) and diet that influenced body weight. These data are in concert with human studies by Corella et al [38] that showed association between the *APOA5* promoter allele −1131T>C and BMI among individuals of the Framingham Heart Study. Furthermore, Lai et al [39] showed interactions between type of dietary fat and the *APOA5* −1131T>C polymorphism such that higher n-6 (but not n-3) polyunsaturated fat diets increased TG levels and modified lipoprotein sizes, resulting in a more atherogenic lipid profile. Overall, multiple contributions of diet, fasting time, and population heterogeneity interact with *APOA5* alleles to alter outcomes of obesity, lipid levels, and heart disease risk factors.

Humans and mice differ in many regards; and in mice, sexual dimorphism in body fat and type 2 diabetes mellitus is known to occur more easily in males, which show greater disease severity in clinical traits than females [40-42]. For wild-type FVB mice fed the HFHS diet, plasma TG levels were higher for males than females; but body weights and levels of plasma glucose, insulin, and cholesterol were comparable between males and females. However, for A5tg mice, sexual dimorphic effects were magnified, as male A5tg mice showed more body weight gain and significant increase in relative inguinal fat pad weight and levels of glucose and insulin. There are other examples for which a genetic modification in genes involved in lipid metabolism or energy balance results in significant interactions with sex as described [43-45]. Thus, it is important to carefully assess the action of new genetic modifications using both sexes and, when possible, multiple genetic backgrounds [41].

Mechanisms by which sexual dimorphism interacts with the *APOA5* transgene are likely to be complex. Sexual dimorphism for leptin responsiveness has been reported for FVB mice [40]. Leptin is a hormone primarily secreted by adipocytes and is known to contribute to body weight regulation through signals to central nervous system that control food intake [46,47]. Reduced leptin levels are expected for mice with reduced insulin resistance [48]. We quantified plasma leptin levels at the end of the study and found essentially no differences between male leptin levels (23 ± 4.0 ng/mL vs 29 ± 2.3 ng/mL for wild-type and A5tg males, respectively), which suggest a lack of contribution through this axis. Alternatively, it is known that the liver, a key tissue mediating lipid and glucose metabolism, has sex-specific gene expression patterns for a variety of transcripts involved in determining fat mass and biotransformation enzymes [49-51]. In our mice, plasma TC levels were higher for males than females ($P < .03$). Because the liver is responsible in large part for lipoprotein production and clearance and is the site of APOA5 synthesis in wild-type mice and these transgenic mice [7], sex-specific factors influencing lipid metabolism in the liver may have contributed to final clinical outcomes.

In summary, since its identification in 2001 as a new apolipoprotein [7,8], APOA5 has generated much interest because of its role in modulating plasma TG levels. There are strong associations between elevated plasma TG levels, obesity, and insulin resistance; and so, we tested whether mice made hypotriglyceridemic by overexpression of human APOA5 would be protected against diet-induced obesity and insulin resistance. There was no improvement in body weight or glucose homeostasis accompanying sustained reductions in plasma TG levels. Sexual dimorphism was evident, as male A5tg mice showed worsened metabolic traits as compared with the wild-type mice. Overall, there was significant interaction between excessive APOA5 expression and sex and diet factors that resulted in lack of protection from obesity and glucose tolerance at least in male mice. These results suggest that caution should be used in considering APOA5 for therapeutic intervention strategies. However, it is difficult to extrapolate these findings directly to humans because major differences in lipid biology exist between these species.

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