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

Peng Zhou [a], Alyssa D. Hummel [a], Cameron M. Pywell [a], X. Charlie Dong [b], Giles E. Duffield [a,*]

[a] Department of Biological Sciences and Eck Institute for Global Health, University of Notre Dame, Notre Dame, IN 46556, USA
[b] Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN 46202, USA

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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.

* Corresponding author.
E-mail address: duffield.2@nd.edu (G.E. Duffield).

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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-