

Rats perinatally exposed to food restriction and high-fat diet show differences in adipose tissue gene expression under chronic caloric restriction

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Keywords: obesity, developmental programming, lipolysis, caloric restriction, lipogenesis

Abbreviations: CEBP α , CCAAT/enhancer-binding protein α ; PPAR γ , peroxisome proliferator-activated receptor γ ; HSL, hormone sensitive lipase; CPT1a, carnitine palmitoyltransferase 1a; FAS, fatty acid synthase; LPL, lipoprotein lipase; FTO, fat mass and obesity associated; BAT, brown adipose tissue

The aim of this study is to analyze how maternal diet during the lactational period influences the adipose tissue response to chronic caloric restriction in offspring. Lactating dams were subjected to one of three treatments: 50% food restriction (FR), ad lib standard chow (AL), or ad lib high-fat diet (HF). Juveniles were first weaned onto standard chow, then in adulthood 50% calorically restricted and maintained at 90% of normal body weight for 60 d. HF animals showed increased percent body fat compared with AL and FR animals despite equivalent body weights. HF animals showed alterations in the balance of adipose tissue lipogenic (FAS, LPL) and lipolytic (HSL) gene expression that may underlie their propensity to maintain fat stores under caloric restriction.

Introduction

Developmental programming is a phenomenon whereby early perinatal nutritional influences have a long-lasting impact on various characteristics in the offspring. Early life food restriction during the suckling period, accomplished by cross-fostering offspring into large litters, results in offspring that remain lean and under-sized compared with control animals.¹ A similar phenomenon is obtained when dams are fed a high fat diet (HFD) during the pregnancy and nursing period, though here the weanlings are heavier, and more prone to weight gain under HFD exposure.² Animals exposed to early-life over-nutrition demonstrate an increased predisposition to diet-induced obesity when exposed to a high-fat diet later in life, indicating that certain elements of this phenotype become visible upon interaction with the postnatal nutritional environment.³

Adipose tissue homeostasis is increasingly recognized as an important factor in metabolic health. The discovery of the adipocytokine leptin, which is secreted in proportion to adipose tissue mass and serves to regulate metabolism both centrally and peripherally,^{4,5} revealed that adipose tissue can exert control on metabolic processes occurring elsewhere. Adipose tissue mass is governed by the balance between lipogenesis and lipolysis occurring in the adipose tissue itself.^{6,7} Hormones such as insulin promote adipose tissue lipogenesis, while leptin, functioning as a signal of adipose tissue sufficiency, inhibits it.⁷ The process

of lipolysis is activated primarily during periods of fasting, and involves the hydrolysis of stored triglycerides into free fatty acids and glycerol. This process is regulated primarily by hormonal factors such as the catecholamines and glucagon.^{6,8}

Under conditions of food restriction, a number of metabolic adaptations take place throughout the body in order to mobilize energy stored in adipose tissue. As the organism becomes more dependent on fatty acid metabolism, the balance of lipolysis and lipogenesis within adipose tissue necessarily changes as a consequence.^{9–11} Interestingly, this adaptation has recently been shown to be altered by gestational caloric restriction, suggesting that adipose tissue is subject to developmental metabolic programming.¹² On the other hand, offspring of obese dams show alterations in adipose tissue metabolism consistent with greater rates of lipogenesis, and this effect is enhanced by postnatal exposure to a cafeteria diet.¹³ Models of early-life over-nutrition and maternal obesity are of increasing relevance to western human populations, for as population-wide obesity rates rise so do the rates of obese and overweight mothers, a state of affairs that may in fact serve to propagate the obesity epidemic across generations.^{14,15}

With these findings in mind, we hypothesized that early life nutrition may also alter the effect of caloric restriction on adipose tissue metabolism. To test this hypothesis, we employed models of early-life over- and under-nutrition in rats and evaluated the effects of chronic food restriction on body composition (Fig. 1). We also analyzed the expression of adipogenic transcription

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Submitted: 03/11/13; Revised: 04/12/13; Accepted: 04/19/13
<http://dx.doi.org/10.4161/adip.24752>

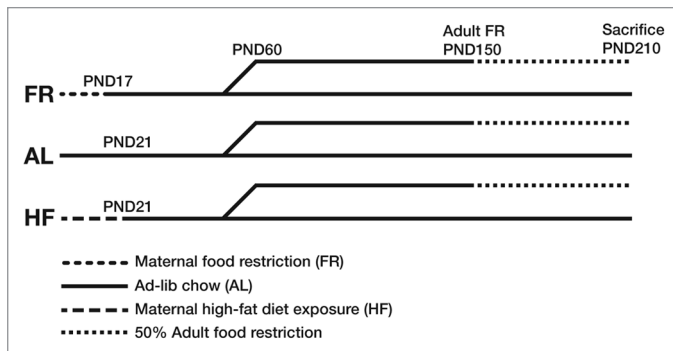


Figure 1. Experimental design. Pregnant dams were fed ad libitum until birth. Pups were randomly cross-fostered on PND1 into three maternal treatments: 50% food restriction (FR), ad lib (AL), and 45% high-fat diet (HF). Offspring were weaned onto ad lib chow. Baseline body weight and food intake measurement began at PND60. At PND120, half of the animals from each group were switched to chronic food restriction (50% of normal ad lib chow intake) until sacrifice at PND210.

factors, lipolytic and lipogenic enzymes, as well as the recently characterized fat mass and obesity (FTO) gene.

Results

There was no interaction between maternal treatment and sex at any time point (Fig. 2A and B). There were no differences in weight between animals consuming chow ad lib and calorically restricted (CR) animals prior to the onset of caloric restriction (Fig. 2C and D). Calorie restriction led to a significant decrease in body weight in all animals after one week. This effect strengthened over the course of calorie restriction, but because animals in that group were maintained at 90% of their ad lib weight, there was no interaction between caloric restriction and maternal treatment at any time point. Ultimately, restricted animals were sacrificed at 90% of their baseline weight, which invariably constituted a significant difference from baseline (Fig. 2C and D).

Cumulative food intake over the five-week baseline period was compared by three-factor ANOVA to exclude the possibility of pre-existing differences between the groups. The ANOVA revealed a main effect of sex, with females generally consuming less food than males. However, there was no significant effect of maternal treatment on food intake at any point in the experiment (Fig. 2E–H). Because food intake was experimentally restricted in calorically restricted animals, their consumption data was not analyzed subsequent to the onset of restriction.

Although FR females consuming ad lib chow had a significantly lower percent body fat in the perigonadal fat pad compared with their AL counterparts, there were no other treatment related effects detected in body composition among animals consuming ad lib chow (Fig. 3A and C). When subjected to caloric restriction, HF males showed significantly elevated fat in the perigonadal, retroperitoneal, and subcutaneous fat pads as compared with their AL counterparts (Fig. 3B). A similar effect was observed in females, with calorically restricted HF females showing significantly elevated perigonadal, subcutaneous, and brown adipose tissue as compared with their AL counterparts.

Because our body composition data suggested that maternal treatment elicited a differential response to caloric restriction in adipose tissue, we explored the expression of several genes involved in adipose tissue metabolism. There were no treatment related differences in leptin expression, though it was higher in males than in females, and downregulated in males by caloric restriction (Fig. 4A). Caloric restriction increased CCAAT/enhancer-binding protein α (CEBP α) expression in all animals, though only AL males showed a significant increase (Fig. 4B). Similarly, peroxisome proliferator-activated receptor γ (PPAR γ) was significantly increased by caloric restriction in all cases except for female FR rats, where this increase did not reach statistical significance ($P = 0.06$; Fig. 4C). Hormone sensitive lipase (HSL) expression was not altered by caloric restriction in females, though it was significantly increased in both AL and HF males exposed to caloric restriction (Fig. 4D). Carnitine palmitoyltransferase 1a (CPT1a) expression was increased by the calorically restricted diet only in AL males and females (Fig. 4E). Fatty acid synthase (FAS) expression was significantly increased by caloric restriction in HF males and females (Fig. 4F). FAS expression was also increased by caloric restriction in AL males, and there was a trend toward a similar increase in FR females ($P = 0.061$) (Fig. 4F). Lipoprotein lipase (LPL) expression was upregulated by CR only in HF males (Fig. 4G). FTO expression was upregulated by CR in AL males and females as well as in HF males (Fig. 4H).

Discussion

Here we demonstrate that a mild model of developmental programming, though not leading to any obvious phenotypic differences under baseline conditions, can affect the metabolic response to an energetic challenge. There exists a great deal of interest in the hypothesis that early-life nutrition can, by virtue of either its nutritive makeup or caloric content, affect adult health.

Animals that are nutritionally deprived during the perinatal period show a thrifty phenotype, with catch-up growth supported largely by increased feed efficiency.¹ Under certain conditions, perinatal under-nutrition can lead to increased susceptibility to obesity and symptoms reminiscent of the metabolic syndrome, particularly when a high-fat diet is introduced postnatally.^{16,17} This effect appears to be dependent on the timing and magnitude of the post-natal leptin surge, as treating pups with leptin eliminates or attenuates many of these metabolic effects.^{17–20} Our model of maternal food restriction did not result in any phenotypic changes to body weight, food intake, or body composition, and these measures were not differentially affected by caloric restriction. Because our study did not challenge offspring with exposure to an obesogenic environment in adulthood, we cannot rule out the possibility that these animals would demonstrate a predisposition to obesity similar to that seen in other studies.¹⁶ It is also possible that our model did not provide a substantial energetic challenge to the pups. This seems unlikely, as previous work has shown that maternal energy restriction of the type we employed results in a 50% reduction in milk volume.²¹ Because

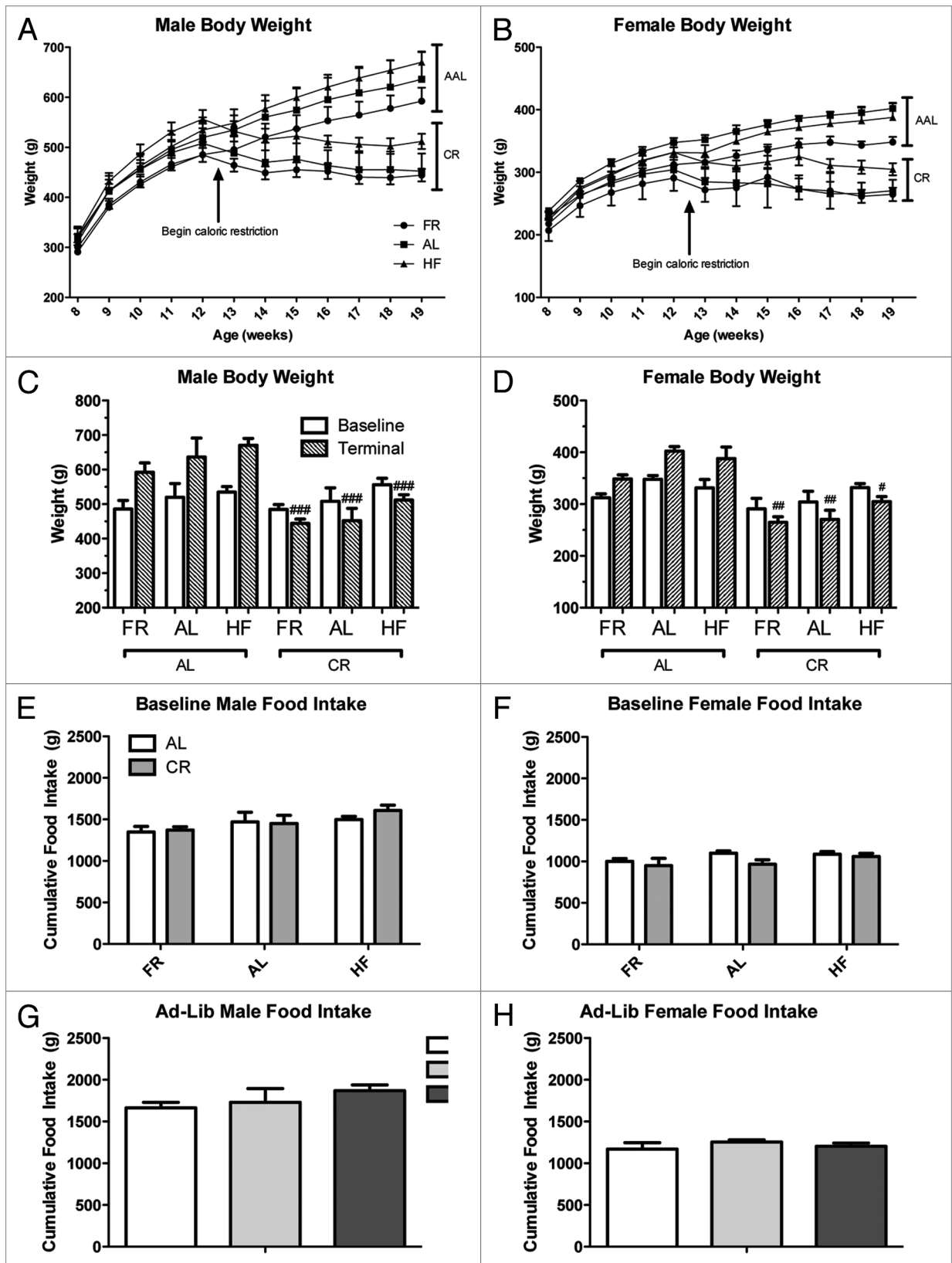


Figure 2. Caloric restriction lead to the expected reduction in body weight in both male and female animals (**A and B**). No differences in body weight were seen between groups before or after caloric restriction in males and females (**C and D**). Food intake did not differ between groups either prior to (**E and F**) the onset of caloric restriction. No differences emerged in food intake among animals that continued to consume ad lib diets (**G and H**). Symbols indicate significant Fisher LSD post-hoc comparisons (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) vs. non-calorically restricted animals from the same sex and maternal treatment.

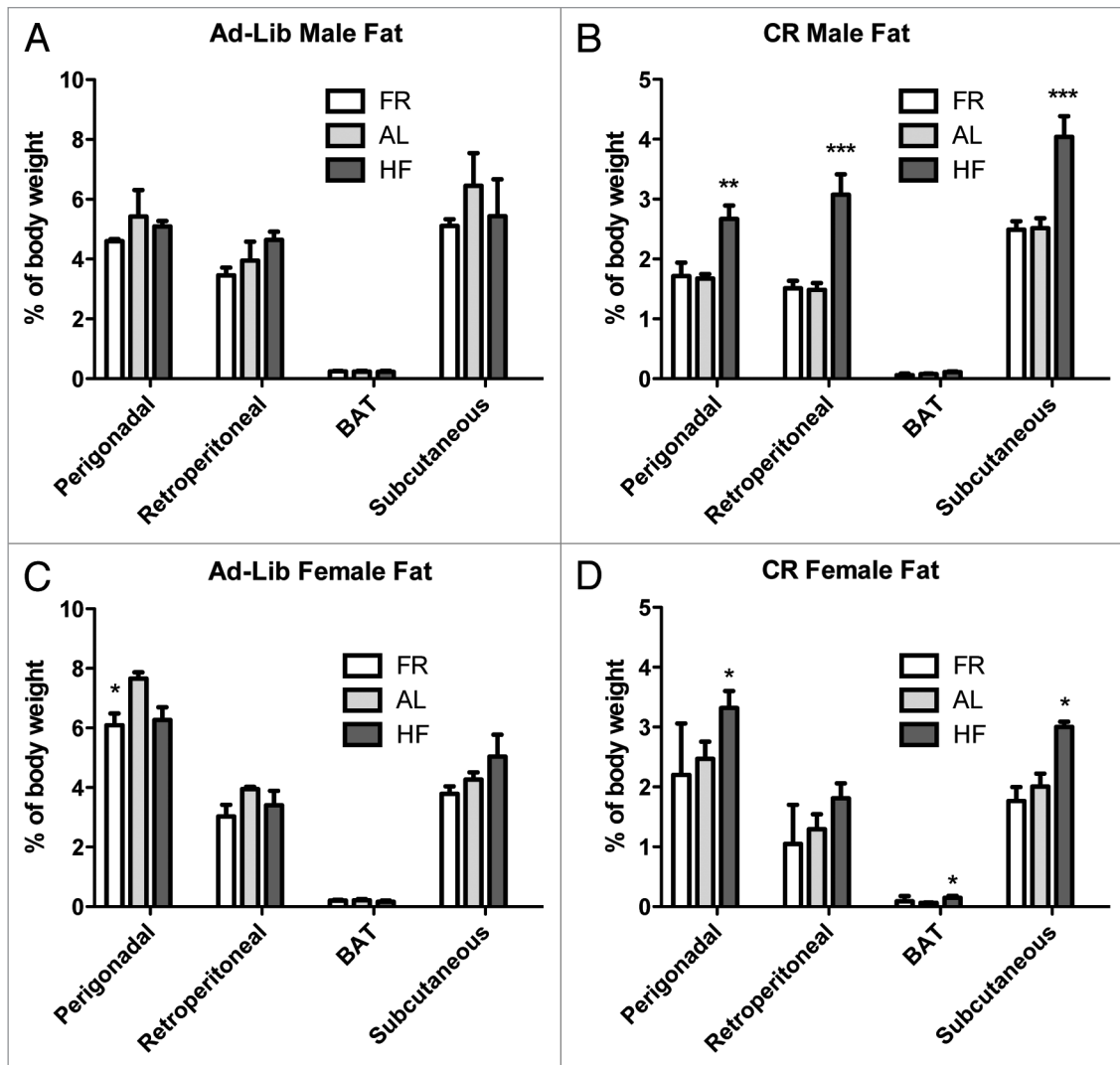


Figure 3. No differences in body fat were seen among males (A) consuming ad lib diets; FR females (C) showed reduced perigonadal fat. Caloric restriction lead to greater relative fat pad mass in HF males (B) and females (D). Symbols indicate significant Fisher LSD post-hoc comparisons (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) vs. AL animals from the same sex and adult diet.

the reduction in milk volume is accompanied by a reduction in lactose concentration, but an increase in fat concentration, the actual caloric density of the milk is increased, though not by enough to mitigate the reductions in volume available to each pup.²¹ There are a variety of methods for implementing early life nutritional modifications that vary with regards to timing (pre-, postnatal, or both), magnitude, and method of the manipulation. Early postnatal energy restriction is often achieved by postnatal cross-fostering of pups into oversized litters.¹ This model has been shown to produce characteristic phenotypic alterations in undernourished offspring including reduced body weight, food intake, circulating leptin, and anorexigenic neuropeptide expression in the ARC.^{1,22,23} While the nutrition of the offspring is no doubt altered in this model, the issue of maternal care becomes an unavoidable confound. Reductions in maternal care affect the offspring's stress axis and feeding responses later in life,^{24,25} and it is not known whether these alterations exacerbate the phenotype

caused by the nutritional modification per se. To circumvent this problem, we directly manipulated the dam's energy intake.

Other studies have demonstrated that maternal HFD exposure has an obesogenic effect on offspring,²⁶⁻²⁸ and it appears that this effect is more pronounced when maternal obesity is induced prior to conception.²⁷ As mentioned earlier, because our study did not include an obesogenic challenge in adulthood, we cannot rule out an interaction between perinatal treatment and the propensity to develop obesity later. In comparison to other studies, our program of HFD exposure was rather mild, as animals exposed maternally to HFD did not show a difference in body weight, fat, or body composition at baseline. However, when subjected to chronic caloric restriction, both males and females in the HF treatment showed a significantly higher percent body fat compared with both AL and FR animals. This occurs in spite of the fact that there were no significant differences in body weight in these animals either before or after caloric restriction.

We reasoned that this effect arose from fat-sparing adaptations in adipose tissue metabolism, and indeed found evidence of sexually dimorphic alterations in the regulation of lipogenic and lipolytic enzymes among these animals.

Caloric restriction resulted in a significant increase in PPAR γ expression in all cases except FR females, where the comparison only approached significance. This finding confirms the results of a previous study that also demonstrated PPAR γ upregulation under caloric restriction.²⁹ The expression of CEBP α was also upregulated in general by caloric restriction, but the magnitude of this effect reached significance only in AL males. This too has been reported as a consequence of prolonged calorie restriction in adult animals.²⁹ PPAR γ and CEBP α are transcription factors critical in the adipogenic program.^{30,31} Chronic caloric restriction also lead to an increase in FAS expression in adipose tissue, though this was limited in males to AL and HF animals, and in females only to HF animals. This increase reflects a tendency for animals to favor fatty acids as a metabolic substrate during calorically restricted conditions, even though the diet remains primarily carbohydrate based.^{10,32} We also noted upregulation of the lipolytic HSL in AL and HF males exposed to caloric restriction. HSL is regulated to an extent by nutritional status, as it constitutes the first rate-limiting step in lipolysis.^{6,33,34} A significant increase in lipolytic gene expression was not seen among females. Both male and female AL animals showed significant increases in CPT1a expression under caloric restriction. This enzyme is involved in shuttling fatty acids across the mitochondrial membrane, a key step in fatty acid β -oxidation. The precise kinetics of lipid metabolism that occur during prolonged calorie restriction are not clear at present; however, the tendency for upregulation of lipogenic factors coupled with an upregulation of lipolytic enzymes seems to suggest an accelerated rate of lipid turnover in the adipose tissue. This is likely supported and initiated by upregulation of the adipogenic transcription factors PPAR γ and CEBP α . It is interesting to note that in spite of possessing upregulated transcription factor expression, FR males did not show any increase in FAS and HSL expression. This may represent a failure to adapt to the metabolic challenge of food-restriction in an effective manner.

Males maternally exposed to HF were unique in that they showed upregulation of LPL under chronic caloric restriction. LPL is involved in the uptake of free fatty acids from triglycerides circulating within chylomicrons or as protein-bound lipids for incorporation into adipose tissue stores.^{35,36} In contrast with HSL, the other lipase studied here, LPL is concerned primarily with fat storage.³⁶ Because this upregulation is unique among HF males, it may be the case that LPL is acting here to maintain or replenish fat stores over and above the putative state of constant turnover we observed. This could account for their tendency to maintain a higher percent body fat than their AL or FR counterparts, though such an interpretation is largely speculative without any measure of enzymatic activity.

We also elected to analyze expression the fat mass and obesity associated (FTO) gene in our animals. Large-scale genome-wide association studies (GWAS) have revealed a common single nucleotide polymorphism (SNP) of FTO gene as possessing a

strong association with body mass index (BMI).^{37,38} FTO is widely expressed in the body, including in adipose tissue, and has been suggested to play a role in adipose tissue lipolysis.³⁹ Moreover, FTO expression in the hypothalamus and liver is altered during early-life over-nutrition in a manner that correlates with future weight gain, suggesting it may play a role in metabolic programming.⁴⁰ Given these findings, we aimed to shed additional light on the function of FTO in lipolysis, and if its regulation is altered in adulthood by early-life conditions. We did not observe a difference in baseline FTO expression; however, we found that it was upregulated by caloric restriction in AL animals of both sexes, as well as HF males. Interestingly, FR animals in both sexes did not show elevated adipose FTO expression under caloric restriction. Studies of FTO expression in human visceral adipose tissue have yielded conflicting results with some reporting a positive,⁴¹ negative,⁴² or no⁴³ relationship between BMI, adiposity, and visceral adipose FTO expression. FTO protein expression in perigonadal adipose tissue was not affected by a 24 h fast in mice.⁴⁴ Similarly, chronic food restriction in rats did not alter FTO expression in the visceral/mesenteric fat pad.⁴⁵ The reason for this discrepancy is not altogether clear, and the peripheral regulation of FTO by nutritional status requires further investigation. The possibility that early-life nutritional manipulations can alter the sensitivity of adipose tissue FTO expression to variations in adult energy balance is, however, an intriguing one.

Though all of the fat pads we measured showed similar trends, we elected to carry out our analysis of gene expression in the perigonadal fat pad, as it shows the highest level of transcriptional change in response to caloric restriction.⁹ The same may not hold for females, as intact females tend to favor utilization of subcutaneous fat stores as opposed to the perigonadal fat pads measured here.⁴⁶ Perhaps indicative of less metabolically active tissue, females generally showed reduced baseline expression or upregulation of genes such as FAS, HSL, CEBP α , and LPL. Our interpretations are thus complicated by these sex differences. Nevertheless, HF females do show alterations in adipose tissue metabolism that could explain their tendency to maintain a higher percentage of body fat under food restriction. Particularly we noted the failure to upregulate CPT1a and HSL as possible contributors to this effect. An additional factor that complicates our analysis of female gene expression is the fact that females were all sacrificed on the day of proestrus, a period during which food intake, body weight, and orexigenic neuropeptide expression all drop significantly.⁴⁷ It is possible that the sexually different effects observed therefore result from regional and cyclic difference in the expression of genes investigated here. Further investigation into sex-specific regulation of adipose tissue metabolism, and how it may be upset by early-life influences will be critical in understanding these effects.

We have presented a model of perinatal food restriction and maternal high-fat diet exposure that appears to generate a sex-specific phenotype with regards to body weight, composition, and adipose tissue gene expression in response to chronic calorie restriction. Our data suggest that males and females maternally exposed to HFD show a response to caloric restriction that favors the maintenance of fat stores, possibly through an altered balance

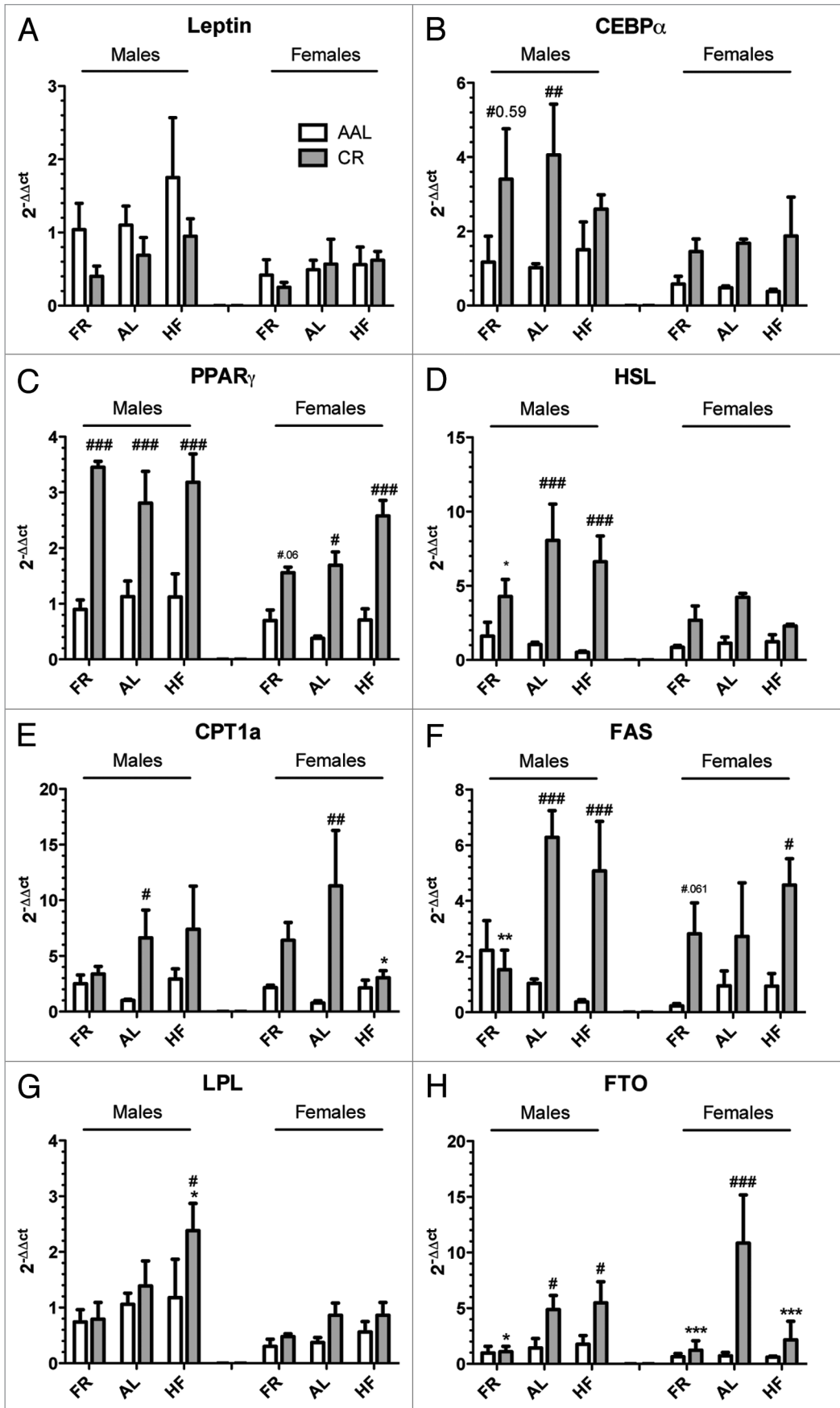


Figure 4 (See previous page). mRNA expression in perigonadal white adipose tissue from ad lib and calorically restricted adult males and females. Symbols indicate significant Fisher LSD post-hoc comparisons (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) vs. AL animals from the same sex and adult diet; (# $P < 0.05$; ## $P < 0.01$; ### $P < 0.001$) vs. non-calorically restricted animals from the same sex and maternal treatment.

of adipogenic and adipolytic gene expression. This may be of relevance to human health, as it illustrates how early-life conditions may modulate the success of dietary food-restriction as a means of losing fat mass. This could constitute an epigenetic mechanism supporting the inheritance of obesity. Further investigation will be needed to clarify energy expenditure, substrate utilization, and adipose tissue enzymatic activity in animals raised under these conditions to further determine the nature of the fat-sparing effect they demonstrate.

Materials and Methods

Virgin female Long-Evans rats were obtained from Charles River and housed pairwise under controlled lighting (12 h light/dark cycle, lights on at 8:00) and temperature (21 °C). All animals had ad libitum access to standard lab chow (Charles River) and tap water. Following a 10-d acclimation period, females were housed with males until pregnancy could be confirmed by the presence of a vaginal plug. The day of birth was considered PND0. To reduce litter confounds that may have occurred due to littermates sharing the same prenatal environment, pups were randomly cross-fostered on PND1. All litters were culled to between 10–12 pups. Control (AL) dams ($n = 2$) were given ad lib access to standard lab chow (3.3 kcal/g). To induce early postnatal under-nutrition, dams (FR) ($n = 2$) were food restricted from PND1 until PND17 by yoking their food intake to half that of an ad lib control animal. Another set of dams (HF) ($n = 2$) was given ad lib access to a high-fat diet containing 45% kcals from fat (Harlan TD.06415, 4.6 kcal/g) from birth through to weaning. At the time of weaning (PND21), four pups of each sex were randomly chosen from each litter group for inclusion in the subsequent analysis. Pups were group-housed (2–4 per cage) for one month with ad lib access to standard lab chow. At PND60 all offspring were single-housed. Body weight and food intake of all offspring was measured daily from this point onward. Rats were randomly assigned to either an ad lib or calorically restricted diet in adulthood. Assignment was balanced such that each post-natal diet had two animals per sex from each litter. Animals in the adult caloric restriction group were fed 50% of their daily food intake from PND150–210, during which time their body weights were brought to, and maintained at, 90% of their normal ad lib values. If body weight fell below this threshold, daily food was temporarily increased to 75% until weight stabilized. All procedures were approved by the Carleton University Animal Care Committee and followed the guidelines of the Canadian Council on Animal Care.

Vaginal smears were collected once daily for at least 8 d prior to sacrifice in female rats. Female rats were sacrificed on the day of proestrus. Animals exhibiting abnormal or arrested cycling were not included in the analysis. Animals were sacrificed by rapid decapitation immediately prior to the time at which food delivery normally took place. Perigonadal fat samples were

rapidly removed and frozen on dry ice before being stored at -80 °C for future analysis. Carcasses were collected and frozen at -20 °C for future dissection. Determination of fat pad weight was accomplished by later thawing these carcasses and dissecting and weighing the perigonadal, mesenteric, retroperitoneal, subcutaneous white adipose tissue, and the inter-scapular brown adipose tissue. The weight of each fat pad was calculated as a percent of the animal's total body weight and used for subsequent statistical analysis.

Perigonadal fat samples were homogenized in 500 μ l TRIzol using the method provided by the manufacturer (Invitrogen) and reverse transcribed using the SuperScript II reverse transcriptase kit with oligo(DT) primers, using the protocol provided by the manufacturer (Invitrogen). cDNA samples were stored at -20 °C for future use. Quantitative Real-Time PCR was performed in a MyiQ Single Color Real-Time PCR Detection System (Bio-Rad Laboratories) using iQ SYBR Green Super Mix (Bio-Rad Laboratories). Ct values were determined automatically by MYiQ software (Bio-Rad). Quantification of transcripts of interest relative to the internal housekeeping control gene β -actin was determined using the $2^{-\Delta\Delta Ct}$ method.⁴⁸ The primer sequences for leptin were: sense 5'-TGTTGGCTTTG GTCCTATCTG T-3', antisense 5'-CGACTGCGTG TGTGAAATG-3'. For CEBP α : sense 5'-GGCGGGAACG CAACAA-3', antisense 5'-TCCACGTTGG CGCTGTTT-3'. For PPAR γ : sense 5'-CTGACCCAAT GGTTGCTGAT TAC-3', antisense 5'-CCTGTTGTAG AGTTGGGTTT TTTCA-3'. For HSL: sense 5'-CCTACTACAC AAATCCC-3', antisense 5'-CTCAAAGAAG AGCACTC-3'. For CPT1a: sense 5'-GTGAGGCCAC TGATGAAGGA AGA-3', antisense 5'-ATTTGGGTCC GAGGTTGACA-3'. For FAS: sense 5'-GAGTCCGAGT CTGTCTCCCG CTTGA-3', antisense 5'-GCCGTGAGGT TGCTGTTGTC TGTA-3'. For LPL: sense 5'-GCGCTCCATC CATCTCTTCA T-3', antisense 5'-GGCAGAGCCC TTTCTCAAAT G-3'. For FTO: sense 5'-AACACCAGGC TCTTACC-3', antisense 5'-CACTTCATCA TCGCAGGAC-3'. For β -actin: sense 5'-GAACCCTAAG GCCAACCGTG-3', antisense 5'-GGTACGACCA GAGGCATACA G-3'.

Adult offspring body weight, composition, food intake, and gene expression data were analyzed with three-way ANOVA with treatment, sex, and adult food restriction status as factors. Significant main and interaction effects were further analyzed with Fisher LSD. Statistical significance was accepted for $P < 0.05$. Data are presented as mean \pm SEM.

Body weight was analyzed at weekly intervals by three-factor ANOVA (maternal treatment \times sex \times adult food restriction status).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

- Remmers F, Fodor M, Delemarre-van de Waal HA. Neonatal food restriction permanently alters rat body dimensions and energy intake. *Physiol Behav* 2008; 95:208-15; PMID:18588905; <http://dx.doi.org/10.1016/j.physbeh.2008.05.021>.
- Parente LB, Aguila MB, Mandarim-de-Lacerda CA. Deleterious effects of high-fat diet on perinatal and postweaning periods in adult rat offspring. *Clin Nutr* 2008; 27:623-34; PMID:18614261; <http://dx.doi.org/10.1016/j.clnu.2008.05.005>.
- Glavas MM, Kirigiti MA, Xiao XQ, Enriori PJ, Fisher SK, Evans AE, et al. Early overnutrition results in early-onset arcuate leptin resistance and increased sensitivity to high-fat diet. *Endocrinology* 2010; 151:1598-610; PMID:20194730; <http://dx.doi.org/10.1210/en.2009-1295>.
- Münzberg H, Huo L, Nillni EA, Hollenberg AN, Bjorbaek C. Role of signal transducer and activator of transcription 3 in regulation of hypothalamic proopiomelanocortin gene expression by leptin. *Endocrinology* 2003; 144:2121-31; PMID:12697721; <http://dx.doi.org/10.1210/en.2002-221037>.
- Thornton JE, Cheung CC, Clifton DK, Steiner RA. Regulation of hypothalamic proopiomelanocortin mRNA by leptin in ob/ob mice. *Endocrinology* 1997; 138:5063-6; PMID:9348241; <http://dx.doi.org/10.1210/en.138.11.5063>.
- Londos C, Brasaemle DL, Schultz CJ, Adler-Wailes DC, Levin DM, Kimmel AR, et al. On the control of lipolysis in adipocytes. *Ann N Y Acad Sci* 1999; 892:155-68; PMID:10842661; <http://dx.doi.org/10.1111/j.1749-6632.1999.tb07794.x>.
- Kersten S. Mechanisms of nutritional and hormonal regulation of lipogenesis. *EMBO Rep* 2001; 2:282-6; PMID:11306547; <http://dx.doi.org/10.1093/embo-reports/kve071>.
- Duncan RE, Ahmadian M, Jaworski K, Sarkadi-Nagy E, Sul HS. Regulation of lipolysis in adipocytes. *Annu Rev Nutr* 2007; 27:79-101; PMID:17313320; <http://dx.doi.org/10.1146/annurev.nutr.27.061406.093734>.
- Li Y, Bujo H, Takahashi K, Shibasaki M, Zhu Y, Yoshida Y, et al. Visceral fat: higher responsiveness of fat mass and gene expression to calorie restriction than subcutaneous fat. *Exp Biol Med (Maywood)* 2003; 228:1118-23; PMID:14610249.
- Bruss MD, Khambatta CF, Ruby MA, Aggarwal I, Hellerstein MK. Calorie restriction increases fatty acid synthesis and whole body fat oxidation rates. *Am J Physiol Endocrinol Metab* 2010; 298:E108-16; PMID:19887594; <http://dx.doi.org/10.1152/ajpendo.00524.2009>.
- Valle A, Sastre-Serra J, Roca P, Oliver J. Modulation of white adipose tissue proteome by aging and calorie restriction. *Aging Cell* 2010; 9:882-94; PMID:20707866; <http://dx.doi.org/10.1111/j.1474-9726.2010.00613.x>.
- García AP, Palou M, Sánchez J, Priego T, Palou A, Picó C. Moderate caloric restriction during gestation in rats alters adipose tissue sympathetic innervation and later adiposity in offspring. *PLoS One* 2011; 6:e17313; PMID:21364997; <http://dx.doi.org/10.1371/journal.pone.0017313>.
- Benkalfat NB, Merzouk H, Bouanane S, Merzouk SA, Bellenger J, Gresti J, et al. Altered adipose tissue metabolism in offspring of dietary obese rat dams. *Clin Sci (Lond)* 2011; 121:19-28; PMID:21288203; <http://dx.doi.org/10.1042/CS20100534>.
- Catalano PM. Obesity and pregnancy—the propagation of a viscous cycle? *J Clin Endocrinol Metab* 2003; 88:3505-6; PMID:12915626; <http://dx.doi.org/10.1210/jc.2003-031046>.
- Baeten JM, Bukusi EA, Lambe M. Pregnancy complications and outcomes among overweight and obese nulliparous women. *Am J Public Health* 2001; 91:436-40; PMID:11236410; <http://dx.doi.org/10.2105/AJPH.91.3.436>.
- Vickers MH, Breier BH, Cutfield WS, Hofman PL, Gluckman PD. Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. *Am J Physiol Endocrinol Metab* 2000; 279:E83-7; PMID:10893326.
- Vickers MH, Gluckman PD, Coveny AH, Hofman PL, Cutfield WS, Gertler A, et al. Neonatal leptin treatment reverses developmental programming. *Endocrinology* 2005; 146:4211-6; PMID:16020474; <http://dx.doi.org/10.1210/en.2005-0581>.
- Vickers MH, Gluckman PD, Coveny AH, Hofman PL, Cutfield WS, Gertler A, et al. The effect of neonatal leptin treatment on postnatal weight gain in male rats is dependent on maternal nutritional status during pregnancy. *Endocrinology* 2008; 149:1906-13; PMID:18187552; <http://dx.doi.org/10.1210/en.2007-0981>.
- Delahaye F, Breton C, Risold PY, Enache M, Dutriez-Casteloot I, Laborie C, et al. Maternal perinatal undernutrition drastically reduces postnatal leptin surge and affects the development of arcuate nucleus proopiomelanocortin neurons in neonatal male rat pups. *Endocrinology* 2008; 149:470-5; PMID:18006626; <http://dx.doi.org/10.1210/en.2007-1263>.
- Yura S, Itoh H, Sagawa N, Yamamoto H, Masuzaki H, Nakao K, et al. Role of premature leptin surge in obesity resulting from intrauterine undernutrition. *Cell Metab* 2005; 1:371-8; PMID:16054086; <http://dx.doi.org/10.1016/j.cmet.2005.05.005>.
- Rasmussen KM. Effects of under- and overnutrition on lactation in laboratory rats. *J Nutr* 1998; 128(Suppl):390S-3S; PMID:9478033.
- Remmers F, Schreuder MF, Gemke RJB, Delemarre-van de Waal HA. Energy intake and resting energy expenditure in adult male rats after early postnatal food restriction. *Br J Nutr* 2008; 99:1149-56; PMID:17925052; <http://dx.doi.org/10.1017/S0007114507843546>.
- Remmers F, Verhagen LA, Adan RA, Delemarre-van de Waal HA. Hypothalamic neuropeptide expression of juvenile and middle-aged rats after early postnatal food restriction. *Endocrinology* 2008; 149:3617-25; PMID:18372335; <http://dx.doi.org/10.1210/en.2007-1388>.
- Penke Z, Felszeghy K, Fernette B, Sage D, Nyakas C, Burlet A. Postnatal maternal deprivation produces long-lasting modifications of the stress response, feeding and stress-related behaviour in the rat. *Eur J Neurosci* 2001; 14:747-55; PMID:11556899; <http://dx.doi.org/10.1046/j.0953-816x.2001.01691.x>.
- Weaver ICG, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, et al. Epigenetic programming by maternal behavior. *Nat Neurosci* 2004; 7:847-54; PMID:15220929; <http://dx.doi.org/10.1038/nn1276>.
- Chen H, Simar D, Morris MJ. Hypothalamic neuroendocrine circuitry is programmed by maternal obesity: interaction with postnatal nutritional environment. *PLoS One* 2009; 4:e6259; PMID:19606226; <http://dx.doi.org/10.1371/journal.pone.0006259>.
- White CL, Purpera MN, Morrison CD. Maternal obesity is necessary for programming effect of high-fat diet on offspring. *Am J Physiol Regul Integr Comp Physiol* 2009; 296:R1464-72; PMID:19244583; <http://dx.doi.org/10.1152/ajpregu.91015.2008>.
- Delahaye F, Lukaszewski MA, Watzet JS, Cisse O, Dutriez-Casteloot I, Fajardy I, et al. Maternal perinatal undernutrition programs a "brown-like" phenotype of gonadal white fat in male rat at weaning. *Am J Physiol Regul Integr Comp Physiol* 2010; 299:R101-10; PMID:20463183; <http://dx.doi.org/10.1152/ajpregu.00604.2009>.
- Zhu M, Lee GD, Ding L, Hu J, Qiu G, de Cabo R, et al. Adipogenic signaling in rat white adipose tissue: modulation by aging and calorie restriction. *Exp Gerontol* 2007; 42:733-44; PMID:17624709; <http://dx.doi.org/10.1016/j.exger.2007.05.011>.
- Rosen ED, Walkey CJ, Puigserver P, Spiegelman BM. Transcriptional regulation of adipogenesis. *Genes Dev* 2000; 14:1293-307; PMID:10837022.
- Kim JB, Spiegelman BM. ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. *Genes Dev* 1996; 10:1096-107; PMID:8654925; <http://dx.doi.org/10.1101/gad.10.9.1096>.
- Karbowska J, Kochan Z, Swierczynski J. Increase of lipogenic enzyme mRNA levels in rat white adipose tissue after multiple cycles of starvation-refeeding. *Metabolism* 2001; 50:734-8; PMID:11398154; <http://dx.doi.org/10.1053/meta.2001.23309>.
- Sztlryd C, Kraemer FB. Regulation of hormone-sensitive lipase during fasting. *Am J Physiol* 1994; 266:E179-85; PMID:8141275.
- Lampidonis AD, Rogdakis E, Voutsinas GE, Stravopodis DJ. The resurgence of Hormone-Sensitive Lipase (HSL) in mammalian lipolysis. *Gene* 2011; 477:1-11; PMID:21241784; <http://dx.doi.org/10.1016/j.gene.2011.01.007>.
- Weinstock PH, Levak-Frank S, Hudgins LC, Radner H, Friedman JM, Zechner R, et al. Lipoprotein lipase controls fatty acid entry into adipose tissue, but fat mass is preserved by endogenous synthesis in mice deficient in adipose tissue lipoprotein lipase. *Proc Natl Acad Sci U S A* 1997; 94:10261-6; PMID:9294198; <http://dx.doi.org/10.1073/pnas.94.19.10261>.
- Frayn KN, Coppack SW, Fielding BA, Humphreys SM. Coordinated regulation of hormone-sensitive lipase and lipoprotein lipase in human adipose tissue in vivo: implications for the control of fat storage and fat mobilization. *Adv Enzyme Regul* 1995; 35:163-78; PMID:7572342; [http://dx.doi.org/10.1016/0065-2571\(94\)00011-Q](http://dx.doi.org/10.1016/0065-2571(94)00011-Q).
- Dina C, Meyre D, Gallina S, Durand E, Körner A, Jacobson P, et al. Variation in FTO contributes to childhood obesity and severe adult obesity. *Nat Genet* 2007; 39:724-6; PMID:17496892; <http://dx.doi.org/10.1038/ng2048>.
- Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 2007; 316:889-94; PMID:17434869; <http://dx.doi.org/10.1126/science.1141634>.
- Wählén K, Sjölin E, Hoffstedt J. The common rs9939609 gene variant of the fat mass- and obesity-associated gene FTO is related to fat cell lipolysis. *J Lipid Res* 2008; 49:607-11; PMID:18048838; <http://dx.doi.org/10.1194/jlr.M700448-JLR200>.
- Caruso V, Chen H, Morris MJ. Early hypothalamic FTO overexpression in response to maternal obesity-potential contribution to postweaning hyperphagia. *PLoS One* 2011; 6:e25261; PMID:21980407; <http://dx.doi.org/10.1371/journal.pone.0025261>.
- Terra X, Auguet T, Porras JA, Quintero Y, Aguilar C, Luna AM, et al. Anti-inflammatory profile of FTO gene expression in adipose tissues from morbidly obese women. *Cell Physiol Biochem* 2010; 26:1041-50; PMID:21220935; <http://dx.doi.org/10.1159/000323979>.
- Klötting N, Schleinitz D, Ruschke K, Berndt J, Fasshauer M, Tönjes A, et al. Inverse relationship between obesity and FTO gene expression in visceral adipose tissue in humans. *Diabetologia* 2008; 51:641-7; PMID:18251005; <http://dx.doi.org/10.1007/s00125-008-0928-9>.
- Samaras K, Botelho NK, Chisholm DJ, Lord RV. Subcutaneous and visceral adipose tissue FTO gene expression and adiposity, insulin action, glucose metabolism, and inflammatory adipokines in type 2 diabetes mellitus and in health. *Obes Surg* 2010; 20:108-13; PMID:19763711; <http://dx.doi.org/10.1007/s11695-009-9952-1>.
- Gao X, Shin YH, Li M, Wang F, Tong Q, Zhang P. The fat mass and obesity associated gene FTO functions in the brain to regulate postnatal growth in mice. *PLoS One* 2010; 5:e14005; PMID:21103374; <http://dx.doi.org/10.1371/journal.pone.0014005>.

45. Wang P, Yang FJ, Du H, Guan YF, Xu TY, Xu XW, et al. Involvement of leptin receptor long isoform (LepRb)-STAT3 signaling pathway in brain fat mass and obesity-associated (FTO) downregulation during energy restriction. *Mol Med* 2011; 17:523-32; PMID:21267512.
46. Shi H, Clegg DJ. Sex differences in the regulation of body weight. *Physiol Behav* 2009; 97:199-204; PMID:19250944; <http://dx.doi.org/10.1016/j.physbeh.2009.02.017>.
47. Olofsson LE, Pierce AA, Xu AW. Functional requirement of AgRP and NPY neurons in ovarian cycle-dependent regulation of food intake. *Proc Natl Acad Sci U S A* 2009; 106:15932-7; PMID:19805233; <http://dx.doi.org/10.1073/pnas.0904747106>.
48. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* 2008; 3:1101-8; PMID:18546601; <http://dx.doi.org/10.1038/nprot.2008.73>.