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Voluntary physical activity modulates self-selection of a high-caloric choice diet in male Wistar rats

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**ABSTRACT**

Physical exercise training has been positioned as a behavioral strategy to prevent or alleviate obesity via promotion of energy expenditure as well as modulation of energy intake resulting from changes in dietary preference. Brain adaptations underlying the latter process are incompletely understood. Voluntary wheel running (VWR) is a self-reinforcing rodent paradigm that mimics aspects of human physical exercise training. Behavioral and mechanistic insight from such fundamental studies can help optimize therapies that improve body weight and metabolic health based on physical exercise training in humans.

To assess the effects of VWR on dietary self-selection, male Wistar rats were given access to a two-component "no-choice" control diet (CD; consisting of prefabricated nutritionally complete pellets and a bottle with tap water) or a four-component free-choice high-fat high-sucrose diet (fc-HFHSD; consisting of a container with prefabricated nutritionally complete pellets, a dish with beef tallow, a bottle with tap water, and a bottle with 30% sucrose solution). Metabolic parameters and baseline dietary self-selection behavior during sedentary (SED) housing were measured for 21 days, after which half of the animals were allowed to run on a vertical running wheel (VWR) for another 30 days. This resulted in four experimental groups (SED CD, SED fc-HFHSD, VWR CD, and VWR fc-HFHSD).

Gene expression of opioid and dopamine neurotransmission components, which are associated with dietary self-selection, was assessed in the lateral hypothalamus (LH) and nucleus accumbens (NAc), two brain regions involved in reward-related behavior, following 51 and 30 days of diet consumption and VWR, respectively.

Compared to CD controls, consumption of fc-HFHSD before and during VWR did not alter total running distances. VWR and fc-HFHSD had opposite effects on body weight gain and terminal fat mass. VWR transiently lowered caloric intake and increased and decreased terminal adrenal and thymus mass, respectively, independent of diet. VWR during fc-HFHSD consumption consistently increased CD self-selection, had an acute negative effect on fat self-selection, and a delayed negative effect on sucrose solution self-selection compared to SED controls. Gene expression of opioid and dopamine neurotransmission components in LH and NAc were unaltered by fc-HFHSD or VWR. We conclude that VWR modulates fc-HFHSD component self-selection in a time-dependent manner in male Wistar rats.
1. Introduction

Obesity is associated with many medical comorbidities, which together result in reduced quality of life, lower life expectancy, and immense social and economic burden [3]. The main driver underlying the global obesity epidemic is the imbalance between energy intake and expenditure, resulting from excessive intake of processed palatable high-caloric diets on one side and general low levels of physical activity on the other side [3,6,16,17,36]. A sedentary lifestyle with low physical activity is associated with greater disease risk [6,36]. Conversely, physical exercise training (i.e. repeated bouts of physical activity with the goal to improve or maintain fitness) is a cheap and easy-accessible non-pharmaceutical strategy that improves myriad aspects of health and well-being across various organ systems [4,10,15,17,35,45]. Because physical exercise training stimulates energy expenditure, it can prevent and treat metabolic diseases, including obesity, type-2 diabetes mellitus, and cardiovascular diseases [4,6,8,15]. A systematic review found no consistent evidence that increased physical activity or exercise training affects energy or macronutrient intake [11], predominantly because it is challenging to assess the lasting effects of physical exercise training on dietary preference in a controlled setting. Insight into the molecular adaptations, signaling pathways, and inter-organ communication underlying the beneficial effects of physical exercise training on energy homeostasis can lead to new or improved weight-loss therapies.

Voluntary wheel running (VWR) is self-reinforcing behavior in wild and laboratory rodents [21,29,33,37,55]. This animal model can be used to gain insight into central adaptations underlying changes in energy homeostasis and dietary self-selection following self-initiated voluntary physical activity, as VWR modulates body weight control by affecting both arms of energy homeostasis (i.e. energy intake and energy expenditure). Although an increase in energy expenditure is an important component of physical activity underlying improvements in body weight control, changes in energy intake can also play a significant role [4,40]. First, rats that are given access to a running wheel generally demonstrate an initial reduction in caloric intake compared to sedentary controls, followed by a relative increase in caloric intake while maintaining a lower body weight [9,12,23,32,38,44,54]. Second, VWR modulates dietary self-selection behavior. For example, VWR in rats decreases self-selection of palatable fat-enriched diets when tested in two- or three-diet choice paradigms with diets varying in macronutrient composition and caloric content [26,31,34,39,47]. Furthermore, when rats are simultaneously introduced to a running wheel and a palatable fat-enriched diet, the reduction in self-selection of the fat-enriched diet over a low-fat diet is so profound that the rats almost completely avoid the fat-enriched diet [34,46]. Notably, avoidance of the fat-enriched diet is maintained after the cessation of VWR [34]. To date, most studies investigating changes in dietary self-selection during VWR have compared two nutritionally complete diets in a two-diet choice paradigm. It therefore remains unclear how VWR modulates self-selection of diets with different components that differ in macronutrient composition. In most societies, consumption of sugar-enriched beverages is very popular, and it is important to understand what the lasting effects of VWR on dietary self-selection are when diets rich in fat or carbohydrates (e.g. fast food) are widely and continuously available.

Despite the profound effects of VWR on dietary self-selection, the neural circuit and molecular mechanisms underlying VWR-mediated changes in dietary self-selection remain elusive. A potential mechanism underlying changes in dietary self-selection during VWR is altered central opioid signaling. For example, opioid signaling in the nucleus accumbens (NAc), a key brain region of the reward-related brain circuitry located in the ventral striatum, is bidirectionally involved in both palatable fat-enriched diet consumption and VWR [19,24,27,28,31,49,56,57]. The lateral hypothalamus (LH), a highly conserved brain region that orchestrates reward-driven and metabolic behavior [25], expresses delta, kappa, and mu opioid receptors that can functionally modulate caloric intake [1,13]. Currently it is not known if the LH opioid system responds to VWR, as has been shown to occur in the NAc [19,56]. Changes in striatal dopamine neurotransmission, via altered dopamine receptor levels, is another potential mechanism underlying changes in dietary self-selection during VWR [21].

In the present study we therefore assessed the effects of VWR on self-selection of the four diet components in a free-choice high-fat-high-sucrose diet (fc-HFHSD) in adolescent/young-adult male Wistar rats. This obesogenic fc-HFHSD allows rodents to choose between several diet components, varying in palatability, fluidity, form, and nutritive content, resulting in strong validity to model behavioral, physiological and molecular adaptations during human diet-induced metabolic dysfunction [50]. To model the effects of exercise training on dietary self-selection in humans pre-exposed to obesogenic multi-component high-caloric diets, male Wistar rats were given access to a nutritionally completely no-choice control diet (CD) or a fc-HFHSD, and diet component self-selection and metabolic behavior was assessed during sedentary and VWR conditions. To provide molecular insight, NAc and LH tissue was isolated to measure opioid- and dopamine-related gene expression following fc-HFHSD and/or VWR, as changes in gene expression might indirectly indicate altered opioid or dopamine neurotransmission in these brain regions. Exploring the processes underlying VWR-mediated changes in dietary self-selection may provide greater understanding how physical exercise training modulates energy homeostasis. Such insight can lead to improved weight-loss therapies based on physical exercise training.

2. Methods and materials

2.1. Ethics

All experiments were approved by the committee of animal health and care, Royal Netherlands Academy of Arts and Sciences (KNAW) and were conducted in accordance with the guidelines and regulations of the European Union directive 2010/63/UE and NIH guidelines for the use of animals for scientific purposes.

2.2. Animal cohorts and diet

Male Wistar WU rats [Crl:WI(WU), Charles River, Germany], weighing 200–240 gr upon arrival (approximately 5–8 weeks of age), were habituated to the temperature (21–23°C), humidity (40–60%) and light-controlled room (12:12 light/dark cycle; ZT12 (19:00) is lights off) animal facility of the Netherlands Institute of Neuroscience for at least seven days to recover from transport stress. A sound system continuously played radio music during the experiment to provide background noise. Rats were group-housed (4/cage) in a polycarbonate type 4 cage (530(l) x 330(w) x 200(h) mm; 1815 cm²; Plexx) with corncob bedding and cage enrichment (gnawing stick and PVC shelter) and had ad libitum access to water and an irradiated nutritionally complete high-carbohydrate control diet (CD; Teklad global diet 2918, 24% kcal from protein, 58% kcal from carbohydrate, and 18% kcal from fat, 3.1kcal/g, Envigo) during acclimatization. After the habitation period, rats were solitary housed and assigned to the sedentary group (SED; housed in a polycarbonate type 3H cage (375(l) x 215(w) x 180(h) mm; 800 cm²; Plexx) without a running wheel) or voluntary running wheel group (VWR; housed in a custom-made cage (422(l) x 422(w) x 475(h) mm, 1781cm²) with a locked running wheel (34cm diameter, 10cm width, 1.668mm/revolution)). For each cage type, rats were further equally divided and given access to the CD or fc-HFHSD. Thus, four experimental groups were included (SED, fc-HFHSD, VWR, and VWR, n=18/group) and all experimental groups were body weight-matched at the start of the experiment. The CD consists of two diet components: a food hopper with CD (3.1kcal/g) and a bottle with tap water. The fc-HFHSD consists of four diet components: a food hopper with CD (3.1kcal/g), a dish with fat (Beef tallow: Osewit/Blanc de Bouef, Vandemoortele, the Netherlands; 9kcal/g) hanging on the inside.
of the cage, a bottle with 30% sucrose solution (commercial grade table sugar dissolved in tap water; 1.2kcal/g), and a bottle with tap water. During the experiment, rats were provided with an aspen wood gnawing stick (Technilab-BMI) for cage enrichment. Intake of all diet components was measured by weighing the hopper/dish/bottle and compare the weight with the previous measurement. Due to a limited number of available running wheel cages, the experiments were conducted in three separate cohorts (initiated March, June and October 2018). Two SED

During the experiment, rats were provided with an aspen wood gnawing available running wheel cages, the experiments were conducted in three separate groups (initiated March, June and October 2018). Two SED

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ZT8, rats were rapidly decapitated under O

2.4. Euthanization and tissue isolation

3.1. Dynamics of VWR behavior

Male Wistar rats were fed a CD or fc-HFHSD and housed without (sedentary; SED) or with a running wheel (VWR), resulting in four experimental groups (SED, CD; VWR, fc-HFHSD). VWR rats had overestimated intake of beef tallow due to excessive spillage and were excluded from caloric intake or diet component preference analysis.

2.3. Energy homeostasis and VWR

Food intake, fluid intake, and body weight were measured 3x/week between ZT5 and ZT9. All diet components were refreshed 2x/week with three and four-day intervals. SED and VWR cage bedding was changed 1x/week. After 21 days of baseline measurements (i.e. with locked wheels), running wheels were unlocked and metabolic parameters were measured for another 30 days. We used 21 days of baseline measurements as our previous studies have demonstrated that this time period is associated with stable intake of the fc-HFHSD components [50]. Rats were not sound isolated from each other and were free to communicate across cages. Wheel revolutions were continuously registered using in-house developed Cage Registration Program (Dep. Biomedical Engineering, UMC Utrecht, The Netherlands), as described previously [20]. Intake of CD, water, and fc-HFHSD components during VWR days 22–49 was compared to the stable caloric intake of the last seven days of the baseline conditions (days 14–21, termed ‘baseline’ (BL) week).

2.4. Euthanization and tissue isolation

After 30 days of VWR, all caloric components, but not water, were removed at ZT0 on the day of euthanasia. From ZT3 to approximately ZT8, rats were rapidly decapitated under O2/CO2 anesthesia and trunk blood was collected in heparinized tubes, centrifuged at 3000rpm for 15’, and stored at –20 °C for further analysis of plasma metabolites or hormones. The brain, right epididymal white adipose tissue (eWAT), perirenal WAT (pWAT), and subcutaneous WAT (sWAT) pads, the intrascapular brown adipose tissue (iBAT) pad, a liver sample, the right gastrocnemius muscle, and kidneys were rapidly dissected, snap frozen in liquid nitrogen, and stored at –80 °C until further use. The left eWAT, pWAT, and sWAT pads, the entire mesenteric WAT (mWAT) pad, both adrenal glands, and thymus were dissected and weighed. After weighing, mWAT pads were quickly frozen snap frozen in liquid nitrogen and stored at –80 °C until further use.

2.5. mRNA extraction and quantitative real-time PCR

Frozen brain sections (200µm) were coronally sectioned on a cryostat and rapidly placed in RNAlater (Ambion, Waltham, MA, USA) and the NAc (bilateral; bregma 2.76 till 0.96) and LH (bilateral; bregma –1.72 till –3.12) [41] were isolated using a 1mm-diameter blunt punching needle. Bilateral tissue samples from the same animal were pooled together. Excessive RNAlater was removed using a tissue before punching were stored at –80 °C until further processing. RNA was isolated from the brain punches by extraction with TriReagent and chloroform. Tissue was homogenized in 300µl TriReagent using an Ultra Turrax homogenizer (IKA, Staufen, Germany), total RNA was purified using an Isolate II RNA Mini Kit (Bioinetics, London, UK). Only RNA values larger than 8.50, as measured with a BioAnalyzer (Agilent, Santa Clara, USA) were included [48]. cDNA synthesis was carried out using equal RNA input (124.44ng; as measured with spectrophotometry (DeNovix DS11; DeNovix, Wilmington, USA) and the transcriptor first-strand cDNA synthesis kit with oligo d(T) primers (Roche Molecular

Biochemicals, Mannheim, Germany). Genomic DNA contamination was controlled for by cDNA synthesis reactions without reverse transcriptase. Gene expression was measured using quantitative real-time pcr (RT-qPCR) with the SensiFAST SYBR no-rox kit (Bioline, London, UK) and Lightcycler® 480 (Roche Molecular Biochemicals); 2µL cDNA was incubated in a final reaction volume of 10µL containing SensiFast and 25ng per primer. Primer sequences were as following: Peptide 

dypylrolyl isomerase A (Pipia, also known as Cyclophilin): F: tgttctcatcatacgctt, G: cctgtagtgacttgctgcc; Hypoxanthine guanine phosphoribosyl transferase (Hprt): F: ccatcataccagcctct; R: tatgctcccctgtggtt; Actin, beta (Actb): F: acaacctctctggactctct; R: ccgactctactccactcactat; Heat shock protein 90 (Hsp90): F: gggccacccctcgtctga, G: accaagtacgcttgctggctag; Ubiquitin-C (Ubc): F: tectgaacttccccatctgtagt; R: gaaatcacaattcaccctctga; Prenephelin (Penk): F: cttcagagacagacagcttga; R: cttgacgtctccagccagtt; Prodynorphin (Pdyn): F: cttgacgctctgtagctctgctcag; R: ccttggaaccattcactctgctgctg; PCR products were analyzed on a DNA agarose gel to confirm a single band of the expected size. All materials were kept under RNase-free conditions throughout the experiment. RT-qPCR quantification was performed using LinReg Software [43]. Expression values of genes of interest were normalized using the geometric mean of four or five reference gene values (Pipia, Hprt, Actb, Hsp90, Ubc). All values were normalized to SED

2.6. Statistics

Data are presented as mean ± SEM. Two group comparisons were performed by two-tailed Student’s t-test. Assessment of effects in experiments involving several conditions was performed using one-, two-, or three-way analysis of variance (ANOVA), with repeated measures where applicable, followed, when appropriate, by Tukey HSD or Sidak’s post hoc tests to adjust for multiple comparisons. A P value < 0.05 was considered significant. See figure legends for statistical details of individual experiments, including statistical tests used, t, F, P-values, and number of subjects or samples tested.

3. Results

3.1. Dynamics of VWR behavior

Male Wistar rats were fed a CD or fc-HFHSD and housed without (sedentary; SED) or with a running wheel (VWR), resulting in four experimental groups (SEDCD, SEDfc-HFHSD, VWRCD, and VWRfc-HFHSD, Fig. 1a, b). The running wheels were locked during the first 21 days and unlocked during the last 30 days (Fig. 1b). Daily dark phase running distances increased during the first two weeks of VWR (i.e. the acquisition phase) and then stabilized around 1400m/night for the remainder of the VWR period (i.e. the maintenance phase), independent of diet (Fig. 1c). In line with the nocturnal nature of rats, most of the running was performed during the dark phase (Fig. 1c; S1a-d). Total VWR distances did not differ between the VWRCD and VWRfc-HFHSD rats (Fig. 1d).

3.2. VWR ameliorates the effects of fc-HFHSD on weight gain and adipose mass

During the 21 ‘wheels locked’ days, all experimental groups demonstrated similar ponderal growth (Fig. 2a, b). During the 30 ‘wheels unlocked’ days, fc-HFHSD consumption increased ponderal growth, whereas VWR decreased ponderal growth (Fig. 2a, c). In line with the effects on body weight, fc-HFHSD consumption increased terminal WAT mass, whereas VWR lowered terminal WAT mass when normalized for body weight (Fig. 2d-g). Similar effects were observed when WAT mass was not normalized for body weight (Figs. S2a-d).
3.3. VWR modulates adrenal and thymus weight

VWR for a longer period is associated with enlarged adrenals, a reliable end-organ measure of HPA axis activation [12,52]. Indeed, VWR was associated with increased terminal adrenal gland weight, independent of diet (Fig. 2h). In line with adrenal hypertrophy, VWR was associated with thymic atrophy, an indicator of chronic glucocorticoid exposure, independent of diet (Fig. 2i).

3.4. Temporal effects of VWR on fc-HFHSD consumption

To study the temporal effects of VWR on fc-HFHSD consumption, we compared the intake of the CD or the four fc-HFHSD components during VWR days 22–49 to the baseline (BL) week (i.e. days 14–21 of the ‘wheels locked’ condition; Fig. 1b). This initial analysis of total caloric intake revealed a substantial and long-lasting effect of VWR on caloric intake in both VWRCD and VWRfc-HFHSD rats compared to their respective SED controls (Fig. S3a-i-iii). However, because VWR generally decreases body weight growth (Fig. 2a), such an analysis, especially during the four VWR weeks, would ignore the substantial effects of body weight on caloric intake. Therefore, we normalized all caloric intake data for body weight. Hence, consumption of the fc-HFHSD resulted in greater total caloric intake than when consuming a CD, independent of housing conditions (Fig. 3a). VWR temporally decreased total caloric intake in VWRCD and VWRfc-HFHSD rats compared to their respective SED controls, and this anorectic effect of VWR appears more pronounced in VWRfc-HFHSD rats (Fig. 3a). As expected, CD intake was lower in fc-HFHSD rats compared to CD rats, independent of housing conditions (Fig. 3b). Furthermore, the pattern of CD intake in VWRCD rats logically mirrors their pattern of total caloric intake, as the CD is their only caloric diet component (Figs. 3a, b). VWRfc-HFHSD rats showed a different CD intake pattern, with CD intake initially being slightly lower (week 1) but then slightly higher (weeks 3 and 4) compared to SEDfc-HFHSD controls (Figs. 3b). Water intake was generally lower during consumption of a fc-HFHSD compared to a CD, independent of housing conditions (Fig. 3c). VWRCD rats showed a slight increase in water intake compared to SEDCD controls (Figs. 3c). Surprisingly, water intake in VWRfc-HFHSD rats was markedly higher compared to SEDfc-HFHSD controls (Figs. 3c). VWRfc-HFHSD rats showed dynamic changes in fat intake, with a strong decrease in fat intake during the first week of VWR compared to SEDfc-HFHSD controls (Figs. 3d). Lastly, VWRfc-HFHSD rats showed consistent lower sucrose solution intake compared to SEDfc-HFHSD controls during the VWR period (Fig. 3e).

3.5. Temporal effects of VWR on fc-HFHSD component self-selection

Self-selection of the fc-HFHSD components during VWR days 22–49 was compared to the baseline (BL) week, when all experimental groups were still housed sedentary (Fig. 1b). Intake of the caloric fc-HFHSD components is shown as percentage caloric intake of the weekly total caloric intake.

Fig. 1. | Experimental timeline and VWR behavior. (a) Experimental diets: two-component control diet (CD): nuggets of nutritionally complete high-carbohydrate CD and bottle of water; free-choice high-fat high-sucrose diet (fc-HFHSD): nuggets of nutritionally complete high-carbohydrate CD, bottle of water, a dish of lard, and a bottle of 30% sucrose solution. The caloric value of each diet component is indicated. (b) Experimental timeline: individually housed male Wistar rats were given the CD or fc-HFHSD and housed without a running wheel (sedentary; SED) or with a running wheel (VWR). Running wheels were locked during days 0–21 and unlocked during days 22–51. BL, baseline week of metabolic measurements during days 14–21. (c) Daily running distance of VWRCD and VWRfc-HFHSD rats (separated into 12 h light phase and 12 h dark phase (dark phase VWR; main effect of time, F29,986 = 18.010, P < 0.00001; one-way ANOVA with repeated measures followed by Tukey’s HSD test). (d) Total running distance during 30 days of VWR (t1,34 = 1.405, P = 0.17 versus VWRfc-HFHSD; t-test). Data are presented as mean ± S.E.M. (c) or as box plots with individual data points indicating the median (line), the interquartile range, and the minimum to maximum values of the data distribution (d). c, d = 18/group.
Fig. 2. | VWR ameliorates the effects of fc-HFHSD on weight gain and adipose mass. (a) Body weight evolution of male SED, SED-HFHSD, VWR, and VWR-HFHSD Wistar rats during 52 days of diet consumption and 30 days of VWR (time x diet interaction, $F_{1,476} = 3.761, P = 0.00060$; time x housing interaction, $F_{1,476} = 29.390, P < 0.00001$). (b) Body weight gain during the ‘wheels locked’ condition (no significant effects) and during (c) the ‘wheels unlocked’ condition (main effect of diet, $F_{1,68} = 9.376, P = 0.0032$, and housing, $F_{1,68} = 46.374, P < 0.00001$; Tukey post hoc: $P_{diet} = 0.0033$, $P_{housing} = 0.00011$). (d-i) Final (d) epididymal white adipose tissue (eWAT; main effect of diet, $F_{1,68} = 55.351, P < 0.00001$, and housing, $F_{1,68} = 24.88, P < 0.00001$; Tukey post hoc: $P_{diet} = 0.00011$, $P_{housing} = 0.00012$), (e) perirenal WAT (pWAT; main effect of diet, $F_{1,68} = 81.611, P < 0.00001$, and housing, $F_{1,68} = 58.303, P < 0.00001$; Tukey post hoc: $P_{diet} = 0.00011$, $P_{housing} = 0.00011$), (f) mesenteric WAT (mWAT; main effect of diet, $F_{1,44} = 48.059, P < 0.00001$, and housing, $F_{1,44} = 65.218, P < 0.00001$; Tukey post hoc: $P_{diet} = 0.00012$, $P_{housing} = 0.00012$), (g) subcutaneous WAT (sWAT; main effect of diet, $F_{1,44} = 9.064, P = 0.0044$, and housing, $F_{1,44} = 20.057, P = 0.00005$; Tukey post hoc: $P_{diet} = 0.0046$, $P_{housing} = 0.00017$), (h) adrenal gland mass (main effect of diet, $F_{1,68} = 14.644, P = 0.00028$; Tukey post hoc: $P_{diet} = 0.00039$), and (i) thymus gland mass (main effect of housing, $F_{1,68} = 7.570, P = 0.0076$; Tukey post hoc: $P_{housing} = 0.0077$), all normalized for final body weight. Data are presented as mean ± S.E. M. (a) or as box plots indicating the median (line), the interquartile range, and the minimum to maximum values of the data distribution (b-i). a-c,e,h,i: $n = 18$; group; f,g: $n = 12$; group.

3.6. Opioid-related gene expression in lateral hypothalamus and nucleus accumbens following VWR and fc-HFHSD

Next, we assessed opioid-related gene expression in LH and NAc to investigate if changes in gene expression are associated with fc-HFHSD and VWR for 51 and 30 days, respectively. However, fc-HFHSD and VWR for this duration did not significantly affect Oprd1 or Oprml expression in the LH (Fig. 5a-c). In the NAc, fc-HFHSD and VWR did not significantly affect Penk, Fdyn, Oprd1, Oprk1, or Oprml expression (Fig. 5d-i).

3.7. Dopamine-related gene expression in nucleus accumbens following VWR and fc-HFHSD

Last, we assessed dopamine-related gene expression in NAc to investigate if changes in gene expression are associated with fc-HFHSD and VWR for 51 or 30 days, respectively. In the NAc, fc-HFHSD and VWR did not significantly affect Drd1a and Drd2 expression (Fig. 5d, j, k).

4. Discussion

Physical exercise training has been positioned as an important non-pharmacological strategy to prevent and/or treat obesity. The current study design to pre-expose rats to the fc-HFHSD before start of VWR was used to model the effects of physical exercise training on dietary self-selection in humans that had been eating unhealthily and turned to physical exercise training to improve their metabolic health and potentially lose weight. We confirm that VWR suppresses body weight gain and WAT accumulation in male Wistar rats during consumption of CD or the obeseogenic fc-HFHSD. More importantly, VWR was associated with intake of less calories. This effect was strongest during the first week and waned during the remaining weeks of VWR. The anorectic effects of VWR appear more substantial during fc-HFHSD consumption compared to CD controls. Lastly, VWR impacted fc-HFHSD component caloric intake. VWR consistently increased CD self-selection during the four VWR weeks compared to SED-HFHSD controls (Fig. 4a). VWR did not significantly change fat or sugar solution self-selection during the first week and waned during the remaining weeks of VWR. The anorectic effects of VWR appear more substantial during fc-HFHSD consumption compared to CD controls (Fig. 4b, c). Indeed, when comparing the caloric intake of each diet component during week 1 to the BL week, VWR during week 1 was associated with increased, decreased, and unaltered preference for CD, fat, and sucrose solution, respectively (Fig. 4d-f).
self-selection in a time-dependent manner. Indeed, VWR had a consistent positive effect on CD self-selection, had an acute negative effect on fat self-selection, and a delayed negative effect on sucrose solution self-selection. Consumption of the fc-HFHSD or VWR for 51– and 30 days, respectively, were not associated with changes in gene expression of components of the opioid- and dopamine circuitry in LH and NAc.

VWR rats demonstrated characteristic bi-phasic running behavior after the running wheels were unlocked, independent of diet, with daily running distances escalating over time during the initial acquisition phase and stabilizing during the maintenance phase [18]. Furthermore, pre-exposure to the fc-HFHSD for 21 days did not significantly affect VWR behavior after unlocking the running wheels. These observations indicate that pre-exposure to the fc-HFHSD did not alter the rewarding properties of VWR in a substantial manner.

After unlocking the running wheels, VWR^fCHFHSD rats show temporal yet substantial decreases in total caloric intake. Such anorectic effects were also observed in VWR^CD rats, but to a lesser extent. Analysis of the percentage caloric intake per fc-HFHSD component revealed that VWR consistently increases CD self-selection, while transiently decreasing fat self-selection. The latter effect occurs predominantly during the first week of running. Thus, our observations generally support previous studies in rats that found that VWR decreases preference for fat-enriched diets [26,31,34,39,47]. Interestingly, our study also found that VWR^fCHFHSD rats did not change self-selection of the 30% sucrose

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**Fig. 3.** Temporal effects of VWR on fc-HFHSD consumption normalized for body weight. (a^iii^) Average total caloric intake of male SED^CD^, SED^fCHFHSD^, VWR^CD^, and VWR^fCHFHSD^ Wistar rats, normalized for body weight, during baseline week (BL; light blue; wheels locked) and VWR weeks 1–4 (wheels unlocked; a^ii^: time x housing interaction, F_{4,128} = 5.346, P = 0.00052; Tukey post hoc: *P = 0.00019, *P = 0.00021, *P = 0.045 versus SED^fCHFHSD^; a^i^: time x housing interaction, F_{4,136} = 41.996, P < 0.00001; Tukey post hoc: *P = 0.00015 versus SED^CD^). (b^iii^) Average control diet (CD) intake, normalized for body weight, time x housing interaction, F_{4,136} = 41.996, P < 0.00001; Tukey post hoc: *P = 0.00015 versus SED^CD^; b^i^: time x housing interaction, F_{4,128} = 5.947, P = 0.0020). (c^ii^) Average water intake, normalized for body weight (c^i^: time x housing interaction, F_{4,136} = 3.265, P = 0.014; c^ii^: time x housing interaction, F_{4,128} = 14.035, P < 0.00001; Tukey post hoc: *P = 0.00016, *P = 0.00019, *P = 0.0035, *P = 0.0029 versus SED^fCHFHSD^). (d) Average fat intake and (e) average sucrose solution intake of SED^fCHFHSD^ and VWR^fCHFHSD^ rats, normalized for body weight (d: time x housing interaction, F_{4,128} = 4.614, P < 0.0016; e: main effect of time, F_{4,128} = 23.652, P < 0.00001; main effect of housing, F_{1,32} = 8.546, P = 0.0063; Tukey post hoc: *P = 0.0005). Data are presented as mean ± S.E.M. a-e: n = 16–18/group.
solution, whereas they did profoundly increase their tap water intake. Thus, even in the presence of simple carbohydrates, the rats shift their intake from fat to CD, without increases in sucrose solution drinking. It is therefore tempting to speculate that not carbohydrates, but protein is an important component driving diet selection processes in this study, as protein is only included in the CD, in addition to carbohydrates. Availability of amino acids, derived from protein intake, is important for proper muscle development following exercise [53]. To date, and to the best of our knowledge, no VWR-mediated dietary self-selection studies have been performed with diets different in protein content. Future studies should focus on determining if protein content drives dietary self-selection during VWR.

To understand how VWR modulates dietary self-selection, it is important to determine the central mechanisms underlying these behavioral changes. First, VWR alters sensitivity to the rewarding effects of morphine [30] and to its analgesic effects [22, 51]. Interestingly, opioid sites involved in hedonic eating were also confined to the NAc shell [42]. Finally, absence of diet and/or VWR effects on NAc opioids and opioid receptor gene expression seems to be in line with a recent study by Lee and colleagues, in which two different rat strains had voluntary access to a running wheel for five weeks, and 2h daily access to a fat-enriched diet [27]. In this study, VWR did not affect DAMGO-induced changes in fat intake when DAMGO was injected in the NAc [27]. The same group also did not find significant differences in DAMGO-driven consumption of a preferred diet in male rats when injected into the NAc [28]. In contrast, female rats showed greater sensitivity to the effects of DAMGO on food consumption when administered to the NAc after VWR [28]. Unfortunately, female rats were not included in this study and future studies should focus on determining how VWR drives fc-HFHSD self-selection in female rodents.

The LH expresses all opioid receptors and is a key brain region involved in the control of reward-driven as well as metabolic behavior [1, 25]. Therefore, we also assessed opioid-related gene expression in this brain region. Again, our exploratory qRT-PCR analysis revealed no significant changes in gene expression of components of the opioid circuitry in LH following 51 days of fc-HFHSD consumption and/or 30 days of VWR. As opioid receptors need to be available on the membrane to mediate signaling within the cell, it remains to be determined how fc-HFHSD and/or VWR modulate cell surface receptor levels.

VWR in male rats is associated with an increase in dark phase plasma corticosterone levels, and this increase is positively correlated with the amount of VWR activity and occurs for approximately three weeks after initiation of VWR [5, 7, 14]. In line with the results of these studies, VWR rats in our study show adrenal hypertrophy and thymic atrophy, indicating chronic glucocorticoid exposure during VWR. Notably, the anorectic effects of VWR are most prominent during the first week of

![Graph](image-url)
VWR during CD or fc-HFHSD consumption, but also appear to last longer during fc-HFHSD consumption. These observations indicate that changes in glucocorticoid signaling may underlie some of the effects of VWR on dietary self-selection [5]. However, more experiments are needed to determine the dynamics of VWR-mediated changes in plasma glucocorticoids as well as the central sensitivity to glucocorticoid signaling in our fc-HFHSD paradigm.

In summary, VWR ameliorates the effects of fc-HFHSD on caloric intake, weight gain and adipose mass gain, and modulates dietary self-selection in a time-dependent manner. VWR and fc-HFHSD for 30 or 51 days, respectively, was not associated with altered gene expression of components of the opioid and dopamine circuitry in LH or NAc. These novel findings further our understanding how VWR modulates caloric intake and diet self-selection when consuming a four-component fc-HFHSD. A better understanding of the underlying mechanisms, both acute- and lasting, may provide important insight and if translatable to humans, can lead to improved weight-gain prevention and weight-loss therapies based on exercise training.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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