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Sex determines effect of physical activity on diet preference: Association of striatal opioids and gut microbiota composition

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Abstract

Previous studies suggest an interaction between the level of physical activity and diet preference. However, this relationship has not been well characterized for sex differences that may exist. The present study examined the influence of sex on diet preference in male and female Wistar rats that were housed under either sedentary (no wheel access) (SED) or voluntary wheel running access (RUN) conditions. Following a 1 week acclimation period to these conditions, standard chow was replaced with concurrent ad libitum access to a choice of 3 pelleted diets (high-fat, high-sucrose, and high-corn starch) in the home cage. SED and RUN conditions remained throughout the next 4 week diet preference assessment period. Body weight, running distance, and intake of each diet were measured daily. At the conclusion of the 4 week diet preference test, animals were sacrificed and brains were collected for mRNA analysis. Fecal samples were also collected before and after the 4 week diet preference phase to characterize microbiota composition. Results indicate sex dependent interactions between physical activity and both behavioral and physiological measures. Females in both RUN and SED conditions preferred the high-fat diet, consuming significantly more high-fat diet than either of the other two diets. While male SED rats also preferred the high-fat diet, male RUN rats consumed significantly less high-fat diet than the other groups, instead preferring all three diets equally. There was also a sex dependent influence of physical activity on both reward related opioid mRNA expression in the ventral striatum and the characterization of gut microbiota. The significant sex differences in response to physical activity observed through both behavioral and physiological measures suggest potential motivational or metabolic difference between males and females. The findings highlight the necessity for further exploration between male and female response to physical activity and feeding behavior.

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Keywords

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1. Introduction

Adequate physical activity has been demonstrated to attenuate the cascade of metabolic maladaptations associated with obesity [1,2]. However, with approximately 90% of adult Americans failing to reach the U.S. Department of Health guidelines for physical activity, the endemic of sedentary lifestyles further contributes to surging obesity rates in the United States [3]. Human studies have revealed critical roles for metabolic and behavioral adaptations to physical activity that may serve to counteract obesity. One of these adaptations may be alteration in food intake [4].

Considering the complex factors that may influence the interaction between physical activity and feeding behavior, animal models have taken a variety of approaches. One of these approaches has been to examine the influence of exposure to physical activity on diet preference. These studies have led to conflicting results, most likely due to methodological approach including type (i.e. forced vs. voluntary) and duration of physical activity, timing of diet assessment (24 h vs short term), composition of diet, and examining only male or only female subjects [5–9]. In particular, the lack of direct investigation of sex differences is problematic. In sedentary conditions, males and females display differences in seeking and consuming palatable diets [10]. In addition, it is well documented that male and female rats demonstrate unique behavioral and metabolic characteristics, including differences in both running behavior [11,12] and corresponding changes in body weight [13]. Therefore, it is predicted that running behavior may produce sex dependent changes in diet selection and general feeding patterns.

While the interaction between physical activity, energy needs, and metabolic function and its influence on feeding behavior has been characterized, the possibility that physical activity may act as a natural reward to alter motivational states toward other rewards (i.e. food) has received less attention. In animal models, the rewarding properties of physical activity (i.e. voluntary wheel running) and palatable diets have both been demonstrated [14,15]. The degree to which two rewarding events influence each other has been explored in other paradigms, including drug self-administration. One interesting outcome is termed reward substitution, a phenomenon that occurs when one reward alleviates the seeking or craving behavior for another reward [16,17]. However, the interaction between the two rewarding events of physical activity and high-fat intake has not been well examined. In the current study, physical activity is predicted to influence high-fat intake by substituting for the rewarding properties of high-fat diet.

It is known from studies on male rats that physical activity and high-fat intake both produce changes in reward-related transcription factors within the ventral striatum [18–20]. Voluntary running is altered following systemic [21] and direct ventral striatum opioid administration in rats selectively bred for high levels of voluntary wheel running [22]. In

addition, palatability-driven feeding is also altered through both systemic [23,24] and direct opioid administration into the ventral striatum [25–27]. Considering the overlap in underlying neurobiology mediating these two rewarding behaviors, it is intriguing to behaviorally characterize potential interactions. Similar to the influence of physical activity on behavior, it is predicted that there will also be changes in the reward pathway.

In addition to potential behavioral and neurological effects, recent studies are beginning to explore how physical activity may mediate health outcomes via alterations of the microbiome. Microorganisms exist in the gastrointestinal tract (also known as the gut microbiota) and regulate physiological systems and their associated behaviors through a gut-brain axis [28,29]. Altered microbiota composition is a factor that may promote obesity by influencing feeding behavior, such as preference for palatable diets [30]. Given the interceding role of gut-brain axis on ingestive behavior, physical activity may serve as a protective factor to prevent maladaptive feeding behaviors. To our knowledge, this is the first study to examine sex dependent changes of the gut microbiota composition in response to physical activity, as well as the interaction between physical activity and resulting diet preference and feeding patterns.

The present study examined the interaction of voluntary wheel running (RUN) or sedentary conditions (SED) home-cage diet selection over a 4 week period in male and female Wistar rats. Changes in opioid-related gene expression within the ventral striatum following this diet selection period were assessed. Lastly, changes in gut microbiota characterization and specific microbial population differences between groups were assessed before and after the introduction of the diet preference assessment phase.

2. Materials and methods

2.1. Subjects and housing

20 male and 20 female outbred Wistar rats (Charles River Breeding, Raleigh, NC) of approximately 28 days of age (weighing 60–75 g and 50–70 g respectively) arrived in the laboratory and were initially pair housed, males and females in separate rooms. Animals remained in a climate-controlled room at a temperature of 22 °C and a 12:12 h light cycle (lights on at 0700). Animal usage and procedures were approved by University of Missouri Animal Care Use Committee.

2.2. Experimental procedure and intervention

At 49 days of age, rats were moved to single housing and assigned to either sedentary (SED) conditions (PVC tubing as environmental enrichment with no wheel access) or wheel access (RUN) conditions in standard Plexiglas cages for the remainder of the study. Tecniplast stainless steel running wheels (34 cm diameter) were placed inside the home cage and running distance and duration was measured daily using Sigma Sport bicycle pedometers. Rats were given 1 week to acclimate to RUN or SED condition with chow diet (Week 0). On day 56 of age, chow was replaced with 3 different pelleted diets (for details see below). All diets were provided simultaneously in divided sections of the cage lid. Location of diets was rotated each day. Daily consumption of each diet was recorded throughout the duration of

the 4 week diet preference test (Week 1–4). Three hours before onset of dark cycle (1600hr), rats were sedated (CO₂), underwent rapid decapitation, brains were rapidly removed, frozen and stored in –80 °C for later dissection in cryostat.

2.3. Diet compositions

Upon arrival, rats were provided with ad lib access to water and standard chow (Lab Diet 5008– 3.5 kcal/gram). The chow was replaced with ad lib access to a choice of three commonly used pelleted diets formulated by Research Diets. These included a (1) high-fat diet (D12492: 5.24 kcal/gram) consisted of 60% fat, 20% carbohydrate and 20% protein; (2) high-sucrose diet (D12450B: 3.85 kcal/gram) consisted of 10% fat, 70% carbohydrate (35% sucrose) and 20% protein; (3) high-corn starch diet (D12450J: 3.85 kcal/gram) consisted of 10% fat, 70% carbohydrate (49% corn starch) and 20% protein (Table 1). Diets introduced at day 56 (Table 2).

2.4. Microbial DNA extraction and quantification

Fecal samples were collected at two time points; the day before introduction of the 3 diet preference test (Week 0), and on the final day of the diet preference test (Week 4). Briefly, one fecal pellet was placed into a sterile 2 mL round-bottom tube containing 800 µL lysis buffer (500 mM NaCl, 50 mM Tris-HCl pH 8.0, 50 mM EDTA, and 4% sodium dodecyl sulfate) and a 0.5 cm diameter stainless steel bead. Samples were mechanically disrupted using a TissueLyser II (Qiagen, Venlo, Netherlands) for 3 min at 30 Hz, followed by incubation at 70 °C for 20 min with periodic vortexing. Samples were centrifuged at 5000 × *g* for 5 min, and the supernatant was then transferred to a sterile 1.5 mL Eppendorf tube containing 200 µL of 10 mM ammonium acetate. Lysates were vortexed, incubated on ice for 5 min, and then centrifuged. Supernatant was transferred to a sterile 1.5 mL Eppendorf tube and one volume of chilled isopropanol was added. Samples were incubated on ice for 30 min and then centrifuged at 16,000 × *g*, at 4 °C, for 15 min. The resulting DNA pellet was washed with 70% ethanol and resuspended in 150 µL Tris-EDTA (10 mM Tris and 1 mM EDTA), followed by addition of 15 µL of proteinase K and 200 µL of AL Buffer. Samples were incubated at 70 °C for 10 min and 200 µL of 100% ethanol was added to the tubes. Samples were mixed by gentle pipetting and the contents transferred to a spin column from the DNeasy kit. The DNA was purified following the manufacturer's instructions and eluted in 200 µL EB buffer.

Quantification DNA concentrations were determined fluorometrically (Qubit dsDNA BR assay, Life Technologies, Carlsbad CA) and purity was assessed via 260/280 and 260/230 absorbance ratios, as determined via spectrophotometry (Nanodrop 1000 Spectrophotometer, Thermo Fisher Scientific, Waltham, MA). Samples were stored at –20 °C until sequencing.

2.5. Library construction and 16S rRNA sequencing

Library construction and sequencing was performed at the University of Missouri DNA Core facility. DNA concentration of samples was determined fluorometrically and all samples were normalized to 100 ng template for PCR amplification. Bacterial 16S rRNA amplicons were generated via amplification of the V4 hypervariable region of the 16S rRNA gene using single-indexed universal primers (U515F/806R) flanked by Illumina standard adapter

sequences and the following parameters: $98^{\circ}\text{C}^{(3:00)} + [98^{\circ}\text{C}^{(0:15)} + 50^{\circ}\text{C}^{(0:30)} + 72^{\circ}\text{C}^{(0:30)}] \times 25 \text{ cycles} + 72^{\circ}\text{C}^{(7:00)}$.

2.6. Informatics

All informatics processing was performed at the University of Missouri Informatics Research Core facility. Assembly, binning, and annotation of DNA sequences was performed at the MU Informatics Research Core Facility. Briefly, contiguous DNA sequences were assembled using FLASH software [31] and culled if found to be short after trimming for a base quality less than 31. Qiime v1.8 [32] software was used to perform *de novo* and reference-based chimera detection and removal, and remaining contiguous sequences were assigned to operational taxonomic units (OTUs) via *de novo* OTU clustering and a criterion of 97% nucleotide identity. Taxonomy was assigned to selected OTUs using BLAST against the Greengenes database (DeSantis et al., 2006 of 16S rRNA sequences and taxonomy. Principal component analyses were performed using $\frac{1}{4}$ root-transformed OTU relative abundance data using Past 3.15 software [33].

2.7. Ventral striatum RNA extraction and cDNA synthesis

Brain sections visibly identified as the ventral striatum, (Bregma 2.2–0.7 mm) (Paxinos and Watson 1998) were sliced coronally with a cryostat (Leica BioSystems). Tissue was collected in 3 mm diameter punch samples at -20°C and then stored at -80°C until processing. Samples were lysed in Trizol using a high speed shaking apparatus for 3 min at 25 Hz (Tissuelyser LT, Qiagen, Valencia, CA) with RNase-free stainless steel beads. RNA was separated according to the manufacturer's instructions (TRIzol, Invitrogen, Carlsbad, CA). A Nanodrop 1000 (Thermo Scientific) was used to quantify the RNA. Quality of RNA was confirmed on a 1% agarose gel.

RNA was reverse transcribed using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Carlsbad, CA).

2.8. Ventral striatum mRNA expression quantification

The cDNA for each sample was assayed in duplicate for target genes using SYBR Green Mastermix (Applied Biosystems Carlsbad, CA). The mRNA was assessed for dopamine receptor 1 (DRD1), dopamine receptor 2 (DRD2), mu opioid receptor 1 (OPRM1), proenkephalin (PENK), and 18S. The mRNA expression values are presented as $2^{-\text{Ct}}$, whereby $\text{Ct} = 18\text{S Ct} - \text{gene of interest Ct}$, and were normalized to MALE SED condition values. The fold changes were calculated from standard error of $2^{-\text{Ct}}$ values.

2.9. Statistical analysis

Analytical procedures were performed using S.A.S. 9.4. All values are presented as mean $\pm$ SEM. Significance for all analyses were set with an alpha value of 0.05. Outcome measures for between-group and within-group comparisons were analyzed using a two-way analysis of variance (ANOVA) [Sex (MALE vs. FEMALE) physical activity condition (RUN vs. SED)].

Within group variables (body weight, running distance) were analyzed using one-way repeated measures ANOVA. Significant effects were followed by Tukey post hoc comparisons. Testing for differences between groups in β -diversity, i.e., community structure, were performed via two-way PERMANOVA of non-transformed OTU relative abundance data, using Past 3.15 software.

3. Results

3.1. Body weight and running distance

3.1.1. Body weight—There was no difference in body weight of the SED vs RUN in either sex prior to the introduction of the voluntary running wheel, Week -1 ($p > 0.05$), as well as during the week of SED vs RUN acclimation prior to introduction of the novel diets, Week 0 ($p > 0.05$) (Fig. 1). Throughout the 4 week diet preference phase, SED weighed more than RUN condition in males ($F_{1,54} 4.6$, $p = 0.04$), but there was no difference in females ($F_{1,54} 1.2$, $p > 0.05$).

3.1.2. Running distance—Daily running distance was higher in females than in males across the 5 weeks of wheel access ($F_{1,72} 35$, $p = 0.0001$) (Fig. 1). Running distance increased over time ($F_{4,72} 27$, $p = 0.0001$) with females increasing running more than males ($F_{4,72} 11$, $p = 0.0001$). Thus, despite running relatively greater distances than the male rats, female rats did not demonstrate the same running associated reduction in weight observed in males.

3.2. Dietary preference assessment

3.2.1. Four week total intake—Intake of each diet was recorded daily and standardized to kilocalories per 100 g body weight (Table 3).

3.2.2. High-fat diet—A two-way ANOVA of high-fat consumption revealed a main effect of sex ($F_{1,36} 15.8$, $p = 0.0003$) but not physical activity condition ($F_{1,36} 2.4$, $p > 0.05$). There was a significant sex by physical activity condition interaction ($F_{1,36} 14.6$, $p = 0.0005$) (Fig. 2). Post hoc analysis demonstrated that male RUN consumed less high-fat diet than both male SED ($p = 0.007$) and female RUN ($p = 0.001$). There was no difference in high-fat diet intake between female RUN and female SED ($p > 0.05$) or between male SED and female SED ($p > 0.05$).

3.2.3. Weekly interval intake—A two-way repeated measures ANOVA revealed a main effect of time ($F_{3,108} 115$, $p = 0.0001$). There was an interaction of time by physical activity condition ($F_{3,108} 5.88$, $p = 0.0009$), but no interaction of time by sex ($F_{3,108} 0.5$, $p > 0.05$) or time by physical activity condition by sex ($F_{3,108} 95$, $p > 0.05$) (Fig. 3). Week 1 high-fat diet intake was significantly higher than Week 4 for all groups ($p = 0.004$). Intake in the SED conditions decreased more over time than the RUN conditions ($p = 0.05$).

3.2.4. High-sucrose diet—A two-way ANOVA of consumption of high-sucrose diet revealed no main effect of sex ($F_{1,36} 0.1$, $p > 0.05$). There was a main effect of physical activity condition ($F_{1,36} 7.11$, $p = 0.01$). There was no interaction of sex by physical activity

condition ($F_{1,36} 1.8, p > 0.05$) (Fig. 2). Post hoc analysis revealed that rats in the RUN condition consumed more high-sucrose diet than SED condition ($p = 0.04$). Weekly interval intake: A two-way repeated measures ANOVA revealed a main effect of time ($F_{3,108} 3.7, p = 0.01$). There was no interaction of time by physical activity condition ($F_{3,108} 1.8, p > 0.05$) or time by sex ($F_{3,108} 73, p > 0.05$) or time by physical activity by sex ($F_{3,108} 1.6, p > 0.05$) (Fig. 3).

3.2.5. High-corn starch diet—A two-way ANOVA of consumption of high-corn starch diet revealed no main effect of sex ($F_{1,36} 2.2, p > 0.05$). There was a main effect of physical activity condition ($F_{1,36} 4.1, p = 0.05$). There was a trend for interaction of sex by physical activity condition ($F_{1,36} 4, p = 0.053$) (Fig. 2). Post hoc analysis revealed that rats in the RUN condition consumed significantly more high-corn starch than rats in the SED condition ($p = 0.05$). Male RUN consumed more high-corn starch than male SED ($p = 0.04$). There was no difference between female RUN and female SED ($p > 0.05$).

3.2.6. Weekly interval intake—A two-way repeated measures ANOVA revealed no main effect of time ($F_{3,108} 2.25, p > 0.05$). There was no interaction of time by physical activity condition ($F_{3,108} 42, p > 0.05$) or time by sex ($F_{3,108} 87, p > 0.05$). There was no time by physical activity condition by interaction ($F_{3,108} 01, p > 0.05$) (Fig. 3).

3.2.7. Caloric intake—A two-way ANOVA of overall caloric intake revealed a main effect of sex ($F_{1,36} 48.5, p < 0.0001$). There was a main effect of physical activity condition ($F_{1,36} 17.5, p = 0.0002$). There was an interaction of sex by physical activity condition ($F_{1,36} 12.7, p = 0.001$) (Fig. 2). Post hoc analysis revealed that females consumed significantly more calories than males ($p = 0.004$). Rats in the RUN condition consumed significantly more calories than the SED condition ($p = 0.005$). Female RUN consumed more calories than female SED and male RUN ($p = 0.005$). There was no difference between male RUN and male SED ($p > 0.05$).

3.2.8. Weekly interval intake—A two-way repeated measures ANOVA revealed no main effect of time ($F_{3,108} 1.9, p > 0.05$). There was no interaction of time by sex ($F_{3,108} 1.09, p > 0.05$). There was a time by physical activity condition interaction ($F_{3,108} 7.7, p = 0.0001$). There was no time by sex by physical activity condition interaction ($F_{3,108} 2.03, p > 0.05$). Post hoc analysis revealed that SED treatment decreased caloric intake over time compared to RUN treatment ($p = 0.005$) (Fig. 3). The above findings indicate that female RUN, compared to male RUN, demonstrated greater overall caloric intake with no detectable change in preference. Lastly, male RUN rats demonstrated a shift in diet preference toward carbohydrate-rich and lower-fat food sources but with no change in overall caloric consumption.

3.3. Correlation between running distance and consumption

A linear regression analysis was used to assess correlations between wheel running distance and intake of any of the 3 diets in both males and females. No significant correlation was found in males for wheel running distance and high-fat diet ($p > 0.05$), high-sucrose diet ($p > 0.05$), and high-corn starch diet ($p > 0.05$). In addition, no significant correlation was

found in females for wheel running distance and high-fat diet ($p > 0.05$), high-sucrose diet ($p > 0.05$), and high-corn starch diet ($p > 0.05$).

3.4. Ventral striatum mRNA expression

3.4.1. DRD1 mRNA expression—A two-way ANOVA for DRD1 mRNA expression revealed no main effect of physical activity condition ($F_{1,33} 0.16$, $p > 0.05$) or sex ($F_{1,33} 0.02$, $p > 0.05$). There was a trend for a physical activity condition by sex interaction ($F_{1,33} 4.09$, $p = 0.051$) (Fig. 4).

3.4.2. DRD2 mRNA expression—There was no main effect of physical activity condition ($F_{1,33} 2.4$, $p > 0.05$) or sex ($F_{1,33} 1.6$, $p > 0.05$) on DRD2 mRNA expression levels. There was no interaction of physical activity condition by sex interaction ($F_{1,33} 2.2$, $p = 0.05$) (Fig. 4).

3.4.3. OPRM1 mRNA expression—There was no main effect of physical activity condition ($F_{1,33} 1.9$, $p > 0.05$) or sex ($F_{1,33} 0.21$, $p > 0.05$) on OPRM1 mRNA expression levels. There was a sex by physical activity condition interaction ($F_{1,33} 7.1$, $p = 0.01$) (Fig. 4). Female RUN expressed greater levels of OPRM1 mRNA compared to female SED ($p = 0.04$). There was no difference between male RUN and male SED.

3.4.4. PENK mRNA expression—There was a trend for a main effect of physical activity condition ($F_{1,33} 3.89$, $p = 0.054$) on PENK mRNA expression levels. There was no main effect of sex ($F_{1,33} 0.28$, $p > 0.05$). There was a physical activity condition by sex interaction ($F_{1,33} 6.27$, $p = 0.01$) (Fig. 4). Female RUN expressed greater levels compared to female SED ($p = 0.05$). There was no difference between male RUN and male SED.

3.5. Characterization of changes to microbiota: bacterial sequencing

To determine if there were sex-dependent or activity-associated differences in the composition of the gut microbiota (GM), fecal DNA was extracted and subjected to 16S rRNA gene amplicon sequencing. Richness and diversity was assessed via the Chao1 and Shannon indices, metrics of α -diversity with differential weight placed on richness and evenness of taxonomic distribution.

Considering Chao1 index, which weights richness over distribution, a significant main effect of time in both males and females ($F_{1,33} 7.6$, $p < 0.009$) ($F_{1,33} 26$, $p < 0.0001$) was observed. A significant effect of physical activity ($F_{1,33} 66$, $p < 0.0001$) was observed only in males. While there was an overall decrease in both males and females from Week 0 to Week 4; male RUN displayed greater richness compared to male SED at both Week 0 ($p < 0.001$) and Week 4 ($p = 0.03$) (Fig. 5A). Comparison of the Shannon indices, which places more weight on the evenness of distribution relative to the Chao1 index, revealed a main effect of time in males and in females ($F_{1,33} 16.4$, $p < 0.001$) ($F_{1,33} 51.2$, $p < 0.001$), respectively. Therefore, like the Chao1 index, both male and female rats displayed clear time dependent decreases in diversity (Figs. 5B). Thus, these data demonstrated that while community evenness decreased similarly in all groups in a time-dependent manner, richness was greater in male

RUN, compared to male SED. Yet there was no difference between female RUN and female SED at either time point.

Notably, the composition of the GM in each group also differed between groups with significant time dependent main effects and several interactions. Visualizing the microbial communities subjectively via stacked bar charts (Fig. 6), there is an apparent decreased relative abundance of certain taxa from Week 0 to Week 4 including *Oscillospira* sp. and unclassified (UC) taxa in family S24-7, and an increased relative abundance of *Akkermansia muciniphila*, *Blautia* sp. and *Bacteroides* sp.

To better visualize and test for differences in overall community structure, principal component analysis (PCA) and permutational multivariate analysis of variance (PERMANOVA) were performed. Ordination of all samples revealed that time had the greatest influence on GM composition as samples from Week 0 and Week 4 separated completely along PC1 regardless of sex and activity level (Fig. 7).

As we were most interested in treatment dependent differences, samples were stratified by sex and subjected to two-way PERMANOVA with physical activity and time as fixed variables. In males, significant main effects of physical activity ($F_{1,33}$ 9.4, $p < 0.001$) and time ($F_{1,33}$ 20.8, $p < 0.001$) were detected. Pairwise comparisons performed via one-way PERMANOVA revealed that male RUN rats were significantly different from male SED at Week 0 ($p = 0.006$) and Week 4 ($p = 0.0002$). In females, only a significant main effect of time was present ($F_{1,33}$ 16.7, $p < 0.001$). Unlike males, there was no difference between female RUN and female SED at either Week 0 or Week 4.

4. Discussion

Considering sex dependent differences in the response to rewards, the current study examined the interaction between the two natural rewards of voluntary wheel running and palatable diets in male and female rats. The present study revealed a sex dependent influence of voluntary wheel running on feeding patterns under a novel three diet choice paradigm that was associated with parallel changes in brain opioid and gut microbiome processes. Both male and female rats that were housed in sedentary conditions (SED), demonstrated a robust preference for the calorically rich and palatable high-fat diet. Male rats allowed access to voluntary running wheels (RUN) consumed significantly less high-fat diet compared to male SED rats but maintained equal overall caloric intake by increasing their levels of both carbohydrate diets. In contrast, female RUN rats still preferred the high-fat diet, displaying no difference from female SED counterparts. While few studies have examined sex differences of voluntary running on feeding behavior, evidence supports a sex difference in response to various other rewarding stimuli, including drugs of abuse [34,35].

One potential explanation of the sex dependent effect of voluntary wheel running in the current study could be a direct effect of metabolic changes due to the disparity in running behavior between males and females [36,37]. However, there was no correlation between running distance and intake of any diet in either males or females. In addition, there was no association between the initial increase and subsequent stabilization of running distance and

diet preference patterns throughout the experiment. In other words, there is no evidence to suggest the sex dependent diet preference in response to voluntary wheel running is related to the sex difference in running distance, suggesting the influence is driven more simply by whether or not rats had access to voluntary wheel running.

A previous study investigating the role of hormones on the influence of physical activity mediating fat preference suggested that males and females demonstrate differences in fat preference regardless of hormonal status and running distance. As observed by Moody et al., using a 2-choice diet paradigm of high-fat vs. chow, access to a voluntary running wheel led to a decrease of high-fat intake in males, yet increase in females. In the same study, ovariectomized (OVX) females displayed the expected decrease in wheel running behavior compared to intact females [38,39]. However, OVX females with access to the running wheel still preferred the high-fat diet, suggesting neither the lack of estrogen nor amount of distance run affects high-fat diet preference in females [40]. The current study utilized a 3-choice diet preference task that included two unique high-carbohydrate diets. While male RUN consumed less high-fat diet compared to male SED, male RUN consumed more of both the high-sucrose and high-corn starch carbohydrate diets, resulting in equivalent levels of overall caloric intake between the male RUN and male SED. However, this pattern of consumption was not observed in females. Female RUN and female SED did not differ in amount of either high carbohydrate consumed, yet female RUN consumed more total calories compared to female SED. These results suggest that the contrast between the male and the female behavioral response to physical activity may be impacted by other processes beyond metabolism, such as that of the reward system influencing feeding behavior.

The opioid system has been shown to regulate both voluntary wheel running and palatable diet consumption. For example, systemic administration of opioid receptor agonists or antagonists respectively increase or decrease voluntary wheel running [21] and palatable diet consumption [23,24,41]. Given the common mechanism through which opioid signaling mediates these behaviors, it is plausible that voluntary wheel running and palatable diets may interact to influence each other. One potential site for this interaction may be the mesolimbic reward pathway, well characterized for its role in mediating rewarding events [42]. Long-term voluntary wheel running produces adaptations to reward related transcription factors in the nucleus accumbens [18,19]. Access to a voluntary running wheel attenuates or prevents development of conditioned rewarding effects of drugs of abuse [43,17] and decreases acquisition and responding rates for nicotine, cocaine, heroin, and methamphetamine in males [44,45,16]. However, the previously mentioned studies assessing the role of opioids and reward behaviors have primarily included only males, while less information is known about the effect in females.

Marked differences in rewarding behavior can be assessed with concurrent measureable changes in mRNA expression in the mesolimbic pathway. In the present study, sex dependent effects of mRNA expression relating to opioidergic signaling in the ventral striatum were observed. Both μ -opioid receptor 1 (OPRM1) and preproenkephalin (PENK) mRNA in the ventral striatum were increased in RUN females compared to SED females, while there was no change in expression level in males. A previous study also showed no change in nucleus accumbens OPRM1 gene expression profiles in male rats following

access to voluntary running wheels and high-fat plus chow diets [46], yet females were not explored.

On the other hand, the lack of mRNA changes in opioid processes in RUN males might be expected due to the robust drop in high-fat diet intake, compared to their SED male counterparts. While the opposite trend has been observed, as chronic exposure to palatable diets and morphine downregulate opioid activity, these studies were conducted in only males and under sedentary conditions [47,20]. Yet a study with both male and female rats [48] reported that while chronic exposure to a cafeteria diet decreased OPRM1 mRNA in the nucleus accumbens of males, there was no change in observed in females. These findings, in addition to previous studies, support the hypothesis of a sex dependent effect of physical activity mediating other reward behaviors.

The present study also assessed the interplay of physical activity and diet preference on gut microbiota. There has been increasing attention to the interactions between the gut microbiota and the central nervous system on feeding behavior [30]. Excess intake of particular diets such as high-fat may drive the growth of specific microbe populations [49]. In turn, these microbes have been shown to induce over-consumption of the preferred diet [30]. Various studies have revealed significant shifts on microbiota composition after exposure to physical activity [50] across different lines of rats [51–53]. In addition, physical activity has been demonstrated to attenuate the high-fat diet-induced alterations in the gut microbiota [54]. This may prevent a cyclic pattern that would otherwise drive further high-fat feeding [55]. However, these studies have been conducted only in males.

Recent evidence suggests that sex may influence the effect of diet on microbiome [56] as well as the gut-brain axis [57,58]. The current findings demonstrate an interaction between sex and physical activity on microbiota composition. First, a divergence in microbiota composition profiles was observed between RUN and SED males, but not females, during Week 0 prior to the diet preference test. Second, this difference continued to persist and increase during the diet preference test. These patterns parallel the feeding patterns that observed. To our knowledge, this is the first study to examine the interaction between diet preference, physical activity, and changes in the microbiota composition in both males and females.

The present data demonstrate that either preventing or permitting rats to voluntarily run in a wheel leads to a profound sex dependent influence on diet preference. Furthermore, the increased striatal mRNA expression of both OPRM1 and PENK in RUN females, compared to SED females, match the well characterized influence of increased opioid signaling underlying rewarding behavior, including high-fat intake and voluntary wheel running. Lastly, the distinctive diet preference patterns were matched by parallel changes in gut microbiome composition. The interactions between diet and physical activity and their influence on microbiota composition extend previous findings by examining novel interactions and revealing robust sex differences.

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Fig. 1.

Average daily running distance and body weight in males and females.

(A) Body weight: There was no difference in body weight between SED and RUN males or females at Week -1 or Week 0. Male SED weighed more than male RUN during the 4 week diet preference phase of the study (Week1-4) (* $p < 0.05$), whereas there was no difference between female RUN and SED rats. (B) Running distance: Females exhibit higher running distance compared to males (* $p < 0.05$).

Fig. 2.

Average daily caloric intake of each diet normalized to 100 g body weight. Caloric intake: RUN females consumed more total calories than SED females and RUN males (* $p < 0.05$ and # $p < 0.05$) respectively.

Fig. 3.

High-fat diet, high-sucrose diet, high-corn starch, & total caloric average intake across 4 weeks. (A) High-fat diet: Male RUN consumed less high-fat diet than male SED (* $p < 0.05$) and female RUN (# $p < 0.05$) across the 4 weeks. (B) High-sucrose diet: Male RUN consumed more high-sucrose diet than male SED (* $p < 0.05$) and female RUN (# $p < 0.05$) across the 4 weeks. (C) High-corn starch diet: Male RUN consumed more high-corn starch diet than male SED (* $p < 0.05$) and female RUN (# $p < 0.05$) across the 4 weeks. (D) Total caloric intake: Female RUN consumed significantly more calories than male RUN (* $p < 0.05$) and female RUN (# $p < 0.05$) during Week 2–4.

Fig. 4.

mRNA levels of 4 different feeding and reward related genes expressed in the ventral striatum. Values represent a normalization to the male SED treatment group. (A) DRD1: There was no difference in expression levels between RUN and SED in males or females. (B) DRD2: There was no difference in expression levels between RUN and SED in males or females. (C) OPRM1: female RUN showed an increased level of OPRM1 expression compared to female SED ($p < 0.05$). (D) PENK: female RUN showed an increased level of PENK expression compared to female SED (# $p < 0.05$).

Fig. 5.

Alpha diversity: (A) Chao1 estimate of phylogenetic diversity and (B) Shannon Diversity Index. (A) Chao1 Index: Male RUN rats displayed greater diversity as measured by Chao-1 index of richness compared to male SED rats at Week 0 (* $p < 0.05$) and Week4 (* $p < 0.05$). There was no difference in females at either time point (B) Shannon Diversity Index: All groups reduced diversity from Week 0 to Week 4.

Fig. 6.
Relative abundance of taxa at Week 0 and Week 4.

Fig. 7.

Principal component analysis (PCA) of community structure: Time had the greatest influence on GM composition in all samples. Male RUN rats were significantly different from male SED at both Week 0 ($p = 0.006$) and Week 4 ($p = 0.0002$), there was no difference between female RUN and female SED at either Week 0 or Week 4.

Table 1

Diet composition.

	<u>High fat diet</u>		<u>High sucrose diet</u>		<u>High corn starch diet</u>	
	<u>#D12492</u>		<u>#D12450B</u>		<u>#D12450J</u>	
	gm%	kcal%	gm%	kcal%	gm%	kcal%
Protein	26.2	20	19.2	20	19.2	20
Carbohydrate	26.3	20	67.3	70	67.3	70
Fat	34.9	60	4.3	10	4.3	10
Total kcal/gm	5.24		3.85		3.85	
Ingredient Casein, 30 Mesh	200	800	200	800	200	800
L-Cystine	3	12	3	12	3	12
Corn Starch	0	0	315	1260	506	2024
Maltodextrin	125	500	35	140	125	500
Sucrose	68.8	275.2	350	1400	68	275
Cellulose, BW200	50	0	50	0	50	0
Soybean Oil	25	225	25	225	25	225
Lard	245	2205	20	180	20	180
Mineral Mix S10026	10	0	10	0	10	0
Di Calcium Phosphate	13	0	13	0	13	0
Calcium Carbonate	5.5	0	5.5	0	5.5	0
Potassium Citrate 1 H2O	16.5	0	16.5	0	16.5	0
Vitamin Mix V10001	10	40	10	40	10	40
Choline Bitartrate	2	0	2	0	2	0
FD & C Blue Dye #1	0.005	0	0	0	0.001	0
FD & C YellowDye #5	0	0	0.005	0	0.004	0
Total	773	4057	1055	4057	1055	4057

Table 2

Time line of diet preference study.

Acclimation week-1		Run/sed (chow only) week 0		Run/sed (3-choice diet) week 1-4			
Age (days)	42-48	49-55	55	56-84	83	84	
Procedure	Acclimation	Run/sed conditions assigned	Fecal sample collection	Diet preference test	Fecal sample collection	Animals sacrificed	

Table 3

Mean daily intake of each diet (kcal per 100 g body weight) $\pm$ SEM.

	High fat	High sucrose	High corn starch	Total calories
MALE SED	24.8 $\pm$ 2.5	3.0 $\pm$ 1.4	1.2 $\pm$ 1.1	28.2 $\pm$ 0.3
MALE RUN	12.4 $\pm$ 2.8 [*]	11 $\pm$ 3.0 [*]	6.4 $\pm$ 2.2 ^{*#}	28.8 $\pm$ 0.9
FEMALE SED	26.2 $\pm$ 3.0	5.0 $\pm$ 1.1	1.6 $\pm$ 0.9	31.6 $\pm$ 0.9
FEMALE RUN	30.3 $\pm$ 4.0	7.2 $\pm$ 2.7	1.9 $\pm$ 1.5	39.4 $\pm$ 1.6 ^{*#}

Average daily intake (kcal per 100 g BW).

^{*}

p < 0.05 compared to male SED.

[#]

p < 0.05 compared to female SED.