Aging Differentially Effects Diet-Induced Obesity and Central Leptin Sensitivity in Rats

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Abstract
In this study we examined whether sex differences in central leptin sensitive young rats disappears in middle-aged rats. As animals age, many gain visceral fat and develop leptin resistance, making them more susceptible to inflammation. Middle-aged rats were fed low-fat (LF) or high-fat (HF) diets for 2 months and during this time were given intra-3rd-ventricular (i3vt) leptin injections in a range of doses. Females had a dose dependent decrease in food intake (FI) in response to i3vt leptin. Males reduced FI after i3vt injection of 5.0 μg leptin but not at any other dose. There was also a higher expression of hypothalamic cytokines that are part of the inflammation cascade in males including IL6, TNFα and XBP1. Females remained sensitive to i3vt leptin and had lower hypothalamic cytokine expression than males. The female rats in both diets had visceral fat percentages similar to that of the males which may mean that age increases fat in this depot in rats. These data indicate that middle-aged rats are in a transition period in terms of hormonal sensitivity that may serve as a model to study age-associated changes. Response patterns in female rats that are cycling but have not reached persistent estrous may be suggestive for explanations of physiological changes in perimenopausal women. These findings are important because aging represents a time when health is impacted by diet, body fat distribution and estrogen levels.

Keywords
Sex Differences; Estradiol; High-Fat Diet; Inflammation; Long Evans Rats; Visceral Fat

1. Introduction

There are several factors that interact in obesity that are part of the current experimental design including sex, diet and age. Consuming high-fat (HF) foods is one of the most important environmental factors leading to obesity [1]. Ingestion of a HF diet leads to hypothalamic leptin and insulin resistance [2, 3]. Obesity is accompanied by chronic inflammation, which is causally linked to leptin and insulin resistance [4-6] and may result in obesity.

When a diet high in saturated fat is consumed free fatty acids (FFAs) increase inflammation by activating toll-like receptor 4 (TLR4) [7]. Chronic activation of pro-inflammatory pathways may be at least partly responsible for obesity-induced insulin resistance and diabetes [4-6]. Pro-inflammatory
cytokines like tumor necrosis factor-alpha (TNFα) and interleukin-6 (IL-6) are elevated in individuals with insulin resistance and diabetes [8, 9]. Despite this, female rats in some experiments have lower levels of inflammatory cytokines than their male controls [10-12].

If the sex differences observed in young rats results from the levels of estrogen in young rats, then aging and the possible increase in inflammation with aging could be a result of lower estrogen levels as women age. One way to test this hypothesis is to measure a known paradigm from young rats like central leptin response in older animals. In a previous experiment sex differences in central leptin demonstrated that female rats were more sensitive to central leptin than their age-matched male controls [13]. Long Evans rats live about 2 years and middle age in our terms would be around 10-12 months old and rats enter estrous senescence around 15 months of age [14]. The rats in this study were 10 -11 months old at the end of the study.

The aim of this study was to determine if diet-induced obesity and central leptin sensitivity remain in middle-aged female and male Long Evans rats. Additionally, because HF diet-induced inflammation leads to central leptin resistance and estrogen is a known anti-inflammatory agent, we sought to determine if middle-aged female rats fed a HF diet will have less hypothalamic inflammation than male rats.

We hypothesized that middle-aged rats will be more susceptible to hypothalamic inflammation that can result in both leptin resistance and diet-induced obesity compared to younger rats. In addition, sex differences observed in younger rats will become less evident in middle age due to declining estrogen levels.

2. Materials and Methods

2.1. Animal Care

All laboratory work was performed at laboratory facilities at the University of North Carolina at Greensboro (UNCG). The UNCG Institutional Animal Care and Use Committee approved all protocols for this experiment.

Age-matched male and female Long Evans rats (8-9 months old, Harlan Labs; Harlan, IN) were individually housed in Plexiglas tubs and maintained on a 12:12-h light-dark cycle in a temperature-controlled vivarium. Upon arrival, they were given 1 week to acclimate to the facility before introduction to sex-specific colony rooms. Rooms were temperature (22 ± 2°C) and humidity controlled and kept on a 12:12 light/dark cycle (lights on at 0400 h). Prior to the start of the experiment, 30 females and 30 males were maintained on a standard laboratory chow (17% fat and 3.1 kcal/g, Harlan Teklad #7012; Indianapolis, IN). Rats had free access to food and water unless otherwise noted. At the start of the experiment, 15 rats of each sex were switched to a high-fat diet (40% fat and 4.54 kcal/g, Research Diets #D03082706; New Brunswick, NJ). Feeding data were converted to kilocalories (kcal) to compare intake between groups.

2.2. I3vt Cannulation

Seven days after arrival, rats were anesthetized with 0.1 ml/100g body weight intraperitoneal (ip) injections of a ketamine (70 mg/kg)/xylazine (2 mg/kg) mixture. Subsequently, 22-gauge guide cannulas (Plastics One Inc., Roanoke, VA) were stereotaxically implanted in the intra- 3rd-cerebral ventricle (i3vt). Briefly, bregma and lambda were situated at the same vertical coordinate and cannula tips were positioned on the midline, 2.2 mm posterior to bregma and 7.5 mm ventral to the dura mater. The cannulas were then fixed to the skull using dental acrylic and anchor screws. Obturators extending 0.5 mm beyond the cannula tract were inserted. When rats returned to their pre-surgical...
body weights, cannula placement was confirmed by i3vt infusion of 10 µg of Neuropeptide Y (NPY) in 1 µl normal saline 3 h prior to the onset of dark and monitoring the rat’s food intake over a 60-min period. Animals that did not eat at least 2 g of chow within 60 min were excluded from the study.

### 2.3. i3vt Injection Protocol

The leptin dose-response consisted of i3vt injections leptin (1.5, 3.5, 5.0 and 7.5 µg/ 1 µl; Human Leptin; CalBiochem, San Diego, CA) or 1 µl vehicle. Using a within-subjects design, each rat randomly received each of the 4 leptin doses and vehicle with a 7 day washout period. On each experimental day, food hoppers were removed from the cages and the animals were weighed 3 h prior to the onset of dark which is the start of the experiment (0 h). At 45 min prior to the onset of the dark, body weights were again recorded and rats received an i3vt injection of vehicle or leptin which was slowly infused with a Hamilton syringe. Food hoppers were returned at the onset of dark and the animals were weighed again the next morning which is the 24 h time.

### 2.4. Leptin Dose Schedule

The changes in 24 h food intake (FI) measured in kcals and change in body weight (BW) change at 24 h were assessed by diet (LF vs. HF), sex (male and female) for the 4 doses of leptin and saline vehicle. The leptin dose response consisted of i3vt injections leptin (1.5, 3.5, 5.0 and 7.5 µg/ 1 µl volume) or 1 µl saline vehicle. Using a within-subjects design, each rat randomly received each of the 4 leptin doses and vehicle with a 7 day washout period. It took 6-7 weeks to complete the experiment from the time of cannulation.

### 2.5. Body Composition

After euthanasia, the skin and subcutaneous fat (pelt) was dissected from the muscle wall and visceral fat (carcass) as previously described [15]. The fat above the muscles measured in the pelt was used as a measure of subcutaneous fat while the fat under the abdominal muscles was used as a measure of visceral fat (VAT). DEXA measurement was performed using a GE Lunar Prodigy Advanced System (GE Healthcare; Milwaukee, WI) and the data were analyzed by Encore 2007 Small Animal software (version 11.20.068). The system was calibrated according to manufacturer’s instructions each day samples were run. Samples were scanned in duplicate to determine fat mass (FM) and lean body mass (LBM) using a protocol previously described [16].

### 2.6. Plasma Analysis

Trunk blood was collected in heparinized tubes and centrifuged immediately following decapitation. Aliquots of plasma were collected and stored at -80°C until analyzed. Plasma was packed on dry ice and sent to the University of Texas Southwestern for specific radioimmunoassay of estradiol (Quest Diagnostics, Inc.; Nichols Institute Diagnostics, San Juan Capistrano, CA).

### 2.7. Gene Expression

The medial basal hypothalamus was preserved in RNAlater and stored for 24 h at 4°C and then stored at -80°C until processed. RNA was isolated using QIAGEN RNAeasy kits (Qiagen, Inc.; Valencia, CA) according to the manufacturer instructions. RNA concentration and purity was assessed by Nanodrop spectrophotometer (Thermo Scientific, ND-1000; Wilmington, DE). 2 ng of RNA for each sample was combined with RNase free H2O and master mix solution (Applied Biosystems; Foster City, CA) and run in a Thermocycler (Applied Biosystems; Foster City, CA) for 2.5 h to obtain cDNA. Then the cDNA was used to determine gene expression via quantitative RT-PCR for TNFα, SOCS3, IL6, and XBP1 using primers from Applied Biosystems.
2.8. Statistical Analysis

Statistical analysis was performed using SPSS (version 18.0) for Windows. Treatment effects and interactions were tested using two-way ANOVA and individual group differences were tested using Dunnett’s T3 post hoc analysis. Data are presented as means with corresponding standard error of the means (SEM) and significance set at p ≤ 0.05.

3. Results

3.1. LF Diet: 24 h Fl/BW Change

Male rats reduced Fl and BW when given the 5.0 µg leptin i3vt injection and reduced BW only when given the 1.5 and 7.5 µg doses (Figure 1). There was no change after the 3.5 µg injection. In contrast, female rats reduced Fl and BW following the 1.5 and 7.5 µg injections of leptin but only reduced Fl following the 3.5 and 5.0 µg leptin doses. That means female rats responded at every dose of leptin, while males were not as consistent. While the amount of weight lost in 24 h was similar in males and females, females (vehicle mean 72.97 kcal) ate fewer kcals than male rats (vehicle mean 101.45 kcal). After central leptin female rats consumed only about 50 kcal in 24 h.

Food intake (Fl) and change in body weight (BW) measured 24 h after i3vt leptin or vehicle injections are graphed for male (n= 15) and female (n=15) rats fed the LF diet (*p< 0.05). In this figure the dose response of male rats can be compared to that of the female rats fed the LF diet.

Abbreviations: LF = low fat

Compared to the results from a previous study using 3-month old males and females, the middle-aged females remained sensitive to central leptin [15]. Three month old males were less sensitive to i3vt
leptin than females and that sex difference remains in this study. The lowest effective dose in our middle-aged males was the 5.0 µg dose which is higher than reported in young males [15] whose lowest effective dose was at the 3.5 µg dose. So while middle-aged males responded to i3vt leptin by losing BW, they lost some sensitivity in that there was no decrease in FI.

### 3.2. HF Diet: 24 h FI/BW Change

When fed the HF diet, males did not reduce FI at any dose of leptin, and reduced BW after the 3.5 and 5.0 µg leptin doses (Figure 2). However, males reduced BW when given the 1.5 and 7.5 µg doses. Surprisingly, females had a clear leptin response on the HF diet, reducing FI after all doses, and reducing BW after the 5.0 µg dose. This resulted in a main effect for sex for FI at 24 h (p<0.05). Generally, rats given a HF diet become leptin resistant. In this protocol, rats were switched to the HF diet on the surgery day and remained on it until sacrifice. Two weeks after surgery, the leptin injections began and were given weekly. That means rats had been fed the diet for 3-7 weeks during the time of the dose response curve. The sex difference remains in that females were more responsive to central leptin, but the kcals eaten were similar in male and female fed the HF diet. Females and males ate the same amount in 24 h (vehicle means 82.57 and 83.69 kcal, respectively) and females reduced this significantly in response to i3vt leptin injection. These data may indicate that middle-aged females fed a HF diet are protected from leptin resistance and that there is some response in males.

![Graph showing 24 h Food Intake and 24 h Body Weight change for HF diet groups](image_url)

**Figure 2: Leptin Dose-Response -HF Diet Groups**

Food intake (FI) and change in body weight (BW) measured 24 h after i3vt leptin or vehicle injections are graphed for male (n=15) and female (n=15) rats fed the HF diet (*p< 0.05). In this figure the dose response of male rats can be compared to that of the female rats fed the HF diet.

Abbreviations: HF = high fat
3.3. Body Composition and Body Fat Distribution

Lean Body Mass (LBM) and fat were measured by DEXA (Table 1) in the carcass and pelt. After euthanasia the pelt which has the skin and its underlying fat is separated from the carcass so that the subcutaneous skin can be measured. The fat that lies under the skin is the subcutaneous adipose tissue (SCAT). The carcass has the muscles and the fat located under the abdominal muscles is the visceral adipose tissue (VAT). The pelt and carcass are separately scanned by DEXA as a method to measure each fat depot.

In the carcass, males had higher LBM than females (p< 0.05). Groups fed the HF diet increased their FM which then reduced carcass LBM. Carcass fat in males was two times that of females on the LF diet. Males fed the HF diet increased carcass fat 50% while females doubled carcass fat when fed the HF diet to a level that matched the LF diet males. Males fed the HF diet increased fat by 50% while females doubled their carcass fat when placed on the HF diet. These data indicate that middle-aged females gained body fat at a rate higher than that of males and that they have higher VAT than male rats. Similarly, males had twice as much fat as females in the pelt area. Pelt LBM was similar in male groups which was three times higher than that of HF females. LF diet females had a small amount of LBM that was only 5% of that of males with averages of 42.67 and 2.11 g respectively.

Body fat was measured in the pelt and carcass, and those values were added to get whole body data (Table 2). Age-matched males weighed 200-300 g more than females at the end of the experiment. When males were fed the HF diet, carcass and pelt body fat increased 50%; conversely females fed the HF diet more than doubled their whole body fat, which incidentally was approximately at the same level of fat in LF males. This same pattern occurred for both pelt and carcass fat, males increased both by 50% and females doubled the fat in each depot when fed the HF diet. Males fed the HF diet increased their % FM by 12%, whereas females increased about 20%. The final two columns (shaded gray) in Table 2 give the location of % FM between the depots. This allows direct comparison of the fat distribution for each group since the transformation to % is similar to the transformation of FI to kcals. Males had more fat in the carcass (VAT) than in the pelt by a 55:45 ratio. This was not changed by the HF diet. Females had more fat in the VAT than males (63% vs. 56%) and this was not changed significantly by the HF diet.

3.4. Plasma Estradiol (E2)

Plasma E2 was measured from blood taken after euthanasia. Female rats were phased biweekly to assure they were still cycling, but were not phased on the day of euthanasia, so the result represent various days of the estrous cycle. We were interested in whether the HF diet would alter E2 values, and it did. Rats fed the LF diet 42.36 pg/ml had higher E2 levels than rats fed the HF diet (32.84 ± 2.61 pg/ml (p=0.049)).

3.5. Quantitative RT-PCR

After euthanasia, the medial basal hypothalamus was dissected and processed for expression of inflammatory cytokines IL6, TNFα and SOCS3 and a marker of intracellular stress, XBP1 by quantitative RT-PCR (Figure 3).
After euthanasia, the medial basal hypothalamus was dissected and processed for expression of inflammatory cytokines IL6, TNFα and SOCS3 and a marker of intracellular stress, XBP1 by quantitative RT-PCR. Statistical differences between groups were performed using Dunnett’s T3 post-hoc analysis in SPSS version 18.0 for Windows. (*p< 0.05).

Abbreviations: low fat (LF), high fat (HF), interleukin 6 (IL6), tumor necrosis factor α (TNFα), suppressor of cytokine signaling 3 (SOCS3), x-box binding protein 1 (XBP1), glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

### 4. Discussion

Central leptin injections reduce food intake and body weight; effects that are dependent upon sex, diet and age. When young-adult (3-month old) Long Evans rats are given i3vt leptin, males respond at higher doses than females indicating that males are less sensitive to central leptin than females [15, 16]. When young rats are put on a HF diet, they become less sensitive to the ability of leptin to reduce food intake [16]. In this experiment, middle-aged male rats fed the LF diet were slightly less sensitive to i3vt leptin than females which was consistent with findings in younger rats. When the rats were fed a HF diet, the females remained sensitive to i3vt leptin.

Males fed the HF diet became leptin resistant, consistent with the literature [17]. This is an important observation because it indicates that the increased caloric intake and gain of body fat in the middle-aged females fed the HF diet occurred in spite of maintained leptin sensitivity. Since plasma E2 levels were normal in the females, the interaction of the leptin and E2 may prevent central leptin resistance. Additive effects could occur since E2 activates intracellular kinases including MAPK, PI3K, Src-K which phosphorylate the STAT3 transcription factor which overlaps leptin’s intracellular cascade [18].

In contrast, males reduced FI after i3vt 5.0 μg leptin and did not reduce FI at any other dose. This is higher than the 3.5 μg effective dose published for young males [15], indicating that age reduced sensitivity to central leptin in male rats. Combined with the higher expression of hypothalamic cytokines in males this may indicate that diet-induced inflammation is not restrained in males as it is in females.
Female groups had lower IL6 expression than males (Figure 3A). This resulted in a main sex effect (p<0.026). Diet did not change this further. There was a diet x sex interaction for TNFα (F(1,24)=5.82, p<0.024; Figure 3B). This resulted from a reduction in TNFα expression in all groups compared to LF males, and an increase in females fed the HF diet when compared to their LF controls. This was similar to what occurred in SOCS3 (Figure 3C). Males fed the LF diet had significantly higher levels of SOCS3 mRNA than all other groups. This resulted in a diet x sex interaction (F(1,24)=5.18, p<0.032). Males fed the HF diet had lower expression of XBP1 than their LF controls (p<0.026; Figure 3D). In contrast, there were no changes to XBP1 in females.

Females had lower expression of IL6, TNFα and SOCS3 than males and this may implicate E2 as a main factor in inflammatory cytokines, regardless of diet. One of the goals of this experiment was to determine whether sex differences observed in younger rats remain in middle-aged rats. They do in the case of inflammatory cytokine expression in the hypothalamus. The genes selected have some control from NFκB since E2 through ERα directly interacts with NFκB.

Retaining central leptin sensitivity with age could explain the persistence of the central effects of leptin working with E2, but the peripheral effects of E2 may have been altered in our study. Rats fed the HF diet started the diet on the day of surgery and remained on it until euthanasia. This was about 2 months. We found that middle-aged females gained more body fat during this time than males. We report for the first time that VAT in our females was similar to that of males, and increased with HF diet. This age-related effect is similar to what is observed in aging women. In young men, excess fat is more likely in the apple pattern (increased VAT) while women tend to gain fat below the waist in the pear shape (increased SCAT) [19]. As women enter middle age and E2 levels decrease, this pattern changes and women gain fat above the waist in the apple pattern [19].

Shown in Table 2, the %FM in males and females increased for the HF diet groups, but females gained 20% more FM in the two months that they were on the HF diet showing a sensitivity to diet-induced obesity. Also shown in Table 2, all groups have approximately 60% of their fat in the visceral depot and only 40% in the SCAT. That is significant because our aged females now have a male fat distribution, regardless of diet. This differs from what is reported in young rats, where males have more VAT than females [15]. However, this age difference is consistent with what occurs in postmenopausal women who accrue more VAT as estrogen levels decline [19]. We interpret this as an age-related change and an indication that estrogen signaling in the fat depot is decreasing.

While plasma E2 levels were not lower than that measured in comparable younger females, the estrogen receptor population and intracellular estrogen signaling could be altered. Rats in the current study were 10-11 months old at the time of euthanasia. According to the literature, female Long Evans rats are still cycling at this age [20] but cycles could be irregular. The females were cycling in our experiment but without a longer history of estrous cycles, we are unable to determine whether the cycles were irregular. Irregular cycle may mean transitioning from 4 d to 5 d cycles. We phased all females in two time points to be sure they were cycling, but did not phase daily. Thus we are only able to report that estrous cycles were regular (4 d length) at those times but do not know if they were irregular at some point during the study.

Estrogen by structure is a steroid hormone and thus can be a factor in decreasing inflammation. This is significant because inflammation, in particular diet-induced inflammation with a saturated fat diet, is a causal factor in central insulin resistance and obesity [3, 15, 17]. The transcription factors measured are controlled by a master controller of inflammation NFκB. NFκB is important for sex differences because 17β-estradiol can block its activation, and the transcription of IL-6, TNFα, and SOCS3 which are all increased by NFκB [21-23]. XBP-1 interacts with E2 and was added to give a snapshot of an additional intracellular cascade. In Figure 3 expression of transcription factors activated by inflammation is presented with the male LF diet group as the control group. Females on both diets
had lower expression IL-6, TNFα, and SOCS3 than the control group by over 50%. This is another indication that central estrogen could have reduced the inflammatory effects of the high-fat diet allowing the females to retain central leptin sensitivity.

There are conflicting data reporting that cytokine expression increases, decreases or remains unchanged with age [24, 25]. There are data reporting increased levels of circulating TNFα and IL-6 [26]. On the other hand, age is associated decreased capacity of the immune system [24]. In addition there is a question about whether increased levels of cytokines, like IL6 is beneficial or detrimental. IL-6 protects neurons against NMDA excitotoxicity in vitro and prevents brain from ischemic attacks in vivo [27]. However, other studies implicate IL-6 in neurodegenerative disorders [28]. Finally, experiments using neuronal cultures show that IL-6 is both neuroprotective and neurotoxic, depending on concentration [29]. Taken together, these data highlight the importance of context in determining functional significance of inflammatory protein expression. It is beyond the scope of this study to interpret whether the increased and decreased expression of the genes measured represent dysfunction or protection. Our goal was to capture changes with aging, and to illuminate the remaining questions.

The changes that occurred with age were mixed in this study. Males had higher hypothalamic cytokine expression and less central leptin sensitivity than females. However, middle-aged females gained more body fat as a percent of BW than males and it resulted in more VAT in females than males. We interpret this as differences in aging in males and females. Females were more sensitive to diet-induced obesity, yet sensitive to central leptin and had greatly reduced cytokine expression than males. Taken together these data indicate that middle-aged females are in a transitional time when some age changes have occurred, and this is a good model for examining sex differences in ingestive behavior in middle-age rats. Particularly in female rats, this age represents cycling females that have not reached persistent estrous and may be comparable to perimenopausal women.

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Disclosures

The authors have no conflicts of interest to declare.

Tables

| Table 1: Body Composition |
|---------------------------------|
| **LF Males** | **HF Males** | **LF Females** | **HF Females** |
| Carcass Fat (g) | 104.22 ± 12.25^a | 156.22 ± 12.88^a | 56.44 ± 8.83^b | 118.15 ± 8.01^a |
| Carcass LBM (g) | 312.22 ± 16.49^a | 270.56 ± 8.55^a | 169.22 ± 5.32^b | 155.08 ± 4.76^b |
| Pelt Fat (g) | 79.78 ± 10.83^ac | 128.11 ± 12.57^a | 32.67 ± 6.87 | 72.23 ± 5.05^c |
| Pelt LBM (g) | 42.67 ± 3.85^a | 47.33 ± 6.12^a | 2.11 ± 1.07^d | 14.23 ± 2.83^c |

Body composition measured by DEXA is presented. After euthanasia, the skin and subcutaneous fat (pelt) was dissected from the muscle wall and visceral fat (carcass) so that visceral (carcass) and subcutaneous (pelt) fat could be measured. Samples
were scanned in duplicate to determine mass of fat and lean body mass (LBM). Data are reported as mean ± SEM. Statistics represent differences in SEM across rows. Values sharing a common letter are not different from one another, p<0.05. Abbreviations: low fat (LF), high fat (HF), lean body mass (LBM).

**Table 2: Body Fat Distribution**

|                | BW (g) | body fat (g) | body % FM | pelt fat (g) | carcass fat (g) | pelt % FM | carcass % FM |
|----------------|--------|--------------|-----------|--------------|-----------------|-----------|--------------|
| **LF Males**   | 574.67 | 184.00 ± 23.08 | 32.02 %   | 79.78 ± 10.83 | 104.22 ± 12.25  | 43.36 %   | 56.64 %      |
| **HF Males**   | 634.28 | 284.33 ± 25.45 | 44.83 %   | 128.11 ± 12.57 | 156.22 ± 12.88  | 45.06 %   | 54.94 %      |
| **LF Females** | 303.32 | 89.11 ± 15.70  | 29.38 %   | 32.67 ± 6.87  | 56.44 ± 8.83    | 36.66 %   | 63.34 %      |
| **HF Females** | 387.89 | 190.38 ± 13.06 | 49.08 %   | 72.23 ± 5.05  | 118.15 ± 8.01   | 37.94 %   | 62.06 %      |

Body composition measured by DEXA. The first three columns represent total values - BW and body fat in grams, and the % of fat in the whole body. The last four columns represent the amount of fat in grams and % fat in the carcass and pelt. Percentage of fat in the pelt and carcass totals 100 % so that the data represent where fat was located for each group, as well as where fat was added as the HF diet groups increased body fat. The pelt % FM is the fat just under the skin or the subcutaneous adipose tissue (SCAT), while the carcass % fat is the fat under the abdominal muscles which is our measure of visceral adipose tissue (VAT). Abbreviations: body weight (BW), fat mass (FM), low fat (LF), high fat (HF).

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