Mice Heterozygous for \textit{Atp10c}, a Putative Amphipath, Represent a Novel Model of Obesity and Type 2 Diabetes$^{1,2}$

Madhu S. Dhar,$^3$ Carla S. Sommardahl,* Tanisa Kirkland,† Sarah Nelson,** Robert Donnell,* Dabney K. Johnson,** and Lawrence W. Castellani‡

Department of Nutrition, University of Tennessee, Knoxville, TN 37996; *University of Tennessee College of Veterinary Medicine, Knoxville, TN 37996; †Office of Biological and Environmental Research and **Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831; and ‡University of California, Los Angeles, CA 90095

ABSTRACT \textit{Atp10c} is a novel type IV P-type ATPase and is a putative phospholipid transporter. The purpose of this study was to assess the overall effect of the heterozygous deletion of \textit{Atp10c} on obesity-related phenotypes and metabolic abnormalities in mice fed a high-fat diet. Heterozygous mice with maternal inheritance of \textit{Atp10c} were compared with heterozygous mice with paternal inheritance of \textit{Atp10c} and wild-type controls. Body weight, adiposity index, and plasma insulin, leptin and triglyceride concentrations were significantly greater in the mutants inheriting the deletion maternally compared with their sex- and age-matched control male mice fed a 10% fat (% energy) diet and female mice fed a 45% fat (% energy) diet. Glucose and insulin tolerance tests were performed after mice consumed the diets for 4 and 8 wk. Mutants had altered glucose tolerance and insulin response compared with controls, suggesting insulin resistance in both sexes. Mice were killed at 12 wk and routine histological evaluations of the liver, pancreas, adipose tissue, and heart were performed. Histological evaluation showed micro- and macrovesicular lipid deposition within the hepatocytes that was more severe in the mutant mice than in age-matched controls. Although sex differences were observed, our data suggest that heterozygous deletion along with an unusual pattern of maternal inheritance of the chromosomal region containing the single gene, \textit{Atp10c}, causes obesity, type 2 diabetes, and nonalcoholic fatty liver disease in these mice. J. Nutr. 134: 799–805. 2004.

KEY WORDS: • mouse chromosome 7 • \textit{Atp10c}, type IV P-type ATPase • insulin resistance

Human obesity and its related disorders including type 2 diabetes, atherosclerosis, nonalcoholic fatty liver disease (NAFLD), and nonalcoholic steatohepatitis are influenced by genetic as well as environmental factors (nutritional and/or hormonal) that can more easily be studied in murine models and then translated into human homologs and phenotypes (1–4). Appropriate mouse models offer many advantages including the availability of inbred strains and genetically altered resources (5). Several polygenic rodent models of obesity and type 2 diabetes have been developed; the underlying genetic factors in these models have been studied by quantitative trait locus (QTL) mapping analysis, and several QTL associated with body weight, body fat, and type 2 diabetes phenotypes have been identified (6,7). These QTL are thought to more closely mimic the presumed polygenic inheritance of obesity and type 2 diabetes in humans than do the single-gene rodent obesity models.

At Oak Ridge National Laboratory (ORNL), we generated and maintain a large collection of mouse stocks that carry radiation-induced chromosomal deletions at the pink-eyed dilution (\textit{p}) locus on mouse chromosome 7 (MMU-7) (8–10). By assessing the body fat of two of the distally extending heterozygous deletions, \textit{p}\textsuperscript{30PUb} and \textit{p}\textsuperscript{30PcOD}, and \textit{p}\textsuperscript{Ipw}, we showed that the \textit{p-linked-obesity-locus 1 (plo 1)} region, on proximal MMU-7 contains gene(s) affecting body fat in these mice (11). \textit{p}\textsuperscript{30PcOD} is the longest distally extending \textit{p} deletion, extending distal to \textit{p}, between ubiquitin protein ligase E3A (\textit{Ube3a}) and the imprinted gene in the Prader-Willi syndrome region \textit{(Ipuw)} (12). On the other hand, \textit{p}\textsuperscript{Ipw}, on the other hand is shorter and its distal breakpoint maps to the chromosomal region between the genes \textit{\gamma-aminobutyric acid receptor (Gabrb3) and Ube3a} (8). Interspecific backcross analysis suggests that \textit{p}\textsuperscript{30PcOD} carries a deletion of \textless1 cm (9,10). Mice heterozygous for either of the two \textit{p} deletions...
have nearly twice the body fat of mice when the deletion is inherited maternally as when it is inherited paternally. Plo 1 overlaps with the critical region of MMU-7 linked to QTDL for body weight/fat and type 2 diabetes phenotypes (11, 12).

Data suggest that the phenotype of the plo 1 locus may be due to haploinsufficiency for a novel type IV P-type ATPase, Atpi10c, mapping between Gabrb3 and Ube3a on MMU-7. Atpi10c, a novel phospholipid translocase, is the only transcript encoding the 5' promoter region of Atpi10c is deleted, whereas in the p30PuB heterozygotes, the complete gene of 21 exons and the 3' flanking region is deleted (Dhar, M., unpublished data).

We and two other groups have also identified and characterized the sequence of the human ortholog, ATP10C on human chromosome 15q11-q13 (12–14). ATP10C maps to the Angelman Syndrome (AS) critical region. AS arises from the loss of maternal allele of the ATP10C gene caused by a 4-Mb maternally inherited deletion, with at least 4 additional genetic loci required for the expression of AS phenotype with an adult-onset obesity appearing at 5–6 mo of age (16). There is also a uniparental disomy mouse model of AS with late-onset obesity reported by Cattanach et al. (17). Several mouse models of AS including p30PuB also show a mild neurobehavioral phenotype with an adult-onset obesity appearing at 5–6 mo of age (16). There is also a uniparental disomy mouse model of AS with late-onset obesity reported by Cattanach et al. (17). Atpi10c/ATP10C is maternally expressed (paternally imprinted) in both mouse and human brains (13, 14, 18). As described above, our data show that Atpi10c is functionally imprinted such that only the maternal allele is expressed and that the loss of the maternal allele causes obesity (11). These studies thus suggest an association of Atpi10c/ATP10C with some forms of obesity and lipid metabolism disorders in both mice and humans.

In the present report, we describe results of studies initiated to characterize the obesity phenotypes associated with p30PuB mutants and to assess the overall effect of the heterozygous deletion of Atpi10c on obesity-related metabolic abnormalities. Experiments to evaluate the response of the mice carrying the heterozygous deletion of Atpi10c to the fat content of their diets were undertaken.

MATERIALS AND METHODS

Mice and diet. Female and male mice carrying the longest distally extending ORNL p deletion, p30PuB, were generated and maintained as described (11). From each cross, a control group of p30PuB mutants (referred to as p30PuB mats) were also included in the studies. The progeny from these crosses were easily genotyped visually because p30PuB contains a darker coat color than p7R75M. In each cross, p7R75M mice were generated by a cross of p7R75M, a fully viable intermediate allele at p, to C57BL/6J, a strain of inbred mice (9). Mice were generated and maintained using this scheme to facilitate genotyping of control and mutants. All mice were age- and sex-matched. Mice were fed a nonpurified diet (Laboratory Rodent Diet, Checkers PMI Nutritional International) until weaning (4–5 wk old). Thereafter, they were weighed and fed commercial rodent diets (D12450B, 20% energy (%E) protein, 70% E carbohydrate and 10% E fat consisting of 225% E of soybean oil and 180% E of lard; and D12451, 20% E protein, 35% E carbohydrate and 45% E fat consisting of 225% E soybean oil and 1597.5% E lard; Research Diets) for an additional 8–12 wk. Mice were killed by CO2 asphyxiation. This project was approved by the Animal Care and Use Committee of the ORNL (Protocol number 0263).

Body weight and adiposity index. Total body weights were measured every 2 wk and body fat measurements were carried out as described earlier (11). When the mice were 12–16 wk old, food was withdrawn at ~1600 h and the mice were killed the next day between 900 and 1200 h. An adiposity index (AI) was calculated for each mouse as the ratio of the sum of the fat pads divided by the weight of the eviscerated carcass (minus the dissected adipose depots). The major organs, liver, spleen, and kidneys were also weighed and the data recorded.

Gluose (GTT) and insulin (ITT) tolerance tests. GTT and ITT were carried out on 8- to 12-wk-old conscious mice fed the high-fat diets for 4 and 8 wk. For GTT, mice were food deprived for ~14–16 h and then a 200 g/L glucose solution was administered i.p.
Tail blood glucose was measured at time 0 (before glucose injection) and at 30, 60, 90, and 120 min after injection with the glucometer (One Touch Ultra, Lifescan).

For ITT, mice were food deprived for 2 h and then 0.375 U/kg of Humulin R (Eli Lilly) was injected i.p. Tail blood glucose was measured at time 0 (before insulin injection) and at 30, 45, 60, 90, and 120 min after injection with the glucometer.

Plasma glucose was measured after mice were food deprived for 2 h as the 0 time values in ITT and after being food deprived for 16–18 h as the 0 time values in GTT. The two sets of values were compared.

**Plasma insulin, leptin, and lipids.** Blood was sampled from the tail of the mice (food-deprived for 16–18 h) after they consumed the 45% fat diet for 4 and 12 wk. Plasma total cholesterol (TC), HDL cholesterol, unesterified cholesterol (UC), and triacylglycerol (TG) concentrations were measured by enzymatic colorimetric assays as described (19). The sum of VLDL and LDL cholesterol was obtained as the difference between TC and HDL cholesterol. Cholesteryl ester (CE) was calculated as the difference between TC and UC.

For plasma insulin and leptin, blood was collected by heart puncture from 12-wk-old mice when they were killed for the body composition analyses described above. Plasma insulin was measured using a rat insulin RIA kit with rat insulin as the standard; leptin was analyzed (Statview Version 4.5). Differences with each group to determine the effects of the genotype on the variables were tested separately. Data were grouped according to the sex, the genotype, and the diets consumed (Fig. 1).

For plasma insulin and leptin, blood was collected by heart puncture from 12-wk-old mice when they were killed for the body composition analyses described above. Plasma insulin was measured using a rat insulin RIA kit with rat insulin as the standard; leptin was analyzed (Statview Version 4.5). Differences with each group to determine the effects of the genotype on the variables were tested separately. Data were grouped according to the sex, the genotype, and the diets consumed (Fig. 1).

**RESULTS**

**Body and fat pad weights.** The female and male p30PUb mats, heterozygous for Apo10c, had significantly greater body weights than the sex- and age-matched control mice (Figs. A, B). At 12 wk of age, when all of the mice were killed, female and male mutant mice weighed 36 and 16% more, respectively, than their corresponding control littersmates. Female p30PUb mats also gained significantly more body weight than their age-matched p30PUb mats (Fig. 1A).

**Histological examination of the heart, liver, and pancreas.** The heart, liver, and pancreas were fixed in 10% buffered formalin. The hearts were transversely sectioned proximal and distal to the atria. The tissues were routinely processed by paraffin embedding, sectioned (5–9 μm), and stained with hematoxylin and eosin. Sections of the liver were also stained with periodic acid-Schiff (PAS) and PAS diastase for evaluation of cytoplasmic carbohydrate deposition. Routine light microscopy was used to examine the tissues.

**Statistical methods.** For all of the analyses, female and male mice were tested separately. Data were grouped according to the sex, the diet, and the genotype of the mice used. Statistical analysis was performed using unpaired t tests comparing mutants and controls in each group to determine the effects of the genotype on the variables analyzed (Statview Version 4.5). Differences with P < 0.05 were considered significant. Values in the text are means ± SD.

**Female p30PUb mats had ~2–2.5 times the actual weights of the individual fat pads and the AIs and ~1–1.5 times the weights of the eviscerated carcasses of both the control lean p30PUb mice and the p30PUb (Table 1, Fig. 2). However, in the male p30PUb mats, the weights of the individual fat pads, the corresponding AIs, and the weights of the eviscerated carcasses were not higher. Male p30PUb mats fed the 10% fat diet for 8 wk and killed for body fat analysis had ~24.5% greater body weight, ~1.5–2.0 times the actual weights of the individual fat pads and the AIs, and ~1.5–2.0 times the weights of the eviscerated carcasses, than the p30PUb controls (Table 1).

**Plasma insulin and leptin.** Compared with the controls, both the female (P = 0.004) and male (P = 0.02) p30PUb mats were hyperglycemic after being food deprived for only 2 h, suggesting an abnormal glucose homeostasis. Female p30PUb mats had ~2.2 times higher insulin (0.229 ± 0.02 pmol/L, P 0.05).

**TABLE 1**

<table>
<thead>
<tr>
<th>Body fat of overnight food-deprived male p30PUb mice after consuming the 10 and 45% fat diets for 8 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% fat diet</td>
</tr>
<tr>
<td>p30PUb</td>
</tr>
<tr>
<td>p30PUb</td>
</tr>
<tr>
<td>p30PUb</td>
</tr>
<tr>
<td>p30PUb</td>
</tr>
<tr>
<td>p30PUb</td>
</tr>
<tr>
<td>Body weight, g</td>
</tr>
<tr>
<td>Eviscerated carcass, g</td>
</tr>
<tr>
<td>Visceral fat, g</td>
</tr>
<tr>
<td>Fat pads, g</td>
</tr>
<tr>
<td>Inguinal</td>
</tr>
<tr>
<td>Epididymal</td>
</tr>
<tr>
<td>Mesenteric</td>
</tr>
<tr>
<td>Retroperitoneal</td>
</tr>
<tr>
<td>Adiposity index</td>
</tr>
</tbody>
</table>

* Values are means ± SD, n = 6–7. * Different from the p30PUb control mice, P < 0.05.
= 0.04) and ~3.0 times higher leptin (26.3 ± 5.70 μg/L, \( P = 0.01 \)) levels than the lean control mice (insulin = 0.102 ± 0.01 pmol/L, leptin = 8.68 ± 2.90 μg/L). Male \( p^{30FU} \) mats on the other hand, did not differ from controls in their blood insulin and leptin levels. However, the blood insulin and leptin levels of the male \( p^{30FU} \) mats (insulin = 0.379 ± 0.02 pmol/L, leptin = 28.7 ± 10.90 μg/L) that consumed the 10% fat diet were significantly higher than those of the corresponding \( p^+ \) controls (insulin = 0.067 ± 0.01 pmol/L, leptin = 2.2 ± 0.75 μg/L).

**Glucose tolerance and insulin resistance.** The obese \( p^{30FU} \) mats had a significantly higher hyperglycemic response to a given dose of glucose and insulin than either the \( p^+p^+ \) or \( p^{30FU} \) mats, suggesting that insulin resistance occurs only with maternal inheritance (Figs. 3, 4). This effect was observed after 4 wk of consuming the 45% fat diet and persisted at 8 wk.

**Liver, pancreas, and heart histology.** Histologic evaluation of the formalin-fixed, paraffin-embedded tissues from all mice fed the 45% fat diet for 8 or 12 wk showed diffuse hepatocyte vacuolar change in the livers (Fig. 6A, B, C). In hematoxylin and eosin, PAS, and PAS with diastase-stained sections, the staining qualities of the hepatocellular vacuoles were consistent with cytoplasmic microvesicular lipid. Cytoplasmic carbohydrates were marginated and nuclei retained
central localization. However, compared with p30PUb pats, the p30PUb mats had a marked progression of this change with more numerous, larger, and more discrete hepatocellular vacuolization consistent with both micro- and macrovesicular lipid deposition after 12 wk (Fig. 6).

The pancreas of p30PUb mats had no abnormalities and did not differ from those of the control mice. The heart valves and aortic walls of the p30PUb mutants and their control littermates showed mild myxomatous change; however, in the mutant mice, this phenotype appeared more pronounced. Female p30PUb pats did not show signs of vacuolar degeneration in hepatocytes even after being fed the 45% fat diet for 12 wk, thus confirming that the fatty liver disease was observed only in p30PUb mats (Fig. 6).

Maternal vs. paternal inheritance of p30PUb. Increased adiposity, impaired glucose tolerance, insulin resistance, and fatty liver disease are observed in p30PUb mats but not p30PUb pats. The body weight and adiposity of p30PUb pats were similar to that of the p+/p+ control mice (Figs. 1A and 2A). Furthermore, after consuming the 45% fat diet for 4 wk, p30PUb mats had normal responses to glucose and insulin tolerance testing (Fig. 3). Histopathological analysis of their livers did not show substantial lipid deposition (Fig. 6C).

DISCUSSION

Obesity is a major contributor to insulin resistance; >80% of individuals with type 2 diabetes are also obese and a substantial number of these patients express abnormalities in insulin sensitivity and glucose metabolism. Type 2 diabetes is also the result of peripheral insulin resistance, hepatic glucose overproduction, and impaired pancreatic insulin secretion. Because the etiology of type 2 diabetes includes obesity and the development of insulin resistance, candidate genes for obesity are candidates for type 2 diabetes as well (4–6).

Glucose homeostasis is tightly controlled by a balance between glucose absorption from the intestine, production by the liver, and uptake and metabolism by the muscle and fat. Thus, insulin resistance may arise due to a defect in insulin signaling in one or all of the three target tissues, i.e., liver, skeletal muscle, and adipose tissue, in which they become less responsive to insulin (20–23). The defect may lie in any of the
steps involving insulin secretion and the final glucose disposal or uptake in the peripheral tissues.

The data presented here suggest that the $p^{30PUb}$ heterozygote mutants represent a novel genetic, diet-induced model of insulin resistance characterized by hyperinsulinemia, hyperglycemia, hyperlipidemia, and obesity in association with glucose intolerance. These studies indicated some sex-specific differences. Body weights of female $p^{30PUb}$ mats increased significantly after consumption of the 45% fat diet for 4 wk. Their fat pads and the eviscerated carcasses were also significantly heavier when they were killed at 12 wk compared with the $p^{30PUb}$ pups. Male $p^{30PUb}$ pups fed the 45% fat diet had significantly increased body weights but not adipose tissue or eviscerated carcass weights compared with their control littermates. Although adiposity and lean carcass weight did not increase, male $p^{30PUb}$ pups fed the 45% fat diet tended to have higher values for these measurements compared with controls ($P = 0.192$–0.122). We hypothesize that this increase in both the control and the $p^{30PUb}$ mice is due to the effect of the 45% fat diet on this background strain because these measurements were significantly higher when they were fed a 10% fat diet. The mutants are maintained on a C57BL/6J background, in which obesity is induced (24). Male C57BL/6J mice tend to be heavier than their female littermates (25). At this age, the effects on the adipose tissue deposition and lean carcass weight from the 45% fat and the mutation are indistinguishable. Potentially, if the male mice were fed the 45% fat diet for a longer period of time, the effect due to the mutation might significantly alter adiposity as seen in the female mice. Similarly, the blood insulin and leptin levels in male $p^{30PUb}$ pups fed the 45% fat diet did not differ from the control mice. However, there were significant differences in the blood insulin and leptin levels in male $p^{30PUb}$ pups fed the 10% fat diet. This can also be attributed to the diet-induced increase in the adipose tissue by the background and the sex of the control mice. It is noteworthy that even though mutant male mice fed a 45% fat diet did not differ from controls in adiposity, glucose and insulin tolerance and hypertriglyceridemia were affected. Even though there are sex differences, the conclusion that maternal inheritance of the deletion of a region of MMU-7 associated with QTL for body weight and type 2 diabetes phenotypes contributes to the above phenotype is unquestionable.

Obesity is strongly associated with insulin resistance manifested by defects in insulin secretion and/or action. The results of the GTT and ITT confirm that the mutant mice are insulin resistant compared with the control littermates. This effect occurred in mice fed the 10 and 45% fat diets; therefore, it is due to the mutation and not the fat content of the diet. The hyperlipidemia in the obese mutant mice was secondary to the development of insulin resistance and obesity; it occurred only when the obese mutants were fed the 45% fat diet for a period of at least 3 mo. This effect was not seen when the mutants were fed the same diet for only 1 mo. Similarly, histopathological analysis showed mild fat infiltration within hepatocytes at 8 wk, which progressed to severe fatty liver disease by 12 wk.

The weight gain and the corresponding increase in adiposity in $p^{30PUb}$ pups did not differ from controls. These data along with the GTT and ITT data show that the phenotypes in $p^{30PUb}$ heterozygotes may be due to the maternal inheritance of the $p^{30PUb}$ deletion.

By positional cloning and molecular analyses, we showed that a murine type IV P-type ATPase, $Atp10c$ and its human ortholog $ATP10C$, encoding a putative phospholipid translocase, is a prime candidate affecting body fat and/or lipid metabolism in mice and humans (11,12). As reported earlier, the $p^{30PUb}$ deletion encompasses the chromosomal region containing three genes, Gabrb3, Atp10c, and Ube3a, whereas the $plo$ 1 region, critical to the changes in the body weight, contains $Atp10c$ and $Ube3a$ only (8,11). Because $Ube3a$ transgenic mice are not obese, it is reasonable to expect that it is not the candidate for obesity or altered glucose and lipid metabolism disorders associated with $p^{30PUb}$ pups (26,27). Thus, the maternal inheritance of $Atp10c$ in the heterozygous deletion mutant mice may be responsible for the altered glucose tolerance, increased adiposity, and NAFLD after consumption of the diet containing 10 or 45% fat for only 8–16 wk. The heterozygous deletion of the complete $Atp10c$ gene leads to defects in glucose homeostasis, clearly affecting the liver and adipose tissue. Whether this is due solely to $Atp10c$ and whether the liver and adipose tissue are the only two tissues affected must be established.

$Atp10c$ is a putative phospholipid translocase and it is conceivable that $Atp10c$ plays a role in lipid trafficking and maintenance of the phospholipid asymmetry and fluidity of the plasma membrane; thus, loss of $Atp10c$ function by maternal deletion (coupled with paternal silencing) upsets the normal membrane milieu and perturbs glucose and lipid metabolism. The exact biological role, however, is not known. It is important to identify the primary biochemical defects and then determine how these subsequently alter metabolism in the whole animal. In the present study, we established the validity of our mice as a novel model for obesity, type 2 diabetes, and NAFLD. Specific metabolic tests to identify metabolic pathway(s) and the target tissue(s) associated with $Atp10c$ will be initiated. These experiments will give us important information about glucose homeostasis in skeletal muscle, adipose tissue, and liver associated with a heterozygous deletion of a novel ATPase in our mouse model.

**ACKNOWLEDGMENTS**

The authors are indebted to Dr. Yie Liu for her critical reading and constructive suggestions for this manuscript. We also thank Dr. Naima Moustaid Moussa for her expert comments and help with the insulin and leptin assays.

**LITERATURE CITED**

A novel ATPase on mouse chromosome 7 is a candidate gene for increased body fat. Physiol. Genomics 4: 93–100.


