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Acute exposure to high-fat diets increases hepatic expression of genes related to cell repair and remodeling in female rats

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ABSTRACT

High-fat diets (HFD) promote the development of both obesity and fatty liver disease through the up-regulation of hepatic lipogenesis. Insulin resistance, a hallmark of both conditions, causes dysfunctional fuel partitioning and increases in lipogenesis. Recent work has demonstrated that systemic insulin resistance occurs in as little as the first 72 hours of an HFD, suggesting the potential for hepatic disruption with HFD at this time point. The current study sought to determine differences in expression of lipogenic genes between sexes in 3-month-old male and female Long-Evans rats after 72 hours of a 40% HFD or a 17% fat (chow) diet. Owing to the response of estrogen on hepatic signaling, we hypothesized that a sexual dimorphic response would occur in the expression of lipogenic enzymes, inflammatory cytokines, apoptotic, and cell repair and remodeling genes. Both sexes consumed more energy when fed an HFD compared with their low fat-fed controls. However, only the males fed the HFD had a significant increase in body fat. Regardless of sex, HFD caused down-regulation of lipogenic and inflammatory genes. Interestingly, females fed an HFD had up-regulated expression of apoptotic and cell repair-related genes compared with the males. This may suggest that females are more responsive to the acute hepatic injury effects caused by HFDs. In summary, neither male nor female rats displayed disrupted hepatic metabolic pathways after 72 hours of the HFD treatment. In addition, female rats appear to have protection from increases in fat deposition, possibly due to increased caloric expenditure; male rats fed an HFD were less active, as demonstrated by distance traveled in their home cage.

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Abbreviations: Acc1, acetyl Co-A carboxylase; Anxa5, annexin 5; Atgl, adipose triglyceride lipase; Bbc3, Bcl-2 binding component 3; Bgn, biglycan; Casp2, caspase 2; Creb1, CAMP-responsive element-binding protein 1; Dgat1, diglyceride acyltransferase; Fas, fatty acid synthase; Fn1, fibronectin; Gata3, GATA binding protein 3; HFD, high-fat diet; Il6, interleukin 6; Jak2, janus kinase 2; LFD, low-fat diet; Madh1, mothers against decapentaplegic homolog 1; Mapk, mitogen-activated protein kinase; Mmp11, matrix metalloproteinase 11; Npy, neuropeptide y; Ppar γ , peroxisome proliferator-activated receptor γ ; Scd1, sterol Co-A desaturase; Serpinh1, serpin peptidase inhibitor clade H member 1; Socs3, suppressor of cytokine signaling 3; Sparc, osteonectin; Srebpc1, sterol regulatory element-binding protein 1; STAT3, signal transducer and activator of transcription 3; Tnf α , tumor necrosis factor α ; Vegf, vascular endothelial growth factor; Xbp1, x-box binding protein.

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1. Introduction

Hepatic lipogenesis contributes significantly to body fat accumulation and metabolic diseases [1]. Previous research has indicated that hepatic lipogenesis plays a part in the development of obesity, and knockdown of hepatic lipogenic pathways can blunt both steatosis and weight gain in nonhuman models [2]. With obesity rates expected to rise to 50% of US adults by 2030, contributing up to 8.5 million additional cases of diabetes, it is imperative that the metabolic disturbances that occur during the onset of obesity are delineated [3].

Recent studies have demonstrated that male rats fed obesogenic high-fat diets (HFD) for 72 hours rapidly develop both insulin and leptin resistance before the development of obesity, as defined previously [4]. Insulin resistance has been observed in the hypothalamus and the amygdala, 2 regions that regulate food intake behaviors [5,6]. Insulin resistance has also been demonstrated in adipose and liver in as little as 72 hours, in correspondence with adiponectin resistance in muscle tissue [7–9]. Such animal models have temporary increases in whole body adiposity and hepatic triglyceride content, steatosis, and serum cytokine levels.

Hepatic insulin resistance results in alterations of normal fuel partitioning, including a marked inability to reduce gluconeogenesis while promoting lipogenesis [10]. High-fat diets promote the development of hepatic insulin resistance, observed to occur at 72 hours, which suggests the potential for increased lipogenesis at this time point. A recent study in male mice, however, indicated opposing results [9]. Although the mice fed the HFD displayed both hepatic insulin resistance and steatosis, they did not show increases in lipogenesis. The potential explanation behind this observation was that hepatic lipogenic enzymes are still capable of responding to substrate-level inhibition because of the influx of dietary fat after 72 hours of an HFD. Increases in hepatic fat content were attributed to increased fat deposition caused by the increased dietary fat.

Most studies investigating the metabolic changes that occur after 72 hours of an HFD have been in male models. Currently, no research is available investigating potential sex differences. It has long been established that females display protection from the mal-effects of HFDs, primarily because of the widespread antiobesogenic role of 17β -estradiol [11]. Preliminary research has demonstrated that estrogens down-regulate hepatic lipogenesis and thus are currently used in the pharmacologic treatment in males with aromatase deficiencies suffering from hypertriglyceridemia and hepatic steatosis [12–14]. It is therefore possible that the observed protection from increased adiposity in females may be, in part, due to a sex difference in hepatic lipogenesis during the early exposure to an HFD. The current study sought to investigate potential sex differences in hepatic gene expression during this period. Owing to observed sex differences in nonalcoholic fatty liver disease, we hypothesized that a sexual dimorphic response would be measured in genes related to lipogenesis, inflammation, and cell injury in 72 hours of an HFD. To assess this, quantitative polymerase chain reaction (PCR) was used to measure the expression of genes in the

above categories in adult male and female Long-Evans rats, a standard diet-induced obesity rodent model. In addition, differences in body composition after 72 hours of an HFD were measured using dual x-ray absorptiometry.

2. Methods and materials

2.1. Animals and housing

Three-month-old male ($n = 15$) and female ($n = 17$) Long-Evans rats were purchased from Harlan Labs (Frederick, MD, USA). Upon arrival, they were given 1 week to acclimate to the facility before introduction to sex-specific colony rooms. Before the start of the experiment, rats were maintained on a standard laboratory chow (17% fat and 3.1 kcal/g, Harlan Teklad #7012, Indianapolis, IN, USA; Table 1). Rats had access to food and water ad libitum throughout the experiment. Rooms were temperature ($22^{\circ}\text{C} \pm 2^{\circ}\text{C}$) and humidity controlled and kept on a 12:12-hour light/dark cycle. At the start of the experiment, within each sex, rats were randomly assigned to HFD (40% fat and 4.54 kcal/g, Research Diets #D03082706, New Brunswick, NJ, USA) or chow (low-fat diet [LFD]; Table 1). Food intake and body weights were measured at the start and end of the experiment. The University of North Carolina at Greensboro Institutional Animal Care and Use Committee approved all protocols for this experiment.

2.2. Determination of estrous cycling

The estrous phase of each rat was determined daily by vaginal lavage, as previously described by Becker et al [15]. Obtained vaginal cells were collected onto glass slides for examination of cell types under a light microscope. Samples were taken at the same time daily until the timing of 2 estrous cycles could be confirmed. When the timing of the estrous cycle was determined for each rat, the experiment was started so that they would be in proestrus on the day of euthanasia. Male rats were handled daily during this period to prevent potential bias.

2.3. Spontaneous physical activity

Measurements of home cage behaviors were performed through real-time video surveillance through HomeCage Scan software (Clever Systems, Inc, Reston, VA, USA). The room was set up with blue backgrounds and red lights for recording during the dark cycle. Animals were given 1-day acclimation to the behavioral room before the start of the study. Cages were changed daily to reduce the amount of potential interference around the rat. To provide an estimate of spontaneous physical activity, the distance traveled in each cage was obtained from the program.

2.4. Body composition

Because no noninvasive methods exist to quantify subcutaneous and visceral fat, postmortem measurement of body composition was performed by dual x-ray absorptiometry. After euthanasia, the skin along with the attached

Table 1 – Diet composition^a

Macronutrient	LFD, Harlan Teklad #7012	HFD, Research Diets #D03082706
Protein ^{b,c} (% kcal)	25	13
Carbohydrate ^{b,d} (% kcal)	58	46
Fat ^{b,e} (% kcal)	17	41
Saturated fat (%)	16	62.5
Monounsaturated fat (%)	26	30.6
Polyunsaturated fat (%)	58	6.9
Total isoflavones (mg/kg) ^f	300-600	0
kcal/g	3.41	4.54
Minerals ^f		
Ca (g/kg)	10.0	7.6
P (g/kg)	7.0	3.6
Na (g/kg)	3.0	1.5
Cl (g/kg)	5.0	2.4
K (g/kg)	8.0	5.4
Mg (g/kg)	2.0	0.8
Fe (mg/kg)	284.11	55.7
Mn (mg/kg)	93.34	15.8
Zn (mg/kg)	63.59	45.2
Cu (mg/kg)	23.06	9.0
I (mg/kg)	2.61	0.30
Se (mg/kg)	0.16	0.23
Vitamins ^f		
A (IU/g)	29.63	46.80
D ₃ (IU/g)	2.39	11.70
E (IU/kg)	114.36	87.8
Choline (mg/g)	2.23	1.2
Niacin (mg/kg)	100.11	35.1
Pantothenic acid (mg/kg)	86.66	18.7
B ₆ (mg/kg)	16.94	8.2
Riboflavin (mg/kg)	13.68	7.0
Thiamine (mg/kg)	95.28	7.0
K (mg/kg)	8.37	0.88
Folic acid (mg/kg)	6.70	2.3
Biotin (mg/kg)	0.77	0.23
B ₁₂ (μg/kg)	91.00	29.0

^a Three-month-old male and female Long-Evans rats were fed diets either high in fat or low in fat for 72 hours.

^b Harlan-Teklad 7012 is a natural ingredient diet. Major macronutrients come from corn, soybean, oat, wheat, and alfalfa ingredients.

^c Protein source for the HFD was casein and cysteine.

^d Carbohydrate source for the HFD was corn starch, maltodextrin 10, and sucrose.

^e Fat source for the HFD was butter with additional soy bean oil.

^f Harlan-Teklad 7012 is an autoclavable diet, which result in some vitamin loss not reflected in this table.

subcutaneous fat (pelt) was dissected from the muscle wall and visceral fat (carcass), as previously described by Clegg et al [16]. Body composition was then performed using a GE Lunar Prodigy Advanced System (GE Healthcare, Milwaukee, WI, USA), and the data were analyzed by Encore 2007 Small Animal software (version 11.20.068; GE, Madison, WI, USA). Both the pelt and the body were scanned in duplicate to determine body fat and lean body mass.

2.5. Hepatic gene expression

After the rats were euthanized, a section of the liver was preserved in RNAlater and stored for 24 hours at 4°C and then

stored at –80°C until processed. RNA was isolated using QIAGEN RNAeasy kits (Qiagen, Inc, Valencia, CA, USA) according to the manufacturer's instructions. RNA concentration and purity were assessed by Nanodrop spectrophotometer (ND-1000; Thermo Scientific, Wilmington, DE, USA). Two micrograms of RNA for each sample was combined with RNase-free H₂O and master mix solution (Applied Biosystems, Foster City, CA, USA) and run in a Thermocycler (Applied Biosystems) for 2.5 hours to obtain complementary DNA (cDNA). The collected cDNA was used to determine gene expression via quantitative PCR for interleukin 6 (*Il6*), tumor necrosis factor α (*Tnf α*), suppressor of cytokine signaling 3 (*Socs3*), acetyl Co-A carboxylase (*Acaca* (*Acc1*)), fatty acid synthase (*Fas*), sterol regulatory element-binding protein 1 (*Srebfl1* (*Srebfl1c*)), sterol Co-A desaturase (*Scd1*), diglyceride acyltransferase (*Dgat1*), peroxisome proliferator-activated receptor γ (*Ppar γ*), neuropeptide y (*Npy*), adipose triglyceride lipase (*Pnpla2* (*Atgl*)), x-box binding protein (*Xbp1*), CAMP-responsive element-binding protein 1 (*Creb1*), janus kinase 2 (*Jak2*), mitogen-activated protein kinase (*Mapk*), signal transducer and activator of transcription 3 (*Stat3*), annexin 5 (*Anxa5*), Bcl-2 binding component 3 (*Bbc3*), caspase 2 (*Casp2*), mothers against decapentaplegic homolog 1 (*Madh1* (*Smad1*)), biglycan (*Bgn*), fibronectin (*), GATA binding protein 3 (*Gata3*), matrix metalloproteinase 11 (*Mmp11*), serpin peptidase inhibitor clade H member 1 (*Serpinh1* [*Hsp47*]), osteonectin (*Sparc*), and vascular endothelial growth factor (*Vegf*) using commercially available TaqMan Gene Expression Assays from Applied Biosystems. Quantitative PCR was performed using a 7900 HT system by Applied Biosystems (Table 2).*

2.6. XBP1 splicing assay

Quantification of spliced *Xbp1* messenger RNA (mRNA) was performed using methods previously described by Hirota et al [17]. Briefly, cDNA was combined with PCR master mix and *Xbp1* primer purchased from Applied Biosystems under the manufacturer's instructions. To create double-stranded cDNA, this mix then underwent 2 PCR cycles in a thermocycler under the following conditions: 94°C for 5 minutes, 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. *PstI* (0.75 U; Promega, Fitchburg, WI, USA) was then added to each sample for 1 hour before quantitative PCR in a 7900 HT system.

2.7. Statistical analyses

Messenger RNA samples from rats were selected for this study from a larger unpublished data set that proved to not be outliers in their respective groups for food intake, body weight change, and body composition. If an animal was an outlier in any one of these measures, it was removed from the current study. Power analysis was performed using G*Power 3 statistical power analysis software (Autenzell, Bayern, Germany) [18]. The final number of animals selected ($n = 32$ from the original $n = 48$) for this study had appropriate power to maintain statistical significance in the above variables. Outlier tests were performed using the GraphPad QuickCalcs outlier calculator available online (GraphPad, San Diego, CA, USA). Final statistical analysis was performed using Statistica

Table 2 – Probes used in real-time PCR

Classification	Gene symbol	Gene name	Context sequence
Endogenous control	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Rn01775763_g1
	18S	45S pre-ribosomal RNA	Rn03928990_g1
Lipid metabolism	ACACA	Acetyl Co-A carboxylase alpha (ACC1)	Rn00573474_m1
	PNPLA2	Patatin-like phospholipase domain containing 2 (ATGL)	Rn01479969_m1
	DGAT1	Diglyceride acyltransferase	Rn00584879_m1
	FASN	Fatty acid synthase	Rn01463550_m1
	Npy	Neuropeptide Y	Rn00561681_m1
	PPAR γ	Peroxisome proliferator-activated receptor γ	
	SCD1	Sterol Co-A desaturase	Rn00594894_g1
	SREBPF1	Sterol regulatory element-binding protein 1	Rn01495769_m1
Inflammation	XBP1	X-box binding protein 1	Rn01443523_m1
	IL6	Interleukin 6	Rn01410330_m1
	SOCS3	Suppressor of cytokine signaling 3	Rn00585674_s1
Cell signaling	TNF α	Tumor necrosis factor α	Rn01525859_g1
	CREB1	CAMP-responsive element-binding protein 1	Rn00578829_g1
	JAK2	Janus kinase 2	Rn00580452_m1
	MAPK	Mitogen-activated protein kinase	Rn00587719_m1
Apoptosis	STAT3	Signal transducer and activator of transcription 3	Rn00562562_m1
	ANXA5	Annexin 5	Rn00565571_m1
	BBC3	Bcl-2 binding component 3	Rn00597992_m1
	CASP2	Caspase 2	Rn00574684_m1
	MADH1	Mothers against decepentaplegic homolog 1	Rn00565555_m1
Cell repair and remodeling	BGN	Biglycan	Rn01529736_m1
	FN1	Fibronectin	Rn00569575_m1
	GATA3	GATA binding protein 3	Rn00484683_m1
	MMP11	Matrix metalloproteinase 11	Rn00564319_m1
	SERPINH1	Serpin peptidase inhibitor clade H member 1	Rn00567777_m1
	SPARC	Osteonectin	Rn01470624_m1
	VEGF	Vascular endothelial growth factor	Rn01511604_m1

Three-month-old male and female Long-Evans rats were fed diets either high in fat or low in fat for 72 hours. Liver samples were removed at sacrifice and stored for quantitative PCR. TaqMan primer sets were purchased from commercially available assays from Applied Biosystems.

(version 7.0) for Windows (StatSoft, Inc., Tulsa, OK, USA). Treatment effects and interactions were tested using 2-way analysis of variance (ANOVA), with the *F* statistic and degrees of freedom provided. Individual group differences were tested using Tukey HSD (Honestly Significant Difference). Data are presented as means \pm SEM, and significance was determined at $P < .05$.

3. Results

3.1. Seventy-two hours of an HFD on food intake and body weight gain

High-fat diet resulted in a significant sex by diet interaction in food intake measured by difference in food weight ($P < .00$; $F_{17,83,1}$). There was no diet effect in males; however, females fed an HFD ate more diet compared with the low fat-fed females ($P < .00$; Table 3). This hyperphagia in the females remained when food intake was normalized for body weight ($P = .01$); however, the male rats fed an HFD reduced their food intake compared with males fed the LFD ($P < .00$; Table 3).

A significant sex by diet interaction was observed in caloric intake ($P = .02$; $F_{6,43,1}$). Females on an LFD had reduced caloric intake compared with males fed LFD ($P < .00$; Table 3). However, this sex difference was attenuated on the HFD, and both sexes significantly increased their caloric intake on

the HFD compared with LFD controls ($P < .00$). When caloric intake was normalized, a significant sex by diet interaction was seen ($P < .00$; $F_{23,30,1}$). The increased caloric intake was lost in HFD-fed males, yet remained within the female diet groups ($P < .00$; Table 3).

The increase in caloric intake caused by an HFD resulted in increased body weight compared with LFD-fed controls ($P < .00$; $F_{24,93,1}$). However, only females on an HFD had a significant increase in body weight gain compared with controls ($P < .05$; Table 3). No difference in body weight was observed between male diet groups presumably because of inexplicable variances, as demonstrated by unusually large SEs in the male rats.

3.2. Body composition

A separate diet and sex effect was observed within subcutaneous (pelt) fat mass ($P = .02$ [$F_{6,85,1}$] for diet and $P < .00$ [$F_{11,34,1}$] for sex). Within the pelt data, only males on an HFD had a significantly higher fat mass compared with all other groups ($P < .05$; Table 3). No other differences were observed.

Within the carcass data, sex resulted in a significant effect, with males displaying an increased amount of lean body mass compared with females ($P < .00$; $F_{79,10,1}$; Table 3). In addition, HFD resulted in an increase in carcass fat mass ($P < .00$; $F_{17,52,1}$). High-fat diet also resulted in a significant increase in carcass fat mass in males and females compared with LFD-fed male rats ($P = .01$). Interestingly, HFD did not result in any

Table 3 – Food intake and changes in body weight and body composition

	LFD males (n = 6)	HFD males (n = 9)	LFD females (n = 8)	HFD females (n = 9)
72-h FI (g)	92.73 ± 2.98 ^a	87.41 ± 2.43 ^{ac}	61.40 ± 2.58 ^b	78.18 ± 2.43 ^c
72-h FI (g)/BW	0.26 ± 0.01 ^a	0.21 ± 0.01 ^b	0.21 ± 0.01 ^b	0.26 ± 0.01 ^a
72-h FI (kcal)	309.70 ± 14.44 ^a	393.10 ± 12.21 ^b	208.25 ± 12.21 ^c	354.93 ± 10.77 ^{ab}
72-h FI (kcal)/BW	0.90 ± 0.05 ^a	0.94 ± 0.04 ^a	0.73 ± 0.04 ^b	1.16 ± 0.04 ^c
72-h BWΔ (g)	4.77 ± 4.97 ^a	15.88 ± 9.52 ^a	1.60 ± 0.98 ^b	16.39 ± 1.25 ^a
LBM (g)	211.80 ± 7.86 ^a	228.43 ± 6.65 ^a	158.29 ± 6.65 ^b	161.11 ± 5.86 ^b
Carcass fat (g)	48.40 ± 3.97 ^a	64.86 ± 3.36 ^b	67.56 ± 2.96 ^{ac}	55.29 ± 3.36 ^{bc}
Pelt fat (g)	43.60 ± 3.84 ^a	57.86 ± 3.25 ^b	38.00 ± 3.25 ^a	41.11 ± 2.86 ^a

Three-month-old male and female Long-Evans rats were fed diets either high in fat or low in fat for 72 hours. Food intake and body weight was measured at the start and end of the experiment. Statistics were performed using 2-way ANOVAs, and individual group differences presented here were measured using Tukey HSD. Means that do not share a common letter indicate statistical difference at $P < .05$. Abbreviations: FI, food intake; BW, body weight; LBM, lean body mass.

significant differences between female diet groups in fat mass or lean body mass within the pelt or carcass weights.

Therefore, within this study, the increase in body weight observed after 72 hours of an HFD in females remains unaccounted for but might have been caused by an additive effect if entire body adiposity was measured as a whole, compared with measuring adipose stores separately. Although rats were randomly assigned to diet, there were no differences in starting weight between diet groups within each sex, suggesting that females were protected from body fat accumulation in this study.

3.3. Spontaneous physical activity

Spontaneous physical activity was estimated by the distance in meters that each rat moved in the cage over a 24-hour time span. A significant sex effect was observed on all 3 days measured and the entirety of the 72 hours ($P = .02$ [$F_{5,72,1}$] for day 1; $P = .01$ [$F_{8,76,1}$] for day 2; $P = .02$ [$F_{6,28,1}$]; $P < .00$ [$F_{16,37,1}$] for the total value). Male rats fed an HFD moved less than female rats fed both an LFD and an HFD ($P < .03$) on day 2 of the experiment (Fig. 1). In addition, this same reduction in distance traveled was seen for the value inclusive of all 3 days. Male rats fed an HFD moved less than females fed either the LFD or the HFD ($P = .03$ and $P = .04$ respectively; Fig. 1).

3.4. Lipogenic gene expression

A significant diet effect was observed in *Fas* gene expression ($P < .00$; $F_{33,86,1}$; Fig. 2A). Both males and females fed HFD had reduced *Fas* expression compared with their respective LFD controls ($P = .006$ and $P = .014$, respectively). Differentially, *Acc1* expression had a significant sex by diet interaction ($P = .02$; $F_{6,43,1}$; Fig. 2A). Females fed an LFD had significantly higher *Acc1* expression compared with all other groups ($P < .02$).

Additional genes associated with hepatic lipogenesis were also measured (Fig. 2A). A significant diet effect was observed in both *Srebp1* ($P < .00$; $F_{12,44,1}$) and *Scd1* ($P < .00$; $F_{23,39,1}$). Both sexes fed an HFD had decreased *Srebp1* and *Scd1* expression compared with the LFD-fed males and females ($P < .03$). Lastly, a significant sex by diet interaction was observed in *Dgat1* expression ($P = .01$; $F_{9,79,1}$). Males of both diets and females fed HFD had lower expression of *Dgat1* compared with female rats fed LFD ($P < .00$), and HFD in both sexes reduced expression of

Dgat1 compared with the LFD-fed males ($P < .00$). Interestingly, we saw a significant up-regulation of *Pparγ* expression caused by HFD ($P < .00$; $F_{8,33,1}$). Because *Pparγ* is an upstream regulator of many lipogenic genes, the up-regulation of this gene might serve as an early marker of future lipogenic changes.

Lastly, previous work has demonstrated that *Npy* is capable of influencing and increasing hepatic release of very low density lipoprotein [19]. In our study, we saw a significant up-regulation of *Npy* expression that was both dependent on HFD ($P < .00$; $F_{11,53,1}$) and the male sex ($P < .00$; $F_{14,09,1}$); Fig. 2A). The significant up-regulation of hepatic *Npy* expression in HFD-fed males may also reflect increased serum content of very low density lipoprotein in our study.

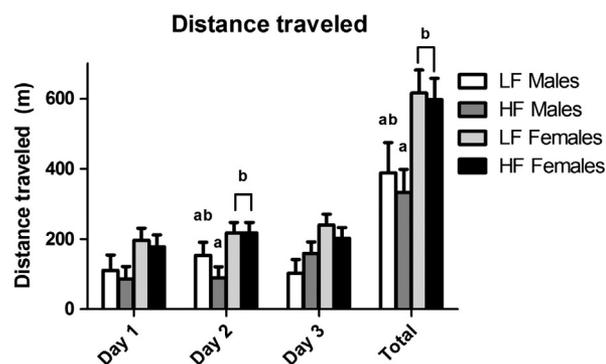


Fig. 1 – Distance traveled within the home cage as measured by HomeCage Scan by CleverSystems Inc (Reston, VA). Three-month-old male and female Long-Evans rats were fed diets either high in fat or low in fat for 72 hours (LF males: n = 6; HF males: n = 9; LF females: n = 8; HF females: n = 9). Rats were given a 24-hour acclimation period to the behavioral room before the start of the study. Data were collected from the computer program in 24-hour intervals throughout the 72-hour study. Distance traveled represents the distance in meters the rat moved within their home cage during each measurement period. Statistics were performed using 2-way ANOVAs, and individual group differences presented here were measured using Tukey HSD. Means that do not share a common letter indicate statistical difference at $P < .05$. Abbreviations: LF, low fat; HF, high fat.

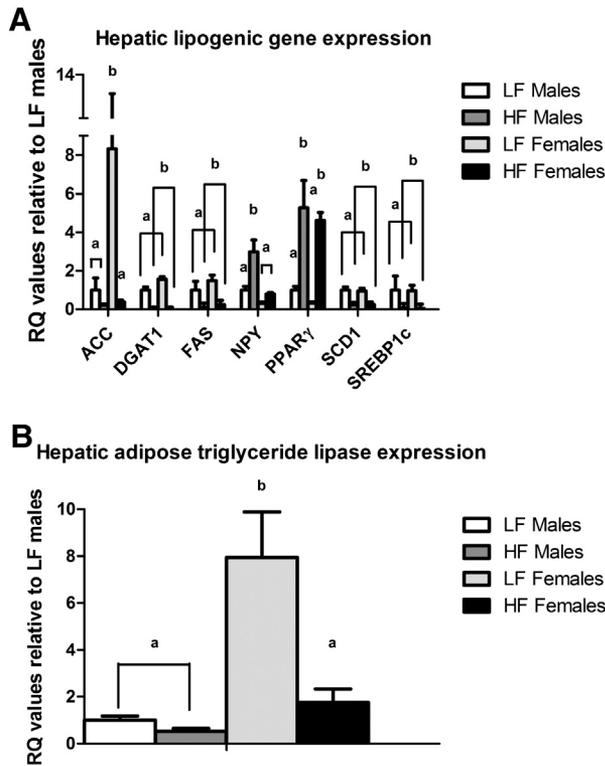


Fig. 2 – Hepatic lipogenic gene expression after 72 hours of high-fat feeding (A) and hepatic adipose triglyceride lipase gene expression (B). Three-month-old male and female Long-Evans rats were fed diets either high in fat or low in fat for 72 hours (LF males: $n = 6$; HF males: $n = 9$; LF females: $n = 5$; HF females: $n = 9$). Data were normalized to GAPDH. Statistics were performed using 2-way ANOVAs, and individual group differences presented here were measured using Tukey HSD. Means that do not share a common letter indicate statistical difference at $P < .05$. Abbreviations: LF, low fat; HF, high fat.

3.5. Lipolytic gene expression

A sex by diet interaction was seen in *Pnpla2* expression ($P < .00$; $F_{17.77,1}$; Fig. 2B). Females fed an LFD had greater lipolytic gene expression compared with all other groups ($P < .00$). The sex differences observed within the low-fat controls was lost during high-fat feeding. This would suggest reduced metabolism of hepatic triglyceride stores for energy use due to HFD, with a greater response in females.

3.6. XBP1 mRNA levels

Corresponding with the reduced lipogenic expression seen in both sexes fed an HFD for 72 hours, both sexes fed an HFD had reduced spliced *Xbp1* mRNA levels compared with LFD-fed males ($P < .0001$; Fig. 3). This observation also resulted in a significant diet effect ($P < .00$; $F_{27.57,1}$). Total *Xbp1* mRNA was also found to have a significant sex effect ($P < .00$; $F_{25.21,1}$). Females fed an HFD had lower total *Xbp1* expression compared with both diet groups in males ($P < .002$).

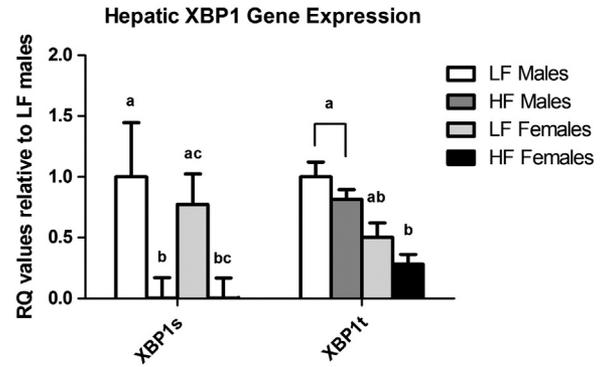


Fig. 3 – Hepatic *Xbp1* gene expression after 72 hours of high-fat feeding. Three-month-old male and female Long-Evans rats were fed diets either high in fat or low in fat for 72 hours (LF males: $n = 6$; HF males: $n = 9$; LF females: $n = 5$; HF females: $n = 9$). *Xbp1s* reflects the spliced, active form of *Xbp1*, whereas *Xbp1t* reflects both forms of *Xbp1*. Data were normalized to GAPDH. Statistics were performed using 2-way ANOVAs, and individual group differences presented here were measured using Tukey HSD. Means that do not share a common letter indicate statistical difference at $P < .05$. Abbreviations: LF, low fat; HF, high fat.

3.7. Cell signaling gene expression

A significant sex by diet interaction was observed in *Creb1* ($P < .00$; $F_{13.76,1}$) and *Jak2* ($P < .00$; $F_{26.78,1}$) expression (Fig. 4). *Creb1* expression was up-regulated in males fed an HFD compared with all other groups ($P < .00$). High-fat diet males reduced *Jak2* expression compared with their LFD-fed controls, whereas HFD females increased their expression ($P < .00$). Both significant sex ($P < .00$; $F_{33.11,1}$) and diet ($P < .00$; $F_{21.05,1}$) effects were seen in *Mapk* expression. In addition, a significant diet effect was observed in *Stat3* expression. In both *Mapk* and *Stat3* expressions, HFD resulted in increased expression in both sexes ($P < .01$).

3.8. Inflammatory gene expression

A significant diet effect was observed in both hepatic *Il6* and *Tnf α* expression ($P = .01$ [$F_{8.51,1}$] and $P < .00$ [$F_{10.74,1}$], respectively; Fig. 5). In both genes, HFD-fed females had reduced gene expression compared with LFD-fed females ($P < .05$). In addition, HFD-fed females had reduced *Tnf α* expression compared with LFD-fed males ($P < .01$). Females on an HFD also had reduced expression of *Socs3* compared with LFD-fed females ($P = .01$). Differentially, *Socs3* had a significant sex by diet interaction ($P = .02$; $F_{6.10,1}$).

3.9. Apoptotic gene expression

Significant diet effects were observed in *Anxa5* ($P < .02$; $F_{6.72,1}$), *Bbc3* ($P < .00$; $F_{11.96,1}$), *Casp2* ($P < .00$; $F_{8.14,1}$), and *Madh1* ($P < .00$; $F_{8.70,1}$; Fig. 6A). High-fat diet resulted in an increase in apoptotic gene expression; however, these were significant only in the females in all genes measured ($P < .05$). Such results indicate a sex-specific enhancement of proapoptotic

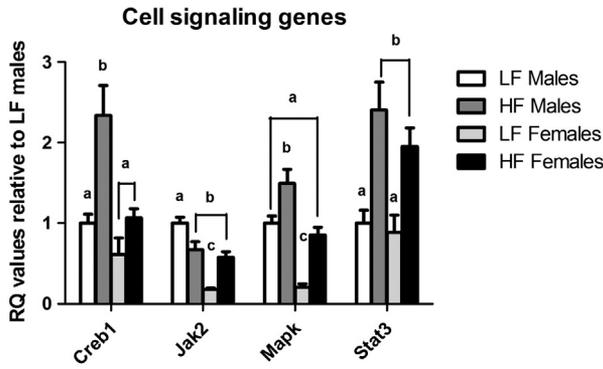


Fig. 4 – Hepatic expression of genes involved with major cell signaling pathways. Three-month-old male and female Long-Evans rats were fed diets either high in fat or low in fat for 72 hours (LF males: n = 6; HF males: n = 9; LF females: n = 5; HF females: n = 9). Data were normalized to GAPDH. Statistics were performed using 2-way ANOVAs, and individual group differences presented here were measured using Tukey HSD. Means that do not share a common letter indicate statistical difference at $P < .05$. Abbreviations: LF, low fat; HF, high fat.

gene expression when rats are given a short-term exposure to HFDs.

3.10. Cell repair and remodeling expression

A significant sex by diet interaction was observed in *Bgn* ($P < .00$; $F_{12,82,1}$) and *Sparc* ($P < .01$; $F_{7,72,1}$; Fig. 6B). Significant diet effects were seen in the expression of *Fn1* ($P < .02$; $F_{6,15,1}$), *Gata3* ($P < .02$; $F_{6,45,1}$), *Mmp11* ($P < .01$; $F_{7,22,1}$), *Serpinh1* ($P < .01$; $F_{7,40,1}$), and *Vegf* ($P < .00$; $F_{10,35,1}$; Fig. 6B). In all genes, HFD feeding resulted in a significant increase in expression in the females

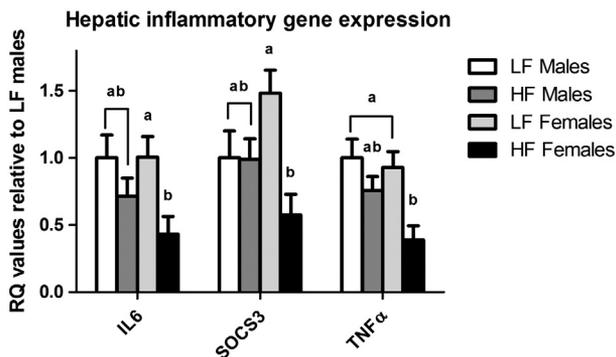


Fig. 5 – Hepatic inflammatory gene expression after 72 hours of high-fat feeding. Three-month-old male and female Long-Evans rats were fed diets either high in fat or low in fat for 72 hours (LF males: n = 6; HF males: n = 9; LF females: n = 8; HF females: n = 9). Data were normalized to GAPDH. Statistics were performed using 2-way ANOVAs, and individual group differences presented here were measured using Tukey HSD. Means that do not share a common letter indicate statistical difference at $P < .05$. Abbreviations: LF, low fat; HF, high fat.

($P < .05$), thus suggesting an acute response of greater hepatic recovery when challenged with an HFD.

4. Discussion

In this study, female rats fed an HFD for 72 hours were protected from increased adiposity in both the visceral and subcutaneous fat depots. Such protection would be expected because it is commonly observed that females experience slower adipose gains compared with males when fed obesogenic diets [11]. This is because female rats consume less energy while expending more energy than similarly treated males, in addition to estradiol-driven actions on adipose, muscle, and hepatic metabolic activity. Interestingly, however, HFD-fed females in our study were not protected from increased caloric intake and hyperphagia. The contradiction between caloric intake and body fat observed in our study requires further exploration. Female rats in this study appeared to be more physically active as suggested by the distance traveled over the experiment. This could suggest that the observed protection could be attributed to differences in caloric expenditure or substrate utilization.

Because hepatic lipogenesis contributes to adiposity and estrogens have been shown to down-regulate lipogenesis, it was hypothesized that the protection from HF-induced increases in adiposity observed in females was, in part, due to reductions in lipogenesis [1,12,14,20]. No sex differences emerged in the lipogenic genes measured including *Fas*, *Sreb1c*, *Dgat1*, and *Scd1*. High-fat diet, regardless of sex, resulted in a down-regulation of most hepatic lipogenic genes compared with LFD controls. These results parallel the findings of a similar study by Ren et al [9] in male C57BL/6 J mice. In their study, mice fed a 60% saturated fat diet had significant hepatic lipid accumulation, steatosis, and insulin resistance after 72 hours of the diet. In addition, the HFD resulted in significant down-regulation of hepatic lipogenesis. In combination with our results, the apparent down-regulation of lipogenesis after 72 hours of an HFD in both sexes supports a product inhibitory effect of dietary fat on hepatic enzymatic activity. Palmitate is a direct inhibitor of *Acc1* function but additionally down-regulates lipogenic gene expression in some models [21,22]. Therefore, our findings suggest that the switch to a stress-induced up-regulation in lipogenesis, a common characteristic of obesity, occurs at a time point beyond 72 hours in both sexes. The up-regulation of *Pparγ* expression in both sexes after 72 hours of HFD indicates this transition toward increased lipogenesis occurred shortly after this period.

Xbp1 has recently been demonstrated to regulate the transcription of lipogenic genes [23]. In brief, *Xbp1* mRNA splicing, often observed during diet-induced stress, leads to activation of its transcriptional properties. Similar to our results, Ren et al [9] found no significant diet-induced activation of *Xbp1* activity. *Xbp1* was chosen because of its documented activation by saturated fat, which was the significant contributor to dietary fat in our diet; activation of *Xbp1* results in up-regulation of hepatic lipogenic enzymes and provides the link between HFDs and increases in lipogenesis [24]. Because estradiol has been shown to

independently regulate both *Xbp1* and hepatic lipogenesis, a sex difference in *Xbp1* activity was hypothesized in our study [12,14,25,26]. This, however, was not observed; the amount of active *Xbp1* mRNA reflected the down-regulation of lipogenic expression in both sexes. However, it is important to mention the amount of total *Xbp1* mRNA (inclusive of both spliced and unspliced variants) was significantly decreased in female rats compared with males. Although it is difficult to interpret what this could reflect physiologically, to our knowledge, we are the first to document a sex difference in total *Xbp1* expression in the liver.

Recent studies have demonstrated that HFDs consumed for 72 hours promote the development of systemic and central insulin resistance [5–9]. Saturated fatty acids, in particular palmitate, induce insulin resistance by activation of inflammatory signaling within cells [27]. *Il6*, *Socs3*, and *Tnf α* all have been associated with induction of hepatic insulin resistance [28,29]. In addition, liver-specific inhibition of *Socs3* resulted in a suppression of hepatic insulin resistance in several models [28,30]. Combined with this knowledge, the HF dietary-induced reduction in hepatic inflammatory genes in our study suggests a potential retention of insulin sensitivity. The HFD in our study did not cause inflammatory gene expression in the liver, therefore greatly reducing the potential for cytokine-induced insulin resistance. This finding is conflicting with the conclusion from a similar study that found that hepatic insulin resistance at 72 hours was dependent on Kupffer cell activation and inflammatory signaling in male mice [31]. One major limitation to our study is the lack of investigation of insulin resistance in females, which has yet to be determined in this model. Although we are the first to report data in females in short-term exposures to HFD, future research needs to investigate differences in insulin signaling between the sexes to help address the above questions.

Previous 72-hour HFD studies found that both serum cytokines and hepatic inflammatory expression are increased, in congruence with insulin resistance in males [7,31]. These observations directly oppose what we observed; however, the difference in inflammation-associated gene expression may be due to differences in the amount of dietary fat between the studies (60% fat compared with 40% fat). The diet used in this study has demonstrated proinflammatory properties during longer intake studies [32]. This diet, being lower in fat compared with other 72-hour studies, might not have been as stressful and thus did not promote inflammatory gene expression. Furthermore, it is also possible that the differences in overall nutrient composition between our HFD and others used in the literature may also be a contributing factor. It is important to note the increased *Stat3* expression caused by HFD in our study. Because *Stat3* is an upstream regulator of the inflammatory cytokines involved in insulin resistance, a progression toward the proinflammatory state could have been occurring and was just not observed during our period with our chosen diet [33].

Lastly, we report a novel finding that 72 hours of HFD results in increased expression of genes related to apoptosis, cell repair, and cell remodeling. Interestingly, HFD up-regulated such genes to a far greater extent in females than in male rats. We did not measure serum alanine transaminase

in our study, which would further support the sexual dimorphic response seen in the liver and thus is a limitation to our work. However, acute apoptotic gene expression can be advantageous in regard to preventing disease and normally occurs within the liver without induction of proinflammatory cytokines [34]. Because this study is an early injury design, the increase in apoptotic gene expression seen in females can be considered a protective mechanism. The proapoptotic effects of estrogen during acute traumas have been previously documented and reviewed in the literature [35,36]. Because an acute up-regulation of apoptotic genes is important during transient injuries, the sex difference observed in our study might serve as a novel pathway by which females are protected from steatosis and hepatocarcinomas [34,37].

To our knowledge, we are the first to demonstrate that both sexes equally down-regulate hepatic lipogenic gene expression after 72 hours of an HFD. This finding opposes what our original hypothesis stated. However, we did measure a greater response caused by the HFD in genes related to inflammation, apoptosis, cell repair, and cell remodeling in female rats. This may suggest a novel mechanism behind the observed sex differences in liver disease. Lastly, both sexes consume the same amount of energy over this time span, ameliorating the sex difference common when rats are fed standard chow. Interestingly, female rats were protected from increases in adiposity in both the visceral and subcutaneous fat deposits. Because this protection was not due to differences in caloric intake or hepatic lipogenesis, why female rats are protected from increased adiposity after 72 hours of an HFD remains to be determined.

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