

Resistance of *Alcat1* Null Mice to High Fat Diet Induced Obesity and Impaired Glucose Tolerance

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Abstract

The mouse acyl CoA: lysocardiolipin acyltransferase (ALCAT1) exhibited lysocardiolipin acyltransferase activity and polyglycerophospholipid acyltransferase activity *in vitro* and therefore was predicted to play a role in polyglycerophospholipid remodeling pathway. To investigate the physiological function of ALCAT1 *in vivo*, *alcat1* null mice were generated. The *alcat1* null mice were challenged with 60 kcal% high fat diet (HFD) to examine ALCAT1's effects on the whole body energy metabolism such as the body weight, tissue content and blood glucose level. Interestingly, the null mice gained less body weight than the controls, and exhibited less fat tissue content while the food consumptions were similar in both mice groups. Lower fasting blood glucose level and less impaired glucose tolerance test in null mice were observed after HFD challenging. Besides, the yeast complementation experiment also suggested that ALCAT1 might function through its acyltransferase activities *in vivo*. In summary, this study suggests that ALCAT1 plays a critical role in regulating energy balance *in vivo*.

Keywords: Acyl CoA, Lysocardiolipin acyltransferase (ALCAT1), Knock out; high fat diet (HFD), Obesity, Impaired glucose tolerance

1. Introduction

Cardiolipin belongs to the family of polyglycerophospholipids and constitutes 20% of total lipid of mitochondria inner membrane (Schlame *et al.* 2000; Hatch, 1998). Polyglycerophospholipids remodeling is a key step for cardiolipin and other polyglycerophospholipids to obtain proper acyl chains *in vivo*. The acyl chain content is very important for the physiological function of cardiolipin and other phospholipids, such as maintaining of the membrane potential and resistance to oxidative stress (Schlame *et al.* 2000; Hatch, 1998; Bogdanov *et al.* 2008; Sen *et al.* 2007; Schlame *et al.* 2009). The mechanism of cardiolipin remodeling is still elusive. The Land's cycle occurring in microsomes is the best-known process of phospholipids remodeling via deacylation and reacylation so far (Eichberg *et al.* 1974). A mouse microsomal enzyme acyl CoA: lysocardiolipin acyltransferase (ALCAT1) was found to possess the acyltransferase activity in the polyglycerophospholipids remodeling (Cao *et al.* 2004; Cao *et al.* 2009). Multiple ALCAT1 orthologs were identified from human to fish, except *Caenorhabditis elegans* and *Saccharomyces cerevisiae*. Northern Blotting analysis showed ALCAT1 highly expressed in mouse muscles, heart and liver. ALCAT1 contains an endoplasmic reticulum (ER) localization motif (KKXX) at its C-terminus and the recombinant ALCAT1 was found to localize in ER of mammalian cells (Cao *et al.* 2004).

ALCAT1 contains a highly conserved phosphate acyltransferase domain (Plsc), which is also found in the TAZ protein that shows lysocardiolipin acyltransferase activity involved in cardiolipin remodeling (Cao *et al.* 2004;

Neuwald, 1997). Wide ranges of phospholipids substrates of ALCAT1 were identified *in vitro* so far. ALCAT1 exhibited substrate specificity for monolysocardiolipin (MLCL), dilyocardiolipin(DLCL), and other members of polyglycerophospholipid including phosphatidylglycerol (precursor of cardiolipin synthesis), and bis (monoacylglycerol) phosphate (an isomer of lysophosphatidylglycerol and a intermediate of cardiolipin) *in vitro*. ALCAT1 is a CoA dependent enzyme and can incorporate 18:2 acyl chain and other polyunsaturated moieties into polyphospholipid (Cao *et al.* 2004; Cao *et al.* 2009). The study of ALCAT1 protein structure indicated that D168 and L169 are critical amino acids potentially involved in its lysophospholipid substrate binding. Damage of cardiolipin content and species caused different type of metabolism situations, such as Barth Syndrome, heart failure, and aging (Zhao *et al.* 2009). The cardiolipin content is linked to the mitochondrial functions of oxidative phosphorylation (Schlame *et al.* 2000; Hatch, 1998; Bogdanov *et al.* 2008; Sen *et al.* 2007; Schlame *et al.* 2009).

It has been reported that high fat diet caused serious diseases including obesity, hypertension and early diabetes (Holl *et al.* 2010; Waqar *et al.* 2010; Hur *et al.* 2010). The early stages of diabetes, such as impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) are common in USA population. About 10 to 15 percent of adults in the United States show one of above conditions. The impaired glucose tolerance is also a risk factor of mortality. Therefore people with impaired glucose tolerance and impaired fasting glucose become an important target group for primary prevention of diabetes (Rao *et al.* 2002; Barr *et al.* 2007). The studies of ALCAT1 *in vitro* suggested that ALCAT1 might play a role in cardiolipin and polyglycerophospholipids remodeling. ALCAT1 exhibits an enzyme activity effectively on a wide range of substrates (polyunsaturated acyl chains and phospholipids) including lysocardiolipin. However, the physiological function of ALCAT1 *in vivo* is largely unknown. To explore how ALCAT1 functions in mouse energy metabolism, a new genetics model of *alcat1* null mouse was generated and the null mice showed stronger catabolic metabolism and resistance to high fat diet induced obesity. Therefore these results suggested ALCAT1 possible positively regulated the anabolic metabolism *in vivo*.

2. Material and Experiment Methods

2.1 Animals

The animals were maintained and handled according to standard animal experimental protocol 2006-053 approved by PSU-COM Institutional Animal Care and Use Committee. Two breeding pairs of *alcat1* knock out mouse (KO) (C57/BL6) (generated by Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN) were transferred from Taconic (Taconic Farms, Inc.) and were housed in standard barrier facilities free of pathogens (Department of Comparative Medicine, PSU-COM). Null mouse founder was backcrossed with wild type (C57/BL6) prior to breeding of the study groups. All animals were maintained on a 12/12 hour light/dark cycle. Every cage housed no more than five mice. Food and water were given *ad libitum*. Control mice (-/+) were littermates of knockout mice (-/-).

2.2 Identification of Knockout *alcat1* Mice with PCR genotyping

The *alcat1* knockout mice were generated following the standard protocols (Hogan, *et al.*, 1994) (Lilly Research Laboratories). The experimental mice were generated from breeding of heterozygous mice and the genotype of progenies was identified by PCR genotyping. PCR genotyping was performed according to the protocol provided by Taconic. Briefly, PCR template, the mice genomic DNA, was isolated from mice tail using a genomic DNA purification System (Promega). Taconic provided the primers' sequence for *alcat1* null mice PCR genotyping and the primers were synthesized in Integrated DNA Technologies (IDT). The primers YS104W1 and YS104S1 are for detecting the wild type allele; the primers N752S and N1131AS are for detecting the knock out allele. Wild type allele generates a 558bp fragment; while the knock out allele generates a 375bp fragment. PCR was performed using Taq polymerase (Promega).

2.3 RNA Isolation and Northern Analysis

Total RNA was isolated from mouse liver using the Trizole protocol (Invitrogen). 10-30ug total RNA per sample was subjected to electrophoresis, and RNA samples were blotted overnight to a Nylon membrane (Roche) (Sambrook and Russell, 2001). ALCAT1 sequence was used as the probe; GAPDH was used as an internal control (a gift from Dr Ji-Yue Zhu's lab, PSU-COM). Probes were radio labeled with [α^{32} P] dCTP (Perkin Elmer) by random primer labeling kit (Invitrogen). The autoradiography was performed by the Typhoon Imager (GE Healthcare Bio-Sciences) and was quantified by ImageQuant software program.

2.4 High Fat Diet Challenging and food consumption

60% fat (kcal %) Rodent Diet (Research Diets, catalogue number: D12492) was used. Knockout mice and control mice, 4-6 weeks old, were provided with high fat diet food *ad libitum* for 8 weeks. Food weights of each cage were

measured every week before mice feeding and after feeding. The average food consumption per mouse in a week was calculated as:

Average Food consumption (g/ week / mouse) = (food weight before a week of feeding – leftover food weight after a week of feeding)/ number of mouse per cage

2.5 Body Weight and Tissue Content Measurements

Body weight measurements were performed respectively before high fat diet challenging and at 2, 4, 5, 6, 7 or 8 weeks with high fat diet challenging. Every mouse was measured individually on an electronic scale (Denville Scientific). After mice were fed with high fat diet for 8 weeks, tissue contents were measured at Mouse Metabolic Phenotyping Center (PSU-COM). Fat tissue/lean tissue/body fluids were measured by EchoMRI 3-in-1 (Echo Medical System) for the un-anesthetized mice.

2.6 Oral Glucose Tolerance Test

Oral Glucose Tolerance Test (GTT) was performed before 60% high fat diet feeding and 8 weeks later after high fat diet feeding, respectively. Mice were fasted for 16-18 hours before the day of the experiment. Unanesthetized mice were orally fed 50% D-glucose with the amount of 1 g/kg body weight with 10³gavage. Blood glucose were measured from tail vein by One-touch glucose meter and One-touch test strips (LifeScan) at time 0, 30, 60, 90, and 120 minutes after glucose administration.

2.7 Yeast complementation

Complementation experiment was performed as previously described with a little revision (Schmidt *et al.* 1997). In brief, the *Ataz* and wild type strains (*w303*) provided by Euroscarf (Euroscarf, German) were transformed with vector pEMBLyes4 with/without *ALCAT1* gene. Positive clones were cultivated in selective liquid SD-URA medium for overnight at 30°C. 10D₆₀₀ cells were washed with 1x phosphate buffer saline (PBS) and suspended into 100ul 1x PBS. 1-5ul sequential 10x diluted cell was dotted on the agar plate of URA selective SD medium with 2% galactose. Plates were incubated in 37°C incubator for 2 days before observing results and taking picture.

2.8 Statistical Analysis

Results were presented as mean ± standard error of the mean (SEM). Results were compared using Student's t-test to assess differences between two genotype groups or two treatment groups. Differences were considered significant when P < 0.05.

3. Results

3.1 Identification of *alcat1* knockout mouse

According to PCR genotyping (Fig 1A), *ALCAT1* expression was examined in liver tissues of mice with different genotypes. Consistent with PCR genotyping, Northern-blotting showed different *ALCAT1* mRNA levels in liver tissues of mice with different genotypes (Fig 1B). Clearly, there was no *ALCAT1* mRNA detected in null mice (-/-); less *ALCAT1* mRNA was present in heterozygous mouse (-/+) than in wild type (+/+); while the internal control of Glyceraldehyde3-Phosphate Dehydrogenase (GAPDH) showed same sample loading in all groups.

3.2 The *alcat1* null mouse showed a smaller body weight than wild type challenged by high fat diet (HFD)

The high fat diet D12492 (Research diets) provides 60% of total energy through diet fat (supplementary data 2). It has been reported that high fat diet D12492 effectively induced phenotypes of obesity and diabetes in C57BL/6 wild type mice (Bush *et al.*, 2001; Shapiro, 2001; Parekh *et al.*, 1998). Therefore, we chose this diet to perform the diet challenging on the *alcat1* null mice, which is in the C57BL/6 background as well. Since there were no different responses identified between heterozygous and wild type littermates to the high fat diet challenging (supplementary data 1), *alcat1* knockout mutation is recessive, thus the heterozygous littermates (+/-) were used as negative controls of *alcat1* null (-/-) mice in all the subsequent experiments.

In male mice, the body weight of *alcat1* null mice (29.6 g ± 0.4) had been significant less than control mice (31.1 g ± 0.4) since the 2nd weeks' HFD feeding (Student's t-test, **P<0.01) (Fig. 2A). Moreover, the difference of body weight between null mice and control mice increased dramatically; the body weight of control mice reached 48.3 g ± 0.4, while null mice was only 41.4g ± 1.5 in the 8th week of HFD feeding (Student's t-test, **P<0.01) (Fig2A). However, the negative control (19.4g ± 0.2) showed similar body weight to that of null males (19.8g ± 0.2) before HFD (Fig. 2A).

The effect of HFD was not so quickly observed in female mice (Fig. 2B). The two groups of female mice showed slight difference in body weight during seven weeks of high fat diet feeding. However, the null female

mice exhibited much smaller body weight than controls in the 8th week of HFD feeding.

3.3 The *alcat1* null mice showed lower fat tissue content after challenged by high fat diet

Within eight weeks of HFD, the male null mice showed 21.9% less fat tissue ($17.8\text{g} \pm 0.48$) than the control mice ($21.7\text{g} \pm 0.53$) (Student's t-test, $**P < 0.01$), while there was small difference of the lean tissue between null mice ($22.1\text{g} \pm 1.2$) and control mice ($25.0\text{g} \pm 0.2$) (Fig. 3A). Also the male null mice (0.07%) contained much less water than control mice (0.2%) (Student's t-test, $**P < 0.01$) (Fig. 3B). Besides, the percentage of lean tissue ($W_{\text{lean}}/W_{\text{body}}$) in male null mice (55%) was a slightly more than that of male control (53%). The female null ($12.5\text{g} \pm 2.2$) showed lower fat weight than the female controls ($17.5\text{g} \pm 2.1$), yet the difference was not statistically significant (Fig. 3C, 3D). And lower percentage of fat tissue ($W_{\text{fat}}/W_{\text{body}}$) in null female (40%) than the controls (47%) was observed.

3.4 The high fat diet food consumption of null mice was similar to the control mice

Body weight differences between *alcat1* null mice and control mice could be attributed to the food consumption difference between mice groups. To test the hypothesis, food consumptions were measured in different genotypes and different genders. However, the food consumptions varied slightly in each group and each week (Table1). Though the null mice consumed a little more than the controls, there were no significant differences in food consumption between two groups.

3.5 The *alcat1* null mice showed better fasting blood glucose level and oral blood glucose tolerance test (GTT) than controls after HFD treatment

It was found that the fasting blood glucose level of the male control mice ($240\text{ mg/dL} \pm 14.5$) elevated much higher than that of male null mice ($158\text{ mg/dL} \pm 7.1$) (Student's t-test, $**P < 0.01$) after HFD feeding, although the two groups showed similar low fasting blood glucose before HFD ($88\text{ mg/dL} \pm 3.9$ and $86\text{ mg/dL} \pm 2.5$, respectively) (Fig 4A, 4B). Likewise, the elevation of fasting blood glucose level of female control ($245\text{ mg/dL} \pm 30.1$) was two folds higher than that of female null mice ($96\text{ mg/dL} \pm 5.9$) with HFD feeding (Student's t-test, $**P < 0.01$) (Fig. 4D), the fast blood glucose before HFD was still alike between control ($74\text{ mg/dL} \pm 3$) and null mice ($78\text{ mg/dL} \pm 6$) (Fig. 4C). It also showed that the level of fasting blood glucose of female mice was lower than that of male mice during the process.

Consequently, null mice exhibited better performance in blood glucose tolerant test than control mice of both male and female after HFD feeding. A much lower blood glucose record ($340\text{ mg/dL} \pm 34$, 30') was found in male null mice, though the male control had reached $553\text{mg/dL} \pm 13$ (30') (Student's t-test, $*P < 0.05$, $**P < 0.01$, 30') (Fig 4B). There was no much difference of GTT between the male control mice ($269\text{ mg/dL} \pm 12$, 30') and the null male mice ($260\text{ mg/dL} \pm 20$, 30') before HFD feeding (Fig 4A). Likely, in GTT test after HFD, the female control demonstrated much higher blood glucose ($482\text{ mg/dL} \pm 30$, 30') than that of female null ($239\text{ mg/dL} \pm 22$, 30') (Student's t-test, $**P < 0.01$) (Fig 4D), while the female control ($282\text{ mg/dL} \pm 30$, 30') was only little higher than that of female null ($204\text{mg/dL} \pm 22$, 30') before HFD feeding (Fig 4C).

3.6 Mouse ALCAT1 protein complemented the growth retardation of *Ataz1* temperature mutant in non-permissive temperature

Mouse ALCAT1 shares a conserved Phosphate acyltransferases (Plsc) domain with yeast *Taz1p* and both of them showed acyltransferases activity. There is no ALCAT1 ortholog in yeast (Cao *et al.* 2004; Gu *et al.* 2004; Testet *et al.* 2005). Protein BLAST was performed between mouse ALCAT1 protein and yeast *Taz1p* protein in amino acids sequences and found four alignments of the two proteins with low but significant similarity scores. The Plsc domain got the highest alignment score among the four alignments in the blast, suggesting the highly conservation of amino acid sequence between two proteins. Loss function of *taz1* in yeast showed a temperature sensitive phenotype that the cell growth was slowed at 37°C due to the compromised membrane stability of mitochondria (Testet *et al.* 2005; Ma *et al.* 2004). Expression of mouse ALCAT1 in *Ataz1* yeast mutant partially rescued the growth retardation at non-permissive temperature, *Ataz1* yeast mutant clearly showed growth defect at 37°C (Fig 5). Six independent complementation experiments were performed and consistent results were observed.

4. Discussion

Mouse ALCAT1, an acyl-CoA acyltransferase, functions in the cardiolipin and other polyglycerophospholipids remodeling pathway *in vitro* (Cao *et al.* 2004; Cao *et al.* 2009). In order to know the role of ALCAT1 in energy balance of the mouse whole body, *alcat1* knockout mice were generated. In consistent with PCR genotyping (Fig. 1A), Northern blotting showed that deletion of 1st exon of ALCAT1 gene completely abolished the ALCAT1 mRNA expression in homozygous mice *in vivo* (Fig. 1B).

It has been reported that the 60kcal% HFD applied in this study can effectively induce obesity in wild type male mice (C57/Bl6) (Bush *et al.* 2001; Shapiro, 2001; Parekh *et al.* 1998). The control male mice showed body weight gaining as wild type littermates when fed with this HFD (supplementary data 1) suggesting that *alcat1* knockout mutation is recessive. Surprisingly, challenged by the same HFD, *alcat1* null male mice exhibited less body weight gaining, less fat tissue content than control males (Fig.2A, Fig.3A, 3B), although their food consumption was found to be close to control mice (Table 1). Similarly, null male mice showed significant less fat tissue and water weight than control mice, while null mice and control mice were similar in their lean tissue contents (Fig.3A, 3B). Clearly, the difference of body weight between null and control mice were mainly attributed to the difference of their fat tissue contents. These results suggested that loss of the function of ALCAT1 caused less energy deposit such as fat store *in vivo*, which may due to high level of thermogenesis, physical activities, and obligatory energy expenditure *in vivo* (Bradford *et al.* 2000).

The fact that male null mice contained more percentage of the lean tissue than the controls indicates more energy expenditure in null mice (Fig2B, 2D). The mitochondrion enriched skeleton muscle in the lean tissue is one of the key factors affecting the energy expenditure: the skeleton muscle is positive associated with the whole body energy expenditure even in resting status (Zurlo *et al.* 1990). Mitochondria in muscle could increase oxidative phosphorylation (OXPHOS) to adapt to the transient high fat diet feeding (within 14 days) and thus limit fat deposition in soleus. However, long-term high fat diet can cause significant decrease in oxidative phosphorylation (OXPHOS) activity in rat muscle (Chanseume *et al.* 2007). The mechanism of ALCAT1 affects the function of mitochondria *in vivo* is not clear yet, it is clear that cardiolipin plays key roles in oxidative phosphorylation, apoptosis and other function of mitochondrial energetic generation (Schlame *et al.* 2000; Bogdanov *et al.* 2008; Schlame and Ren, 2009). Depletion of cardiolipin content and species have been known to be linked to mitochondria oxidative stress and aging response *in vitro*, which indicated the potential of mitochondrial inner membrane was lost and may lead to oxidative phosphorylation uncoupling, depletion of ATP and activation of apoptotic cascade in the tissue (Sen *et al.* 2007; Schlame and Ren, 2009). Moreover, loss function of mouse phospholipase PLA2 gamma which was involved in the cardiolipin metabolism was reported to be resistant to high fat diet induced obesity and insulin resistance *in vivo*. The iPLA2gamma (-/-) knockout mice showed significantly decrease in State 3 respiration of skeleton muscle due to the uncoupling of phosphorylation and oxidative. Moreover, the iPLA2gamma (-/-) knockout mice kept an increased fatty acid oxidation in adipose tissue under long term high fat diet treatment (Mancuso *et al.* 2010; Malhotra *et al.* 2009; Zachman *et al.* 2010). Therefore, it is quite possible that ALCAT1 negatively affects mouse whole body energy expenditure through its indirect disturbance of the process of oxidative phosphorylation of mitochondria.

The *alcat1* null mice exhibited a better performance in GTT and fasting blood glucose level when challenged with high fat diet indicating its resistance to HFD induced hyperglycemia (Fig.4). The elevation of fast blood glucose and impaired glucose tolerance are the signs of pre-diabetes or hyperglycemia (Rao *et al.* 2002). It was reported that this kind of high fat diet could induce obesity accompanied with insulin resistance and elevated blood glucose level in wild type male mice. Due to high fat diet, the mitochondria dysfunction leads to hyperglycemia, insulin resistance and other disorder of metabolism such as diabetes, heart failure and oxidative stress, which was reported in many researches (Holl *et al.* 2010; Waqar *et al.* 2010; Jornayvaz *et al.* 2010; Hur *et al.* 2010; Bush *et al.* 2001; Shapiro, 2001; Parekh *et al.* 1998). However, loss function of iPLA2 gamma in mouse causing cardiolipin content decrease in muscle mitochondria exhibited insulin sensitive and normal glucose tolerance phenotype in HFD feeding (Mancuso *et al.* 2010; Malhotra *et al.* 2009; Zachman *et al.* 2010). The *alcat1* null mice (both male and female) showed close to normal glucose tolerance test (GTT) and fasting blood glucose level (both male and female) after HFD feeding (Fig 4), suggesting *alcat1* null mice were insulin sensitive and may not impair the fatty acid oxidization and function of insulin receptors (Bush *et al.* 2001; Shapiro, 2001; Parekh *et al.* 1998).

This report also showed gender difference of mice in their response to HFD feeding. Generally speaking, regarding body weight and fat tissue content, male mice were more sensitive to HFD while female mice were not (Fig. 2, Fig. 3); and the male fasting blood glucose level and GTT test were more sensitive to HFD than those of female (Fig. 4). These results are consistent with the previous finding that the higher efficiency of energy expenditure in female than male in mice was due to the effect of sex hormone (Dubuc *et al.* 1993; Wiedmer *et al.* 2004).

As to the biochemical function of ALCAT1 *in vivo*, the complementation experiment showed ALCAT1 partially complemented the function of yeast Taz1p *in vivo* (Fig.5). The yeast *taz1* gene is well known homologue of human taffazzin whose mutation is tightly linked to the Barth Syndrome; loss function of *taz1p* in yeast caused mitochondria membrane instability (Testet *et al.* 2005; Ma *et al.* 2004; Ma *et al.* 2004). So our complementation

result suggested that it was very likely that ALCAT1 functioned as an Acyl-CoA acyltransferase *in vivo*. The low but significant alignment score in the Plsc domain between ALCAT1 and yeast Taz1p suggested function conservation of the Plsc domain of the two genes. ALCAT1 partially complemented the stability defects of mitochondria membrane in Δtaz mutant indicating that ALCAT1 could be involved in phospholipids content of mitochondria membrane such as polyglycerophospholipid remodeling. Since loss function of ALCAT1 could change the profile of phospholipids *in vivo*, including cardiolipin and other lipid 2nd messengers, which would therefore affect the potential gradient across the mitochondrial inner membrane, destabilize the electronic transport chain complexes, uncouple oxidative phosphorylation and reduce ATP synthesis, thus whole body energy generation would be potentially decreased (Lamson *et al.* 2002; Ritz and Berrut, 2005; Schlame and Ren, 2009). Due to the possibility of less usable energy generated in mitochondria, *alcat1* null mice gained less body weight and fat tissue compared to the negative controls.

In summary, the data presented above suggest that loss function of ALCAT1 in mice enhanced the energy expenditure *in vivo*. Therefore, the mouse ALCAT1 might play a critical regulatory role in inhibiting energy consumption. However, the specific substrate of ALCAT1 *in vivo* is still missing and the detailed mechanism of ALCAT1 in mice energy metabolism needs further investigation.

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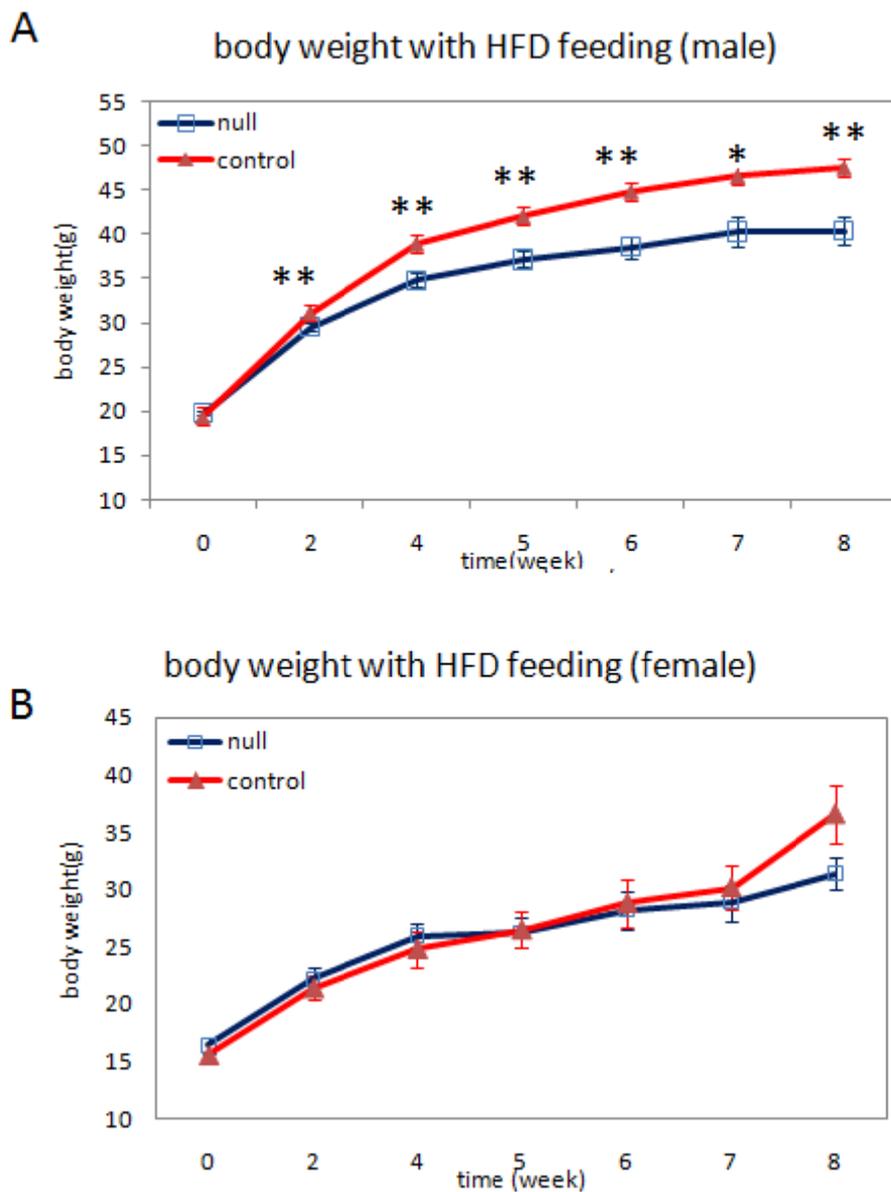


Figure 2. The measurement of mouse body weight with high fat diet feeding.

A. Male mouse; B. Female mouse. --□--, ALCAT1 null mouse; --▲--, control mouse; for each group, the number of tested individual mouse is 4; *, $P < 0.05$; **, $P < 0.01$. Error bars are standard errors.

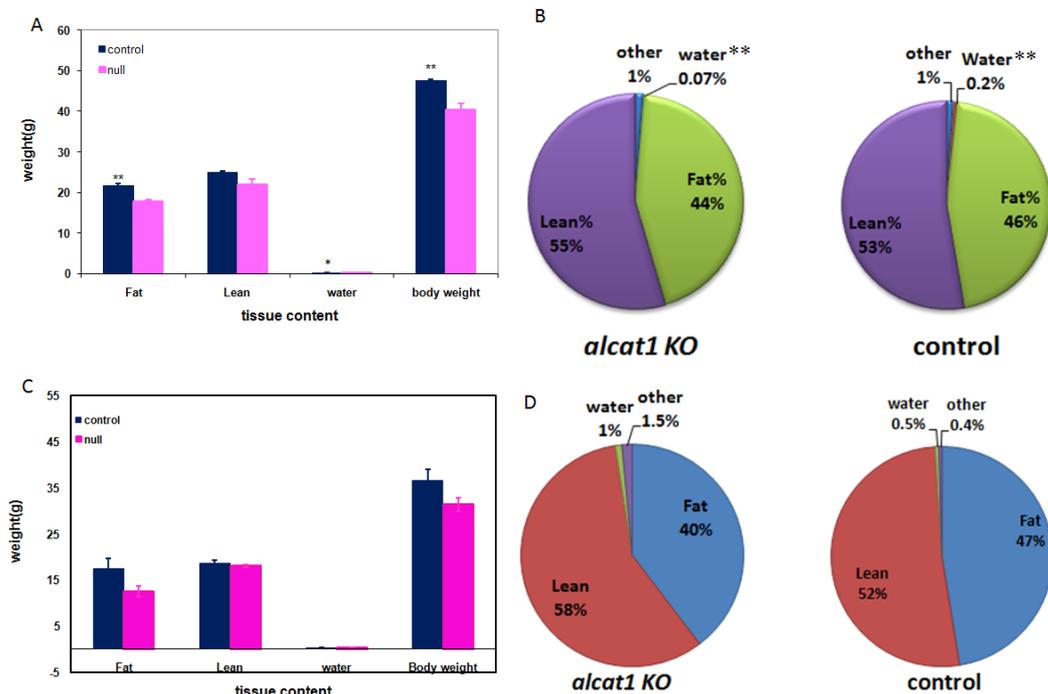


Figure 3. The measurement of mouse tissue content by EchoMRI 3-in-1 after high fat diet challenge.

A. Male mouse tissue content in weight; B. The percentage of male mouse tissue content; C. Female mouse tissue content in weight; D. The percentage of female mouse tissue content. *, P<0.05; **, P<0.01. Error bars are standard error.

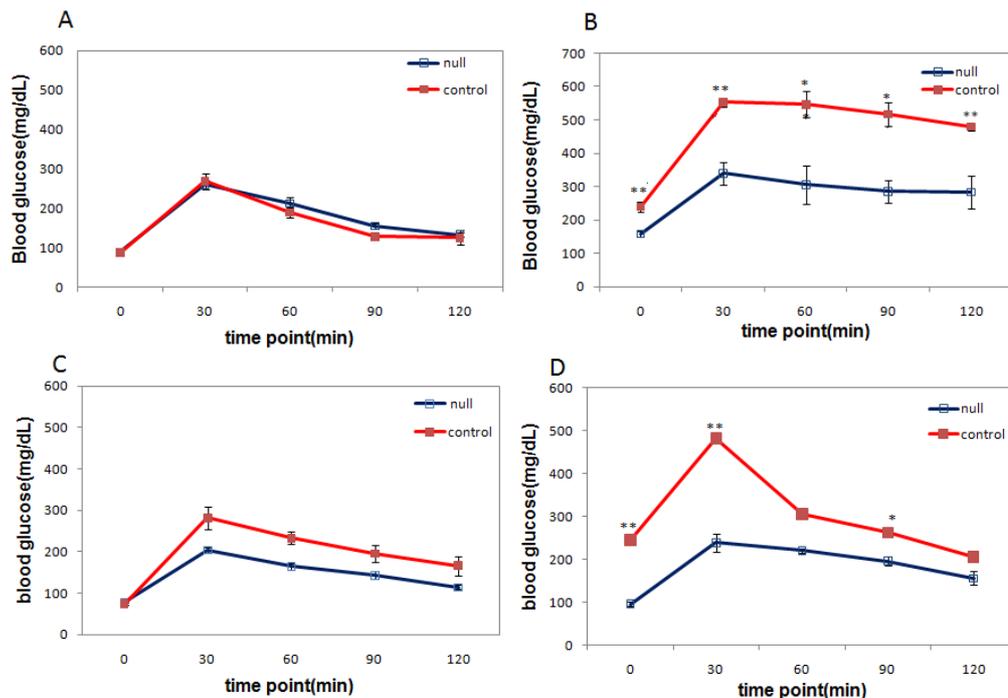


Figure 4. The oral Glucose Tolerance Test (GTT) before and after HFD challenge in mouse.

A. GTT of male mouse before HFD challenge; B. GTT of male mouse after HFD challenge; C. GTT of female mouse before HFD challenge; D. GTT of female mouse after HFD challenge. --□--, *alcat1* null mice; ---■--, control; for each group, the number of tested mouse is 4; Error bars are standard errors.

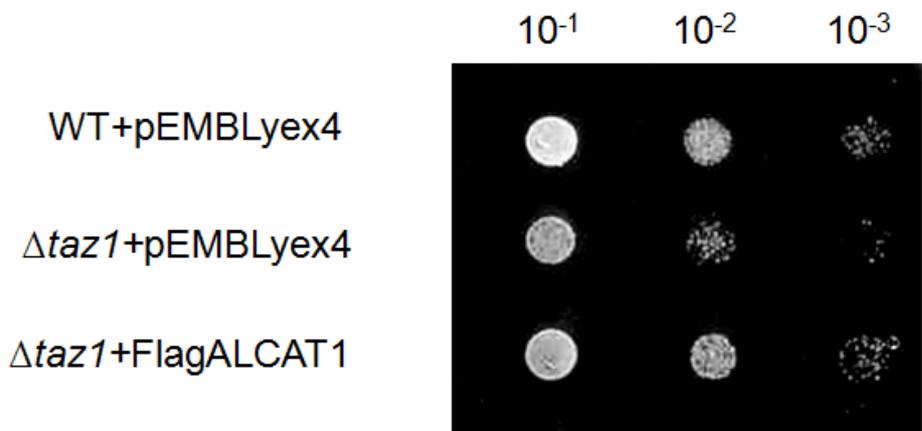
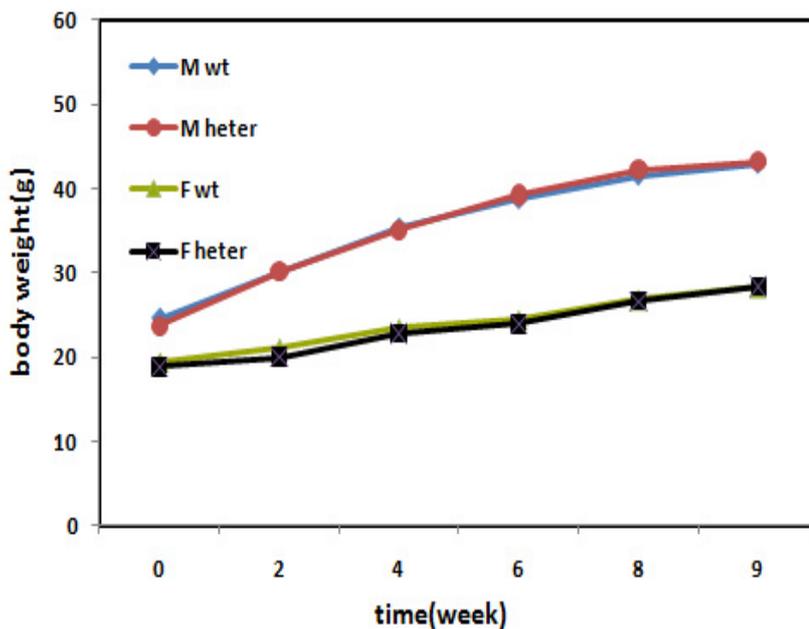


Figure 5. The mouse ALCAT1 gene complemented yeast $\Delta taz1$ mutant. Mouse ALCAT1 transformed in yeast temperature sensitive $\Delta taz1$ mutant as described above, complementation experiment was performed at 37°C; n=6.



Sup 1 The formula of 60% high fat diet (D12492).

The Research Diets Inc. provides the 60% high fat diet (D12492), which can be found on line: http://www.taconic.com/user-assets/Documents/Taconic_D12492.pdf.

Sup. 2 60% high fat diet formula (D12492)

	gm%	Kcal%
Protein	26.2	20
Carbohydrate	26.3	20
Fat	34.9	60
Total		100
	kcal/gm	
	5.24	
Ingredient	gm	Kcal
Casein, 80 Mesh	200	800
L-Cystine	3	12
Corn Starch	0	0
Maltodextrin 10	125	500
Sucrose	68.8	275.2
Cellulose, BW200	50	0
Soybean Oil	25	225
Lard*	245	2205
Mineral Mix S10026	10	0
DiCalcium Phosphate	13	0
Calcium Carbonate	5.5	0
Potassium Citrate, 1 H ₂ O	16.5	0
Vitamin Mix V10001	10	40
Choline Bitartrate	2	0
FD&C Yellow Dye #5		
FD&C Red Dye #40		
FD&C Blue Dye #1	0.05	0
Total	773.85	4057

Formulated by E. A. Ulman, Ph.D., Research Diets, Inc., 8/26/98 and 3/11/99.

The body weight of wild type (+/+) and *alcat1* heterozygote (-/+) littermates in response to high fat diet.

Mice were fed with high fat diet for the indicated period of time and body weight was shown. The mouse numbers in each group is 8.

*Typical analysis of cholesterol in lard = 0.95 mg/gram.