Adipocyte-Specific Deletion of Lamin A/C Largely Models Human Familial Partial Lipodystrophy Type 2

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Mechanisms by which autosomal recessive mutations in Lmna cause familial partial lipodystrophy type 2 (FPLD2) are poorly understood. To investigate the function of lamin A/C in adipose tissue, we created mice with an adipocyte-specific loss of Lmna (LmnaADKO). Although LmnaADKO mice develop and maintain adipose tissues in early postnatal life, they show a striking and progressive loss of white and brown adipose tissues as they approach sexual maturity. LmnaADKO mice exhibit surprisingly mild metabolic dysfunction on a chow diet, but on a high-fat diet they share many characteristics of FPLD2 including hyperglycemia, hepatic steatosis, hyperinsulinemia, and almost undetectable circulating adiponectin and leptin. Whereas LmnaADKO mice have reduced regulated and constitutive bone marrow adipose tissue with a concomitant increase in cortical bone, FPLD2 patients generally have heterozygous or compound heterozygous variants affecting exons 8 and 11 of Lmna. Variants in exon 8, including position R482, are associated with 80% of FPLD2 cases and produce a severe lipodystrophic phenotype (10–14). Strong disease phenotypes have also been described for autosomal-dominant non-R482 single missense mutations and for homozygous pathogenic variants; less severe metabolic disease phenotypes have been associated with mutations in other Lmna exons (15,16). Although Lmna mutations alter functional aspects of nuclear structure, chromosome organization, and transcription factor activity, mechanisms by which lamin A/C mutations cause lipoatrophy remain incompletely understood.

Overexpression of wild-type lamin A or R482 mutants in 3T3-L1 cells is sufficient to inhibit preadipocyte differentiation (2). Transgenic mice that overexpress the most common FPLD2 Lmna mutation (R482Q) in adipose tissue have characteristics similar to the human phenotype, including loss of adipose tissue, glucose intolerance, insulin resistance, and fatty liver; however, these phenotypes develop slowly and are relatively mild, even on a high-fat diet (HFD) (1). On the other hand, precursor cells isolated from lamin−/− mice also have diminished capacity for adipogenesis, as do induced pluripotent stem cells lines derived from patients with FPLD2 (5,17), and mice with global deletion of Lmna lack white adipose tissue (WAT).

Lipodystrophy syndromes are rare diseases characterized by generalized or partial loss of adipose tissues, accompanied by metabolic alterations secondary to insulin resistance including lipodystrophy-related diabetes, hyperlipidemia, and nonalcoholic fatty liver disease (1–9). The most common form of monogenic lipodystrophy is familial partial lipodystrophy type 2 (FPLD2), which is caused by autosomal dominant mutations in the Lmna gene encoding nuclear lamins A and C. FPLD2 patients generally have heterozygous or compound heterozygous variants affecting exons 8 and 11 of Lmna. Variants in exon 8, including position R482, are associated with 80% of FPLD2 cases and produce a severe lipodystrophic phenotype (10–14). Strong disease phenotypes have also been described for autosomal-dominant non-R482 single missense mutations and for homozygous pathogenic variants; less severe metabolic disease phenotypes have been associated with mutations in other Lmna exons (15,16). Although Lmna mutations alter functional aspects of nuclear structure, chromosome organization, and transcription factor activity, mechanisms by which lamin A/C mutations cause lipoatrophy remain incompletely understood.

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Unfortunately, whole-body lamin-/- mice are a challenging experimental model to interpret, as they are runted, have severe musculoskeletal defects, and die by 6–8 weeks of age (3,4). Thus, more sophisticated animal models are required to evaluate mechanisms of lipoatrophy, since both gain- and loss-of-function approaches for lamin A/C mutations cause impaired adipogenesis of cultured cells and depletion of adipose tissue in mice.

Herein we report that mice with selective deletion of floxed Lmna in adipocytes (Lmna^ADKO) develop functional WAT and brown adipose tissue (BAT) depots postnatally but lose the vast majority of adipocytes coincident with sexual maturity. While both male and female Lmna^ADKO mice develop lipoatrophy and metabolic dysfunction, effects are more pronounced on an HFD and in females, mirroring the sex differences observed in FPLD2 patients. Although the phenotype of Lmna^ADKO mice is highly correlated with clinical signs of FPLD2, some characteristics are more reminiscent of acquired generalized lipoatrophy. In addition, cortical bone mass is increased in Lmna^lox/lox mice, whereas we observed reduced bone mass in FPLD2 patients. Adipogenesis of primary Lmna-deficient precursors is not impaired; however, adrenergically stimulated lipolysis of resulting adipocytes is slightly increased. Together, these data in mice demonstrate that loss-of-function syndromes (LD-Lync; clinical trial reg. no. NCT03087253, clinicaltrials.gov). DEXA data, available from 24 R482 patients and 18 control individuals matched by age, sex, and BMI, were evaluated for body composition, as well as bone variables. Statistical comparisons between matched cohorts were made using the Wilcoxon rank sum test, and data are presented as mean ± SD. Values were considered to be statistically significant when P < 0.05. DEXA parameters were also evaluated from 24 patients from the R482 group and 18 from the control group.

**Cell Culture**

Primary mesenchymal stem cells from Lmna^FL mice were isolated, cultured, and differentiated as previously described (21,22). For Cre-mediated deletion of Lmna, precursors were treated with 1 × 10^10 adenoviral particles/mL expressing either GFP (Adeno-GFP) or Cre recombinase (Adeno-Cre) for 48 h in serum-free media. Adenoviral treatment was begun 1–2 days after plating cells and completed prior to induction of adipogenesis. Oil Red O staining to visualize neutral lipid accumulation was performed as described previously (23).

**Immunofluorescence**

For Mac2/Cav1 immunofluorescent staining, freshly isolated posterior subcutaneous WAT (psWAT) and gonadal WAT (gWAT) were minced into small pieces (~5 mg) and fixed overnight in 1% paraformaldehyde at 4°C. The tissues were then washed three times with PBS, incubated in blocking solution (PBS, 5% BSA, and 0.3% Triton X-100) for 1 h at room temperature, and incubated in primary antibodies (diluted 1:300 in blocking solution) overnight at 4°C. The tissues were washed three times with blocking solution and then incubated with Alexa Fluor–conjugated secondary antibodies (diluted 1:300 in blocking solution) for 1 h at room temperature, protected from light. The tissues were washed two times with PBS, stained with 150 nmol/L Hoechst in PBS for 10 min, washed two times with PBS, and immediately imaged using confocal microscopy (Nikon A1). Antibody information is listed in Supplementary Table 1.

**Analyses of Bone and Bone Marrow Adipose Tissue**

Bones from Lmna^FL and Lmna^ADKO mice were fixed in 10% neutral buffered formalin for 24 h and then washed...
three times with water and placed in Sorenson phosphate buffer (0.1 mol/L; pH 7.4) as previously described (24,25). Tibiae were placed in a specimen holder 19 mm in diameter and scanned over their entire length using a microcomputed tomography (μCT) system (μCT100 Scanco Medical). Scan settings were as follows: voxel size 12 μm, 70 kVp, 114 μA, 0.5-mm AL filter, and integration time 500 ms. Density measurements were calibrated to the manufacturer’s hydroxapatite phantom. Analyses were performed using the manufacturer’s evaluation software and a threshold of 180 for trabecular bone and 280 for cortical bone. Osmium tetroxide staining and quantification of bone marrow adipose tissue (BMAT) by μCT were performed as described previously (24,25).

Statistics for Animal Studies

Data are presented as mean ± SD unless otherwise noted and were analyzed by the two-tailed Student t test or ANOVA, unless otherwise indicated. Data collected using indirect calorimetry are presented as mean ± SEM and were analyzed by ANCOVA using the CalR platform with lean body mass as a covariate. Differences were considered significant at P < 0.05 or as indicated in figure legends.

Data and Resource Availability

All reagents and data from this article are available from the corresponding author upon request.

RESULTS

*Lmna* Deletion in Adipocytes Causes Loss of Adipose Tissue

To investigate the importance of lamin A/C in adipose tissue, we developed a model to delete *Lmna* in adipocytes (*Lmna*ADKO) by breeding Adipoq-Cre mice with mice containing floxed *Lmna* alleles (*Lmna*FL) (Supplementary Fig. 1A–E). We confirmed through genotyping and immunoblot analyses that lamin A/C is lost specifically from adipocytes of sWAT in mice at 4 weeks of age (Supplementary Fig. 1B and E). To determine whether recombination of exons 10 and 11 results in a truncated lamin protein, we infected cultured mesenchymal precursors from *Lmna*FL mice with an adenovirus expressing Cre recombinase (Adeno-Cre) or GFP (Adeno-GFP) prior to adipogenesis. Immunoblot analyses with an N-terminal antibody to lamin A/C revealed a robust band in lysates from Adeno-GFP–infected cells but not from Adeno-Cre–infected cells, even at high exposure (Supplementary Fig. 1C). We then asked whether the mRNA expression of truncated *Lmna* might be altered in cells treated with Adeno-Cre and observed that mutant *Lmna* was expressed comparably to wild-type *Lmna* mRNA (Supplementary Fig. 1D). These data suggest that the truncated protein is rapidly degraded. At 12–14 weeks of age, visual inspection revealed that male *Lmna*ADKO mice have a striking loss of WAT, including psWAT, anterior sWAT, and gWAT (epididymal or ovarian) depots (Fig. 1A and Supplementary Fig. 1F). In addition, interscapular BAT was smaller and whiter in appearance. The livers of *Lmna*ADKO were enlarged and paler in color (Fig. 1A and Supplementary Fig. 1F). Adipocyte-specific deletion of *Lmna* did not substantially affect total body weight of either sex (Fig. 1B) but, in females, led to a change in body composition, with decreased fat mass and elevated lean mass (Fig. 1C). Consistent with visual inspection, loss of lamin A/C in adipocytes led to a profound reduction in weight of psWAT, gWAT, and mesenteric, pericardial, renal, and retroperitoneal WAT in both sexes (Fig. 1D and E and Supplementary Fig. 1F; data not shown). Although male mice had reduced BAT and increased liver weight, significant differences in weights of these tissues were not observed in female mice (Fig. 1F and G). Heart, pancreas, and spleen did not have grossly altered appearances, and although heart weights were increased in both sexes, weights of pancreas and spleen were unaltered (Supplementary Fig. 1G–I). Histological analyses of WAT remnants from *Lmna*ADKO mice revealed an almost complete loss of adipocytes, along with an increase of fibrotic stroma (Fig. 1H). In both sexes, the BAT of *Lmna*ADKO mice acquired a whitened phenotype with the appearance of large, unilocular adipocytes (Fig. 1H). Liver showed mild hepatosteatosis, and dermal adipocytes were completely absent (Fig. 1H). We did not detect a phenotype in heterozygous *Lmna*FLO mice (data not shown), consistent with previous findings that heterozygous global null *Lmna* mice do not have lipatrophy or metabolic dysfunction (26). Taken together, these data demonstrate that loss of lamin A/C in adipocytes causes a lipatrophic phenotype in mice.

On a Chow Diet, *Lmna*ADKO Mice Exhibit Only Mild Metabolic Dysregulation

FPLD2 patients have significant metabolic complications including clinical diabetes and nonalcoholic fatty liver disease (7–9); thus, we next investigated effects of adipocyte-specific loss of lamin A/C on whole-body metabolism in mice. Surprisingly, glucose tolerance of *Lmna*ADKO mice was not different between genotypes, although area under the curve suggested glucose intolerance in female mice (Fig. 2A and B and Supplementary Fig. 2A). Whereas fasting blood glucose concentrations were elevated in *Lmna*ADKO mice of both sexes (Fig. 2C), glucose and insulin concentrations were only higher in random-fed female mice (Fig. 2C and D). Adiponectin was undetectable in the serum of *Lmna*ADKO mice (Fig. 2B), but there was only a trend toward reduced leptin concentrations (Supplementary Fig. 2B). Adipocyte-specific deletion of *Lmna* led to increased serum triacylglycerols in female mice and decreased glycerol in both sexes (Fig. 2F and G). We next measured circulating glycerol concentrations following administration of isoproterenol. *Lmna*ADKO mice of both sexes had lower concentrations of serum glycerol than *Lmna*CTRL mice.
at baseline and a blunted increase in response to isoproterenol, indicating an impaired capacity for lipolysis (Supplementary Fig. 2D and G). However, normalization of glycerol concentrations to total WAT weight suggests the adipocytes present may have been highly lipolytic (Supplementary Fig. 2E and H). To evaluate whether mice develop more severe metabolic complications with age, we performed glucose and insulin tolerance tests in female
Figure 2—LmnaADKO mice display mild metabolic dysregulation on a normal chow diet. A and B: Glucose tolerance tests for male (A) and female (B) LmnaFL and LmnaADKO mice 14 weeks of age. C and D: Circulating glucose (C) and insulin concentrations (D) in LmnaFL and LmnaADKO mice 12–14 weeks of age with ad libitum feeding or following a 16-h fast. E–G: Circulating adiponectin (E) and concentrations of triacylglycerol (F) and glycerol (G) in serum of LmnaFL and LmnaADKO mice 12–14 weeks of age. H: Insulin tolerance test for female LmnaFL and LmnaADKO mice ~6 months of age. I: Respiratory exchange ratio (VCO2/VO2) for female LmnaFL and LmnaADKO mice ~6 months of age; statistical analysis was by ANCOVA with lean body mass as a covariate; data presented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001. F, female; M, male; ns, not significant.
LmnaFL and LmnaADKO mice at ~6 months of age and found that while LmnaADKO mice were highly insulin resistant, their glucose tolerance did not differ from that of controls (Fig. 2H and Supplementary Fig. 2I). Taken together, these results indicate that LmnaADKO mice have a mild dysregulation of glucose and lipid metabolism that is more pronounced in females than in males.

Dramatic energy balance alterations have been observed in other mouse models of lipodystrophy, including a “flattened” respiratory exchange ratio (RER), hyperphagia, metabolic inflexibility in switching between oxidation of carbohydrates and fat, and changes in energy expenditure (27–30). We therefore used CLAMS analyses to investigate energy balance in female LmnaFL and LmnaADKO mice ~6 months of age. RER in the LmnaADKO animals remained constant at ~0.9 throughout the light and dark cycles, in stark contrast to the cyclical RER observed in control animals (Fig. 2I). In keeping with these observations, LmnaADKO mice had significantly lower rates of oxygen consumption than LmnaFL mice during the light cycle and were metabolically inflexible, with an almost constant oxidation of fat and glucose throughout the light and dark cycles (Supplementary Fig. 2J–L). Food consumption, water consumption, and physical activity were not altered in LmnaADKO animals (Supplementary Fig. 2M; data not shown).

Adipocyte-Specific Deletion of Lmna Causes Loss of BMAT and Increased Cortical Bone
Whether lipoatrophy extends to BMAT depends on the underlying cause; BMAT is lost in patients with mutations in AGPAT1 or seipin but is maintained in patients with mutations in caveolin or cavin1 (31). In FPLD2 patients, MRI scans suggest that BMAT is also comparable to that of healthy controls (32). To determine whether adipocyte-specific loss of lamin A/C influences BMAT, we histologically evaluated the marrow niche following bone decalcification. We observed a striking loss of regulated bone marrow adipocytes in the distal femur and proximal tibia (Fig. 3A and B and Supplementary Fig. 3A) and a surprising decrease in constitutive BMAT of distalibia and caudal vertebra (Fig. 3C and D), which generally are resistant to depletion (25). Indeed, quantification with osmium staining revealed a significant reduction in distal tibial BMAT of both male and female animals (Supplementary Fig. 3B). Clinical correlations often show a reciprocal relationship between BMAT and bone mass (33). μCT analysis of distal tibia revealed increased cortical area and cortical thickness in female LmnaADKO mice (Fig. 3E–G), although no differences were observed in trabecular bone variables of proximal tibia (Supplementary Fig. 3C). Together, these data indicate that in mice, lamin A/C is required for development or maintenance of regulated and constitutive BMAT and that loss of BMAT is associated with an increase in cortical bone, although the systematic effects could not be excluded.

FPLD2 Patients With R482 Mutations Have Reduced Fat Mass, Bone Mass, and Bone Mineral Density
To determine whether FPLD2 patients also have increased bone, we retrospectively evaluated 24 FPLD2 patients with the R482 mutation and 18 control patients without lipodystrophy, matched for age, sex, and BMI. Average age of the control and R482 groups was ~50 years, with ~70% women in both cohorts (Supplementary Fig. 4A). Unlike LmnaADKO mice on a normal chow diet, the human population had substantial metabolic dysfunction; 76.2% of R482 patients had type 2 diabetes, and 71.4% were diagnosed with fatty liver, whereas 23.8% and 19.0% of controls were diagnosed with type 2 diabetes and fatty liver, respectively. All patients with R482 mutations had dyslipidemia, compared with 40.5% of patients in the control group. Serum triglycerides were significantly higher in R482 patients (Supplementary Fig. 4B), and HDL cholesterol levels were lower in this group (Supplementary Fig. 4A). Although patients with R482 pathogenic variants were not different in weight, height, BMI, trunk mass index, or fat-free mass index (Fig. 4A and Supplementary Fig. 4A), the fat mass index and trunk fat mass index were significantly lower in the R482 group (Supplementary Fig. 4A and C). As expected, fat mass of FPLD2 patients was significantly lower in arms, legs, trunk, and total body (Fig. 4A). Interestingly, bone mineral density was lower in R482 patients (Fig. 4B), and bone mass of the arms, legs, trunk, and total body were also significantly lower in patients with R482 mutations (Fig. 4C–F). These data indicate a discordance between effects on bone of lamin A/C deficiency in LmnaADKO mice and global mutations of LMNA on R482 in humans. Thus, reduced bone mass in humans may be secondary to metabolic dysfunction or perhaps R482 mutation in osteoblasts or other nonadipocyte populations.

HFD Worsens Lipodystrophy of LmnaADKO Mice
Based on mouse models with chronic (34–36) or acute (37,38) deficiency of adipose tissue, LmnaADKO mice on a chow diet have a surprisingly mild metabolic phenotype. Thus, we next tested whether an HFD would exacerbate the metabolic phenotype, given that inability to store excess energy is a well-established stimulus for disrupting metabolic homeostasis (39). Due to the sex-specific differences in phenotype observed earlier, we conducted these experiments in female animals. Although female LmnaADKO mice only tended toward less weight gain during 5 weeks of HFD feeding (Fig. 5A), these mice lacked detectable WAT depots, including psWAT, gWAT, and mesenteric and pericardial WAT (Supplementary Fig. 5A; data not shown). Livers of LmnaADKO mice following HFD feeding were significantly heavier (Fig. 5B and Supplementary Fig. 5A) and, by histological analyses, showed lipid droplet voids indicative of hepatic steatosis (Fig. 5C). BAT of LmnaADKO mice following HFD feeding also showed evidence of fibrosis and had a whitened appearance, presumably due to the
Adipocyte-specific deletion of Lmna causes loss of regulated and constitutive BMAT and increases cortical bone. A–D: Representative histologic sections stained with hematoxylin-eosin for distal femur (A), proximal tibia (B), distal tibia (C), and caudal vertebra (D) from LmnaFL and LmnaADKO mice 12–14 weeks of age. Scale bar 100 μm. E: Representative μCT scans of the distal tibiae from LmnaFL and LmnaADKO mice 12–14 weeks of age. F and G: Cortical bone area per total bone area (F) and cortical thickness (G) in LmnaFL and LmnaADKO mice 12–14 weeks of age. *P < 0.05. F, female; M, male.
Fat mass, bone mass, and bone mineral density are reduced in patients with R482 mutations in the LMNA gene. 

**Figure 4**—Fat mass, bone mass, and bone mineral density are reduced in patients with R482 mutations in the LMNA gene. A: Fat mass and total mass of arms, legs, trunk, and total body in control and R482 patients. B: Bone mineral density in control (n = 18) and R482 patients (n = 24). C–F: Bone mass in control (n = 18) and R482 patients (n = 24) in the arms (C), legs (D), trunk (E), and total body (F). *P < 0.05, **P < 0.01, ***P < 0.001. BMD, bone mineral density.
presence of large unilocular lipid droplets (Fig. 5C). It was challenging to identify even a few adipocytes in psWAT or dermal WAT of LmnaADKO mice following HFD feeding (Fig. 5C). In addition, we observed a striking loss of regulated and constitutive BMAT in HFD-fed LmnaADKO mice (Supplementary Fig. 5B–E), which was again correlated with higher cortical bone area fraction and thickness and increased trabecular thickness (Supplementary Fig. 5F–M). As predicted, the metabolic phenotype of LmnaADKO mice worsened with HFD feeding. Circulating glucose concentrations were elevated in fasted and random-fed states (Fig. 5D), whereas serum insulin concentrations were higher only in random-fed LmnaADKO mice (Fig. 5E). Adiponectin and leptin were essentially undetectable in LmnaADKO mice after 5 weeks of HFD feeding (Fig. 5F and G). Circulating triacylglycerol concentrations showed an upward trend (Fig. 5H), whereas serum glycerol concentrations were decreased (Fig. 5I), in female mice with adipocyte-specific Lmna deletion. Taken together, placing LmnaADKO mice on an HFD stimulates profound lipodystrophy, characterized by dysfunction in glucose and lipid homeostasis, which is highly reminiscent of lipodystrophy in humans with R482 mutations.

Adipose Tissue Develops in Young LmnaADKO Mice Postnatally and Is Progressively Lost With Aging

Patients with FPLD2 exhibit relatively normal adipose tissue development until puberty, at which point adipocyte tissue progressively deteriorates and/or is redistributed from the limbs to the head and neck (7,8,14). Thus, we next explored whether young mice with adipocyte-specific Lmna deletion develop adipose tissues postnatally. When observed at 4 weeks of age, psWAT and gWAT appeared normal upon gross examination (Fig. 6A and Supplementary Fig. 6A). Total body weight was unaffected by loss of lamin A/C in adipocytes at 4 weeks of age (Supplementary Fig. 6B). Although weight of psWAT mass was not different in either sex at 2 weeks of age, female LmnaADKO mice had reduced psWAT at 4 weeks of age, and both sexes had less psWAT at 6 weeks of age, compared with LmnaFL controls (Fig. 6B). In histological examination of psWAT and gWAT, gross abnormalities were not detected (Fig. 6C), but histomorphometric analysis revealed smaller adipocytes in female but not male LmnaADKO mice at 4 weeks of age (Fig. 6D).

Livers of LmnaADKO mice at 4 weeks of age did not have an obvious difference in appearance (Fig. 6A and C and Supplementary Fig. 6A), and their weights were not statistically increased (Supplementary Fig. 6C). Whereas BAT appeared grossly similar between LmnaFL and LmnaADKO mice at 4 weeks of age (Fig. 6A), histological analyses revealed an increase in lipid droplet size at that time (Fig. 6C). Weights of gWAT and spleen were not different between LmnaFL and LmnaADKO mice at 4 weeks of age (Supplementary Fig. 6D and E). On the basis of our observation of reduced circulating glycerol concentrations in adult LmnaADKO mice (Supplementary Fig. 2C), we measured responses to isoproterenol in LmnaFL and LmnaADKO mice at 4 weeks of age. There were no differences in concentrations of circulating free glycerol at baseline between experimental groups, but LmnaADKO mice displayed a decreased lipolytic response to isoproterenol treatment at 60 min (Supplementary Fig. 6F).

We also observed an apparent increase in number of stromal cells in psWAT and gWAT of LmnaADKO mice at 4 weeks of age, which we hypothesized represented immune cell infiltration (Fig. 6C). However, FACS analysis of stromal vascular cells did not reveal differences in number of CD45+ or CD31+ cells between genotypes (Supplementary Fig. 6G and H). We also performed immunofluorescent staining to observe macrophages (Mac2) and adipocytes (Cav1) in psWAT tissue of LmnaFL and LmnaADKO mice at 6 weeks of age but did not detect marked differences in number of Mac2+ cells between genotypes or in frequency of crown-like structures, a hallmark of adipocyte turnover (Fig. 6E). These data suggest that increased appearance of stromal vascular cells may be secondary to reduced size of adipocytes. We did, however, confirm our previous observation of smaller adipocytes in psWAT and gWAT depots of young LmnaADKO mice. Furthermore, LmnaADKO macrophages were larger and more clustered (Fig. 6E). Together these results demonstrate that adipose tissue develops postnatally in LmnaADKO mice and is progressively lost starting at ~4 weeks of age and continuing through sexual maturity, which closely mirrors FPLD2 disease progression in humans.

Lmna Deletion Does Not Affect Adipogenesis of Primary Mesenchymal Precursors

To interrogate molecular mechanisms underlying loss of adipose tissue in LmnaADKO mice, we induced deletion of Lmna in mesenchymal precursors isolated from LmnaFL mice with an adenovirus expressing either Cre recombinase (Adeno-Cre) or GFP as a negative control (Adeno-GFP). PCR confirmed efficient recombination of the Lmna floxed allele with Adeno-Cre treatment (Supplementary Fig. 7A). Lmna deletion had no effect on adipogenesis or morphology of mature adipocytes even when cultured for up to 28 days (Fig. 7A), despite almost complete loss of lamin A/C protein (Fig. 7B).

Lamin A/C Deficiency Increases Lipolysis of Cultured Adipocytes

Next, we asked whether Lmna deletion in cultured adipocytes affects cellular function. We hypothesized that loss of lamin A/C in adipocytes might increase lipolysis based on the decrease in size of adipocytes in psWAT of LmnaADKO mice (Fig. 6D). Although lipolysis at baseline or after treatment with forskolin was not altered, we did observe a slight increase in glycerol secretion in Lmna-deficient adipocytes following stimulation with
**Figure 5**—HFD challenge exacerbates the lipodystrophic phenotype of Lmna<sup>ADKO</sup> mice. 

**A:** Body weights of female Lmna<sup>FL</sup> and Lmna<sup>ADKO</sup> mice over the course of 5 weeks of HFD feeding. 

**B:** Weights of psWAT, liver, and spleen in Lmna<sup>FL</sup> and Lmna<sup>ADKO</sup> mice after 5 weeks of HFD feeding. 

**C:** Representative histologic images of psWAT, BAT, liver, and skin of Lmna<sup>FL</sup> and Lmna<sup>ADKO</sup> mice stained with hematoxylin-eosin. Scale bars 100 μm. 

**D and E:** Circulating glucose (**D**) and insulin concentrations (**E**) in HFD-fed Lmna<sup>FL</sup> and Lmna<sup>ADKO</sup> mice fed ad libitum or following an 8-h fast. 

**F:** Immunoblot of circulating adiponectin in serum of HFD-fed Lmna<sup>FL</sup> and Lmna<sup>ADKO</sup> mice. 

**G–I:** Circulating leptin (**G**), triacylglycerol (**H**), and glycerol (**I**) concentrations in serum of HFD-fed Lmna<sup>FL</sup> and Lmna<sup>ADKO</sup> mice. *P* < 0.05.
Figure 6—Adipose tissue develops postnatally in LmnaADKO mice and is progressively lost with aging. A: Representative images of indicated organs from female LmnaFL and LmnaADKO mice 4 weeks of age. B: psWAT tissue weight at the indicated time points in LmnaFL and LmnaADKO mice. C: Representative histologic images of the indicated organs stained with hematoxylin-eosin from female LmnaFL and
isoproterenol or norepinephrine (Fig. 7C). Effects of lipolytic stimuli on glycerol release were correlated with corresponding increases in phosphorylated hormone-sensitive lipase, as well as perilipin and phosphorylation of protein kinase A substrates (Fig. 7D). Whereas total hormone-sensitive lipase was increased in adipocytes lacking lamin A/C, changes in ATGL or adiponectin were not observed (Fig. 7D). We then investigated fatty acid uptake in Lmna-deﬁcient cultured adipocytes and found that functional lamin A/C is not required for entry into adipocytes of palmitic acid (Supplementary Fig. 7D). On the basis of our observation of increased circulating glucose and insulin concentrations in LmnaADKO mice (Fig. 5D and E), we considered whether Lmna deletion in cultured adipocytes would affect cellular responses to insulin stimulation; however, no differences were observed in baseline or insulin-stimulated glucose uptake or in stimulation of phosphorylated AKT (Supplementary Fig. 7B and C). These findings indicate that lamin A/C is not required for adipogenesis, but loss of lamin A/C does increase sensitivity of adipocytes to lipolytic stimuli.

**DISCUSSION**

Taken together, our ﬁndings demonstrate that adipocyte-speciﬁc loss of lamin A/C in mice yields a phenotype that closely mirrors that observed in human FPLD2 patients. In both cases, development of adipose tissues appears to occur normally, but depots are progressively lost during sexual maturation, with almost complete depletion at older ages. Lipoatrophy in LmnaADKO mice is accompanied by ectopic lipid accumulation in BAT and liver, as well as increased circulating glucose and insulin and decreased circulating concentrations of adiponectin, leptin, and glycerol. Furthermore, these mice demonstrate a striking metabolic inflexibility and appear unable to switch substrate oxidation between lipids and carbohydrates to meet their energetic needs. The metabolic dysfunction observed in LmnaADKO mice is not as extreme as that observed in FPLD2 patients or in other mouse models of lipoatrophy (34–38), even after 5 weeks of HFD; however, it is likely that a longer-term feeding of HFD would further worsen the impaired glucose and lipid homeostasis.

The major discrepancies between LmnaADKO mice and FPLD2 patients are the extent of lipodystrophy and effects on bone. Whereas LmnaADKO mice have increased cortical bone, we show herein that FPLD2 patients have widespread loss of skeletal bone mass and bone mineral density. We speculate that older LmnaADKO mice on a long-term HFD might have greater metabolic dysfunction and a corresponding loss of cortical and trabecular bone. Alternatively, increased bone in LmnaADKO mice may be secondary to reductions in regulated and constitutive BMAT, another variable that differs between LmnaADKO mice and FPLD2 patients. A vast majority of FPLD2 cases are caused by a single point mutation (R482) in the Lmna gene, which is predominantly believed to act in a dominant-negative manner (40,41). Although Lmna haploinsufﬁciency does not cause either lipatrophy or metabolic disease (26), the close parallels between LmnaADKO mice and FPLD2 patients suggest that the R482 mutation likely causes a loss of lamin A/C function.

Important questions that remain to be answered include the bases for progressive loss of WAT and BAT and whether sexual maturity plays a causative role, given the timing and sexual dimorphism. While it may be that postweaning development of adipocytes requires lamin A/C, and that adipocyte attrition simply reﬂects normal turnover without adipocyte replacement, our data provide strong support for a model in which lamin A/C is not necessary during adipogenesis for the embryonic/early postnatal development of adipose tissues. This idea is supported by our observation that lamin A/C is not required for adipogenesis of cultured primary mesenchymal precursors. We considered whether loss of lamin A/C might increase targeting of mature adipocytes by immune cells, but our evaluation of stromal vascular cell populations and tissue macrophages did not provide evidence to support this notion. Finally, it could be that lamin A/C deﬁciency is required for maintenance of the mature adipocyte phenotype and that apparent attrition is the result of negative energy balance or adipocyte death; however, our experiments suggest that mice lacking functional lamin do not have higher energy expenditure or altered food intake on a chow diet. Additional experiments will be required to distinguish between these possibilities, but the smaller adipocyte size observed in 4-week-old LmnaADKO mice and elevated lipolytic response to adrenergic stimuli of cultured adipocytes support a catabolic metabolic mechanism.

In summary, we report the novel ﬁnding that loss of lamin A/C in adipocytes causes a phenotype in mice similar to human FPLD2. Although the underlying genetics differ greatly between LmnaADKO mice and FPLD2, and opposing effects on bone and BMAT are observed, this is the ﬁrst mouse model that mimics many of the characteristics of human FPLD2. LmnaADKO thus serves as a promising model to investigate molecular mechanisms that cause loss of progressive adipose tissue.

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**LmnaADKO** mice 4 weeks of age. Scale bars 100 µmol/L. D: Histomorphometric quantiﬁcation of adipocyte size in LmnaADKO and LmnaADKO mice 4 weeks of age. E: Confocal ﬂuorescent micrographs of the indicated organs in LmnaADKO and LmnaADKO mice 6 weeks of age stained for macrophages (Mac2; red), adipocytes (Cav1; green), and cell nuclei (Hoechst; blue). *P < 0.01.
Figure 7—Lmna deletion does not affect adipogenesis of primary precursors. 

A: Primary Lmna<sup>fl</sup> mesenchymal stem cells were treated with adeno-GFP or adeno-Cre prior to differentiation. Representative images at the indicated time points after induction of adipogenesis, with either brightfield microscopy or after staining neutral lipid with Oil Red O. Scale bars 50 μmol/L. 

B: Immunoblot of indicated proteins in mature adipocytes. 

C: Glycerol concentrations in conditioned media of mature adipocytes (n = 3) following treatment for 2 h with vehicle (DMSO), forskolin (5 μmol/L), isoproterenol (1 μmol/L), or norepinephrine (1 μmol/L). 

D: Immunoblot for the indicated proteins in mature adipocytes following 15-min treatment with vehicle (DMSO), forskolin (5 μmol/L), isoproterenol (1 μmol/L), or norepinephrine (1 μmol/L). 

*P < 0.05. HSL, hormone-sensitive lipase; pHSL, phosphorylated hormone-sensitive lipase.
in FPLD2, as well as to develop new therapeutic strategies for lipodystrophy.

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