Biochemical and Biophysical Research Communications 451 (2014) 374–381

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

High fat diet rescues disturbances to metabolic homeostasis and survival in the *Id2* null mouse in a sex-specific manner

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ARTICLE INFO

Article history:
Received 21 July 2014
Available online 7 August 2014

Keyword:
Lipid accumulation
Survivorship
Glucose tolerance
Adipogenesis
Hepatic steatosis
Sexual dimorphism

ABSTRACT

Inhibitor of DNA binding 2 (ID2) is a helix-loop-helix transcriptional repressor rhythmically expressed in many adult tissues. Our previous studies have demonstrated that *Id2* null mice have altered expression of circadian genes involved in lipid metabolism, altered circadian feeding behavior, and sex-specific enhancement of insulin sensitivity and elevated glucose uptake in skeletal muscle and brown adipose tissue. Here we further characterized the *Id2*−/− mouse metabolic phenotype in a sex-specific context and under low and high fat diets, and examined metabolic and endocrine parameters associated with lipid and glucose metabolism. Under the low-fat diet *Id2*−/− mice showed decreased weight gain, reduced gonadal fat mass, and a lower survival rate. Under the high-fat diet, body weight and gonadal fat gain of *Id2*−/− male mice was comparable to control mice and survival rate improved markedly. Furthermore, the high-fat diet treated *Id2*−/− male mice lost the enhanced glucose tolerance feature observed in the other *Id2*−/− groups, and there was a sex-specific difference in white adipose tissue storage of *Id2*−/− mice. Additionally, a distinct pattern of hepatic lipid accumulation was observed in *Id2*−/− males: low lipids on the low-fat diet and steatosis on the high-fat diet. In summary, these data provides valuable insights into the impact of *Id2* deficiency on metabolic homeostasis of mice in a sex-specific manner.

1. Introduction

Inhibitor of DNA binding (*Id*) genes, encode helix-loop-helix (HLH) transcription factors lacking a DNA binding domain, that act as dominant negative regulators of basic HLH transcription factors [1,2]. The *Id* gene family includes four genes (*Id1-4*), which are involved in the regulation of many biological processes, including the cell cycle, circadian rhythms and adipocyte differentiation [1–4]. Recent studies have revealed a role for *Id1* in the regulation of insulin secretion and β-cell differentiation [5], and *Id4* in adipocyte differentiation and adipose tissue formation [6].

ID2 is rhythmically expressed in many mammalian tissues and is involved in the input pathway, core clock function and output pathways of the circadian clock [3,7,8]. ID2 contributes to the output pathways of the circadian clock as demonstrated in *Id2*−/− mice by the altered expression profiles of clock controlled genes in the liver, including those involved in lipid metabolism [3]. Moreover, studies have shown that absence of *Id2* results in impaired adipogenesis *in vitro* and that *Id2*−/− mice have reduced gonadal white adipose tissue (WAT) and lipid content in the liver [3,4]. Our previous findings demonstrated that *Id2*−/− mice exhibit altered feeding and locomotor rhythms, sex- and age-dependent enhanced glucose tolerance and insulin sensitivity, and sex-dependent elevated glucose uptake in skeletal muscle and brown adipose tissue [9]. It is well known that risk, development and manifestation of obesity, metabolic syndrome and insulin-resistance are sexually dimorphic [10,11]. Here we extend our studies on the characterization of the *Id2*−/− mouse metabolic phenotype under energy-rich diet challenge in a sex-specific context.

2. Materials and methods

2.1. Animals

The generation and husbandry of *Id2*−/− mice, and determination of genotypes, was performed as described previously [7,9]. *Id2*+/+ wild-type (WT) and *Id2*−/− mice were on a mixed background for breeding purposes: 129sv/C57BL6J/FBVN [7,9]. Mice

Abbreviations: HFD, high fat diet; HLH, helix-loop-helix; iBAT, interscapular brown adipose tissue; Id2, Inhibitor of DNA binding 2; LAF, lower abdominal fat; LFD, low fat diet; TG, triglyceride; WAT, white adipose tissue; ZT, Zeitgeber time.

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http://dx.doi.org/10.1016/j.bbrc.2014.07.106
0006-291X/

were maintained on a regular chow diet (Kal – 22:23:55 by % calculation as fat: protein: carbohydrate; Teklad Global diet 2919) provided *ad libitum*, and with sterile water containing antibiotic [3,7,9]. All mice were housed in laboratory cages at temperature of 20°–21 °C and humidity of 50–65% under a 12:12 light:dark (LD) cycle with lights on at Zeitgeber time (ZT) 0 and lights off at ZT12. Starting at age wk 8–10, mice were fed *ad libitum* with either a low fat diet (LFD; Kal – 12:22:66; Harlan Laboratories: 2916) or a high fat diet (HFD; Kal – 45:20:35, Research Diet Inc.: D12451). Littermate mice were used as control animals, assignment to diet groups was randomized, and apart from the change in diet all other conditions were as detailed above. All animal experiments were approved by the University of Notre Dame Animal Care and Use Committee and performed in accordance with NIH Guidelines for the Care and Use of Laboratory Animals.

For the growth curves, 48 WT and 39 *Id2*−/− mice were weighed weekly for 17 wk. For the survival rate, the percentage of surviving animals over a period of 20 wk was determined. The *in vivo* body fat composition of WT and *Id2*−/− mice was measured ranging from age 12–19 wk. Mice were aged 32–34 wk at end of experiment when tissues were harvested for phenotyping.

2.2. Glucose tolerance tests

After 18 wk on LFD or HFD, mice were subjected to glucose tolerance testing. Mice were fasted overnight (16 h) and given an intra-peritoneal injection of D-glucose (1.5 g/kg of body mass) at ZT4. Subsequent blood glucose were measured at 0, 10, 20, 30, 60, 90, and 120 min from a distal tail vein as previously described [9].

2.3. Fat mass estimation

In vivo X-ray Micro-Computed Tomography (MicroCT) was used to quantify percent body fat (see Supplementary information for full methods)

2.4. Analysis of serum and liver lipid and endocrine panels

At the age of 32–34 wk, and 22 wk of feeding experiment, serum and liver were harvested at ZT4 for lipid and endocrine analysis, and conducted at the UC Davis Mouse Metabolic Phenotyping Center (MMPC; Davis, CA) (see Supplementary information for full methods).

2.5. Tissue mass and histology

At the end of the feeding experiment, gonadal WAT deposits and interscapular brown adipose tissue (iBAT) were excised and weighed. Cryostat cut WAT sections were prepared and stained with hematoxylin-eosin as described [9]. For lipid analysis, cryostat cut liver sections were stained for Oil-red-O and hematoxylin-eosin, as described [12]. Multiple images were captured at 20× and 10× magnification for WAT and liver, respectively, using a Nikon 90i wide field microscope with a Nikon DS-Fi1 digital camera. To measure WAT cell area and liver lipid accumulation, three to five sections from each animal were analyzed manually using NIH ImageJ software and using methods previously described [9,12].

2.6. Statistics

Data were analyzed using Sigma Plot 12.0 software (Chicago, IL) to run two-factor ANOVA, two-factor repeated measures (RM) ANOVA and three-factor ANOVA with genotype, sex and diet as the independent variables. Tukey's *post hoc* tests were performed when significant ANOVA results between factors were revealed. Where necessary, data were natural log, square root or ranks transformed to correct for non-normal distributions. The survival rate data were analyzed by using χ^2 analyses (and Fisher's exact tests) for trend (Prism 5.0, Graphpad, La Jolla, CA).

3. Results

3.1. High fat diet ameliorates Id2−/− male mice phenotype and survival rate

The lean and lower body mass phenotype of *Id2*−/− mice reported in our previous studies raised the question as to whether this animal phenotype is affected by a HFD [3,7,9]. 8–10-wk-old *Id2*−/− mice and their WT littermates were put on LFD and HFD for 22 wk. WT mice gained weight indistinguishably on both diets, presumably due to their mixed genetic background, which may cause resistance to diet-induced obesity on a HFD [13] (Fig. 1A). Conversely, *Id2*−/− males gained more weight and became heavier on HFD than on a LFD by the end of experiment (Time (T), $p < 0.001$; Diet (D), n.s.; Interaction (I), $p < 0.05$) (Fig. 1A). However, this pattern of weight gain was not observed in *Id2*−/− females (T, $p < 0.001$; D, n.s.; I, n.s.) (Fig. 1A). When the body weights of WT and *Id2*−/− mice were normalized to their initial weight, the growth rate of *Id2*−/− males was found to be lower than their WT counterparts when fed the LFD (T, $p < 0.001$; Genotype (G), n.s.; I, $p < 0.01$) (Fig. 1B). However, under HFD, *Id2*−/− males showed nearly the same growth rate as their WT littermates (T, $p < 0.001$; G, n.s.; I, n.s.). *Id2*−/− females grew significantly less than WTs under LFD (T, $p < 0.001$; G, $p < 0.01$; I, $p < 0.001$) and exhibited a lower growth rate than WTs under HFD (T, $p < 0.001$; G, n.s.; I, $p < 0.05$) (Fig. 1B). We also monitored the survival rate of the WT and *Id2*−/− mice during the 20 wk experiment. Surprisingly, none of the *Id2*−/− males died on the HFD, whereas the survival rate of *Id2*−/− males on LFD dropped to under 65%; and only 56% and 67% of *Id2*−/− females on LFD and HFD, respectively, reached the age of 20 wk, (χ^2 test for trend: HFD male, $p < 0.001$, different from other 3 groups) (Fig. 1C). The remaining three *Id2*−/− groups were not different from one another (χ^2 test for trend, n.s.). There was no significant decline in body weight prior to death, as compared with susceptible *Id2*−/− mice groups, measured over 4 wk prior to death (two factor ANOVA, n.s.). Note that all WT mice survived irrespective of sex and diet.

3.2. High fat diet modulates Id2−/− male mice glucose homeostasis

Our previous study revealed that male, but not female, *Id2*−/− mice exhibit an enhanced glucose tolerance when fed with a regular chow diet (22% kcal from fat) [9]. To assess the consequences of diet on *Id2*−/− mouse glucose homeostasis, glucose tolerance tests were performed on these mice after 17 wk on LFD or HFD. *Id2*−/− males had an enhanced glucose tolerance under LFD compared to WTs (Time (T), $p < 0.001$; Genotype (G), $p < 0.001$; Interaction (I), n.s.) (Fig. 2A). In contrast, no difference in glucose tolerance between male *Id2*−/− and WT mice on HFD was observed (T, $p < 0.001$; G, n.s.; I, n.s.) In the female mice, an enhanced glucose tolerance was observed in *Id2*−/− mice fed with either LFD (T, $p < 0.001$; G, $p < 0.001$; I, n.s.) or fed with HFD (T, $p < 0.001$; G, $p < 0.001$; I, n.s.) (Fig. 2B).

3.3. Modulation of fat storage of Id2−/− male mice under high fat diet

The apparent weight gain of *Id2*−/− males under HFD raised a question as to whether the increased body mass in these animals was associated with an increase in fat mass. Therefore, we exam-

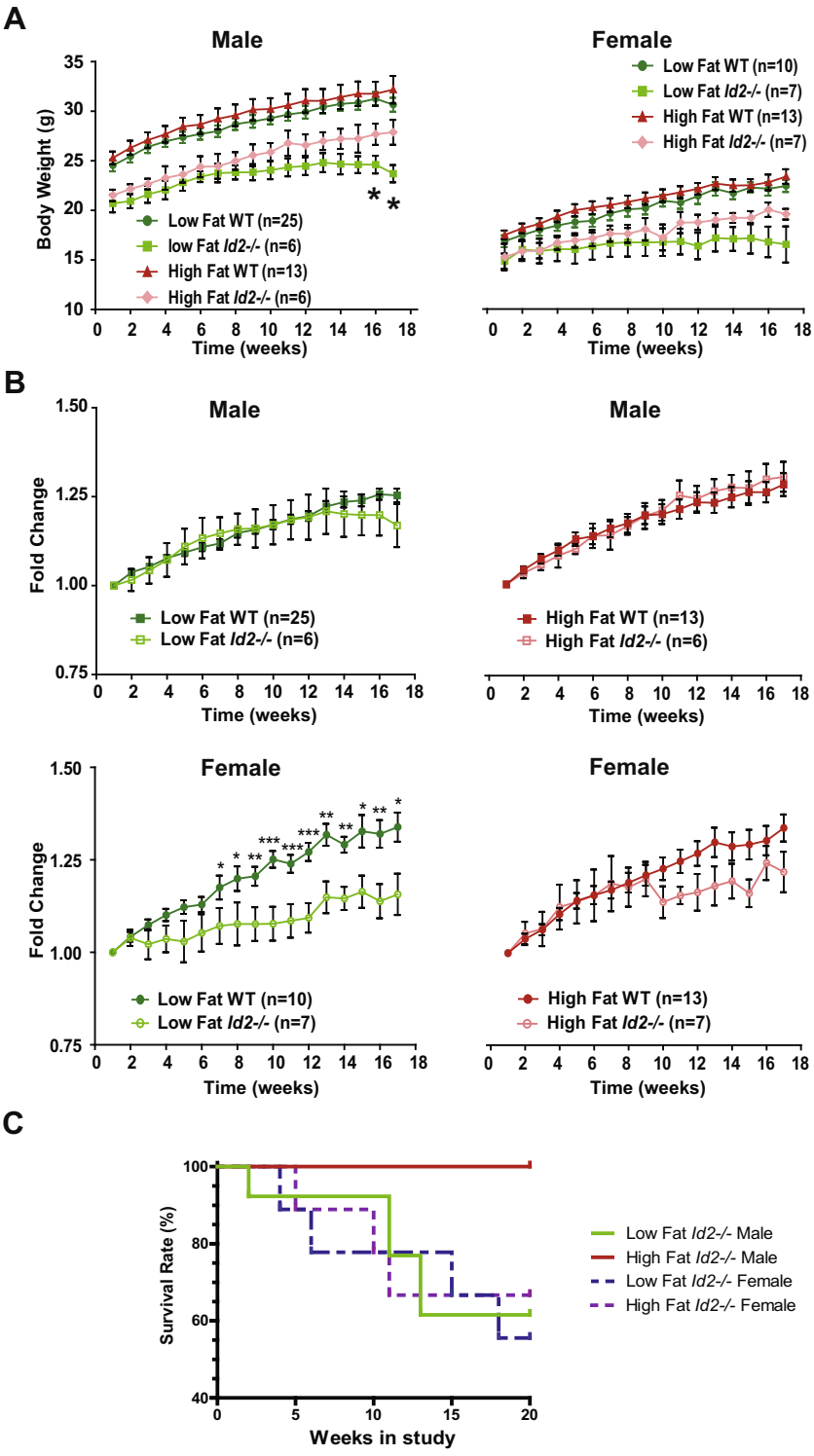

Fig. 1. Sex- and diet specific regulation of growth and survival rate in *Id2*−/− mice. (A) Weekly body weight measurements of WT and *Id2*−/− male (left) and female (right) mice fed with low fat diet (LFD) or high fat diet (HFD) over 17 wk. (B) Upper: Growth rate (fold change) of WT and *Id2*−/− male (Left: LFD; Right: HFD). Lower: Growth rate (fold change) of WT and *Id2*−/− female (Left: LFD; Right: HFD). Values are mean ± S.E.M. RM-ANOVA were performed followed by Tukey's post-hoc tests, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. (C) Percentage survival rate of the WT and *Id2*−/− mice during the 20 wk experiment.

ined and quantified the amount of fat tissue present in WT and *Id2*−/− animals fed the LFD and HFD. A representative 3D visual display of fat deposits in WT and *Id2*−/− males is shown in Fig. 3A. Analysis by MicroCT revealed no difference in fat/total volume ratio between *Id2*−/− and WT males fed with LFD, whereas *Id2*−/− males on HFD had a significant increase in this ratio compared to WTs (Genotype (G), n.s.; Diet (D), $p < 0.001$, Interaction (I), $p = 0.05$) (Fig. 3B). There was no difference in fat/total volume ratio between WT and *Id2*−/− females when fed with LFD. However, *Id2*−/− females had a significantly lower fat/total volume ratio compared to WTs when fed with HFD (G, $p < 0.01$.; D, $p < 0.05$, I, $p < 0.05$) (Fig. 3B). The trends observed from global body

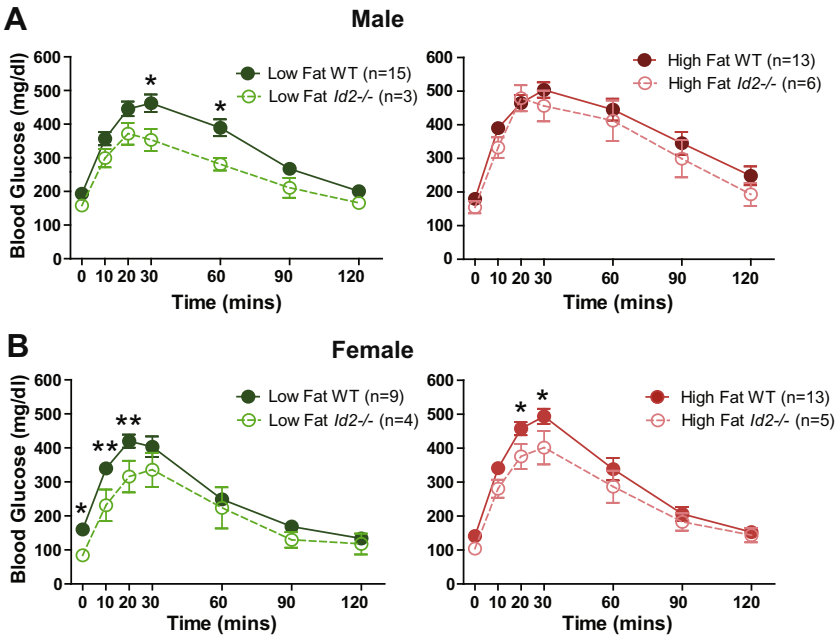

Fig. 2. Sex-specific modulation of *Id2*−/− mice glucose homeostasis under high fat diet. (A) Glucose tolerance tests of WT and *Id2*−/− male mice (Left: LFD; Right: HFD). (B) Glucose tolerance tests of WT and *Id2*−/− female mice. (Left: LFD; Right: HFD). Values are mean ± S.E.M. Two-factor ANOVA were performed followed by Tukey's post-hoc tests, $^{*}p < 0.05$, $^{**}p < 0.01$.

fat of WT and *Id2*−/− mice fed with the two diets were consistent with those of the lower abdominal fat (LAF) trends (male: G, n.s.; D, $p < 0.01$; I, n.s.; female: G, $p < 0.01$; D, n.s.; I, n.s.) (Fig. 3B). Moreover, at the end of the experiment, at wk 22, perigonadal adipose tissue in WT and *Id2*−/− mice was harvested and weighed (Fig. 3C). The weight ratio of fat from gonadal WAT was lower in *Id2*−/− males than that from WT males fed with the LFD. However, these differences were not observed in *Id2*−/− male fed with HFD (G, $p < 0.05$; D, $p < 0.001$; I, n.s.). *Id2*−/− females had a lower gonadal WAT weight ratio in both diets when compared to WT females (G, $p < 0.001$; D, $p < 0.05$; I, n.s.). No difference in iBAT weight ratios was observed between WT and *Id2*−/− mice when fed with LFD and HFD (two factor ANOVA: n.s.) (Fig. 3C). The actual weight trends of gonadal WAT and iBAT were comparable to those of weight ratio trends (Supplemental Fig. 1A and C). Histological evaluation of gonadal WAT revealed no significant difference between genotype, although the trends of adipocyte size in the female LFD group were comparable to our previous study, but less pronounced (Supplemental Fig. 1B) [9].

3.4. High fat diet modulates lipid metabolic homeostasis of Id2−/− mice

At the end of the 22 wk of controlled diet, and at the age of 32–34 wk, serum and liver were collected from mice following overnight fasting. Table 1 shows the lipid and endocrine parameters measured for these surviving mice. No difference of serum triglyceride (TG) levels was observed between genotypes in either males and females when fed with LFD or HFD (Table 1, Supplemental Fig. 2A). However, the serum total cholesterol levels were significantly lower in *Id2*−/− males fed with HFD (Table 1, Fig. 4A). Also, serum LDL cholesterol levels were lower in *Id2*−/− males fed with HFD in comparison with diet and sex-matched WTs (Table 1, Fig. 4A). A similar pattern was observed in serum HDL cholesterol levels, although this pattern was less pronounced and did not reach statistical significance (Table 1, Supplemental Fig. 2A). There was no significant difference in insulin levels between genotype, sex and diet (Table 1, Supplemental Fig. 2B). However, *Id2*−/− females were found to have elevated glucagon levels when fed with HFD (Table 1, Supplemental Fig. 2B). The serum leptin levels were found to be consistently lower in *Id2*−/− mice, irrespective of diet and sexes. However, the reduction between male *Id2*−/− and WT mice fed with HFD was not observed to be significant in *post hoc* analysis (Table 1, Fig. 4A). The *Id2*−/− mice serum adiponectin levels were not significantly different from WTs when fed with LFD and HFD (Table 1, Supplemental Fig. 2B). The liver total cholesterol levels in males showed a similar pattern across experimental groups as serum total cholesterol, but not reaching statistical significance (Table 1, Fig. 4B). Surprisingly, although the liver TG levels in *Id2*−/− males fed with LFD were significantly lower than WTs, there was striking accumulation of TG in *Id2*−/− males when fed with HFD compared to WTs (Table 1, Fig. 4B). Furthermore, these differences were observed in males only. Oil-red-O staining of liver sections confirmed this distinct pattern of lipid accumulation in *Id2*−/− male mice: a depletion of lipids on LFD but significantly higher levels of lipid on HFD, as compared to WTs (Fig. 4C).

4. Discussion

In this study, we have explored the effects of ID2 deficiency on energy metabolism in mice. Loss of ID2 inhibited weight gain in animals exposed to a LFD. This could be attributed, in part, to reduced adiposity, as shown in the current study and prior work, as well as impaired adipogenesis, and increased energy expenditure from iBAT and skeletal muscle [3,4,9]. High fat diet moderately ameliorates the gaining weight capacity of *Id2*−/− male mice only. This sexual dimorphism may be attributed to multiple factors, such as the sex difference in the fat deposition and storage, hormones, and metabolism [14,15]. Interestingly, we also found that the survival rate of *Id2*−/− male mice was significantly improved by a HFD. One possibility might be amelioration of body weight loss and reduced fat mass, as mice with no WAT fat (A-ZIP/F-1 mice) show a high mortality rate [16]. Moreover, no significant decline in body weight prior to death was found. It is also plausible

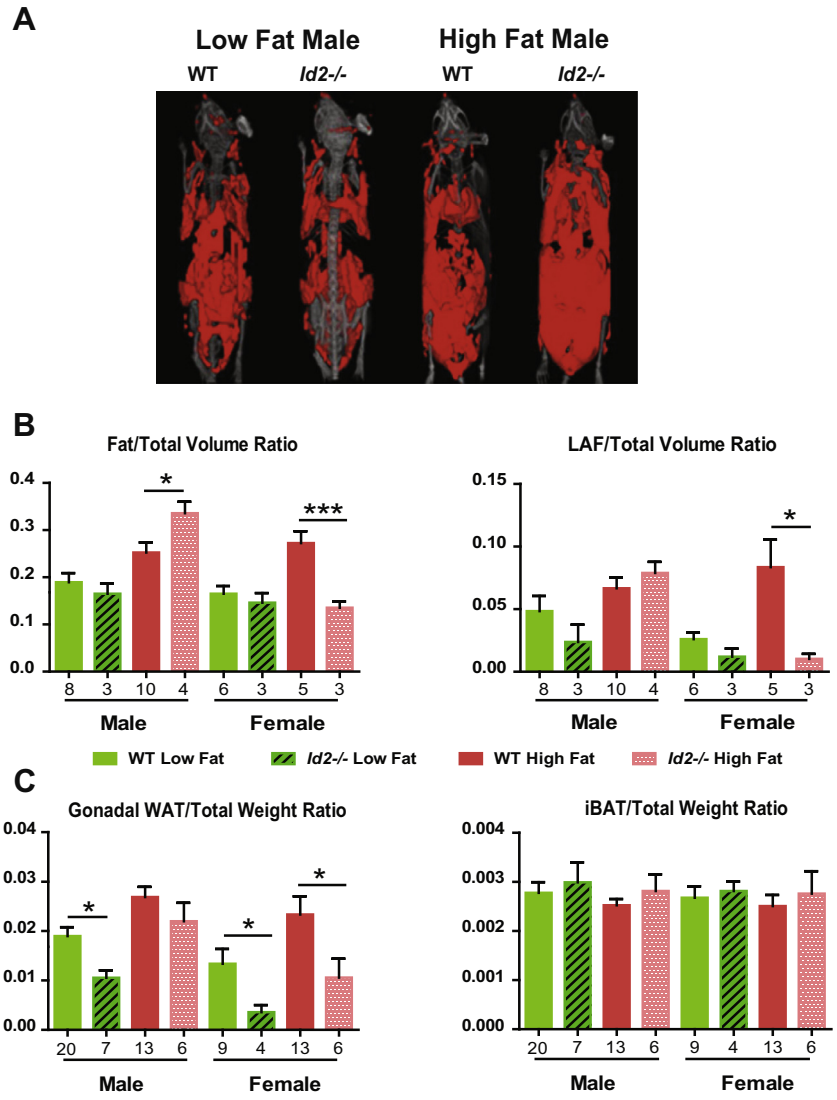

Fig. 3. Sex-specific modulation of fat storage of *Id2*−/− mice under high fat diet. (A) MicroCT representative 3D visual display of global fat deposits of WT and *Id2*−/− male mice. (B) MicroCT examination of the ratio of total fat to body volume (left) and of lower abdominal fat (LAF) volume to total volume (right) from WT and *Id2*−/− mice fed with LFD or HFD. (C) Ratio of weight of gonadal white adipose tissue to total body mass (left) and of weight of iBAT to total body mass (Right) from WT and *Id2*−/− fed with LFD and HFD. Values are mean ± S.E.M. Two factor-ANOVAs were performed followed by Tukey's post-hoc tests, $^{*}p < 0.05$, $^{***}p < 0.001$.

that the cause or contribution to the sudden occurrence of death was due to heart failure; it has been reported previously that *Id2*−/− mice have an abnormal cardiac morphology and intraventricular conduction [17]. Since HDL cholesterol levels are particularly higher in *Id2*−/− male mice on HFD compared to other groups of *Id2*−/−mice, this may help improve their cardiac function.

In this study, both female and male *Id2*−/− mice showed increased glucose tolerance, except for HFD males. MicroCT analysis revealed sex differences in total fat storage of *Id2*−/− mice under HFD. Moreover, assessment of gonadal WAT mass suggests that there are increased visceral fat depots in *Id2*−/− male mice under HFD, whereas there is less stored in visceral fat in females on both diets. Furthermore, serum leptin levels were consistent with the appropriate WAT/body mass ratio. Since previous studies show a clear association between visceral fat accumulation and insulin sensitivity, increased visceral fat depots in *Id2*−/− males might contribute to a loss of enhanced glucose tolerance [18,19]. In addition, hepatic steatosis may also compromise glucose metabolism in the HFD-fed *Id2*−/− mice. Our previous findings also revealed that increased energy expenditure is compensated in *Id2*−/− male mice through increasing food intake and reducing locomotor activity [9]. However, an energy-rich diet may undermine this compensation effect in a sex-specific manner, which may contribute to fat accumulation, loss of the lean phenotype and loss of enhanced glucose tolerance.

Interestingly, despite the lack of a marked change in circulating adiponectin levels, ectopic lipid accumulation occurred in the liver of *Id2*−/− mice in a sex-specific manner. *Id2*−/− male liver showed an opposite effect on TG levels under low and high fat diets. This observation could be explained in part by earlier findings that *lipoprotein lipase* (*Lpl*) was found to be up-regulated in the liver of *Id2*−/− mice under regular chow diet in a time-specific manner [3]. Hepatic-specific overexpression of LPL leads results in hepatic steatosis [20,21]. Another possibility for the steatosis is the spillover of fat from adipose tissue, as the total fat mass is increased in the *Id2*−/− male mice.

According to our data, we propose the following possible mechanisms to explain the sexual dimorphism observed in *Id2*−/− mice. First, female *Id2*−/− mice have higher adiponectin levels than their

Table 1
Serum and liver lipid/endocrine parameters. Serum and liver lipid and endocrine parameters were analyzed as described in Section 2. Values are mean ± S.E.M. Three factor ANOVAs were performed with sex, diet and genotype as independent variables. n.s., non-significant. Two factor-ANOVA were performed followed by Tukey's post-hoc tests, [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$, and significantly different values highlighted in bold underline in table.

	Male				Female				Three factor ANOVA statistic		
	LFD		HFD		LFD		HFD		Sex	Diet	Genotype
	WT	*Id2−/−*	WT	*Id2−/−*	WT	*Id2−/−*	WT	*Id2−/−*	Male vs female	LFD vs HFD	*Id2−/−* vs WT
Serum											
Triglyceride (TG; mg/dl)	105.6 ± 11.4	86.3 ± 33.7	94.4 ± 12.3	62.5 ± 8.5	93.8 ± 13.9	89.5 ± 13.6	88.1 ± 6.7	125.6 ± 33.5	n.s.	n.s.	n.s.
Total cholesterol (TC; mg/dl)	165.5 ± 11.2	127.2 ± 7.8	198.5 ± 14.6	**141.5 ± 11.2[a]**	119.0 ± 8.2	103.8 ± 13.1	127.9 ± 6.7	120.7 ± 10.4	*p < 0.001*	n.s	*p < 0.01*
HDL cholesterol	59.2 ± 6.5	48.4 ± 5.6	80.0 ± 7.8	69.2 ± 4.9	42.5 ± 6.3	53.8 ± 10.7	42.0 ± 5.5	37.0 ± 7.4	*p < 0.01*	n.s.	n.s.
									p < 0.001 (within HFD)	*p < 0.05* (within male)	
LDL cholesterol (mg/dl)	48.2 ± 3.6	39.7 ± 2.8	58.0 ± 4.2	**43.2 ± 2.6[a]**	35.0 ± 2.9	30.7 ± 4.3	37.9 ± 2.1	35.5 ± 2.4	p < 0.001	n.s.	*p < 0.05*
Insulin (ng/ml)	0.8 ± 0.1	0.9 ± 0.4	0.7 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.5	1.1 ± 0.3	0.6 ± 0.4	n.s.	n.s.	n.s.
Glucagon (pg/ml)	92.1 ± 18.1	69.3 ± 6.8	59.5 ± 14.4	48.0 ± 5.6	90.2 ± 15.8	206.6 ± 88.3	64.9 ± 9.6	**102.1 ± 20.4[a]**	*p < 0.01*	*p < 0.01*	n.s.
Leptin (ng/ml)	2.8 ± 0.3	**0.8 ± 0.4[a]**	4.1 ± 0.9	2.6 ± 0.6	3.7 ± 0.7	**0.6 ± 0.2[b]**	5.9 ± 0.9	**2.1 ± 0.8[b]**	n.s.	*p < 0.01*	*p < 0.001*
Adiponectin (μg/ml)	7.2 ± 0.3	8.2 ± 1.8	6.9 ± 0.4	8.0 ± 0.8	13.2 ± 0.7	12.1 ± 0.7	14.2 ± 0.8	12.7 ± 2.0	*p < 0.001*	n.s.	n.s.
Liver											
TG (mg/g tissue)	14.4 ± 1.8	**4.2 ± 0.6[c]**	15.0 ± 2.1	**44.5 ± 9.4[c]**	22.1 ± 5.0	28.4 ± 1.9	23.5 ± 3.7	15.8 ± 7.4	n.s.	n.s.	n.s.
									p < 0.001 (within LFD-*Id2−/−*)	*p < 0.001* (within male-*Id2−/−*)	*p < 0.001* (Diet × Genotype × Sex)
									p < 0.001 (within HFD-*Id2−/−*)	*p < 0.05* (within female-*Id2−/−*)	
TC (mg/g tissue)	2.0 ± 0.1	1.9 ± 0.0	2.8 ± 0.2	2.4 ± 0.1	2.0 ± 0.1	2.4 ± 0.1	2.8 ± 0.1	2.8 ± 0.3	*p < 0.001*	*p < 0.001*	n.s.
									p < 0.001 (within *Id2−/−*)	*p < 0.001* (within WT)	*p < 0.05* (within male)
										p < 0.001 (within *Id2−/−*)	

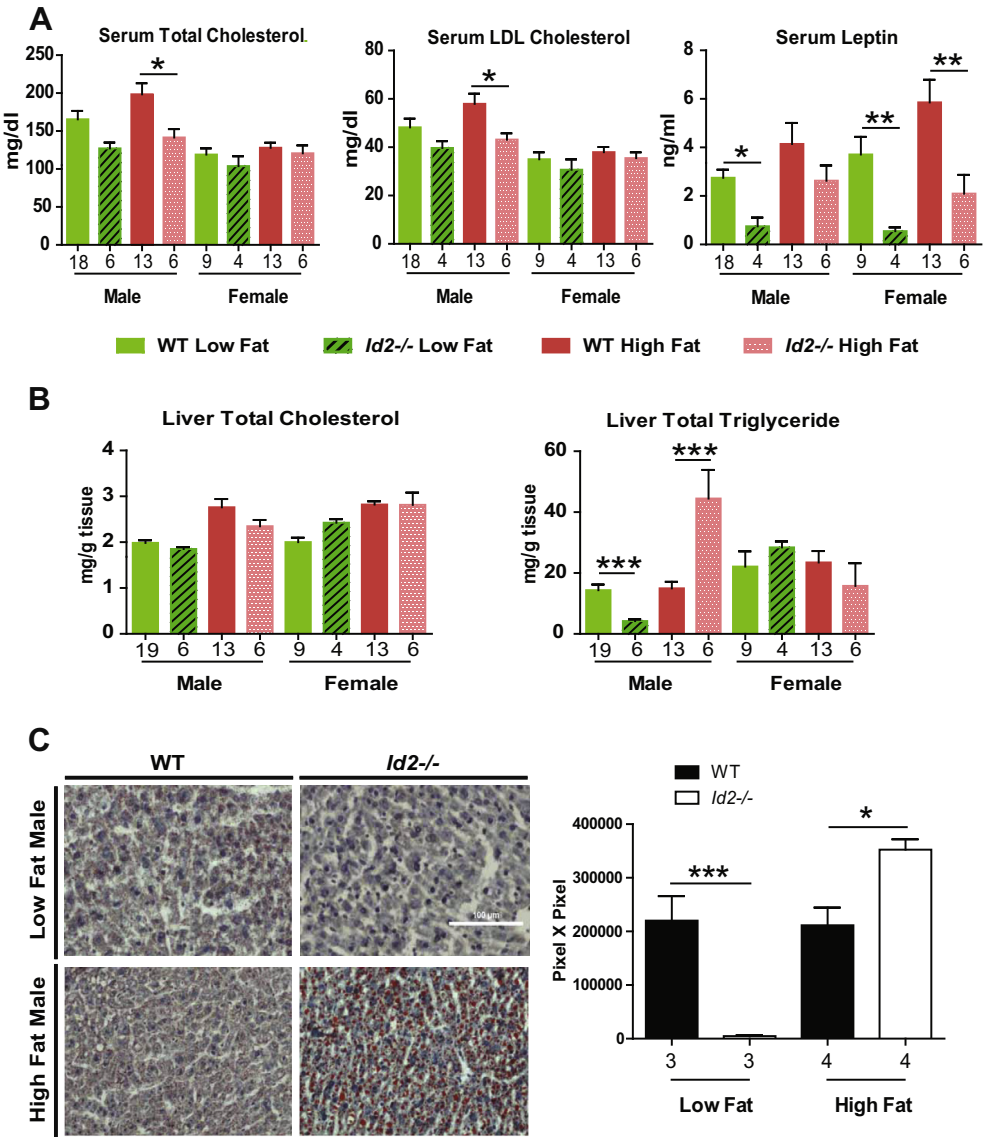

Fig. 4. High fat diet differentially modulates lipid metabolic homeostasis of *Id2*−/− male mice. (A) Serum total cholesterol (left), LDL cholesterol (middle) and leptin (right) of WT and *Id2*−/− under LFD or HFD. (B) Liver total cholesterol (left) and triglyceride (right) of WT and *Id2*−/− under LFD or HFD. (C) Representative Oil-red-O and hematoxylin-eosin stained liver section from WT and *Id2*−/− male mice liver fed with LFD or HFD. Scale bar, 100 μm. Oil-red-O quantification values, mean ± S.E.M., on the right. Two factor-ANOVAs were performed followed by Tukey's post-hoc tests, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

male counterparts. Since adiponectin is an important adipokine that regulates systemic insulin sensitivity and lipid metabolism [22], female *Id2*−/− mice maintain glucose tolerance and moderate levels of hepatic lipid contents even on high-fat diet. Second, an increase in circulated glucagon in *Id2*−/− females may also improve lipid homeostasis in adipose tissue and liver lipid degradation [23]. In addition, sex-specific adipocyte programing [9,14] and differences in hepatic lipid uptake and export might play important roles as well. Given that *Id2* is implicated in regulating circadian clock output [3,9], it is also possible that the *Id2*−/− mouse phenotypes could be a result of poor intra- and inter-organ temporal coordination of metabolic processes, including central nervous system control and intestinal lipid absorption [24,25]. These data and other pieces of evidence suggest that *Id2*−/− mice have defects in adipogenesis and lipid metabolism, which might include disturbances to rhythmic and non-rhythmic regulation of genes and processes necessary for adipocyte differentiation and function, lipid synthesis/uptake, metabolism and/or storage [26,27]. In conclusion, our current data and earlier reports suggest that ID2 is a potential therapeutic target for treating metabolic disorders. Further, these data reinforce the relevance of sex-specific analyses in studying models of metabolic function as they pertain to human health and disease.

Acknowledgments

The work was supported from grants to GED from NIGMS (R01-GM087508) and AHA (10SDG4030011). The authors are grateful to the Freimann Life Science Center for assistance with animal care and training, the Notre Dame Integrated Imaging Facility (W.M. Leevy and S. Chapman) for assistance with CT and histology, and the UC Davis MMPC (NIDDK grant U24-DK092993) for phenotyping services.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.07.106.

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