Impact of age on host responses to diet-induced obesity: Development of joint damage and metabolic set points

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Abstract

Background: Osteoarthritis is one of the leading causes of pain and disability worldwide, and a large percentage of patients with osteoarthritis are individuals who are also obese. In recent years, a series of animal models have demonstrated that obesity-inducing diets can result in synovial joint damage (both with and without the superimposition of trauma), which may be related to changes in percentage of body fat and a series of low-level systemic inflammatory mediators. Of note, there is a disparity between whether the dietary challenges commence at weaning, representing a weanling onset, or at skeletal maturity, representing an adult onset of obesity. We wished to evaluate the effect of the dietary exposure time and the age at which animals are exposed to a high-fat and high-sucrose (HFS) diet to determine whether these factors may result in disparate outcomes, as there is evidence suggesting that these factors result in differential metabolic disturbances. Based on dietary exposure time, we hypothesized that rats fed an HFS diet for 14 weeks from weaning (HFS Weanling) would demonstrate an increase in knee joint damage scores, whereas rats exposed to the HFS diet for 4 weeks, starting at 12 weeks of age (HFS Adult) and rats exposed to a standard chow diet (Chow) would not display an increase in knee joint damage scores.

Methods: Male Sprague-Dawley rats were fed either an HFS diet for 14 weeks from weaning (HFS Weanling) or an HFS diet for 4 weeks, starting at 12 weeks of age (HFS Adult). At sacrifice, joints were scored using the modified Mankin Criteria, and serum was analyzed for a defined subset of inflammatory markers (Interleukin-6, leptin, monocyte chemoattractant protein-1, and tumor necrosis factor α).

Results: When the HFS Weanling and HFS Adult groups were compared, both groups had a similar percent of body fat, although the HFS Weanling group had a significantly greater body mass than the HFS Adult group. The HFS Weanling Adult diet groups had significantly higher serum proinflammatory mediators (Interleukin-6, leptin, monocyte chemoattractant protein-1, and tumor necrosis factor α) as compared to the Chow group. Although knee joint damage scores were low in all 3 groups, we found, contrary to our hypothesis, that the HFS Adult group had statistically significant greater knee joint damage scores than the Chow and HFS Weanling groups. Furthermore, we observed that the HFS Weanling group did not have significant differences in knee joint damage scores relative to the Chow group.

Conclusion: These findings indicate that the HFS Weanling animals were better able to cope with the dietary challenge of an HFS diet than the HFS Adult group. Interestingly, when assessing various serum proinflammatory markers, no significant differences were detected between the HFS Adult and HFS Weanling groups. Although details regarding the mechanisms underlying an increase in knee joint damage scores in the HFS Adult group remain to be elucidated, these findings indicate that dietary exposure time maybe less important than the age at which an HFS diet is introduced.

Keywords: Adult exposure; High-fat, high-sucrose diet; Rat obesity model; Serum biomarkers; Weanling exposure

1. Introduction

Rates of obesity are rising in developed and developing countries. The onset and progression of this epidemic have become most pronounced over the past 40 years. The reasons for this rise include increased sedentary lifestyles, changes in diet, and increased genetic susceptibility. The World Health Organization estimates that approximately 1.9 billion adults worldwide are overweight, with 600 million being obese. In the United States, obesity rates have more than doubled since the 1980s, with 42% of adults being overweight or obese, and 40% of children being overweight or obese. Despite numerous efforts to address the obesity epidemic, prevalence rates have continued to rise.

The impact of obesity on joint health is of particular concern, as obesity is a risk factor for osteoarthritis, one of the most common joint diseases. Osteoarthritis affects millions of people worldwide, causing pain and disability, and is the leading cause of musculoskeletal disability. The prevalence of osteoarthritis increases with age, and the risk is higher in individuals with obesity. The relationship between obesity and osteoarthritis is complex, involving both mechanical and metabolic factors.

Mechanical factors include increased joint loading due to increased body weight, which can lead to cartilage damage and osteoarthritis. Metabolic factors include systemic inflammation, which can contribute to joint damage. Chronic low-level systemic inflammation is common in obesity, and it has been hypothesized that this may contribute to the development of osteoarthritis.

Current research aims to elucidate the mechanisms underlying the link between obesity and osteoarthritis, with the ultimate goal of developing effective prevention and treatment strategies. This study evaluated the effect of dietary exposure time and age at which animals are exposed to an HFS diet on joint damage scores.

Materials and Methods

Male Sprague-Dawley rats were fed either an HFS diet for 14 weeks from weaning (HFS Weanling) or an HFS diet for 4 weeks, starting at 12 weeks of age (HFS Adult). At sacrifice, joints were scored using the modified Mankin Criteria, and serum was analyzed for a defined subset of inflammatory markers (Interleukin-6, leptin, monocyte chemoattractant protein-1, and tumor necrosis factor α).
rapid increase in obesity rates can be attributed, at least in part, to dietary factors and more sedentary behavior in people of all ages. For adults, diets of processed foods containing high levels of fat and sucrose have become more common, along with less physically demanding occupations and a lack of consistent exercise (occupational or recreational), all of which have led to caloric imbalances. These imbalances have resulted in a deleterious combination contributing to the onset of obesity and its consequences, such as type 2 diabetes (T2D), metabolic syndrome, cardiovascular diseases, fatty liver, joint pain, and compromised skeletal muscle.\(^2\) It has been suggested that there is a “set point” that regulates not only body weight but also metabolic health.\(^3\) Thus, the timing of the onset and progression of obesity as a skeletally mature adult or as an older adult (e.g., postmenopause in a female) could have consequences that differ from obesity onset in childhood.

The onset of obesity in childhood and adolescence is particularly concerning because many young individuals are now experiencing cardiovascular alterations, T2D and metabolic syndrome, which were previously seen typically only in older adults.\(^4\) The onset of obesity in childhood is thought to lead to health compromises not unlike those seen in adults with obesity. Puberty appears to be a critical transition time with respect to how obesity-inducing conditions impact host systems.\(^5\) Furthermore, there are known transgenerational effects resulting from parental diets that can induce deleterious musculoskeletal consequences in young individuals.\(^6\) Research regarding the timing of the onset of obesity and its consequences in infants and prepubescent children, however, is not well established. In contrast, the direct effects of obesity-inducing diets on weanling rodents\(^7\)-\(^9\) and the indirect effects via the maternal diet on offspring\(^10\)-\(^12\) have been more fully studied, although these studies have focused mainly on insulin/diabetes, metabolism, or brain development. Early exposure to obesity-inducing diets has been shown to lead to early adaptive changes in musculoskeletal tissues.\(^13\)-\(^19\) This raises the question of whether younger rats can transiently adapt to diets with high fat and high sucrose levels and be protected against other sequelae of musculoskeletal dysregulation in the same way.\(^9\)

Over the past several years, we developed and characterized a rat model of diet-induced obesity using adult male rats that were, on average, 12 weeks of age when started on an obesity-inducing diet, which consisted of a high-fat and high-sucrose (HFS) content.\(^2\),\(^13\)-\(^19\) In this model, after 12 weeks on the HFS obesity-inducing diet, knee joint damage, muscle-specific changes, onset of insulin resistance, development of T2D, and onset of local and systemic biomarker patterns were observed.\(^2\),\(^14\),\(^19\),\(^20\) After 28 weeks on the HFS diet, the incidence and severity of the knee joint damage had progressed.\(^13\),\(^17\) As such, this HFS rat model, as well as other model systems,\(^21\),\(^22\) have shown that starting an obesity-inducing diet during adulthood leads to reproducible compromises in multiple host systems that resemble many features of diet-related obesity in humans. In line with this thinking, it has been suggested that long-term exposure to an obesity-inducing diet from weaning can subsequently alter the development of post-traumatic osteoarthritis (OA) in rodents.\(^23\),\(^24\) There is a gap in understanding as to whether a transient protection observed in rats fed an obesity-inducing diet from weaning may ultimately be overridden and result in joint damage. Recent findings on the development of the gut and microbial environment suggest that the timing of exposure to obesity-inducing diets may be important in the development of obesity-related complications.\(^25\) Understanding the differential adaptive phenomena that result from weanling versus adult HFS diet onset is critical in that it can help to inform better planning of experimental approaches, as well as to inform therapeutic strategies to manage obesity and its consequences.

Therefore, the purpose of the present study was to evaluate the onset of knee joint damage following exposure of male rats to an HFS diet using 2 experimental approaches. We aimed to assess knee joint damage following short-term and standard-term diet exposure in a scenario where rats were the same age at the time of sacrifice but were fed an HFS diet for different time periods. Previously, we have observed damage in the quadriceps muscles of male rats fed for 4 weeks on an HFS diet,\(^15\) and we, therefore, considered 4 weeks to be a reasonable short-term diet exposure to use in the present study and to allow for a comparison to a “standard” 12- to 14-week obesity-induction period.\(^26\) Our hypothesis was that rats fed an HFS diet for 14 weeks from weaning (HFS Weanling, 3 weeks of age) would demonstrate knee joint damage, whereas rats exposed to the HFS diet for 4 weeks starting at 12 weeks of age (HFS Adult) and rats exposed to a standard chow diet (Chow) would not have knee joint damage. We hypothesized that this result would be due to the shorter dietary exposure of the HFS Adult group to the HFS diet compared to the HFS Weanling group.

### 2. Materials and methods

#### 2.1. Dietary intervention and body composition

A total of 22 male Sprague-Dawley rats were allocated to either an HFS diet group (45% sucrose, 40% fat; custom Diets \#103915 and \#102412,\(^15\) Dyets Inc., Bethlehem, PA, USA) or Chow control-diet group (13% fat, 3.15% sucrose; Lab Diet 5001; Dyets Inc.). Rats were purchased from a specific pathogen-free facility (Charles River Laboratories) and maintained at the University of Calgary with standard monitoring thereafter. A total of 6 rats were fed an HFS diet ad libitum from 3 weeks of age (HFS Weanling), with an additional 6 rats commencing the diet at 12 ± 1 weeks of age (HFS Adult). The Chow group (Chow, \(n = 10\)) served as the control group and comprised rats from both feeding challenges (Chow Weanling, \(n = 4\), and Chow Adult, \(n = 6\)). All rats consumed their respective diets until 16 weeks of age. At this time, body mass (g) and body composition (body fat percentage, by Dual-energy X-ray Absorptiometry, Hologic QDR 4500; Hologic Inc., Bedford, MA, USA) were measured prior to sacrifice, as described previously.\(^15\) All procedures were approved by the University of Calgary Life and Environmental Sciences Animal Care Committee.

#### 2.2. Preparation of knee joints

Knee joints were harvested from all rats, fixed in 10% neutral buffered formalin (HT501128-4 L; Sigma Chemical Co., St. Louis,
Serial, sagittal plane sections (8 μm thickness) were obtained using a Leica RM 2165 (Leica Biosystems Nussloch GmbH, Wetzlar, Germany) microtome. Sections were mounted onto Super Frost plus slides (12-550-15; Fisher Scientific Inc., Waltham, MA, USA), and sampling was done to represent approximately every 80 μm throughout the joint. Slides were stained sequentially with hematoxylin, fast green and safranin-O stains (Fisher Scientific Inc.) using an auto stainer (Leica ST 5010). Further details regarding knee joint preparation can be found in Collins et al.13 Stained slides were scored using modified Mankin Criteria.13,17 Osteoarthritis Society International subscores for bone changes and synovial thickening were also determined for each joint.

The total modified Mankin score for each rat represents the cumulative Mankin scores (medial tibial plateau, medial femoral condyle, lateral tibial plateau, lateral femoral condyle, retro-patellar surface, femoral groove, and meniscus) and Osteoarthritis Society International subscores27 (synovium, bone) for each rat. All Chow rats consumed the same diet for the entire study, so these rats were pooled when making between-group comparisons (Chow, HFS Weanling, and HFS Adult) in modified Mankin scores to improve the statistical power of this analysis. Of note, the 2 HFS groups considered here were evaluated as part of 2 separate cohorts at 2 separate times. However, for consistency, the same graders assessed the histology images considered here, and images of slides were revisited to ensure valid comparisons. Additional outcomes related to the HFS Adult group can be found in Collins et al.13

2.3. Serum cytokine analyses

At the time of harvest, blood was obtained from all rats, and serum was prepared and stored as described previously.15 Aliquots of the serum were assessed for biomarkers using either a 27-plex protein array (rats fed from weaning; Eve Technologies Corp., Calgary, AB, Canada) or a custom subset of these markers (4-plex for interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor α (TNF-α), and leptin) using the Luminex platform technology (Eve Technologies Corp.). Of note, we evaluated TNF-α, but the serum levels for many animals were below the lower limit of detection and, therefore, were not reported due to the lack of reliability. As a consequence, only 3 cytokines were compared in this analysis. Cytokine measurements were conducted on separate plates for the HFS Weanling and HFS Adult groups. For this reason, we are unable to make direct comparisons between these groups. Therefore, cytokine measurements for the HFS Weanling and HFS Adult groups are presented as fold-changes compared to their respective Chow groups, with error bars representing percent error relative to the average.

2.4. Statistical analysis

A Kruskal-Wallis H test (one-way analysis of variance on ranks) was performed to determine whether there were any statistically significant differences in body mass, percent body fat, and knee joint damage scores among the 3 groups (Chow, HFS Weanling, and HFS Adult), with Dunn post hoc tests used to assess between-group differences.

Independent t tests (α = 0.05) were used to compare the cytokine measurements for the HFS Weanling and HFS Adult groups relative to their respective Chow groups. Analyte values that were outside of the curve or out of range were adjusted accordingly. Values outside of the curve were extrapolated based on their assigned fluorescence intensity values, whereas out-of-range values were designated as the lowest value obtained for that particular analyte.

To understand systemic contributors to the Mankin scores, Spearman correlations were run between body fat, body mass, and Mankin score across all rats, α = 0.05. All statistical analyses were performed in SPSS (Version 21.0; IBM Corp., Armonk, NY, USA) (α = 0.05), and figures were created in GraphPad (Version 23.0; Graphpad Prism, San Diego, CA, USA).

3. Results

3.1. Body composition

HFS Weanling group animals demonstrated significant increases in body mass and body fat compared to the Chow group (Fig. 1). Although HFS Adult group animals had significantly more body fat and body mass relative to the Chow group animals, they had reduced body mass compared to the HFS Weanling group animals (Fig. 1).
3.2. Modified Mankin score

After 14 weeks of HFS feeding, the HFS Weanling group and Chow group animals had similar overall modified Mankin knee joint scores (Fig. 2 and Fig. 3), despite higher body mass in the HFS Weanling group compared to the Chow animals. In contrast, the HFS Adult group animals had greater knee joint damage, as defined by increased modified Mankin scores, when compared to the Chow group animals. Furthermore, HFS Weanling group animals had significantly lower modified Mankin scores compared to HFS Adult group animals ($p = 0.0043$, Table 1).

3.3. Serum cytokine profiles

The HFS Weanling group animals had statistically lower serum MCP-1 levels compared to the Chow Weanling group (Fig. 4A). In contrast, the HFS Adult group animals had similar serum marker profiles (MCP-1, IL-6, and leptin) compared to the Chow Adult group animals (Fig. 4B).

3.4. Relationships between body composition and modified Mankin scores

There was a significant positive relationship between body fat percentage and the modified Mankin scores for the analysis, including the HFS Adult and Chow group animals. When the HFS Weanling group animals were included in this analysis, the relationship became nonsignificant (Fig. 5).

4. Discussion

The primary result of this study was that 4 weeks of exposure to an HFS diet in the HFS Adult group animals led to significant increases in knee joint damage, whereas 14 weeks of exposure to the same diet in the HFS Weanling group animals did not produce increased knee joint damage scores when compared to the corresponding Chow group.

A dietary challenge (HFS Adult and HFS Weanling) resulted in a similar increase in percent body fat but a smaller increase in body mass in the HFS Adult compared to the HFS Weanling group animals. Body fat percentage and body mass have been positively associated with increased joint damage and OA progression in previous research. Although there was no difference in any of the cytokine markers measured in the HFS Adult group, there were significant decreases in a number of cytokine markers (e.g., MCP-1) in the HFS Weanling group animals when compared to the corresponding Chow control group. These data suggest that the HFS Weanling group animals may be better equipped to adapt to the challenge of an HFS diet compared to the HFS Adult group, allowing the young animals to mitigate joint damage. We assume that this mitigation of joint damage is transient, given previous data demonstrating joint damage with long-term exposure to diet challenge from weaning. We anticipate that with a longer exposure to the HFS diet than was given in the present study, this protection in the HFS Weanling group animals would eventually be overridden. Nonetheless, the present data contribute important novel insights into the complicated interactions among the timing, age, and duration of obesity-inducing diets and the development of knee joint damage. Specifically, our results suggest that the relation between an HFS diet and knee joint damage in young rats may differ conceptually.

![Fig. 2. HFS Adult group animals exhibit greater knee joint damage scores than the HFS Weanling and Chow groups. HFS Weanling and Chow groups had similar modified Mankin scores, while the HFS Adult group had significantly greater modified Mankin scores relative to the Chow group, despite the short exposure time to the HFS diet. Raw data are shown, and lines indicate the median ± 95% confidence intervals. $* p < 0.05$ compared with other groups, using a Kruskal-Wallis analysis of variance with the Dunn correction for multiple comparisons (Chow, $n = 10$; HFS Adult, $n = 6$; HFS Weanling, $n = 6$). HFS = high-fat and high-sucrose.](image1)

![Fig. 3. Representative medial tibial plateau safranin-O and fast green histology images of Chow-fed (A), HFS Adult (B), and HFS Weanling (C) animals reveal increased damage in HFS adult animals. HFS = high-fat and high-sucrose. Images were taken at 10 ×; scale bar indicates 200 μm.](image2)
Table 1
Mankin subscores that compose the modified Mankin score reveal increased medial tibial plateau damage in HFS Adult animals.

|                | Medial tibial plateau | Medial femoral condyle | Lateral tibial plateau | Lateral femoral condyle | Patella | Groove | Synovium | Bone | Meniscus |
|----------------|-----------------------|------------------------|------------------------|-------------------------|---------|--------|----------|------|----------|
| Chow           | 4 (0–7)               | 0 (0–6)                | 0 (0–6)                | 0 (0–5)                 | 0 (0–9) | 0 (0–4) | 2 (0–3)  | 1 (1–3) | 0 (0–1)  |
| HFS Adult      | 6 (0–11)*             | 0 (0–0)                | 6 (0–12)*              | 2 (0–11)                | 0 (0–10)| 0 (0–2) | 2 (1–3)  | 2 (1–4) | 1 (0–3)  |
| HFS Weanling   | 6 (0–6)               | 0 (0–0)                | 0 (0–0)                | 0 (0–0)                 | 0 (0–0) | 0 (0–4) | 2 (1–2)  | 1 (1–1) | 0 (0–0)  |

Note: Data are shown as median (range).
* p < 0.05 compared with Chow group.
Abbreviation: HFS = high-fat and high-sucrose.

Fig. 4. HFS Weanling group animals had significantly lower serum MCP-1 levels compared to the Chow Group animals (A), with no significant differences seen between the HFS Adult and control group animals (B). The figures display fold-changes relative to their respective Chow groups, with error bars representing % error. * p < 0.05 using an independent t test. HFS = high-fat high-sucrose; IL-6 = Interleukin-6; MCP-1 = monocyte chemoattractant protein-1.

Fig. 5. Chow Adult rats and Adult HFS rats showed a significant positive relationship between body fat (A) and a trend toward a significant relationship with body mass (B) and knee joint modified Mankin score. When the HFS Weanling rats were added to the analysis, relationships between body fat (C) and body mass (D) and knee joint modified Mankin score became nonsignificant. Spearman correlations calculated between the Chow Adult and Adult HFS group rats (A and B) and all rats (C and D) (Chow, n = 10; HFS Adult, n = 6; HFS Weanling, n = 6). HFS = high-fat and high-sucrose; NS = nonsignificant.
compared to previously reported relations between increased body fat and joint damage in adult rats, particularly with regard to the timing of the HFS challenge onset.\textsuperscript{13,14,28,30} Based on the metrics used in this study, despite an exposure time to the HFS diet that was 3.5 times longer in the HFS Weanling group (14 weeks) compared to the HFS Adult group animals (4 weeks), the 14 weeks of HFS exposure in the young rats did not result in global increases in knee joint damage scores. Of note, the final 4 weeks of the 14-week exposure in the HFS Weanling group encompassed the 4-week exposure experienced by the HFS Adult group animals. Previously, we observed measurable increases in knee joint damage scores that were positively related to the percentage of body fat\textsuperscript{14} and speculated that low-level systemic inflammatory mediators derived from visceral fat were key components in the onset and progression of obesity-associated OA.\textsuperscript{5} However, in this study, the increased knee joint damage (i.e., modified Mankin scores) in the HFS Adult group occurred independent of increases in any systemic markers (e.g., MCP-1, IL-6, and leptin) measured in the serum. These results beg the question as to the underlying mechanisms driving knee joint damage in the HFS Adult group animals, especially in view of the fact that the greater increases in body mass in the HFS Weanling compared to the HFS Adult rats did not lead to an increase in joint damage scores. This is in contrast to reports in which changes in proinflammatory markers and increased body mass resulted in knee joint OA, as reviewed in Berenbaum et al.\textsuperscript{28}

Leptin has been widely implicated in OA pathogenesis,\textsuperscript{31,32} although it does not seem to induce cartilage damage directly.\textsuperscript{22} It is not surprising that fold-change levels for leptin were similar in HFS Adult and HFS Weanling group animals because leptin is thought to be secreted in proportion to body fat,\textsuperscript{13} and percent body fat values were similar for the 2 HFS groups. Here, levels of leptin do not seem to explain the differential joint damage scores in Adult versus Weanling group animals. However, we did not assess expression of leptin receptors in various tissues and, thus, cannot determine whether leptin was equally functional in the 2 HFS groups. Fold-change levels for MCP-1, a mediator implicated in OA onset and progression,\textsuperscript{34–36} have previously been observed to be elevated in serum in HFS diet-induced OA,\textsuperscript{14} although this was not the case in the present study. This may indicate that the HFS Weanling and HFS Adult group animals were either not under significant metabolic stress or that adipose tissue or other metabolic tissues were able to compensate for the metabolic challenge.\textsuperscript{37,38}

Across many of the mediators measured in the extended 27-plex assay evaluating the HFS Weanling group animals, reduced levels for proinflammatory mediators were observed, which may allow for anti-inflammatory mediators to act more effectively. However, this speculation remains to be tested directly. Other preliminary analysis has shown a loss in muscle integrity for some muscles in the HFS Adult (4 weeks)\textsuperscript{15} and the HFS Weanling (14 weeks) group animals evaluated here (MacDonald et al., unpublished observations). Moreover, some aspects of the responses of the young rats to the diet, as they progress through maturation and puberty, are different from the responses of the adult, skeletally mature rats exposed to the HFS diet postmaturation, even for the short time of 4 weeks.

We observed previously that a short-term metabolic challenge can result in a rapid compromise of vastus lateralis muscle integrity and elevated tissue-level inflammation, likely resulting from systemic inflammation and alterations in the gut microbiota following short-term exposure to a high-fat/high-sucrose diet.\textsuperscript{5} Findings from the present study partially oppose these previous findings because knee joint damage was present despite no increases in the specific markers of systemic inflammation in serum that were evaluated here. However, other markers were increased in HFS Adult animals at this timepoint and earlier timepoints\textsuperscript{15} but were not able to be compared in this context because of the limited number of analytes evaluated in the HFS Weanling animals. Obesity induction thus appears to be a dynamic process that includes compensation across tissues and systems. Specifically, compromised muscles have been purported to be a key risk factor for OA onset and progression, but the precise cross-talk between joints and muscles and the mechanisms by which muscle weakness induces or contributes to joint loading and OA remains to be clarified and is an exciting area for future research.\textsuperscript{2,39,40}

We cannot rule out changes later in life with prolonged HFS exposure initiated at weaning, a time when joint damage is more likely to occur. A recent study suggested that osteochondral lesions are induced by 9 weeks of high-fat diet (45% fat, 0% sucrose) exposure in male Sprague-Dawley rats from weaning.\textsuperscript{41} However, the male rats used in the present study were on the HFS diet for 14 weeks (weaning to adulthood), and, thus, the previous findings\textsuperscript{41} were not confirmed, although the diets were different (high-fat alone vs. high-fat, high-sucrose). The intervention diet used in the present study had a higher energy density due to fat content (kcal) and higher energy derived from simple carbohydrates (kcal) compared to a 45% (kcal) fat diet. Subtle differences between diets may result in differential regulatory feedback in response to metabolic dysregulation that could yield different outcomes. Spontaneous osteochondral lesions can occur in Sprague-Dawley rats,\textsuperscript{12} osteochondral lesions may be exacerbated by a high-fat diet,\textsuperscript{41} and OA can occur secondarily to osteochondral lesions in both humans and animals.\textsuperscript{42}

Interestingly, although the knee joint damage following a dietary change in the obesity-inducing diet would appear to be severely detrimental to the adult rats, these rapid changes can be eliminated by coexposure of the rats to either moderate aerobic exercise or a microbiome-modifying prebiotic when the obesity-inducing diet is initiated.\textsuperscript{20} Thus, although the onset of knee damage in the HFS Adult group is rapid and progressive, the underlying mechanisms driving these changes need to be elucidated. It is important to note that the detrimental changes to the knee observed in this study can be prevented by exercise (host variables) and prebiotics (microbiota variables) initiated at the same time as the HFS diet.\textsuperscript{21} Because adult animals show distinctly different metabolic
and body fat changes when exposed to the HFS diet used here over long time periods,2,13–15,19,20 more careful characterization of early exposure compared to adult exposure, in addition to time-course studies and studies with male and female rats, may facilitate a more complete understanding of these relationships and the role of puberty in their regulation. In the future, preventive interventions and details regarding the molecular and physiologic adaptations to early exposure to obesity-inducing diets from weaning may provide insight into therapeutic strategies or alternative approaches to address the metabolic and nutritional insults induced by adult exposure to HFS diets.

There are several limitations to this study that need to be considered in the interpretation of our results. First, we did not have a short-term (4-week) exposure group that started at weaning to directly compare either the duration of HFS dietary exposure or the timing of onset of the HFS challenge. Furthermore, due to the exploratory nature of this study, we did not assess many outcomes that may have strengthened the understanding of the effects observed here, such as the microbiome, synovial fluid, a more comprehensive profile of serum mediators, and other musculoskeletal changes that might have provided key protective features or pathways activated in weanling rats. Also, we had a limited number of rats in each group and evaluated only male rats due to the preliminary nature of this study. Because there are known sex differences in programming and early exposure to HFS diets,6,44 future studies should evaluate female rats to evaluate the relationships studied here. Moreover, because the cohorts were from 2 separate batches of rats that were evaluated at different times, there are potentially seasonal effects and batch effects at play in this study. The present analysis does not detail osteochondral changes, so ongoing analysis should aim to evaluate this aspect directly. It is possible that the induction of degradative knee joint changes is mitigated when prolonged exposure to an HFS diet is initiated at the time of weaning due to the body’s ability to adapt its nutrient set-point during maturation. However, further studies are needed to understand this phenomenon more completely.

5. Conclusion

Exposure to 14 weeks of an HFS diet did not induce overt increases in joint damage in young male rats exposed to an HFS diet from the time of weaning. In contrast, rats fed the same HFS diet when they were adults showed significant increases in knee joint damage scores despite a much shorter exposure time to the diet than the young rats, although details regarding the mechanisms involved remain to be elucidated. Although little is known about the implications of adult versus weaning onset of obesity and the musculoskeletal consequences thereof, these findings indicate that childhood and adult onset of obesity may manifest differently in hosts’ responses to dietary challenges. Such an interpretation may help to explain why rats exposed to adult-onset obesity are at greater risk for spontaneous joint damage than rats exposed to an HFS diet from the time of weaning.

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Authors’ contributions

KHC and GZM designed, executed, and analyzed all experiments and drafted the article; JLR assisted with experiment design, tissue collection, analysis, and revision of the article; RAS assisted with tissue collection, analysis, and revision of the article; WH, DAH, and RAR were responsible for supporting conception and design of studies, interpretation of the data, and critical revision of the article. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Competing interests

The authors declare that they have no competing interests.

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