Naltrexone alters cardiovascular function following acute forced swimming in mice

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Purpose   Naltrexone (NTX) is an opioid antagonist that can reverse the physiological effects of opioid receptors when bound. Opioid receptors have been found to play a role in cardiovascular (CV) function, and thus, binding of NTX may alter CV activity at rest and in response to acute and chronic exercise (EX). We hypothesized that opioid receptor blockade will alter the typical CV responses following acute EX.

Methods   We assessed the effects of opioid receptor blockade on CV function via echocardiography in mice following an acute bout of forced swimming (FSw), a model of rodent EX. We administered opioid receptor antagonist, NTX, or saline in mice before FSw and in the absence of an FSw perturbation. Furthermore, we assessed how NTX can influence maximal EX capacity on a rodent treadmill.

Results   Our data shows that NTX administration does not decrease maximal EX capacity in mice ($P > 0.05$). However, NTX attenuated cardiac output following FSw (FSw = 52.5 ± 2.5 ml/min vs. FSw + NTX = 32.7 ± 5.2 ml/min; $P < 0.05$) when compared with saline control (33.5 ± 3.8 ml/min). Further, the administration of NTX in the non-EX condition significantly ($P < 0.05$) reduced ejection fraction.

Conclusion   These data suggest that normal opioid receptor activation is necessary for typical CV function following FSw. *Cardiovasc Endocrinol Metab* 11: 1–7 Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc.

Introduction

The production of endogenous opioids both during and following exercise (EX) has been well-established [1–3]. The dopaminergic system regulates mood, well-being, and is involved in several physiological functions [4]. These psychoactive characteristics (euphoria, analgesia, and addiction) tend to be blunted by opioid receptor blockade [1–3,5] with drugs such as naloxone and naltrexone (NTX).

NTX is a synthetic opioid antagonist, which is prescribed to decrease the desire to consume opiates and alcohol. Prior studies in rodents have also shown that NTX can reduce weekly wheel running totals [6], and human studies have shown that NTX can attenuate some of the psychological benefits of EX [7]. Opioid receptor activation is key to regulating our bodies' response to stress [8] and may also be responsible for some of the cardiovascular (CV) adaptations resulting from chronic EX [8–10].

Obesity is the second leading cause of preventable death in the USA behind tobacco smoking [11]. According to the NIH, in 2013–2014 over 70% of American adults above the age of 20 were overweight with 35% considered obese based on BMI [12]. Obesity is a precursor for issues such as heart disease, stroke, various cancers, diabetes, and kidney diseases [13].

NTX in combination with bupropion is prescribed for weight loss. Bupropion is a dopamine and norepinephrine reuptake inhibitor that is used for the treatment of depression and smoking cessation [14]. While the exact mechanism is uncertain, NTX/bupropion combination results in a significantly greater weight loss than either medication or behavior modification alone [14].

It is common knowledge that EX is an effective intervention for body weight management and obesity prevention. EX is a positive stress and results in an increase in cardiac output (CO) [15]. The EX-mediated increase in CO is facilitated by both an increase in heart rate (HR) and stroke volume (SV), which is EX intensity- and duration-dependent [15]. In aerobically trained young adults, NTX increased HR reactivity and mean arterial blood pressure both during and following EX [16]. This

study by McCubbin et al. [16] may demonstrate that opioid receptor activation is important for EX-mediated CV adaptations to occur. Despite this, the effects of NTX on CV function immediately following acute EX (EX recovery) have not been clearly established. Therefore, we are unsure if the combination of EX and NTX has the potential to be problematic when combined for weight loss. The purpose of this study was to assess CV function via echocardiography (ECHO) on mice following an acute bout of forced swimming (FSw), which we used as a model of EX.

Methods

Animal model

All experiments were approved by the Rutgers University Institutional Animal Care and Use Committee and were conducted in accordance with the Public Health Service Policy regarding the Humane Care and Use of Laboratory Animals. Sixteen (8 week old; 24–26 g) male C57-BL6 WT mice (Charles Rivers Laboratories) were used for ECHO. Mice were required to complete all four ECHO sessions to be included in the data analysis. Eight separate mice (8 week old; 24–26 g) were assessed for maximal EX capacity but did not undergo ECHO. All mice were housed under the standards of Rutgers University. Mice were provided ad libitum access to food and water and followed a 12-h light/dark cycle. Animals were acclimatized to the facility for 1 week before experiments began.

For our ECHO experiments, we utilized FSw as a model of EX. Each mouse served as their own control and was experimented under each of the following conditions: control (CON), FSw, NTX injection without FSw (NTX), and NTX injection prior to FSw (FSw + NTX). Following the 1-week acclimatization period, mice underwent 1 week of swimming familiarization. ECHO measurements were randomized and all four trials took place over the course of 3 weeks. Mice that died during the study were not included in our data analysis.

Graded exercise testing

In a separate cohort of mice ($n = 8$), we assessed maximal EX capacity using a treadmill with and without NTX using a graded exercise test (GXT). All mice were subjected to a practice trial 3 days before the experiment to adapt to the treadmill testing environment. Food was withdrawn 3 h before EX. At the time of the GXT, mice began at 4 m/min with a gradual speed increase of 2 m/min every 2 min until exhaustion; treadmill grade remained constant at a 10% incline. Exhaustion was defined as spending 10 unbroken seconds on the electric stimulus platform without attempting to reengage the treadmill belt.

Forced swimming

As we performed previously [5], mice were placed in cylindrical tanks (30-cm height × 20 cm diameters) for the FSw. Tanks were filled with room temperature (23–25 °C) tap water. Water levels were marked and maintained at 15 cm from the bottom to ensure water volume consistency across mice. To ensure the water temperature was maintained at room temperature for the animals, the tank was placed on a heating pad and monitored throughout.

Before experiments, mice were familiarized by performing daily swimming sessions over the course of 1 week with sessions gradually lengthened from 5 to 25 min. During these sessions, mice were monitored by one or two observers; swimming was terminated at any sign of distress (failure to maintain swimming or floating). Following acclimatization, mice were challenged with a 50-min bout of swimming/wading, an adequate time shown to induce opioid-mediated hyperphagia [5,17]. Mice that completed over 30 min of FSw underwent an ECHO. Mice unable to swim at least 30 min were omitted from the FSw condition and did not undergo ECHO.

Naltrexone administration

Mice received an injection (i.p) of saline (CON and FSw) or NTX mixed with saline (NTX and FSw + NTX) (4.0 mg/kg, Sigma Aldrich; St. Louis, Missouri, USA). Conditions were randomized for each mouse. As we have done previously, 15 min after injections, mice were challenged with FSw. NTX injection did not change FSw time, all mice were able to swim past 30 min our set criteria for a valid EX bout.

Echocardiography

Anesthesia was induced using 3% isoflurane and followed by a maintenance dose at 1–2% of ISO and adjusted to keep each mouse's respiratory rate between 80 and 100 breaths per minute. Isoflurane was administered using a rodent vaporizer via a small nose cone. Anesthesia depth was determined by immobility and the absence of right paw withdrawal following probing. Sedated animals were placed in the supine position on a prewarmed platform and limbs were taped over metal electrocardiogram leads for echocardiographic imaging. The ultrasound transducer was placed slightly left of the sternum over the fourth and sixth ribs. Body temperatures were carefully monitored and maintained as close to 37 °C as possible for the duration of the procedure.

The high-frequency VisualSonics Vevo 2100 system (VisualSonics, Toronto, Canada) was used to capture images. Two-dimensional-guided M-mode image recordings were made using the parasternal short-axis view at the level of the papillary muscles. Three consecutive beats were measured 5 min after anesthesia, and the average of these measurements was taken for analysis. Left ventricular (LV) internal systolic and diastolic diameters were measured using the American Society of ECHO leading-edge technique [18]. Vevo 2100 software was used to calculate fractional shortening (%), ejection

fraction (%), and SV (ml). Stroke index and cardiac index were calculated to ensure differences in LV function were not influenced by body weight.

Data analysis
A power analysis using G*Power 3.1.9.4 [19] was performed to determine the number of animals needed for each group. A power analysis was performed for a two-way repeated measures analysis of variance. A large expected difference was expected before and after FSw and blockade with NTX for ECHO. Therefore, a large effect size was set to $F = 0.40$. Our power analysis was run for mice serving as their own control for four experimental conditions [Saline (control) Fsw, NTX, FSw + NTX]. Power was set to 0.80 and significance was set at a $P > 0.05$. Based on previous experiments performed in our laboratory, we anticipated a very low mortality rate of less than 10% coupled with a high success rate.

A Student's *t*-test was run to compare differences between two groups. A two-way repeated measures analysis of variance was used to assess the differences between multiple groups and a Tukey's test was used for post hoc analysis. Statistical analysis was performed in SPSS (IBM; version 25.0; Armonk, New York, USA). A P-value of less than 0.05 ($P < 0.05$) was considered significant. Data are shown as mean ± SEM.

Results
Naltrexone has no effect on mouse maximal treadmill exercise capacity
NTX did not affect maximal EX capacity in mice using a GXT. Administration of NTX did not significantly decrease maximal running distance or maximal work between CON or NTX groups during the GXT ($P > 0.05$; Fig. 1a and b). The average swim time for NTX treated mice was 45.9 ± 1.2 min vs. CON (49.8 ± 2.2 min). There was no difference in swim time between mice under CON or NTX conditions ($P > 0.05$).

Naltrexone attenuates exercise-mediated increases in cardiac output following forced swimming
We next examined the effects of NTX on HR recovery following a bout of FSw. We found a significant effect of treatment (NTX) on HR ($F = 4.362$; Df = 45; $P < 0.05$). FSw significantly increased HR under CON conditions (CON = 276 ± 11 bpm vs. FSw = 360 ± 21 bpm; $t = 2.8$; $P < 0.05$); however, these effects were attenuated with the addition of NTX (FSw + NTX = 253.3 ± 30.2 bpm; Fig. 2a). There was also a significant effect of treatment (NTX) on SV ($F = 5.66$; Df = 45; $P < 0.05$; Fig. 2c). Mice receiving NTX had a decrease in both HR and SV, compared with mice following FSw without NTX, however, these changes were NS (Fig. 2b and d).

CO was significantly elevated following FSw ($F = 17.12$; Df = 45; $P < 0.05$; Fig. 3a). FSw increased CO under control conditions (CON = 33.5 ± 3.8 µl/min vs. FSw = 52.5 ± 2.5 µl/min; $P < 0.05$). The addition of NTX decreased CO relative to both conditions (NTX = 16.0 ± 2.5 µl/min vs. CON) (NTX + FSw = 32.7 ± 5.2 µl/min vs. NTX; $F = 26.83$; Df = 45; $P < 0.05$). To ensure that changes in CO and SV were not due to weekly changes in body weight, we normalized both SV and CO into indices (i.e. cardiac index and stroke index) (Fig. 4a and b). Our findings remained unchanged after normalization.

Effects of naltrexone on left ventricular function
EX with either saline or NTX had no impact on other variables involved with LV function (Table 1). However, we did observe that NTX in the absence of FSw significantly lowered ejection fraction when compared with the saline-only condition ($F = 3.71$; $P < 0.05$). Fractional shortening was not different between conditions ($P > 0.05$).

Discussion
These data demonstrate that NTX administration: (a) does not decrease maximal EX capacity, however (b) blunts increases in HR following FSw, (c) decreases SV

Fig. 1

Administration of NTX did not alter maximal exercise capacity for (a) maximal running distance or (b) maximal work performed during a graded exercise test (GXT). $N = 8$/group. Data shown as mean ± SEM. NTX, naltrexone.

Fig. 2
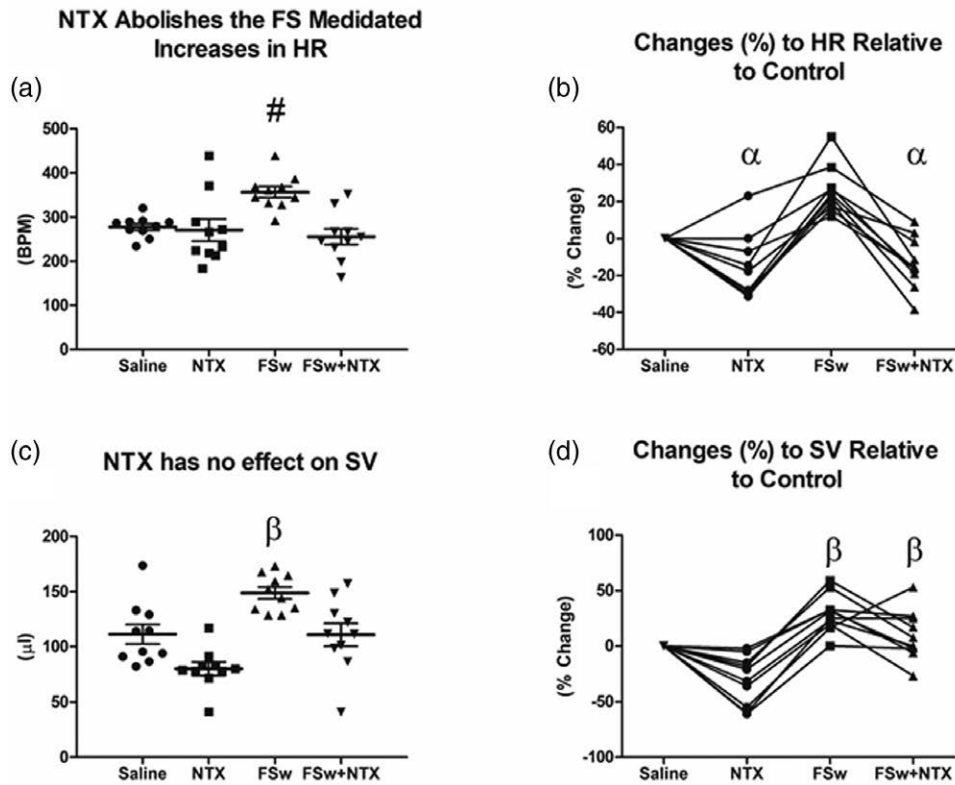
(a) FSw increased HR these effects were attenuated with NTX (b) NTX given with and without FSw decreased HR relative to saline (c) NTX altered SV in sedentary and mice who underwent FSw. (d) There was a treatment effect of NTX relative to saline on SV. $N = 10$/group. $P < 0.05$; α vs. FSw; β vs. saline. Data shown as mean ± SEM. FSw, forced swimming; HR, heart rate; NTX, naltrexone; SV, stroke volume.

Fig. 3
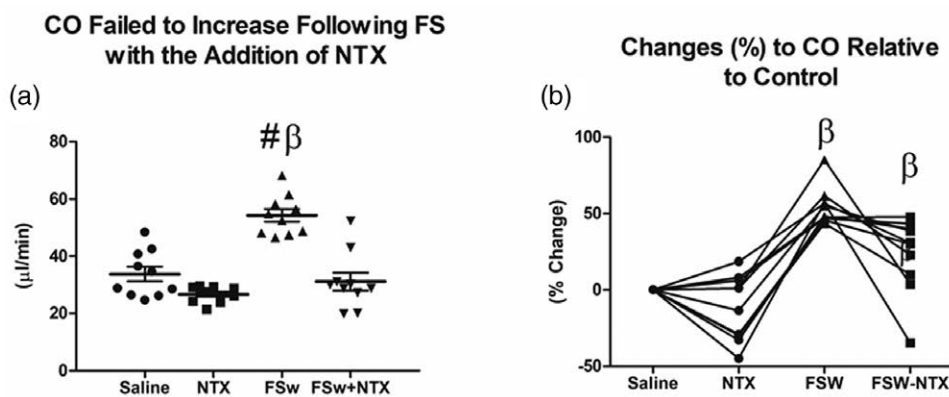
(a) There was a significant effect of FSw and NTX on CO. (b) NTX decreased CO when made relative to saline. $P < 0.05$; $N = 10$/group. β vs. saline; # vs. NTX. Data shown as mean ± SEM. CO, cardiac output; FSw, forced swimming; NTX, naltrexone.

in the absence of and following FSw, and (d) decreases CO following FSw and at rest. The observed reduction in CO immediately following EX may negatively influence EX recovery, as increased CO and, thus, blood flow to skeletal muscle facilitate substrate replenishment and cellular homeostasis following EX [20]. Previous work administering NTX appeared to alter HR reactivity in EX-trained individuals, supporting the hypothesis that

Fig. 4
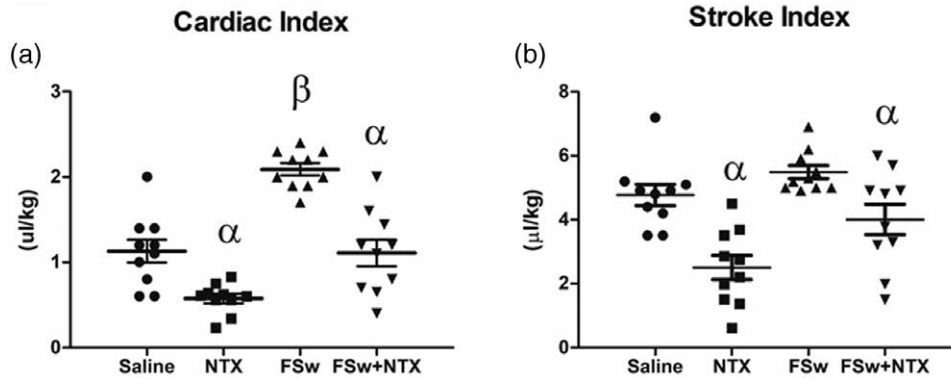
There were no differences when data were made relative to body weight. Cardiac index and stroke index were similar to cardiac output and stroke volume. $P < 0.05$; $N = 10$/group. β vs. saline; α vs. FSw. Data shown as mean ± SEM.

Table 1 Effects of exercise and naltrexone on ejection fraction, left ventricular internal dimension in diastole and systole, and fractional shortening

Condition	EF (%)	LVIDd (mm)	LVIDs (mm)	FS (%)
Control	63 ± 0.04	3.38 ± 0.14	2.24 ± 0.82	34.4 ± 3.4
FSw	62 ± 0.06	3.62 ± 0.05	2.27 ± 0.16	37.5 ± 3.9
NTX	54 ± 0.04*	2.96 ± 0.34	2.09 ± 0.33	32.6 ± 3.9
FSw + NTX	58 ± 0.04	3.35 ± 0.20	2.25 ± 0.17	33.2 ± 3.3

Values are mean ± SEM.
EF, ejection fraction; FS, fractional shortening; LVIDd, left ventricular internal dimension in diastole; LVIDs, left ventricular internal dimension in systole.
*$P < 0.05$.

inadequate perfusion may prolong recovery from an EX bout [16]. Therefore, the combination of EX and NTX, such as in the case of a weight loss regimen may be problematic.

Although HR reactivity was not the primary focus of this experiment, the authors of this study hypothesized that a primary mechanism responsible for EX-induced changes to CV function is influenced by opioid blockade. Although not measured, neural mechanisms, specifically the arterial baroreflex, are involved in making rapid changes in HR and blood pressure during EX [20]. A previous study demonstrated NTX protects from hypotension in rats by decreasing the baroreflex sensitivity [21] and, thus, can be a mechanism responsible for the augmented HR during recovery. The hypothalamic-pituitary-adrenal (HPA) axis is a well-studied component of endocrine function during EX, acting to modulate hormone and neurotransmitter release in a manner dependent on intensity and duration [22–25]. Dopamine release is modulated by EX [26] and its presence has long been known to significantly change CO and SV [27]. In human male subjects, the administration of NTX blunted HR increases associated with alcohol intoxication and reward-stimulating outcomes of alcohol consumption linked to dopamine release, likely due to the ability of NTX to influence the HPA axis [28]. Despite not measuring adrenocorticotropic hormone or

cortisol levels, one can hypothesize the duration and intensity of our EX model can stimulate the HPA axis. This is supported by evidence that forced EX induces stress that increases levels of corticosterone compared with animals provided with free wheel running, which is the equivalent of physical activity [29].

Furthermore, when delivered chronically via minipumps, NTX downregulated opioid receptor mRNA expression in Wistar rats who underwent an aortocaval fistula to induced cardiac overload [30]. This was accompanied by a decrease in central venous pressure and LV end-diastolic pressure. These improvements along with attenuated systolic and diastolic pressures and improvements in LV function may indicate a cardiodepressant and protective effect during pathological volume overload.

It is well established that, during aerobic EX, the heart also undergoes acute pressure overload. Repeated pressure overload via EX is responsible for eccentric cardiac hypertrophy, which increases SV and CO over time. Therefore, although the rats studied by Dehe *et al.* [31] were observed chronically, the results of the study may provide another potential mechanism for the cardiodepressant effects demonstrated in our study. It is possible that NTX can cause an acute downregulation of cardiac opioid receptors during EX and, therefore, alter LV function. During pathological pressure overload, this

effect would be of benefit and potentially decrease the risk/rate of LV hypertrophy and improve cardiac function, chronically. However, when combined with chronic EX, it is possible that NTX can interfere with positive EX adaptation. Previous findings from our laboratory demonstrate that the endogenous opioid system plays a role following FSw [5]. NTX appears to alter typical patterns of fludeoxyglucose-18 uptake in the brain following acute FSw. This study provides further evidence that dopaminergic signaling plays a role during EX and in recovery from EX. It appears that opioid receptor blockade with NTX may alter the response to EX in multiple organ systems. Our study is not without limitation. First, we used only male mice. Female mice have enhanced EX capacity [31] and our more likely to become addicted to various substances [32,33], this may indicate a potential difference in dopaminergic signaling. These two variables alone warrant a follow-up study focusing on female mice. The differences in EX capacity and addiction appear to be mediated through estrogen as these effects are attenuating in ovariectomized mice [31,33]. Therefore, it may be that NTX would have a greater effect on female mice relative to males.

Second, we used FSw as our primary means of EX for our study. Forced EX is known to cause stress and therefore release stress-associated hormones, which act as a confounding variable [34]. Third, we did not measure HR during GXT or FSw. Thus, we are unsure if HR during EX followed the same pattern as post-EX. Fourth, we did not measure the body temperature of our mice during FSw or during ECHO. Fluctuations in body temperature are known to have an influence on HR in rodents [25]. Finally, the use of anesthetics, although common in rodent ECHO, makes it more difficult to translate our findings to a human population. Further, the interaction between NTX and isoflurane may lead to confounding effects on our experiments.

In conclusion, it appears that dopaminergic signaling plays a role in the CV systems recovery from EX. Despite the mechanism involved a blunted CV response following EX could potentially decrease the beneficial effects of EX. Future studies should focus on identifying the specific mechanisms that are responsible for this occurrence. Specifically, exploring changes to cardiac opioid receptors may be particularly prudent. If these data are able to be translated to a human population it may provide insight between the interaction of drugs prescribed containing NTX and their combined use with a regular EX regimen.

Acknowledgements

Conflicts of interest
There are no conflicts of interest.

References
1　Harber VJ, Sutton JR. Endorphins and exercise. *Sports Med* 1984;**1**:154–171.
2　Stagg NJ, Mata HP, Ibrahim MM, Henriksen EJ, Porreca F, Vanderah TW, Philip Malan T Jr. Regular exercise reverses sensory hypersensitivity in a rat neuropathic pain model: role of endogenous opioids. *Anesthesiology* 2011;**114**:940–948.
3　Hamer M, Karageorghis CI. Psychobiological mechanisms of exercise dependence. *Sports Med* 2007;**37**:477–484.
4　Le Merrer J, Becker JA, Befort K, Kieffer BL. Reward processing by the opioid system in the brain. *Physiol Rev* 2009;**89**:1379–1412.
5　Qadiri Q, York H, Muth BJ, Longoria CR, Campbell SC, Guers JJ. The addition of naltrexone alters cerebral glucose uptake following acute forced swimming. *Physiol Behav* 2021;**228**:113199.
6　Li G, Rhodes JS, Girard I, Gammie SC, Garland T Jr. Opioid-mediated pain sensitivity in mice bred for high voluntary wheel running. *Physiol Behav* 2004;**83**:515–524.
7　Craft LL, Perna FM. The benefits of exercise for the clinically depressed. *Prim Care Companion J Clin Psychiatry* 2004;**6**:104–111.
8　Headrick JP, See Hoe LE, Du Toit EF, Peart JN. Opioid receptors and cardioprotection - 'opioidergic conditioning' of the heart. *Br J Pharmacol* 2015;**172**:2026–2050.
9　Dickson EW, Hogrefe CP, Ludwig PS, Ackermann LW, Stoll LL, Denning GM. Exercise enhances myocardial ischemic tolerance via an opioid receptor-dependent mechanism. *Am J Physiol Heart Circ Physiol* 2008;**294**:H402–H408.
10　Michelsen MM, Støttrup NB, Schmidt MR, Løfgren B, Jensen RV, Tropak M, *et al.* Exercise-induced cardioprotection is mediated by a bloodborne, transferable factor. *Basic Res Cardiol* 2012;**107**:260.
11　National Institutes of Health (NIH). Overweight & obesity statistics. 2017. www.niddk.nih.gov/health-information/health-statistics/overweight-obesity#prevalence [Accessed 20 December 2021].
12　World Health Organization (WHO). Obesity and overweight. 2020.
13　Jakicic JM, Otto AD. Physical activity considerations for the treatment and prevention of obesity. *Am J Clin Nutr* 2005;**82**:226S–229S.
14　Khan SR, Berendt RT, Ellison CD, Ciavarella AB, Asafu-Adjaye E, Khan MA, Faustino PJ. Bupropion hydrochloride. *Profiles Drug Subst Excip Relat Methodol* 2016;**41**:1–30.
15　Gleim GW, Coplan NL, Nicholas JA. Acute cardiovascular response to exercise. *Bull N Y Acad Med* 1986;**62**:211–218.
16　McCubbin JA, Cheung R, Montgomery TB, Bulbulian R, Wilson JF. Aerobic fitness and opioidergic inhibition of cardiovascular stress reactivity. *Psychophysiology* 1992;**29**:687–697.
17　Davis JM, Lamb DR, Yim GK, Malven PV. Opioid modulation of feeding behavior following repeated exposure to forced swimming exercise in male rats. *Pharmacol Biochem Behav* 1985;**23**:709–714.
18　Gottdiener JS, Bednarz J, Devereux R, Gardin J, Klein A, Manning WJ, *et al.*; American Society of Echocardiography. American Society of Echocardiography recommendations for use of echocardiography in clinical trials. *J Am Soc Echocardiogr* 2004;**17**:1086–1119.
19　Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav Res Methods* 2009;**41**:1149–1160.
20　Hoppeler H, Billeter R. Conditions for oxygen and substrate transport in muscles in exercising mammals. *J Exp Biol* 1991;**160**:263–283.
21　Crisafulli A, Marongiu E, Ogoh S. Cardiovascular reflexes activity and their interaction during exercise. *Biomed Res Int* 2015;**2015**:394183.
22　Huang YP, Lin MT, Chen JS, Wu PY. Naltrexone protects against hypotension, hyperthermia, and beta-endorphin overproduction during heatstroke in the rat. *J Pharmacol Sci* 2005;**97**:519–524.
23　Davies CT, Few JD. Effects of exercise on adrenocortical function. *J Appl Physiol* 1973;**35**:887–891.
24　Luger A, Deuster PA, Gold PW, Loriaux DL, Chrousos GP. Hormonal responses to the stress of exercise. *Adv Exp Med Biol* 1988;**245**:273–280.
25　Park E, Chan O, Li Q, Kiraly M, Matthews SG, Vranic M, Riddell MC. Changes in basal hypothalamo-pituitary-adrenal activity during exercise training are centrally mediated. *Am J Physiol Regul Integr Comp Physiol* 2005;**289**:R1360–R1371.
26　Lin TW, Kuo YM. Exercise benefits brain function: the monoamine connection. *Brain Sci* 2013;**3**:39–53.

27 Horwitz D, Fox SM 3D, Goldberg LI. Effects of dopamine in man. *Circ Res* 1962;**10**:237–243.

28 Peterson JB, Conrod P, Vassileva J, Gianoulakis C, Pihl RO. Differential effects of naltrexone on cardiac, subjective and behavioural reactions to acute ethanol intoxication. *J Psychiatry Neurosci* 2006;**31**:386–393.

29 Svensson M, Rosvall P, Boza-Serrano A, Andersson E, Lexell J, Deierborg T. Forced treadmill exercise can induce stress and increase neuronal damage in a mouse model of global cerebral ischemia. *Neurobiol Stress* 2016;**5**:8–18.

30 Dehe L, Shaqura M, Nordine M, Habazettl H, von Kwiatkowski P, Schluchter H, *et al.* Chronic naltrexone therapy is associated with improved cardiac function in volume overloaded rats. *Cardiovascular Drugs and Therapy* 2021;**35**:733–743.

31 Oydanich M, Babici D, Zhang J, Rynecki N, Vatner DE, Vatner SF. Mechanisms of sex differences in exercise capacity. *Am J Physiol Regul Integr Comp Physiol* 2019;**316**:R832–R838.

32 Fattore L, Fadda P, Fratta W. Sex differences in the self-administration of cannabinoids and other drugs of abuse. *Psychoneuroendocrinology* 2009;**34**(Suppl 1):S227–S236.

33 Kerstetter KA, Ballis MA, Duffin-Lutgen S, Carr AE, Behrens AM, Kippin TE. Sex differences in selecting between food and cocaine reinforcement are mediated by estrogen. *Neuropsychopharmacology* 2012;**37**:2605–2614.

34 Hannon JP. Effect of temperature on the heart rate, electrocardiogram and certain myocardial oxidations of the rat. *Circ Res* 1958;**6**:771–778.