

# Adipose-specific deletion of *autophagy-related gene 7 (atg7)* in mice reveals a role in adipogenesis

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Edited by Arnold J. Levine, Institute for Advanced Study, Princeton, NJ, and approved October 8, 2009 (received for review June 2, 2009)

White adipocytes have a unique structure in which nearly the entire cell volume is occupied by one large lipid droplet. However, the molecular and cellular processes involved in the cytoplasmic remodeling necessary to create this structure are poorly defined. Autophagy is a membrane trafficking process leading to lysosomal degradation. Here, we investigated the effect of the deletion of an essential autophagy gene, *autophagy-related gene 7 (atg7)*, on adipogenesis. A mouse model with a targeted deletion of *atg7* in adipose tissue was generated. The mutant mice were slim and contained only 20% of the mass of white adipose tissue (WAT) found in wild-type mice. Interestingly,  $\approx 50\%$  of the mutant white adipocytes were multilocular. The mutant white adipocytes were smaller with a larger volume of cytosol and contained more mitochondria. These cells exhibited altered fatty acid metabolism with increased rates of  $\beta$ -oxidation and reduced rates of hormone-induced lipolysis. Consistently, the mutant mice had lower fed plasma concentrations of fatty acids and the levels decreased at faster rates upon insulin stimuli. These mutant mice exhibited increased insulin sensitivity. The mutant mice also exhibited markedly decreased plasma concentrations of leptin but not adiponectin, lower plasma concentrations of triglyceride and cholesterol, and they had higher levels of basal physical activity. Strikingly, these mutant mice were resistant to high-fat-diet-induced obesity. Taken together, our results indicate that *atg7*, and by inference autophagy, plays an important role in normal adipogenesis and that inhibition of autophagy by disrupting the *atg7* gene has a unique anti-obesity and insulin sensitization effect.

diabetes | differentiation | knockout | metabolism | obesity

Obesity, which is associated with type II diabetes, atherosclerosis, hypertension, and cancer, is reaching a pandemic level in the developed world. Essentially, obesity results from an imbalance between energy intake and energy expenditure (1, 2). Clinically, obesity is characterized by increased mass of white adipose tissue (WAT), which stores excess energy in the form of triglyceride (TG) and serves as a major energy reservoir in mammals. In addition, WAT is an endocrine organ that is central to energy homeostasis regulation. It integrates metabolic signals and in turn regulates systemic energy balance by secreting adipokines, including leptin, adiponectin, and tumor necrosis factor (TNF)- $\alpha$  (1, 3).

Consistent with its energy storage function, a mature white adipocyte has a unique structure in which almost the entire cytoplasm is occupied by one large (10–200  $\mu\text{m}$  in diameter) unilocular TG-rich lipid droplet, while the rest of cytoplasm occupies negligible space. Extensive studies have elegantly revealed that a transcriptional network involving PPAR $\gamma$  plays a central role in orchestrating adipogenesis, the differentiation process that generates mature adipocytes from fibroblast-like preadipocytes (4–7). The development of the highly specialized cellular structure of white adipocytes requires massive cytoplasmic remodeling. This aspect of adipogenesis, however, has not been well-studied, and the cellular and molecular mechanisms underlying this remodeling process remain unclear.

Autophagy is a major cellular degradation process involving intracellular membrane trafficking toward the lysosome (8, 9).

Autophagy is initiated by the emergence of double-membrane vesicles, known as autophagosomes, which engulf a portion of the cytoplasm. The autophagosome then delivers its cargo to the lysosome for degradation. Over the last decade, the molecular machinery of autophagy has been identified in both yeast and mammals. Most of the genes encoding components of autophagy machinery, named autophagy-related genes (*atg*), have been characterized (10, 11). Targeted deletion of essential autophagy genes in mice has revealed the various important functions of autophagy, including tumor suppression, neuronal protection, neonatal survival [(8–10) and references therein], as well as differentiation of erythrocytes (12, 13), lipid droplet formation, and lipid metabolism (14, 15) in hepatocytes.

The ability of autophagy to facilitate massive cytoplasmic degradation prompted us to investigate its possible involvement in adipogenesis. More than 25 years ago, an increased level of autophagosomes was observed when differentiating 3T3-L1 cells were analyzed morphologically with electron microscopy by Novikoff et al. (16). Recently, we showed that targeted deletion of *atg5*, an essential autophagy gene, markedly reduced white adipogenesis efficiency of the primary mouse embryonic fibroblasts (MEFs) (17). However, the *atg5*<sup>-/-</sup> mice die at birth (18), and white adipogenesis in mammals mainly occurs postnatally. Thus, the *in vivo* role of autophagy in white adipogenesis remains undefined.

The *atg7* gene encodes an E1-like enzyme that is specifically involved in autophagosome formation and is essential for autophagy (11). To rule out the possibility that an autophagy-independent function of *atg5* might be required for adipogenesis, and more importantly, to establish that autophagy is involved in normal adipogenesis *in vivo*, in the present study, we have investigated the requirement of *atg7* in adipogenesis in the primary MEF model and in a mouse model with a targeted deletion of *atg7* in adipose tissue. The *atg7*<sup>-/-</sup> primary MEFs exhibit drastically reduced adipogenesis efficiency. Moreover, the adipose-specific *atg7* conditional knockout mice show striking phenotypes in the structure of adipocytes and exhibit an interesting combination of anti-obesity/anti-diabetic metabolic features. This study provides strong *in vivo* evidence that *atg7*, and by inference autophagy, is critical for normal adipogenesis.

## Results

***Atg7*<sup>-/-</sup> Primary MEFs Exhibited Drastically Reduced Adipogenesis Efficiency and Adipose-Specific *atg7* Knockout Mice Had Drastically Reduced White Fat Mass and Reduced Body Weight.** The wild-type and *atg7*<sup>-/-</sup> primary MEFs were induced for adipocyte differentiation, and the efficiency of adipogenesis was compared by micro-

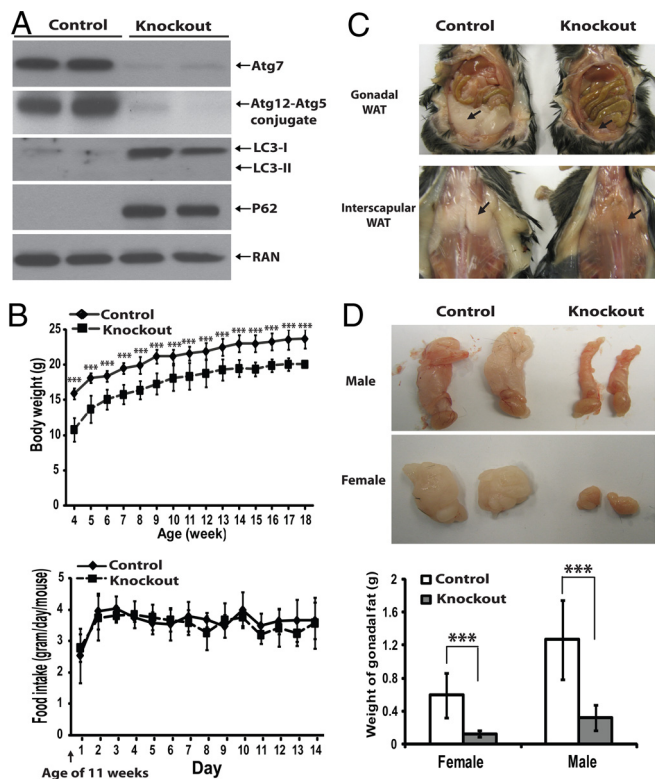
Author contributions: Y. Zhang and S.J. designed research; Y. Zhang, S.G., R.B., and Y. Zhao performed research; M.K. contributed new reagents/analytic tools; Y. Zhang, S.G., and S.J. analyzed data; and S.J. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at [www.pnas.org/cgi/content/full/0906048106/DCSupplemental](http://www.pnas.org/cgi/content/full/0906048106/DCSupplemental).



**Fig. 1.** Adipose-specific *atg7* knockout mice exhibited reduced body weight and WAT mass. (A) Immunoblotting analyses of lysates of white adipose tissue (female, uterine WAT) from control (*atg7 flox/flox*) and adipose-specific *atg7* conditional knockout (*atg7 flox/flox; ap2-Cre*) mice using indicated antibodies (Atg12-Atg5 conjugate was detected with an anti-Atg12 antibody). (B) Upper, body weight chart for control (female,  $n = 12$ ) and *atg7* conditional knockout (female,  $n = 11$ ) mice from 4–18 weeks. Lower, a 2-week food intake chart for control (female,  $n = 6$ ) and *atg7* conditional knockout (female,  $n = 6$ ) mice starting from week 11. (C) Representative pictures of control and *atg7* conditional knockout mice at the age of 20 weeks, showing gonadal (Upper) and interscapular (Lower) white adipose tissue (WAT) as indicated by arrows. (D) Representative pictures of gonadal fat pad (uterine fat in female and epididymal fat in male) and quantification (Lower) from control (male,  $n = 10$ ; female,  $n = 12$ ) and *atg7* conditional knockout (male,  $n = 5$ ; female,  $n = 6$ ) mice at the age of 18–20 weeks. \*\*\*,  $P < 0.001$ , Student's *t* test.

scopic analysis as well as by quantification of lipid droplet formation. As shown in Fig. S1, the *atg7*<sup>-/-</sup> primary MEFs mirrored the phenotypes of *atg5*<sup>-/-</sup> MEFs and exhibited a drastically reduced efficiency of adipogenesis. These results support that an autophagy defect is responsible for the adipogenesis defect observed in these cells.

White adipogenesis in mammals mainly occurs postnatally; however, both the straight *atg5*<sup>-/-</sup> and *atg7*<sup>-/-</sup> mice die at birth (18, 19). To study the *in vivo* role of autophagy in adipogenesis, we generated an adipose-specific *atg7* knockout mouse model by crossing *flox-atg7* mice (19) with the *aP2-cre* mice, in which CRE expression is under the control of an adipose tissue-specific aP2 (fatty acid binding protein 4, FABP4) promoter (20). The homozygous *atg7 flox/flox/aP2-cre* F2 mice were born in normal Mendelian ratios. The ablation of *atg7* expression in white fat tissue was nearly complete (Fig. 1A). The deficiency of autophagy in adipose tissue was confirmed by three independent parameters (21) (Fig. 1A): (1) absence of LC3-II even in the presence of high levels of LC3-I, indicating an absence of steady-state levels of autophagosome formation; (2) accumulation of a common substrate of autophagic degradation, p62, indicating a lack of functional autophagic degradation (autophagy flux); and (3) undetectable levels of Atg5-Atg12 conjugate, a complex required for autophagosome forma-

tion. As the *atg7* conditional knockout mice grew, they were visibly slim, seemingly more active, and shivered more frequently than their control wild-type littermates, but otherwise appeared normal. Both the male and female homozygous *atg7* conditional knockout mice appeared to be infertile and failed to produce any offspring.

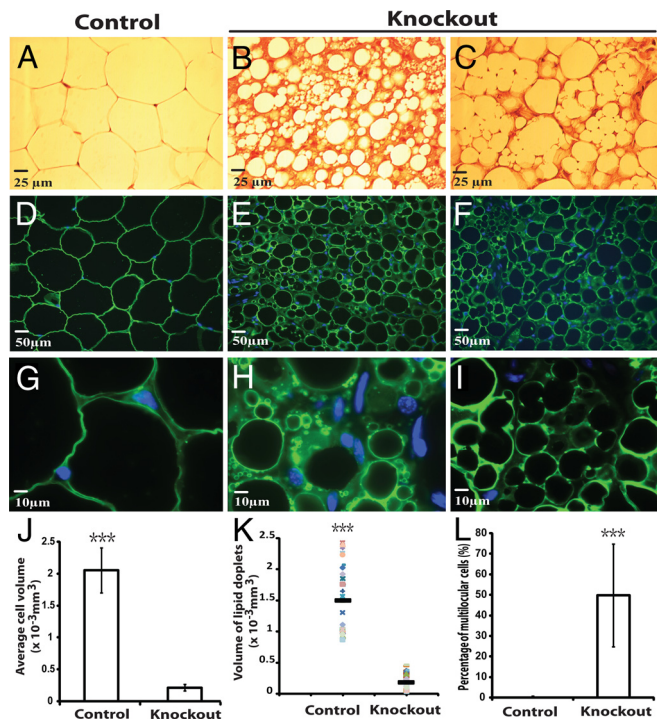
Body weight was compared between the *atg7* knockout mice and their littermates after weaning (at 3 weeks of age). As shown in Fig. 1B upper panel, the average body weight of the *atg7* adipose-specific knockout mice was  $\approx 12$  g at the age of 4 weeks vs.  $\approx 16$  g in the control *atg7* wild-type mice. The difference in body weight was maintained and found to be statistically significant through 18 weeks of age when the experiment was stopped. Similar results were obtained with the male mice. Interestingly, the total food intake rates (per animal) were almost identical between the *atg7* knockout and control mice, as shown in Fig. 1B (Lower), suggesting either a reduced efficiency in energy storage or an increased energy expenditure rate, or both, in the *atg7* conditional knockout mice.

The fat tissue in the mice was analyzed. Fig. 1C shows the gross appearance of gonadal fat pads as well as white fat tissue in the scapular region, in which a striking reduction of fat mass in the *atg7* conditional knockout mice was evident. The white adipose tissue in other regions of the mutant mice, including retroperitoneal fat and inguinal fat deposits, showed a similar extent of reduction in mass. Fig. 1D shows that the gonadal fat pads of the *atg7* conditional knockout mice (uterine fat in female and epididymal fat in male) were typically 20% of the mass of those found in the control *atg7* wild-type littermates. Importantly, other organs in the *atg7* conditional knockout mice did not appear to have any defects and the weight of liver, heart, lungs, kidneys, and brain did not exhibit any significant difference from those in the control *atg7* wild-type mice. Together, these results reveal that deletion of the *atg7* gene in adipose tissue has a profound impact on the mass of WAT deposits in adult mice.

**Atg7 Knockout WAT Contained Smaller Adipocytes and Had Large Populations of Multilocular Cells with Significant Amounts of Cytoplasm, but Exhibited No Apparent Changes in Adipocyte-Specific Gene Expression.** Histological analysis of gonadal fat was performed. Fig. 2 shows the results of uterine fat pad analysis. Hematoxylin and eosin (H&E) staining of tissues showed that wild-type WAT (Fig. 2A) was morphologically homogeneous and exhibited a typical structure in which almost the whole cell was occupied by one large lipid droplet, while cytoplasm was essentially undetectable. In contrast, the *atg7* knockout white adipocytes were heterogeneous (Fig. 2B and C). The mutant cells were smaller (Fig. 2J) and a large population of the cells contained a significant amount of cytoplasm (Fig. 2B and C, stained in red). Immunofluorescence microscopy was performed with perilipin antibody, which labels the membrane of the lipid droplets in the cells (Fig. 2D–I). While all of the wild-type adipocytes were unilocular (containing only one lipid droplet) (Fig. 2D and G),  $\approx 50\%$  of the *atg7* knockout adipocytes were multilocular (containing multiple lipid droplets) (Fig. 2E, F, H, I, and L). On average, the size of the lipid droplets in the mutant adipocytes was smaller (Fig. 2E, F, H, I, and K). Similar results were obtained from the epididymal fat pad analysis of male mice.

Quantitative PCR was performed to compare the mRNA levels of the important adipocyte-related genes, including *gpam*, *cebpa*, *pparg*, *fabp4*, *ucp1*, *agpat2*, and *plin*. As shown in Table S1, there is no significant change in the expression pattern of these genes between the *atg7* knockout white fat and the wild-type control. It was noteworthy that although the *atg7* knockout WAT gained a number of phenotypical features of brown fat, including multilocular lipid droplets, increased cytoplasmic volume, and enriched mitochondria content (Fig. 3), it did not show a significant increase in expression of the brown fat-specific genes including *ucp1*, *cidea*, *elov13*, and *prdm16* (Table S1). In addition, no change was observed in mRNA of enzymes involved in lipolysis, *lipe* and *pnpla2* (Table S1).

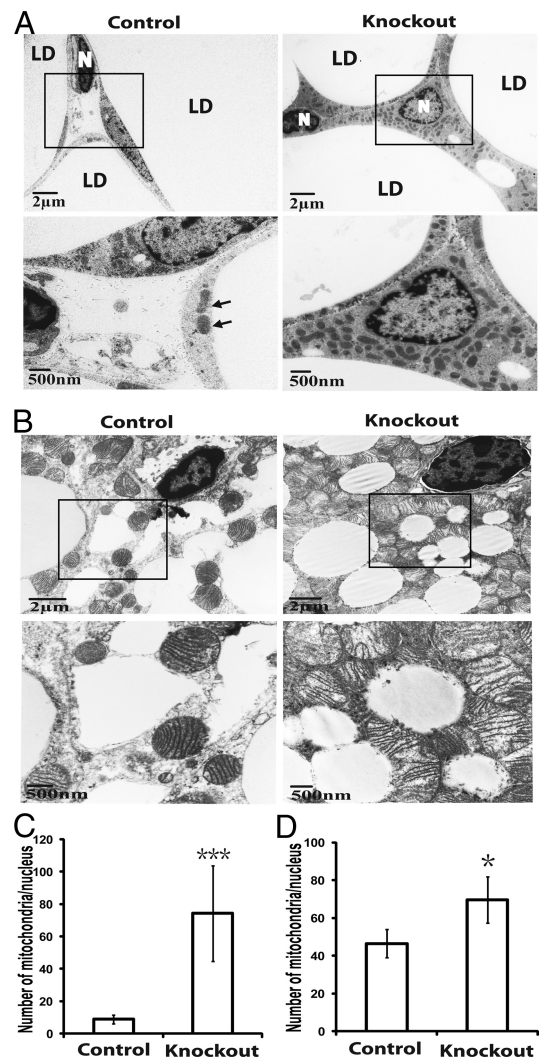




**Fig. 2.** Histological and immunofluorescence analysis of gonadal WAT from control and *atg7* conditional knockout mice. (A–C) Representative microscopic pictures of H&E stained sections of uterine WAT from control (*atg7<sup>flox/flox</sup>*, A) and adipose-specific *atg7* conditional knockout mice (*atg7<sup>flox/flox</sup>; aP2-Cre*, B and C). (D–I) Representative microscopic pictures of immunofluorescence assays of uterine WAT from control (D and G) and *atg7* conditional knockout mice (E, F, H, and I) with Perilipin A antibody. D–F were pictures of low magnification and G–I were pictures of high magnification. (J–L) Quantification of average cell volume, lipid droplet volume, and percentage of multilocular cells, as indicated, of uterine WAT from control and *atg7* conditional knockout mice. \*\*\*,  $P < 0.001$ , Student's *t* test. The data show representative results of tissues from six pairs of female mice (control and *atg7* knockout).

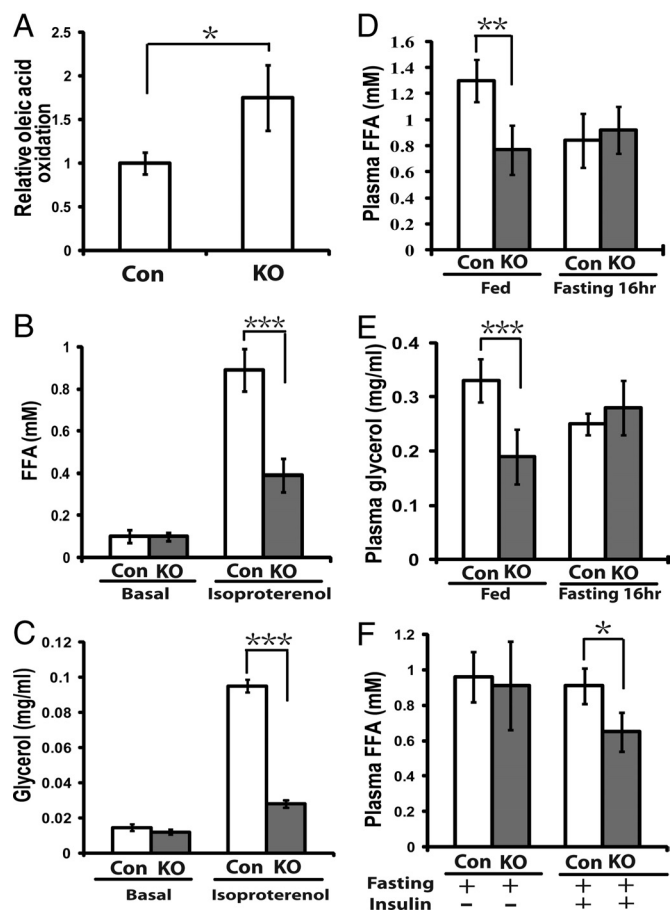
***Atg7* Knockout Adipose Tissues Had Increased Mitochondria Content.** Mitochondria play an important role in lipid metabolism and autophagy is critical for mitochondria elimination (22). We therefore analyzed mitochondria levels in adipose tissues of wild-type and *atg7* knockout mice. Fig. 3A and B show electron microscopic pictures of the white and brown adipocytes, respectively. The wild-type white adipocytes contained a limited volume of cytoplasm and relatively few mitochondria (Fig. 3A, *Left*). In contrast, the *atg7* knockout adipocytes contained a larger cytoplasmic volume and a dramatically increased number of mitochondria (>8-fold increase, Fig. 3A and C). High levels of mitochondria were also observed in the mutant brown adipocytes (Fig. 3B and D). The cytoplasm of almost every single *atg7* knockout brown fat cell was densely packed with mitochondria to such an extreme extent that, except for the lipid droplets, no mitochondria-free area, or other cellular structures could be easily identified (Fig. 3B, *Right*).

***Atg7* Knockout Adipocytes Had Increased  $\beta$ -Oxidation Rates, Reduced Lipolysis Rates, and the Adipose-Specific *atg7* Knockout Mice Exhibited Lower Fed Plasma Levels of Fatty Acids and Accelerated Rates of Fatty Acid Reduction in Response to Insulin.** Mitochondria are the site of  $\beta$ -oxidation and mitochondria play an important role in lipid metabolism. We compared free fatty acid (FFA) catabolism in the wild-type and *atg7* deleted adipocytes isolated from mice by measuring the rates of  $\beta$ -oxidation. The  $1\text{-}^{14}\text{C}$ -labeled oleic acid was added into the culture medium of adipocytes and the release of  $^{14}\text{C}$ -labeled  $\text{CO}_2$  was quantified. As shown in Fig. 4A, the primary adipocytes from the mutant mice exhibited almost



**Fig. 3.** Adipose-specific *atg7* knockout mice accumulated more mitochondria in WAT and BAT (brown adipose tissue). (A) Electron microscopic pictures of adipocytes from uterine WAT of control and adipose-specific *atg7* knockout mice. Selected regions in low magnification images (within the squares) are shown below in high magnification. LD, lipid droplet; N, nucleus; arrows indicate mitochondria in the control tissue sample. (B) Representative electron microscopic pictures of interscapular BAT from control and *atg7* conditional knockout mice. Selected regions in low magnification images (within the squares) are shown below in high magnification. (C and D) Quantification of mitochondria in WAT and BAT of control and adipose-specific *atg7* knockout mice, respectively. Mitochondria number was counted in 25 random cells and expressed as the number of mitochondria per nucleus. \*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$ , Student's *t* test.

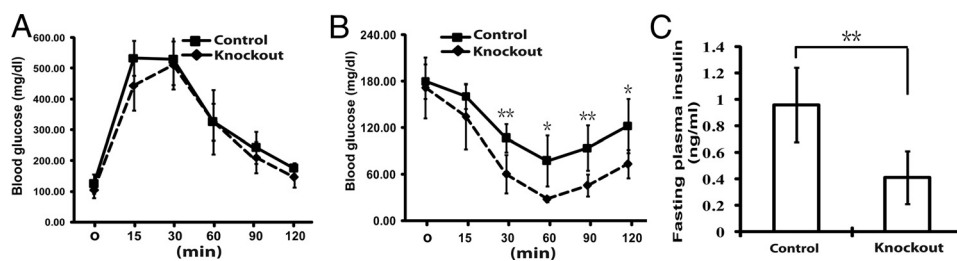
double the rates of oleic acid oxidation found in wild-type cells. We then analyzed lipolysis rates in the isolated adipocytes (Fig. 4B and C). As shown in Fig. 4B and C, while no difference was observed between the basal lipolysis rates of the isolated mutant and wild-type adipocytes, the hormone induced lipolysis rate of the mutant cells was lower than that of the wild-type cells. We further measured the plasma levels of fatty acids in the wild-type and mutant mice. As shown in Fig. 4D and E, the mutant mice exhibited significantly lower fed levels of plasma fatty acids and glycerol, even though no significant difference in fasting FFA levels was observed. Furthermore we compared the responsiveness of plasma FFA concentrations toward insulin stimuli. As shown in Fig. 4F, 30-min post insulin injection, the plasma concentration of free fatty acids in the mutant mice declined to a much lower level than in the wild-type mice.



**Fig. 4.** Fatty acid oxidation and lipolysis analysis of the adipose-specific *atg7* conditional knockout mice. (A)  $\beta$ -oxidation analysis of white adipocytes isolated from control (CON,  $n = 3$ ) and *atg7* conditional knockout mice (KO,  $n = 4$ ).  $1\text{-}^{14}\text{C}$  labeled oleic acid was added to the medium containing isolated adipocytes ( $5 \times 10^5$ ), and  $^{14}\text{CO}_2$  trapped by the filter paper soaked with hyamine hydroxide was measured after a 3 h incubation by a scintillation counter. (B and C) Lipolysis analysis of white adipocytes from control (CON,  $n = 3$ ) and *atg7* conditional knockout (KO,  $n = 4$ ) mice. Adipocytes ( $5 \times 10^5$ ) from control and *atg7* conditional knockout mice were incubated in medium for 2 h in the absence or presence of  $10 \mu\text{M}$  isoproterenol. Free fatty acid (FFA) (B) and glycerol (C) levels were then measured. (D) Plasma FFA levels of fed and fasting (16 h) mice (Con,  $n = 6$ ; KO,  $n = 6$ ). (E) Plasma glycerol levels of the fed and fasting (16 h) mice (Con,  $n = 6$ ; KO,  $n = 6$ ). (F) Plasma FFA levels of the mice (fasted for 5 h) before and 30 min after i.p. injection of insulin (0.75 U/kg, Con,  $n = 6$ ; KO,  $n = 6$ ). \*,  $P < 0.05$ , \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ . Student's *t* test. These data are representative results from two independent experiments.

**Adipose-Specific *atg7* Knockout Mice Exhibited Increased Insulin Sensitivity.** Lipatrophy in mice typically leads to the abnormal deposition of triglyceride in liver and muscles instead, which in turn

**Fig. 5.** Adipose-specific *atg7* conditional knockout mice exhibited increased insulin sensitivity. (A) Glucose tolerance tests. Control mice (male,  $n = 6$ ) and *atg7* conditional knockout mice (male,  $n = 6$ ) were fasted overnight before receiving an i.p. injection of 2 g/kg glucose and blood glucose concentration was measured at indicated time points. (B) Insulin tolerance tests. Control mice (male,  $n = 6$ ) and *atg7* conditional knockout mice (male,  $n = 6$ ) were fasted for 5 h before receiving an i.p. injection of 0.75 U/kg insulin and blood glucose concentration were measured at indicated time points. (C) Plasma insulin levels of the control (male,  $n = 6$ ) and *atg7* conditional knockout mice (male,  $n = 6$ ) fasted for 5 h. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ , Student's *t* test. These data are representative results from two independent experiments.



**Table 1. Metabolic parameters of control and *atg7* conditional knockout mice**

Parameters	Control	Knockout	<i>P</i> value*
Glucose (mg/dl), fed	146.25 $\pm$ 12.66	150.40 $\pm$ 18.31	0.13
Glucose (mg/dl), fasting 16 hr	90.17 $\pm$ 27.02	96.67 $\pm$ 11.89	0.60
Lactate (mM), fed	2.28 $\pm$ 0.25	2.00 $\pm$ 0.25	0.10
Lactate (mM), fasting 16 hr	1.48 $\pm$ 0.42	1.67 $\pm$ 0.21	0.34
Cholesterol (mg/ml), fed	2.62 $\pm$ 0.59	2.80 $\pm$ 0.74	0.68
Cholesterol (mg/ml), fasting 16 hr	2.64 $\pm$ 0.47	1.51 $\pm$ 0.46	0.008
Triglyceride (mg/ml), fed	0.85 $\pm$ 0.32	0.42 $\pm$ 0.13	0.01
Triglyceride (mg/ml), fasting 16 hr	0.33 $\pm$ 0.05	0.19 $\pm$ 0.04	0.0005

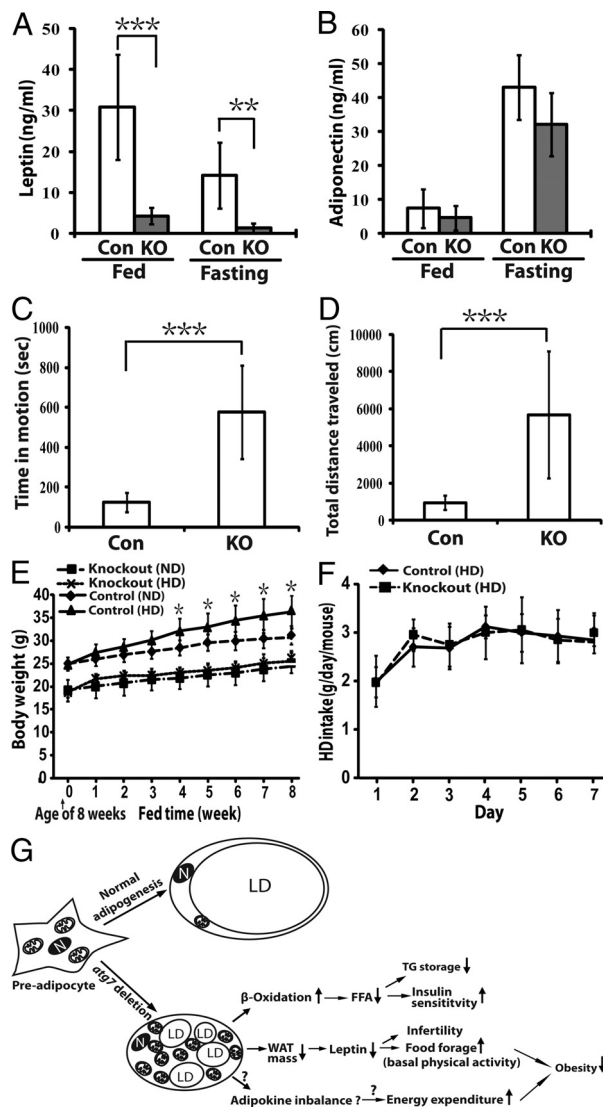
\*Control:  $n = 6$ ; Knockout:  $n = 6$ . Student's *t* test.

causes insulin resistance (20). Surprisingly, unlike other lipatrophy mouse models, the *atg7* conditional knockout mice did not exhibit abnormal fat deposition in liver and muscles. We then measured insulin sensitivity in these mice by performing glucose tolerance and insulin tolerance tests. As shown in Fig. 5A (basal levels), as well as Table 1, no significant difference in fasting and fed plasma glucose levels was observed between the control *atg7* wild-type and the *atg7* conditional knockout mice. In addition, the mutant mice exhibited no significant difference in response to glucose in glucose tolerance tests compared to the wild-type mice (Fig. 5A), suggesting that the insulin secretion function of the pancreatic B cells of the mutant mice in response to glucose elevation was normal. Importantly, the mutant mice exhibited significantly increased sensitivity to insulin as measured by insulin tolerance tests (Fig. 5B). Consistent with increased insulin sensitivity, the fasting plasma insulin levels were lower in the mutant mice (Fig. 5C). These results show that the *atg7* conditional knockout mice had significantly increased insulin sensitivity.

**Adipose-Specific *atg7* Knockout Mice Exhibited Markedly Decreased Leptin Levels, Reduced Plasma Concentrations of Triglycerides and Cholesterol, Increased Basal Physical Activities, and Were Resistant to High-Fat-Diet-Induced Obesity.** The plasma levels of leptin and adiponectin were measured. As shown in Fig. 6A, the fed and fasting plasma levels of leptin in the mutant mice were only  $\approx 10\%$  the levels found in the wild-type mice. Interestingly, no significant difference was observed in the plasma adiponectin levels between the mutant and wild-type mice (Fig. 6B). The plasma concentrations of other metabolites were also measured (Table 1). Significantly, the adipose-specific *atg7* knockout mice also had reduced plasma concentrations of triglycerides and fasting levels of total cholesterol.

The mutant mice had a similar rate of food intake compared to the wild-type mice (or even an increased rate if normalized against body weight), yet they accumulated less WAT mass, suggesting an increased rate of energy expenditure. We directly compared the basal physical activities of the wild-type and the *atg7* conditional knockout mice using an animal activity monitor equipped with computer-linked infrared sensors that detect the movement of





**Fig. 6.** The adipose-specific *atg7* conditional knockout mice exhibited altered adipokine secretion, increased basal physical activity, and were resistant to high-fat diet induced obesity. (A) Leptin levels of the fed and fasted (5 h) control ( $n = 6$ ) and *atg7* conditional knockout mice ( $n = 6$ ). (B) Adiponectin levels of the fed and fasted (5 h) control ( $n = 6$ ) and *atg7* conditional knockout mice ( $n = 6$ ). The data from (A) and (B) are representative results from two independent experiments. (C and D) Open Field Test of mouse activity. 12 wild-type and 12 *atg7* conditional knockout mice were analyzed with VersaMax animal activity monitor (Accuscan) for a period of 20 min. Time in motion (C) and total distance traveled (D) were recorded. (E) Body weight chart of control (*atg7*<sup>flox/flox</sup>, male,  $n = 9$ ) and adipose-specific *atg7* conditional knockout mice (*atg7*<sup>flox/flox</sup>; *aP2-cre*, male,  $n = 6$ ) fed with normal diet (ND) or high fat diet (HD) from the age of 8–16 weeks. (F) One-week HD food intake chart of control (male,  $n = 6$ ) and *atg7* conditional knockout (male,  $n = 6$ ) mice starting from week 14. (G) A working model summarizing the phenotypes of the adipose-specific *atg7* knockout mice and the proposed underlying mechanisms. Detail was described in Discussion. \*,  $P < 0.05$ , \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ , Student's *t* test. LD, lipid droplet; N: nucleus; TG, triglyceride; ↑, upregulation; ↓, downregulation.

small animals in a defined open field (open-field test). As shown in Fig. 6 C and D, the mutant mice exhibited dramatically increased basal physical activities, as manifested by increased time in motion and increased total distance traveled during the testing period.

We further investigated if the adipose-specific *atg7* knockout mice were more resistant to high-fat diet induced obesity. The age-matched control and *atg7* conditional knockout mice were provided with a high-fat diet starting at the age of 8 weeks and

continuing for 2 months. The body weight of each mouse was measured weekly. Fig. 6E shows the body weight chart. As expected, the wild-type mice gained  $\approx 20\%$  more body weight when fed with the high-fat diet during this 2-month period as compared to mice fed with a normal diet. In contrast, the mutant mice fed with the high-fat diet gained almost no additional weight compared to those fed a normal diet. Again, little difference in food intake was observed between the control *atg7* wild-type mice and the *atg7* conditional knockout mice (Fig. 6F).

## Discussion

White adipocytes possess a unique cellular structure which contains a unilocular lipid droplet occupying almost the whole cell volume. In this report, we have investigated the functional role of autophagy in adipogenesis in vivo by generating and characterizing a mouse model in which an essential autophagy gene, *atg7*, is deleted in adipose tissue. Our study has demonstrated that *atg7* deletion has a profound impact on the structure of white adipocytes in mice. The mutant mice developed white adipose mass that is only about one-fifth that found in the wild-type mice by the age of 18 weeks. While the total number of white adipocytes in the mutant mice appears similar to the wild-type mice, these adipocytes suffer severe morphological abnormalities, including reduced cell size, drastically increased cytoplasmic volume with higher mitochondria content, and a strikingly high percentage of cells (50%) containing multilocular lipid droplets. Despite the fact that the *atg7* knockout white adipocytes possess a number of characteristics of normal brown adipocytes, including containing multiple smaller lipid droplets instead of one large one, they do not express significant levels of *Ucp1* and other brown fat genes such as *elov13*, *cidea*, and *prdm16*. In addition, the multilocularity of the mutant cells is not caused by an increase in lipolysis, since the mutant adipocytes exhibited no change in basal lipolysis rate and even a reduction in hormone induced lipolysis (Fig. 4 B and C). These phenotypes thus underscore the importance of the function of *Atg7* in the formation of the highly specialized cellular structure during maturation of white adipocytes.

Most mouse models with lipoatrophy exhibit hyperlipidemia, hyperglycemia, and insulin tolerance as a result of defects in lipid storage and also show a consequent misdeposition of lipid in liver and muscles. Surprisingly, the adipose-specific *atg7* knockout mice are euglycemic and are more sensitive to insulin. It appears that the structural changes of the mutant white adipocytes have led to interesting functional changes. Consistent with higher mitochondria content, the mutant adipocytes have increased rates of fatty acid  $\beta$ -oxidation. Moreover, the mutant adipocytes exhibit reduced hormone-induced lipolysis rates. Together, the changes of lipid metabolism in the mutant WAT may have led to the altered lipid homeostasis observed in the mutant mice, which exhibit decreased fed plasma levels of FFA and accelerated rates of FFA reduction upon insulin stimulus. In turn, the systemic changes of FFA homeostasis likely contribute to the enhanced insulin sensitivity (illustrated in Fig. 6G).

Another striking phenotype of the adipose-specific *atg7* knockout mice is resistance to high-fat diet induced obesity. This must be either a result of increased energy expenditure or reduced energy utilization/storage efficiency or both. Indeed, the mutant mice are hyperactive [increased basal physical activity (Fig. 6 C and D)]. The adipose-specific *atg7* knockout mice have drastically reduced plasma leptin levels, likely a result of the reduced WAT mass. It is likely that the reduction of leptin contributes to the hyperactivity phenotype (23) as well as infertility (3). However, the reduction of leptin alone cannot account for the phenotype of resistance to high-fat diet induced obesity, since a lack of leptin expression in mice with normal adipogenesis (the *ob/ob* mice) results in obesity (24). The exact mechanism underlying this phenotype remains unclear, but the answers may lie in the uniqueness of the *atg7*-deleted white adipocytes, which may have a reduced efficiency of

