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# Exercise protects against high-fat diet-induced hypothalamic inflammation

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ABSTRACT

Hypothalamic inflammation is a potentially important process in the pathogenesis of high-fat diet-induced metabolic disorders that has recently received significant attention. Microglia are macrophage-like cells of the central nervous system which are activated by pro-inflammatory signals causing local production of specific interleukins and cytokines, and these in turn may further promote systemic metabolic disease. Whether or how this microglial activation can be averted or reversed is unknown. Since running exercise improves systemic metabolic health and has been found to promote neuronal survival as well as the recovery of brain functions after injury, we hypothesized that regular treadmill running may blunt the effect of western diet on hypothalamic inflammation. Using low-density lipoprotein receptor deficient (*ldlr* −/−) mice to better reflect human lipid metabolism, we first confirmed that microglial activation in the hypothalamus is severely increased upon exposure to a high-fat, or "western", diet. Moderate, but regular, treadmill running exercise markedly decreased hypothalamic inflammation in these mice. Furthermore, the observed decline in microglial activation was associated with an improvement of glucose tolerance. Our findings support the hypothesis that hypothalamic inflammation can be reversed by exercise and suggest that interventions to avert or reverse neuronal damage may offer relevant potential in obesity treatment and prevention.


## 1. Introduction

Over the last decade, research has established that obesity is associated with a state of moderate but chronic inflammation. Such subacute inflammatory processes are characterized by increased tissue-specific as well as circulating levels of interleukins and cytokines, a phenomenon which is also believed to participate in the development of many obesity-associated comorbidities [1]. Intriguingly, pro-inflammatory cytokines are also produced in the hypothalamus, a key site for regulation of food intake, body weight, and energy expenditure [2,3]. This process of hypothalamic inflammation has recently been proposed as a critically important phenomenon occurring during consumption of a western diet (WD) and one which may further promote obesity [4]. This increase in a hypothalamic inflammatory response is detectable as early as 1 week of WD feeding and coincides with excessive energy intake and hypothalamic insulin or leptin resistance [5,6]. Emerging evidence points to a plethora of potentially underlying pathways and molecular processes including endoplasmatic reticular stress, toll-like receptor (TLR) and c-Jun N-terminal kinase (JNK) signaling [7–11].

Microglial cells, the quiescent macrophages of the central nervous system (CNS), have been reported to be involved in the mediation as well as the compensation of inflammation-associated damage [12]. Microglia are particularly sensitive to even modest homeostastic imbalances in their microenvironment and increased microglia activity is a well established sign of an inflammatory response [13]. Upon WD feeding, morphology of these cells transforms from small cell bodies with fine ramified processes to an activated pro-inflammatory phenotype associated with enlarged cell bodies and highly ramified processes, producing cytokines and interleukins which in turn induce inflammation and cell death [5,6,14,15]. Moreover, microglial cells can also be activated by diverse pro-inflammatory factors or cytokines, including metabolic hormones such as leptin, which stimulate the production of IL1β, TNFα and IL6 in microglia. All of those events have been found to be capable of affecting metabolism [16–18]. Activated microglia continue to accumulate in the mediobasal hypothalamus over months of WD feeding, thus perpetuating hypothalamic inflammation and potentially posing a constant challenge that chronically impairs metabolic homeostasis [5]. Thus, it becomes increasingly apparent that hypothalamic inflammation and microglial activation may represent a key process in the pathogenesis of chronic metabolic disease caused by exposure to WD.

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It is unknown whether WD-induced activation of microglia is reversible. Although specific lifestyle interventions and therapeutic approaches have been reported to decrease circulating inflammatory markers in overweight and obese patients [19], it is unclear whether such influences would also translate into reduced hypothalamic inflammation, and as a consequence of improved systemic metabolism. Since the risk of developing obesity and its comorbidities is inversely associated with regular physical activity [20], we hypothesized that regular running exercise may blunt the activation of hypothalamic microglia by WD exposure. Using low density lipoprotein receptor deficient mouse (*ldlr* −/−) as a relevant genetic mouse model for the metabolic syndrome, we report for the first time that moderate amounts of regular exercise prevent WD-induced microglial activation in the hypothalamus even in the absence of body weight loss. The observed reduction in hypothalamic inflammation was associated with improved metabolic function.

## 2. Materials and methods

### 2.1. Mice and exercise regime

Age-matched male low density lipoprotein receptor deficient (*ldlr* −/−) mice (The Jackson Laboratories, Bar Harbor, ME) were housed in a pathogen-free environment with all animal care and experimental procedures conforming to institutional guidelines for animal experiments and approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Cincinnati. After randomization for body weight, all mice were accustomed to treadmill running for 3 days as described previously [21–23] and then an exercise exhaustion test was performed in all mice. Next, mice were divided into two groups and fed the D12079B western diet from Research Diets (New Brunswick, NJ) containing 17 kcal% protein, 42% carbohydrate, and 41 kcal% fat as well as a high-fructose corn syrup sweetened beverage (a popular commercial soda). The 'runner' group (n = 10) was subjected to a 30-min treadmill running (Simplex II metabolic rodent treadmill equipped with an electrostimulator, Columbus Instruments, Columbus, OH) during the first 4 h of the dark phase of the circadian cycle, five times a week with an inclination of 10% and a speed of 5 m/min for 30 min for 26 weeks, resulting in a covered distance of 150 m/day. The 'sedentary' group (n = 6) was not forced to run except during exercise exhaustion tests. For exhaustion tests during the first four hours in the dark phase of the circadian cycle at weeks 0 and 25 of the study, animals ran on the treadmill tilted 25% uphill starting at a warm-up speed of 14 m/min for 6 min. Every subsequent 2 min, the speed was increased by 2 m/min until mice were exhausted. Exhaustion was defined as the inability of the animal to return to running within 10 s after direct contact with an electric-stimulus grid. Running time was measured and running distance calculated. Distance is the product of time and speed of the treadmill. Outside of the training schedule, all mice had unlimited access to food and soda. Food and soda intake were measured daily during the resting period (last two hours of the light cycle) for 1 week in individually housed mice and presented as cumulative caloric intake at week 24 of the study and in the metabolic cages throughout their circadian cycle during the measurements of indirect calorimetry on weeks 20 to 22 (data not shown). For energy balance measurements at week 20 of the study, mice were acclimated to respiratory chambers for 4 days before measurements. Energy expenditure, respiratory quotient, food intake, fluid intake, and locomotor activity were measured simultaneously over a 24-h period using a customized 32-cage, Indirect Calorimetry System combined with Drinking and Feeding Monitor and TSE ActiMot system (TSE-Systems, Germany) as described previously [24,25]. Mice were not exercised during ongoing measurements of indirect calorimetry.

### 2.2. Biochemical assays

An intraperitoneal glucose tolerance test (ipGTT) was performed by injection of glucose (2 g/kg, 50% wt/vol. D-glucose [Sigma, St Louis, MO, USA] in 0.9% wt/vol. NaCl) after a 6-h fast. Tail blood glucose levels [mmol/l] were measured with a glucometer (TheraSense Freestyle) before (0 min) and at 15, 30, 45, 60 and 120 min after injection. Plasma insulin levels were measured with the Ultra Sensitive Rat Insulin ELISA kit (Crystal Chem, Chicago, IL) using rat insulin as the standard in mice that had been fasted for 15 h. HOMA Index has been calculated as described previously [26,27]. Circulating levels of adipokines and inflammatory markers leptin, TNF-a, IL6, INF-g, IL-1 alpha, PAI-1 and MCP1 have been measured at the end of the study using the LINCOplex Kit (Millipore, Billerica, MA).

### 2.3. Immunohistochemistry

At the end of the study, all groups of mice were decapitated and brains were immerse-fixed in 4% paraformaldehyde 0.1 M phosphate-buffered saline (PBS, pH 7.4) at 4 °C for 48 h. Brains were then equilibrated 48 h with 30% sucrose in 0.1 M Tris-buffered saline (TBS, pH 7.2). Immunohistochemistry and quantitative analysis for allograft inflammatory factor 1 (iba1) were performed on coronal brain sections (30 μm) throughout the hypothalamus. After rinsing in 0.1 M TBS, whole-brain sections were incubated with rabbit anti-iba1 primary antibody (Synaptic System, Goettingen, Germany) at 1:1000 dilution overnight at 4 °C. Sections were then rinsed and incubated in biotinylated secondary antibody (horse anti-rabbit IgG, Vector, CA, USA) for 1 h; subsequently, sections were rinsed and incubated in avidin–biotin complex (ABC, Vector) for 1 h. The reaction product was visualized by incubation in 1% diaminobenzidine with 0.01%

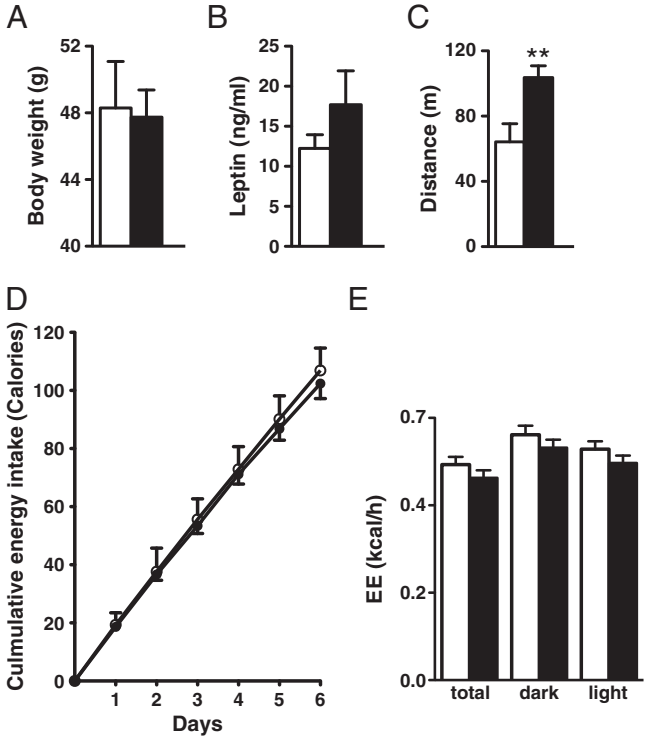


**Fig. 1.** Moderate exercise does not change body weight, circulating leptin levels, food intake and energy expenditure, but improves endurance performance in *ldlr*−/− mice fed a western diet. Body weight (A), circulating leptin levels (B), distance covered during exercise exhaustion test (C), energy intake (D), and energy expenditure (E) in sedentary (open bars, n = 6) and exercised (closed bars, n = 10) *ldlr* −/− mice on western diet. Values are expressed as means ± SEM. $^{**}P<0.005$ compared with the sedentary groups.

hydrogen peroxide for 7 min. Sections were mounted on gelatin-coated glass slides, dried, dehydrated in graded ethanol series, cleared in xylene, and cover-slipped for observation by light microscope.

### 2.4. Quantitative analysis

All quantitative analyses were performed under blinded conditions and confirmed by at least two independent researchers. For each mouse, two to three sections in the middle portion of the arcuate nuclei (ARC) within the mediobasal hypothalamus were selected and images were captured by a computerized image analysis system consisting of an Axioskop color video camera (Carl Zeiss International, Thornwood, NY). Both sides of the ARC were manually outlined with an area of 0.03 $mm^2$ on each side. iba1 immunoreactive (ir) microglia were manually counted throughout the ARC, and expressed as number of cells per $mm^3$. The average of iba1-ir microglia from each mouse was then calculated and expressed as the mean ± SEM from each group.

### 2.5. Statistical analyses

Quantitative data are presented as mean ± SEM. Values were analyzed for statistically significant differences applying two-tailed, unpaired t tests. $P<0.05$ was considered significant (GraphPad Prism,

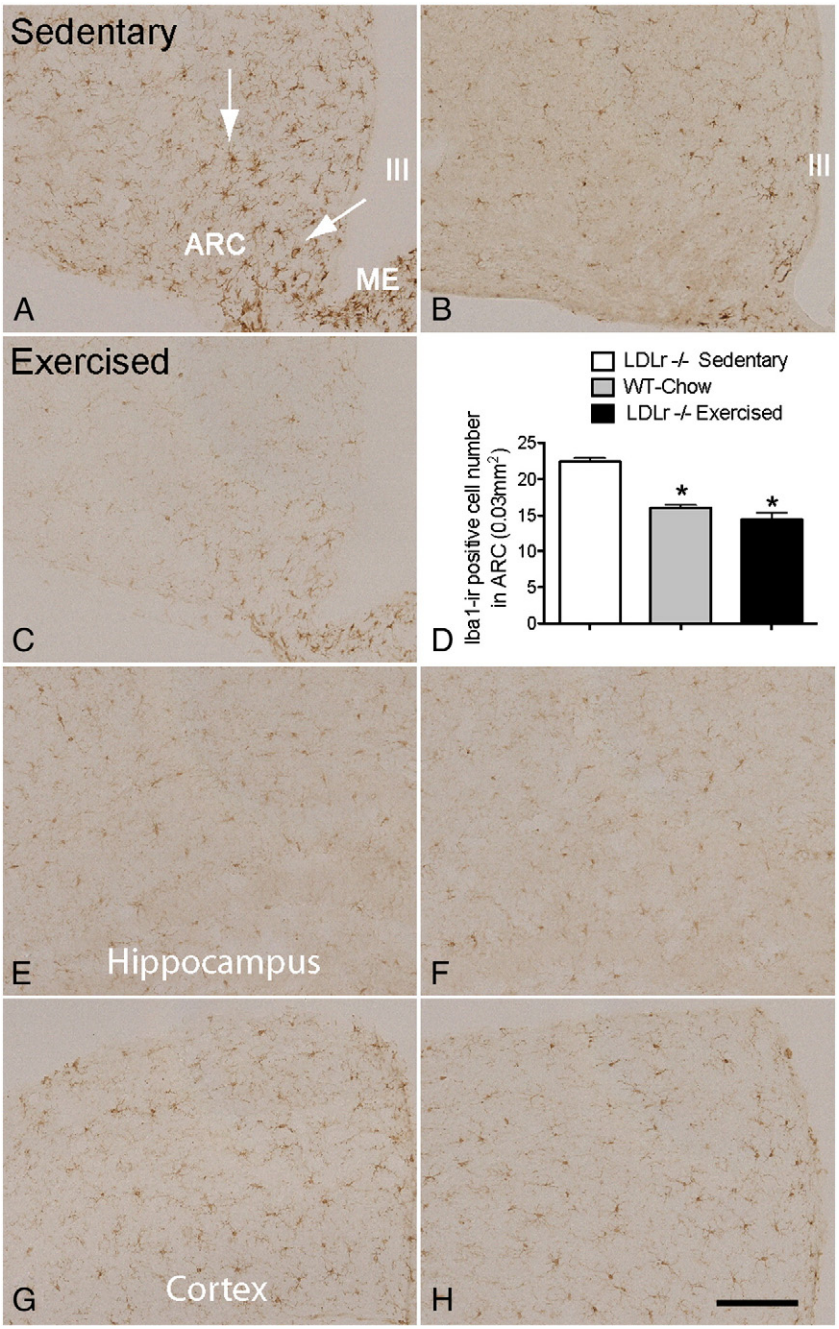


**Fig. 2.** Microglial activation is markedly blunted specifically in the arcuate nucleus (ARC) in the hypothalamus of exercised *ldlr* −/− mice fed a western diet. Iba1-immunopositive cells in the ARC of sedentary mice fed a high fat diet (n = 6) (A) are significantly increased compared to chow fed WT mice (B), and exercise largely blunted this diet-induced increase (C and D), This changes only take place in the ARC, but not in the hippocampus (E and F) and the cortex (G and H) of exercised mice (n = 10). ARC, arcuate nucleus; ME, median eminence. Arrows indicate some of the activated microglia. Scale bar: 50 μm.

GraphPad Software, La Jolla, CA, USA; SigmaStat, Systat Software, San Jose, CA, USA).

## 3. Results

### 3.1. Moderate regular exercise enhances endurance performance independently of body mass in ldlr−/− mice on WD

Throughout the study, we determined body weight in sedentary and exercised *ldlr*−/− mice fed a WD. As expected, such moderate exercise did not lead to a significant difference in body weight between the two groups of animals (Fig. 1A). In addition, based on our finding that circulating leptin levels, a surrogate marker for body fat mass, were not changed in exercised mice (Fig. 1B), we assumed that body composition did not likely differ significantly between the groups. Furthermore, cumulative caloric intake and energy expenditure was not different between the two groups of mice when they were not subjected to treadmill running (Fig. 1D and E) although muscular energy expenditure should have been increased during the treadmill running in the exercised group. To ascertain whether our exercise regimen did indeed enhancing muscular function, we performed exercise-exhaustion tests on treadmills in all mice. As expected, endurance capacity was significantly increased in exercised *ldlr*−/− mice compared to sedentary *ldlr*−/− mice fed a WD (Fig. 1C).

### 3.2. Moderate regular exercise reduces hypothalamic microglial activation independently of body mass in ldlr−/− mice on WD

For the immunohistological detection of activated microglia we used the well-established macrophage/microglia-specific calcium-binding protein allograft inflammatory factor 1 (iba1). This protein is involved in the Rac signaling pathway and its expression is markedly increased upon microglial activation [28]. Iba1 is specifically expressed in activated microglia and is regarded a well established surrogate parameter for microglial activation and commonly used to detect and compare microgliosis in numerous species [29]. Our quantitative analysis of brain sections revealed a marked increase in microglial activation specifically in the ARC of the mediobasal hypothalami in brains from sedentary *ldlr*−/− mice fed a WD (Fig. 2A). As an additional sign of microglial activation, we found that the iba1-ir positive cells also displayed the typical pro-inflammatory morphology with enlarged cell bodies and thickened processes. As a reference for physiologically normal positive iba1 immunohistological staining, we included brain slides from wt mice fed chow diet (Fig. 2B). Conversely, we found that regular and moderate treadmill running of *ldlr*−/− mice fed a WD considerably decreased the iba1-ir cell number (Fig. 2C) and the morphological transformation of ARC microglia (Fig. 2C and D). Importantly, we did not observe exercise-induced differences in microglial activation in other regions of the brain, including the hippocampus (Fig. 2E and F) or the cortex (Fig. 2G and H). Thus, our results indicate that regular and moderate exercise prevents WD-induced inflammation specifically in hypothalamic ARC regions which play a pivotal role in regulation of energy balance.

### 3.3. Moderate regular exercise improves glucose homeostasis independently of body mass in ldlr−/− mice on WD

To determine whether the body weight-independent improvement in hypothalamic inflammation caused by moderate and regular exercise is associated with improved systemic glucose homeostasis, we analyzed glucose metabolism in sedentary and exercised *ldlr*−/− mice fed a WD. Although fasting glucose and insulin levels were similar between the two groups (Fig. 3A and B), we detected a trend towards improved HOMA Index values, a clinical

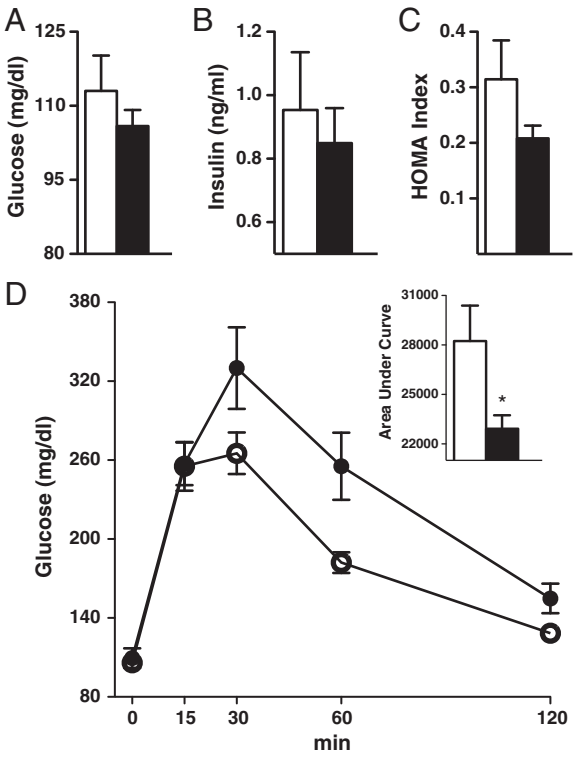


**Fig. 3.** Moderate exercise improves glucose turnover in *ldlr*−/− mice fed a western diet. Fasting glucose (A), insulin levels (B) and HOMA Index (C) in sedentary (open bars, n = 6) and exercised (closed bars, n = 10) *ldlr*−/− mice on western diet (n = 6–10). Intraperitoneal glucose tolerance test (*D*) in sedentary (closed circles) and exercised (open circles) *ldlr*−/− mice on western diet. Values are expressed as means ± SEM. *$P<0.05$.

surrogate marker for insulin sensitivity (Fig. 3C) [26,27]. To further determine into detail glucose turnover rates, we performed an intraperitoneal glucose tolerance test (ipGTT), a commonly clinical test used to determine insulin sensitivity. We found that the response to ipGTT was significantly improved in exercised *ldlr*−/− mice as compared to sedentary *ldlr*−/− mice fed a WD (Fig. 3D). Exercise-induced improvement in glucose homeostasis was not associated with changes in circulating inflammatory markers TNF, IL6, INF gamma, INF-1alpha, PAI-1, and MCP1, which again highlights the moderate character of our exercise challenge (Table 1).

## 4. Discussion

We report for the first time that very moderate amounts of regular exercise are effective in reducing diet-induced hypothalamic inflammation in mice. During the study exercised mice covered an approximate total distance of 19.5 km, averaging about 0.15 km/day. This exercise regimen is significantly below the threshold of 0.5 km/day, which has been established as necessary for the running-induced activation of signaling molecules that influence learning and memory in

**Table 1**
Circulating cytokines were not changed by exercise regimen in *ldlr*−/− mice fed a western diet. Fed cytokine levels in sedentary and exercised *ldlr*−/− mice after 26 weeks of western diet exposure and treadmill exercise.

| Cytokine in pg/ml | Sedentary *ldlr*−/− | Exercised *ldlr*−/− |
|---|---|---|
| Interferon gamma | 15.84 ± 8.76 | 14.9 ± 8.74 |
| Interleukin 6 | 22.76 ± 2.75 | 25.29 ± 2.19 |
| Interleukin 1 alpha | 7.99 ± 0 | 10.51 ± 5.94 |
| Tumor necrosis factor alpha | 2 ± 0 | 4.67 ± 2.67 |
| Monocyte chemoattractant protein 1 | 41.1 ± 22.75 | 50.26 ± 15.1 |
| Plasminogen activator inhibitor type 1 | 3027 ± 815 | 4478 ± 1327 |

rodents [30]. Furthermore, the fact that exercised mice exhibited similar energy intake and expenditure, body weight and fat mass compared to sedentary mice, underscored the importance of exercise on normal hypothalamic function independently of changes in energy balance and body composition. We thus postulate that even subthreshold levels of regular exercise unable to affect energy balance or hallmark signaling pathways may be beneficial for the prevention of diet-induced hypothalamic dysfunction.

We also observed that the exercise regimen used within this study enhanced insulin-action at target tissues such as skeletal muscle, adipose tissue and liver, independently of changes in body weight. Several underlying causes may be responsible for the improved response to ipGTT in the exercised animals: since in our study this finding was associated with a reduction in diet-induced hypothalamic inflammation it is plausible that restored hypothalamic function had led to an improved control over systemic metabolic processes such as insulin sensitivity in liver, muscle and adipose tissue. However, a parallel possibility would be that a similar reduction of macrophage presence and activation in other tissues had similar impact on these endpoints. Kawanishi et al. have recently reported that exercise training inhibits inflammation in adipose tissue of mice by suppressing macrophage infiltration and phenotype switching from the inflammatory M1 subtype to the anti-inflammatory M2 subtype [31]. In humans it has been shown that low-intensity exercise (such as walking 10,000 steps a day, three times a week) also increased M2 markers like PPARγ/PGC-1α/β, and Th2 cytokines of circulating leucocytes [32]. Thus, exercise-mediated reduction in macrophage/microglial activation may constitute a novel anti-inflammatory benefit of low-intensity exercise. However, our results do show that circulating inflammatory markers were not changed in our just moderately exercised mice pointing to a more hypothalamo-centric mechanism as the more likely explanation.

For these intervention studies, we chose the forced-treadmill running regimen for its advantageous resemblance to human physical training and objective outcome measures as it allows for more precise determinations of the correlation between amounts of exercise and potential benefits. Since treadmill running also forces activity, it reflects more realistically the attitude of many human subjects toward exercise, which is more often driven by health demands as a rationale decision rather than spontaneous action or pleasure. Since voluntary running often results in extreme distances covered and speeds maintained by rodents [33,34], voluntary wheel running may model only those exceptional humans who are highly motivated to engage in frequent, sustained physical activity, such as endurance athletes or leisure exercise "addicts". Our results suggest that even very moderate levels of regular forced exercise may have a beneficial effect on WD-induced dysregulation of hypothalamic functions [5].

The fact that by covering the same distance, the forced-running paradigm enhances significantly more neurogenesis in rodents as compared to the voluntary running paradigm [33], indicates not only that these two forms of exercise have inherently different effects on brain and behavior, but also that the therapeutic potential of the forced-running paradigm for hypothalamic dysfunction may be broader than that of the voluntary regimen. Among other considerations, our choice was based on the recent finding that forced, but not voluntary exercise, effectively induces neuroprotection in rodent stroke models [35]. The authors of that study argue that exercise with a stressful component, rather than voluntary exercise or stress alone, may be better able to improve clinical outcome. Although forced exercise acutely elevates corticosterone levels to a greater extent than voluntary exercise [36], there is a mounting body of evidence that the hypothalamic-pituitary-adrenal axis (HPA) adapts to chronic running, such that either voluntary or forced running no longer elevates corticosterone levels compared to non-runners after several weeks of training [33,37].

The recent finding that forced exercise does not enhance microglial activation in the dentate gyrus of the hippocampus has been of particular interest for this study [33]. Based on the finding that stress-induced elevation of glucocorticoids actually increases microglial activation [38] and proliferation [39], our findings underscore the possibility that chronic forced exercise may actually not affect HPA-mediated microglial activation. A very recent report suggesting that cancer-induced anorexia may lead to a pro-inflammatory state in the hypothalamus, which is prevented by treadmill endurance training [40], underscores the pivotal role of physical activity in the maintenance of normal hypothalamic function. This is supported by the present finding where we observed no differences in the hippocampus when comparing sedentary and exercised mice, while preventing WD-induced microglial activation in the ARC. Wu et al. have recently reported that the unfolded protein response (UPR), an adaptive response pathway that maintains endoplasmatic reticulum (ER) homeostasis upon luminal stress, is activated in skeletal muscle during exercise and adapts skeletal muscle to exercise training [41]. Since ER stress appears to represent a crucial player in the development of hypothalamic inflammation [7–11], we hypothesize that adaptation to exercise-induced UPR may offer an alternative mechanism to improve hypothalamic inflammation and prevent dysfunction of hypothalamic control over systems metabolism.

## 5. Conclusions

A sedentary lifestyle and omnipresent exposure to high-caloric "western diets" (WD) are thought to be responsible for a massive rise in the prevalence of obesity and diabetes. WD feeding induces inflammatory signaling not only in several peripheral tissues such as adipose depots, but also in the hypothalamus, causing local resistance to both insulin and leptin. Interventions that prevent or even reverse hypothalamic inflammation during WD feeding therefore offer opportunities for the prevention and treatment of obesity and diabetes. The present results underscore the potential of regular moderate exercise to protect against diet-induced metabolic damage by preventing diet-induced hypothalamic inflammation.

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