A pilot study of metabolic fitness effects of weight-supported walking in women with obesity

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Abstract

Background

This is an exploratory pilot study of novel technology enabling people with mobility disability to walk with minimal effort, in the "sedentary range". The study's premise is that impairment of the leading physical activity of daily living, walking, is a major contributor to a dysmetabolic state driving many prevalent "civilization diseases" associated with insulin resistance.

Methods

We explore within-subject changes in standard oral glucose tolerance (OGT) tests including metabotropic molecules after 22 twice-weekly, 30-minute bouts of weight-supported light-moderate physical activity in 16 non-diabetic obese, otherwise healthy, reproductive-age, volunteer women walking on an "anti-gravity" lower-body positive pressure (LBPP) treadmill.

Results

Subjects had reference base-line fasting plasma glucose and triglycerides (TG) but 2-hr OGT insulin levels of 467 ± 276 pmol • liter^{-1} (mean± S.D.) indicating nascent insulin resistance, compared to post-study 308 ± 179 (p = 0.002). Fasting TG decreased from 0.80 ± 0.30 mmol • liter^{-1} to 0.71 ± 0.25 (p = 0.03). Concomitantly plasma total ghrelin decreased from 69.6 ± 41.6 pmol • liter^{-1} to 56.0 ± 41.3 (p = 0.008). There were no statistically significant changes in body weight or any correlations between weight change and cardiometabolic markers. However, there were robust positive correlations between changes among different classes of peptides including C-reactive protein–Interleukin 6, leptin–adiponectin, β-endorphin–oxytocin and orexin A (r^2 = 0.48–0.88).

Conclusion

We conclude that brief, low-dose physical activity, walking on an anti-gravity LBPP treadmill may improve cardiometabolic risk, exhibiting favorable changes in neuro-regulatory peptides without weight loss in people with problems walking.

Introduction

Mobility disability, the leading form of disability, is increasing in step with epidemic obesity [1, 2]. Although life-style interventions require sustained moderate—high levels of physical activity [3, 4], persons with overweight/obesity are physically impaired in their ability to walk, the leading energy-consuming activity of daily living (ADL). Thus, life-style change regimens with diet and exercise recommendations, the primary therapeutic and preventive interventions for numerous degenerative diseases including obesity, osteoarthritis and cardio-pulmonary insufficiency, are rarely maintained long enough to be effective over the long term [3, 5, 6] owing to the fact that exercise is hard and painful.

Availability of a NASA developed anti-gravity treadmill offloading body weight using lower body positive pressure (LBPP) [7], prompted us to test whether use of this treadmill would engender known positive metabolic effects of increasing physical activity [8]. We recruited a convenience sample of 16 sedentary, obese, non-diabetic, reproductive-age volunteer Black women, culturally disinclined to lose weight [9]. Therefore we chose an activity level well below life-style guidelines [10], enabling comfortable, safe ambulation according with ADL levels of painless exertion within the "sedentary range" unlikely to cause weight loss [4; 11; 12; 13].

Most interventions are implemented late in cumulative disease processes when the magnitude and the rapidity of recommended interventions exceed the speed with which calories have been stored [14; 15]. Such relatively large and rapid changes (low calorie diets, moderate-high intensity exercise) cause counter-regulatory 'stress' as maladaptive non-homeostatic dysautonomic responses, similar to mechanisms of insulin resistance [16]. Whereas exercise (exertion) is beneficial in physically fit individuals [17], dose-response levels are circumstantial and highly variable owing to intrinsic and environmental influences.

Although the "sedentary range" demonstrates that increasing physical activity is uncoupled from appetite, interpreted as "dysregulation of energy intake" supporting recommendations to exceed the sedentary range [13], we posit that uncoupling of appetite and food intake from exertion might be beneficial. Lower levels of physical activity within the sedentary range, by being undetected, imperceptible or "dysregulated", might expend sufficient energy without exertion that challenges lean body mass [14] or sensitizes appetite [12;18;19]. At the same time, accumulating evidence supports positive effects of low intensity activity [20–23]. Whereas most guidelines propose moderate to high intensity exercise to lose weight, there is little research investigating metabolic effects of lower levels of energy expenditure without weight loss [13; 20, 21] and, to our knowledge, none on brief, low-amount, activity-of-daily living level ambulation.

This pilot study implies that an investment of 30 minutes of lower-body pressure (weight-offloading) walking twice weekly for 10–12 weeks is sufficient to significantly reduce nascent insulin resistance independent of weight change in non-diabetic reproductive-age Black women.

Methods

Subjects

Volunteers among hospital employees and their families (not related to or supervised by the investigators) were recruited through word-of-mouth at SUNY Downstate Medical Center, a convenience sample. They were offered participation in a study of "metabolic fitness" defined as "the ability of the body to use energy from dietary sugars and fats". Eligible were otherwise healthy overweight–obese (BMI 28–50; mean ± SD: 35 ± 7), weight-stable, untrained, non-diabetic, pre-menopausal Black women aged 18 to 56 years (40 ± 11) (S1 Table).

Participants were willing and able to commit to 2–3 times weekly 30-minute bouts of exercise on the weight-supporting treadmill for 12 weeks (more than 20 bouts) comfortably and painlessly using the treadmill. They were not compensated but received a thorough metabolic work-up described below, signing consent forms covering all aspects of this study approved by the Institutional Review Board of SUNY Downstate Medical Center specifically for this study (IRB# 12–044). Investigators blinded to accrued phenotypic data performed all assays and measurements, treadmill supervision and statistics.

Exclusion criteria were shift-work and strenuous work-related functions, participation in dieting or exercise programs the previous 3 months, surgical treatment of obesity, being pregnant or planning pregnancy, being smokers, taking medications known to affect energy balance or having musculo-skeletal or other conditions incompatible with using the treadmill. We excluded subjects with known diabetes, hypertension and dyslipidemia and those using psychotropic medications potentially affecting appetite regulation, oral contraceptives or steroids of any type. They consumed no or only minimal alcohol.

Baseline history, physical and activity assessment. A structured 45-50-minute interview based on questionnaires exploring demographic and socio-economic factors, food insecurity, household stressors and a thorough medical history was conducted. Anthropometric measures included weight, height, BMI, waist and hip circumferences. Blood pressure and heart rate were measured after subjects had been seated for 5 minutes. Taking into consideration the cultural habits and beliefs of our population, there was no "weighing in" or mention of body weight, diet or dieting during visits to the laboratory. Most subjects were administrators engaged in typically sedentary work. Daily activity was exclusively measured pre- and post-study using a wrist-worn Fit-Bit accelerometer for two 3-day periods one of which included one non-working weekend day (S1 Methods).

Fasting morning blood and oral glucose tolerance tests

An in-dwelling ante-cubital venous catheter was placed with the patient seated in a comfortable recliner. At time 0 of a standard 75 g oral glucose tolerance test (OGTT) blood was drawn for determination of glucose and insulin, HOMA-IR, GLP-1, GIP, free fatty acids (FFA), C-peptide, fasting C-reactive peptide (CRP), Interleukin-6 (IL-6), TNFα, leptin, total ghrelin, total adiponectin, GIP, glucagon, triglycerides (TG), HDL-cholesterol, oxytocin (OXT), β-endorphin and orexin A (ORA). During OGTT at 15, 30, 60, 90 and 120 minutes blood was drawn for glucose, insulin, C-peptide, GLP-1 and FFA, allowing calculations at 2 hours and area under the curve (AUC). Blood chemistry details are provided in Supporting Methods (S2 Methods).

Weight-supporting treadmill

The lower-body positive pressure (LBPP) treadmill (AlterG) [24] consists of a stationary heavy metal frame with a movable gantry that can be raised to the level of a user's waist and is

attached to an inflatable clear-plastic malleable "skirt" surrounding the electric treadmill. (S1 Fig) The waist of the skirt is encircled with one portion of a zipper corresponding to a zipper around the waist of neoprene shorts, worn by the subject. The treadmill's mechanism covertly measures the person's weight, enabling determination of a percentage of body weight to be off-loaded by treadmill pump inflation increasing pressure on the lower body. The maximum pressure exerted is equal to that of standing in water in a pool ($\sim$ 1 psi; 51.7 mmHg; 70 cm H_2O; 6.9 kPa) [25].

Treadmill protocol

Off-loading was set at 40% reduction of body weight, subjects thus walking on the treadmill at 60% of their weight throughout the study, a level approximating long-term weight loss after successful metabolic surgery. Speed set by the subject, by adjusting treadmill speed on the dashboard, allowed completion of a 30-minute session. Initial incline was set at 0°. Subjects were encouraged to increase the speed with time and staff gradually increased the incline every 3 sessions to 3, 5, 7, and a maximum of 10%. Time, distance, speed, treadmill incline and % weight offloaded at the 25-minute mark was recorded after each session. After completion of 20–24 sessions OGTT with fasting blood tests, similar to before the exercise regimen, were performed at least more than 24 hours after the last session, sufficient to rule out acute effects of this brief (30-minute) low-intensity, infrequent (twice weekly) incremental exertion.

Energy expenditure. We calculated metabolic equivalents (METs) of energy expenditure according to standard equations [26], but adjusted for weight in our study of weight-supported ambulation. We used the Harris-Benedict Equation for RMR to improve the accuracy of MET estimates to determine "corrected METs" when offloading body weight with the LBPP treadmill [27].

Corrected METs, kilocalories (kcal) and Watts (W) expended per session according to ACSM formulas, total energy expended per week as MET minutes and kcal expenditure were used to compare our effort intensity at 60% body weight with CDC and ACSM exercise recommendations at full body weight [28] adding up total energy expended by each subject during all sessions (S1 Methods).

Statistics

Data are expressed as means ± standard deviations (SD), except as noted. Within-subject differences pre- and post-intervention were compared using paired sample t-tests or Wilcoxon's rank-sum tests based on a one-sample Kolmogorov-Smirnov test against a normal distribution. The exploratory nature of this pilot study with 16 subjects precluded co-varying for multiple variables in the pre-post intervention analyses. Similarly, we have not corrected for multiple testing. We calculated Pearson correlation coefficients between relevant variables and used Spearman correlations when relationships were not linear owing to the variance in this relatively small population. Statistical analyses used SPSS version 23 (SPSS, Chicago, IL).

Results

Baseline characteristics and proteomics

According to our inclusion and exclusion criteria and the characterization in Methods, these 16 subjects were overweight/obese and sedentary but were not diabetic, dyslipidemic or hypertensive. Mean weight was 93.9 ± 13.6 kg, waist circumference 108 ± 22 cm and blood pressure 113/74 mmHg (S1 Table). There were no statistically significant correlations between BMI and baseline molecules. In addition to traditional diabetes and dyslipidemia biomarkers, we

analyzed an array of incretins, inflammatory cytokines, adipokines and neurometabolic peptides individually known to be responsive to exercise and lower-body positive-pressure. We found several novel baseline correlations between these different classes of peptides, relevant to our post-study outcomes (Table 1A and 1B).

Table 1. Baseline correlations of plasma A. cardio- and B. neuro-metabolic and appetitive molecules.

A			
*Glucose AUC**		r	p
	Glucose, 2-hr +	0.648	0.007
	Insulin AUC +	0.671	0.004
	C-peptide, AUC +	0.800	0.0001
	GIP	0.506	0.046
	Ghrelin	0.675	0.004
	Orexin-A	-0.558	0.025
	Oxytocin	-0.527	0.036
	Beta endorphin	-0.504	0.047
Insulin			
	GLP-1	0.704	0.002
	Glucagon	0.838	0.0001
	GIP	0.581	0.018
	Triglyceride	0.778	0.0001
GLP-1			
	Glucagon	0.802	0.0001
Interleukin-6			
	Insulin	0.574	0.020
	C-Peptide	0.474	0.06
	TNF-Alpha +	0.591	0.016
Triglyceride			
	TNF-Alpha	0.508	0.045
	Interleukin-6	0.482	0.058
	Glucagon	0.648	0.007
HDL-cholesterol			
	Ghrelin	0.621	0.01
	Glucagon +	0.572	0.021
B			
Leptin			
	Orexin A	-0.512	0.042
	Oxytocin	-0.578	0.019
	Beta endorphin	-0.624	0.010
Ghrelin			
	Adiponectin +	-0.453	0.078
Orexin A			
	Oxytocin	0.889	0.0001
	Beta endorphin	0.705	0.002
Beta endorphin			
	Oxytocin	0.821	0.0001

*2-hr after oral glucose (OGTT); AUC: area under curve

** Correlations are Pearson unless noted; + Spearman Correlation

Physical activity

Subjects ambulated for 30 minutes 2–3 times weekly (mean 2.2) for 11 weeks on the Alter-G treadmill at 60% body weight without complications. They walked/jogged a mean distance of 2 miles (3.2 km) each session at a mean rate of 4.3 mph. The weekly addition of 4 miles corresponded to a 19% increase in weekly ambulation from 21 to 25 miles. The average level of intensity was 8.5 METs calculated as corrected METs at 60% body weight, representing the lower end of recommended weekly expenditure (Table 2) [28].

Cardio- and neuro- metabolic changes

There were no statistically significant before-after mean differences in body weight, waist circumference or blood pressure. Eight subjects gained (range: 1.0–7.0 kg) and 6 lost weight (range: 2.2–9.5 kg), two exhibiting no change. Subjects, who had lost weight, disclosed *post hoc* during informal follow-up interviews that they had, on their own, dieted during and for several months following the study, although instructed to "eat as usual". Nevertheless, there were no statistically significant differences in outcomes between those with weight loss and those with weight gain. The mean duration between last exercise bout and blood testing was 9.8 days (range 28 hours—44 days), with no statistically significant relationships between time post last session and any outcome described in the following sections.

Dynamic Metabolic Testing (OGTT). After completion of the program, the 75-gram oral glucose challenge showed statistically significant decreases in both 2-hour (Fig 1B) and incremental *insulin* responses (34% [p = 0.002] and 15% [p = 0.022] respectively) concomitant with a significant 15% decrease in 2-hour C-peptide (p = 0.033) and trend towards a 19% decrease in 2-hour GLP-1 (p = 0.06; Table 3), together supporting reduced insulin resistance. There were only marginal, not statistically significant decreases in fasting, 2-hour and incremental *glucose* area.

Fasting cardiometabolic and inflammatory molecules. Several differences in fasting plasma parameters were noted comparing before to after program completion. Glucose, in the normal range decreased slightly (p = 0.17) while there was a 19% numerical, not statistically significant reduction in fasting plasma insulin (p = 0.36) (Fig 1A). There were no statistically significant changes in adiponectin which increased 2.6% (p = 0.61) or decreases in C-peptide, GIP and glucagon (S2 Table), with no significant change in fasting GLP-1. There was a trend toward increased leptin (12%; p = 0.084).

Triglycerides, not elevated in any subject at baseline, decreased 11% (p = 0.03) (S2A Fig) with discordant changes in free fatty acids. HDL cholesterol increased minimally (S2 Table).

Changes in inflammatory cytokines varied between subjects according to base-line status, undetectable in some, divergent in others. C-reactive protein (CRP) was elevated in 7 subjects

Table 2. Energy expenditure during twice-weekly LBPP treadmill sessions (mean ± SD).

Speed per session (mph)	4.3 ± 1.2
Distance per session (miles)	2.0 ± 0.55
Treadmill Grade (%)	6.4 ± 0.4
Days for completion of study	80 ± 28
Number of sessions	22 ± 2
Intensity (Corrected METs) [1]	8.5 ± 2.8
Corrected MET[1] minutes per week	512 ± 131
Total energy expended (MET minutes)[1]	5874 ± 1725

[1] at 60% of body weight (BW) [Ref. 27]

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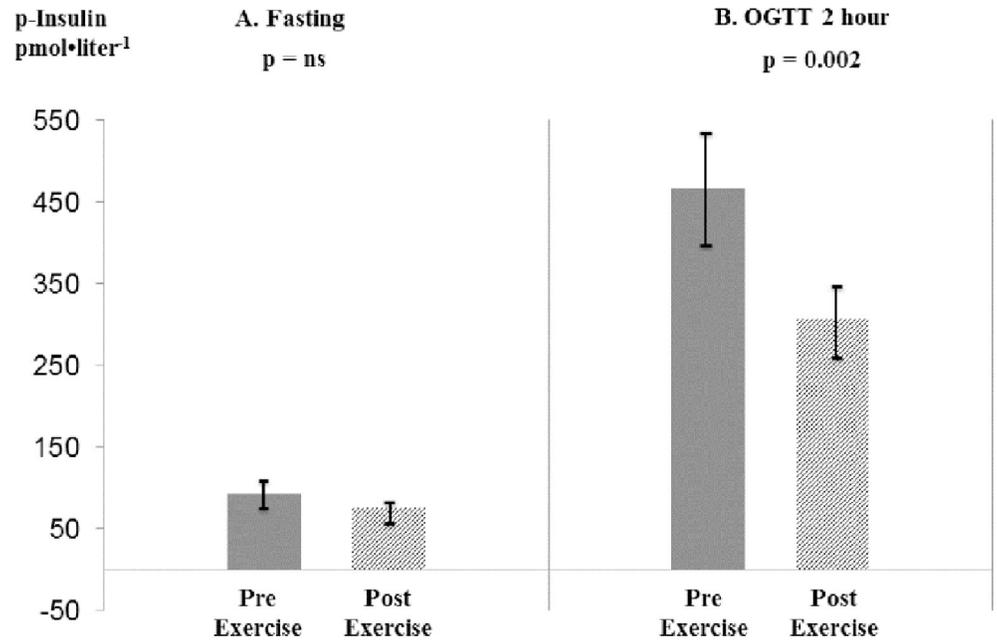
Fig 1. A. Fasting p-insulin. B. OGTT 2-hour p-insulin pre- and post- weight supported ambulation (n = 16; mean ± s.e.m.).

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in whom it decreased by 25% (p = 0.052). TNFα levels decreased in 7 subjects with detectable levels pre-exercise. Anti-inflammatory Interleukin-6, detectable at baseline in 8 subjects (mean 7.4 ± 4.9 pg/ml) increased 58%, not statistically significant post study (mean 11.6 ± 16.3; p = 0.170).

Fasting neuropeptides. Plasma ghrelin had decreased by 20% at the end of the study (p<0.008; S2B Fig) whereas there were no statistically significant changes in orexin A, which increased numerically 25% (p = 0.169), oxytocin 27% (p = 0.348) and β-endorphin 24%

Table 3. Changes in plasma cardio-, neuro-metabolic and appetitive molecules before (Pre-) and after (Post-) bodyweight-supported walking (mean ± SD).

		Pre	Post	Change	P
2 Hour Post Oral Glucose (OGTT)					
Glucose	mmol.liter^{-1}	6.4±2.2	6.0±1.4	-0.4±1.1	0.19
Insulin	pmol.liter^{-1}	467 ± 276	308 ± 179	-160 ± 174	0.002
C-Peptide	nmol.liter^{-1}	1.21 ± 0.46	1.03 ± 0.41	-0.18 ± 0.31	0.03
GLP-1	pmol.liter^{-1}	12.57 ± 5.3	10.18 ± 5.1	-2.39 ± 4.7	0.06
Fasting					
Glucose	mmol.liter^{-1}	5.0 ± 0.9	4.9 ± 0.9	-0.1 ± 0.5	0.17
Insulin	pmol.liter^{-1}	92 ± 59	76 ± 46	-17 ± 70	0.36
Triglyceride	mmol.liter^{-1}	0.80 ± 0.30	0.71 ± 0.25	-0.10 ± 0.16	0.03
Interleukin-6	pg.ml^{-1}	7.37 ± 4.87	11.62 ± 16.27	4.30 ± 11.8	0.17
Adiponectin (total)	µg.ml^{-1}	11.7 ± 4.6	12.0 ± 5.1	0.3 ± 2.3	0.61
Leptin	µg.liter^{-1}	31.0 ± 14.1	34. 6 ± 12.8	3.57 ± 7.7	0.08
Ghrelin	pmol.liter^{-1}	69.65 ± 41.60	55.97 ± 41.27	-13.68 ± 18.0	0.008
Orexin-A	pmol.liter^{-1}	150 ± 42	187 ± 91	37 ± 110	0.20
Oxytocin	pmol.liter^{-1}	89.3 ± 37.2	113.3 ± 85.8	24.0 ± 99.1	0.35
Beta endorphin	pmol.liter^{-1}	103 ± 40	128 ± 58	25 ± 67	0.16

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(p = 0.156). These relatively large within-subject numerical increases were statistically insignificant by 2-tailed t-tests owing to the relatively small numbers of subjects.

Correlations between before-after changes. There were no statistically significant correlations between body weight change or total energy expended (MET minutes) and decreases in OGTT glucose, 2-hr insulin, C-peptide, fasting triglycerides or ghrelin. Significant correlations were present between total MET minutes and decrease in glucoregulatory 2-hr GLP-1 (r = -0.679; p = 0.004) and the numerically small (0.3 µg/ml) increase in adiponectin (r = 0.471; p = 0.066). Changes in 2-hour GLP-1 were unrelated to changes in other appetitive peptides (ghrelin, Orexin A, β-endorphin).

Although parametric statistical analyses did not detect significant before-after changes in many peptides, the changes were remarkably highly and consistently correlated. Intercorrelations between drops in gluco-regulatory 2-hr glucose, related to TNFα, and 2-hr insulin and C-peptide were significant; 2-hr insulin, in turn was highly correlated with rises in β-endorphin, orexin A and oxytocin (Table 4). Baseline levels of leptin, marginally correlated with 2-hr insulin (r = 0.38; p = 0.143), predicted the reduction in 2-hr insulin (r = 0.566; p = 0.022). Significant correlation between increases in fasting leptin and adiponectin (r = 0.718; p = 0.002; Fig 2) reflected improved insulin sensitivity concordant with significant reductions in insulin and C-peptide. HDL-cholesterol change correlated with FFA AUC, leptin and adiponectin but not with any inflammatory cytokines (Table 4).

We found previously not reported highly significant correlations between changes in before-after exercise *neuro-endocrine* peptides in agreement with the pre-exercise correlations and unrelated to total energy expended, weight changes or any phenotypic data (Table 4).

Altogether, these findings suggest before-after reductions in gluco-regulatory molecules reflected in correlations between glucose, insulin, C-peptide and incretins during OGTT. These reductions correlate with inflammatory markers, adipokines and neuroregulatory, appetitive peptides.

Discussion

Physical activity

Cardio-metabolic factors. The preliminary findings of this unique study challenge the premise of the preponderance of studies and guidelines for treating and preventing diabesity, namely that body weight is of primary importance for the disease and requires intermediate to high levels of frequent physical activity to improve performance and to cause weight loss [29]. Our sedentary subjects did not lose weight from the low level of exertion in the sedentary range and, indeed, there were no associations between energy expended or weight loss or gain and improved cardio-metabolic metrics such as insulinemia, glucose tolerance markers and plasma triglycerides earlier described in literature supporting diet and exercise guidelines.

Robust reductions in plasma triglycerides in the normal range in our Black subjects, normally with lower levels than those with other geographic ancestry [30,31], are significant in the context of increased recognition of low-moderate triglyceride levels as cardio-metabolic risk factors [32] furthermore supporting the need to redefine blood lipid standards for Black people [31]. Hyperinsulinemia has long been known as a driver of hepatic triglyceride synthesis [33], which accords with our present finding of concomitant reductions of OGTT insulin and fasting plasma triglycerides after our low intensity ambulation which otherwise is found after intensive exercise [34, 35]. Our related findings of correlations between fasting leptin and adiponectin and reductions of 2-hr insulin and C-peptide reflecting improved insulin sensitivity might imply less resistance of leptin receptor exposed to increased levels of leptin.

Table 4. Correlations between changes in cardio- and neuro-metabolic molecules.

	r	p[*]
Glucose, 2-hr[*]		
Insulin, 2-hr[*]	0.445	0.084
TNF α	0.631	0.009
Leptin	0.482	0.059
Ghrelin	0.457	0.076
Orexin A	-0.502	0.048
Oxytocin	-0.422	0.103
Beta Endorphin[+]	-0.517	0.037
Insulin, 2-hr[*]		
C-Peptide, 2-hr[+]	0.538	0.031
Triglyceride	0.357	0.174
Leptin	0.475	0.063
Orexin A	-0.594	0.015
Oxytocin	-0.690	0.003
Beta Endorphin	-0.487	0.056
C-Peptide, 2-hr[*]		
C-Reactive Protein	0.451	0.079
GLP-1, 2-hr[*]		
Glucagon	0.555	0.026
IL-6	0.462	0.071
GIP		
Glucagon	0.580	0.019
C-Reactive Protein	0.532	0.034
HDL		
Leptin[+]	0.547	0.033
Adiponectin[+]	0.587	0.015
CRP		
IL-6[+]	0.689	0.003
Adiponectin		
Leptin	0.719	0.002
Orexin A		
Oxytocin	0.935	0.0001
Beta Endorphin	0.737	0.001
Oxytocin		
Beta Endorphin	0.783	0.0001

[*] 2 hours after oral glucose tolerance test (OGTT)

AUC area under curve of OGTT

[+] Spearman correlation; Pearson correlations unless noted

Our findings regarding *inflammatory markers* are similar to those of other studies of obese populations, although we generally excluded subjects with advanced disease sufficient to manifest detectable elevations of cytokines measured by routine methods. Our subjects were hyperinsulinemic, without impaired fasting glucose (with the exception of 3) or glucose tolerance, a pre-cursor to pre-diabetes. Nevertheless 7 subjects had detectable elevated CRP and 7 had elevated TNFα, all of whom reduced their levels post study, implying reduced inflammation associated with adiposity Three subjects did have impaired glucose tolerance (IGT) which
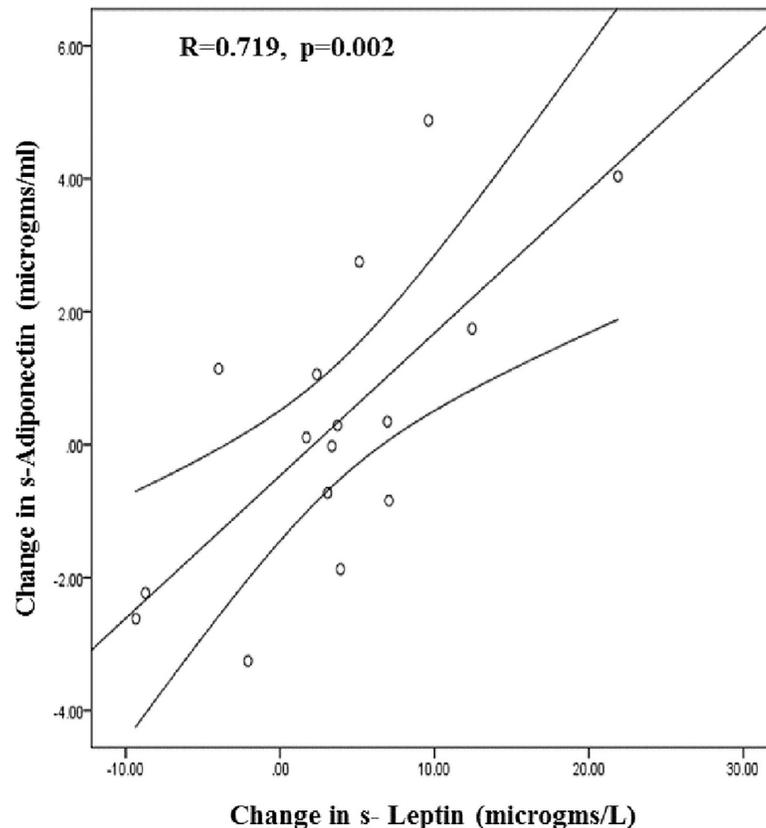
Fig 2. Pearson correlation of changes (Δ) in s-adiponectin and s-leptin, pre- and post- weight supported ambulation. [Y = 0.47+0.21X; r = 0.719; p = 0.002. Fit line 95% CI for the mean. (n = 16)].

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normalized post-study independent of weight changes. These three were among the subjects that increased post-study levels of the anti-inflammatory IL-6, known to be beneficially elevated by exercise [20, 36].

Neuro-endocrine peptides. Remarkably, the low amount of low intensity activity combined with mild lower-body positive pressure was associated with effects on a wide array of cholinergic regulatory central and peripheral peptides. There was a substantial statistically significant reduction in fasting levels of orexigenic ghrelin, a rapid sensor of fluctuations in nutrient stores that was not associated with statistically insignificant increases in 'satiety' peptides leptin and β-endorphin or orexin A shown in exercise studies [37–41]. Owing to the large statistically insignificant related increases in plasma orexin A, oxytocin and β-endorphin in our underpowered study, we performed a median split analysis, which exhibited statistically robust differences (all p<0.01; not shown), consistent internally and with exercise and baro-physiology literature. Since glucose is the primary substrate in brain, skeletal muscle and whole-body metabolism our finding of a strong relationship between the reduction of glucose area under the curve and ghrelin reduction (p<0.004) is interpreted as a primary response to ambulation preceding transition to lipid and amino acid utilization in the sedentary range. In addition, there were no relations between fasting plasma ghrelin and fasting and glucose-stimulated levels of the pleiotropic neuro-endocrine brain-gut peptide GLP-1. Although it is synthesized in the ileum and mediates incretin effects associated with insulinotropic pancreatic β-cell secretion exhibited in this study, GLP-1 is also known for its central effects suppressing appetite and increasing neurogenesis [20, 42], here demonstrating discordance between its peripheral

and central effects, possibly related to improved GLP-1 receptor function. Fasting and OGTT 2-hr GLP-1 have been shown by others to respond differentially to exercise [41].

The robust correlations between leptin and β-endorphin, orexin A and oxytocin and among these latter peptides are intriguing, given their respective roles in energy balance, neurogenesis and mood [43–46]. Although not the subject of this study, documented beneficial effects of exercise on anxiety and mood disorders [21, 47, 48] might be expected, related to our neuro-peptide changes after low-intensity exercise, especially in the absence of pain, a powerful mediator of mood. Informally all of our subjects have later spontaneously expressed their enjoyment of this exercise, especially those with experience with conventional treadmills. Given the problem of adherence to conventional exercise programs, it is conceivable that LBPP activity might be useful in the context of neuro-psychiatric studies in subjects with mobility disability [18]; the brain-gut peptides in this study participate in cholinergic circuits that modulate appetite suppression on downstream targets in the hypothalamus [49] and affect pancreatic structure, function and release of neurotrophic peptides other than GLP-1.

Our heterodox findings of improved glucoregulation with triglyceride decrease, mild effects on cytokines and adipokines but a robust effect on the orexigenic peptide ghrelin in the absence of weight loss accompanying brief, ambulation raises questions about putative mechanisms. We speculate that improvements in substrate utilization such as glucose disposal, lipolysis or "protein sparing" are not likely to be primary determinants, but rather synergistic with autonomic nervous system activation/conditioning ("stress buffering") [17] and/or release of key molecules through mild compression and use of lower extremity muscles known to have beneficial cardiovascular [50, 51], angiogenic and metabolic effects associated with restored autonomic balance.

Baro-physiology

Standing, *per se* improves cardiometabolic risk when used as a break in sedentary activity [21, 52] as do exercise "snacks" [53]. The compression exerted by the lower-body positive pressure treadmill during 40% off-loading is similar to standing in a swimming pool unrelated to muscle use. We have demonstrated decreased heart rate during weight offloading during standing [54], recently shown by others during increasing levels of exertion during LBPP [55]. We speculate that there is synergy between standing and positive lower body pressure which would cause local hypoxia increasing muscle perfusion and oxygen uptake [56, 57] as in pre-conditioning, thus potentiating the effects of the low intensity exercise improving mitochondrial function. Together with the described effects on the different classes of peptides, we posit that lower-body positive pressure increases parasympathetic tone in the cranio-sacral division of the autonomic nervous system, also reducing dysautonomia. Although not studied in these subjects ample evidence supports effects of LBPP on sympathetic tone, hemodynamics, oxygenation and metabotropic molecules [34, 38, 40, 41, 49, 50, 53, 56].

Public health perspective

A recent review of obesity identifies that "decreasing time spent in occupational physical activities and displacement of leisure-time physical activities with sedentary activities" contributes to epidemic obesity. "Lack of effective and accessible life-style programs that can be administered locally or remotely at low cost to diverse populations" explain why "only a fraction of patients for whom treatments are indicated actually receive them" [3]. Among those that are treated, only a minority complete programs and fewer still sustain benefits [5, 6].

Our findings imply that it is possible to improve metabolic fitness in underserved populations with high prevalence of diabesity, barriers to exercising and cultural resistance to losing

weight. The study coincides with reports of adverse effects of outdoor exercise in inner-city environments [58]. The indoor exercise in this study is provided by a safe and convenient weight-supporting lower-body pressure treadmill proven effective for ambulation in orthopedic and neurologic rehabilitation.

Effects of this magnitude on hyperinsulinemia, plasma triglycerides and ghrelin are exhibited after more time-consuming and intense volitional life-style changes or after metabolic operations recommended for type 2 diabetes [59]. The cost of an anti-gravity treadmill is well within the range of these modalities. This treadmill is safe and user-friendly and does not need supervising staff. Furthermore, it requires substantially less personal investment of time to use and is cost-effective since a single treadmill can be used by many subjects (e.g. in a family, therapeutically or preventively) over a long period, thus providing return on the initial investment regardless of payer.

Limitations

This within-subject before-after study has no controls. The premise for using a body weight-supporting, lower-body positive pressure "anti-gravity" treadmill in people with chronic obesity is that walking at a normative pace (for able-bodied women of the same age) is painful and hard for obese subjects. They cannot and will not painlessly and effortlessly engage in walking on a conventional treadmill or, indeed, walking as an activity of daily living, unless paid or convinced of the utility of doing so in conflict with their own experiences (and extant published evidence). Recruiting such subjects is not ethically compatible with equipoise, where both investigator and subject are cognizant of the lack of efficacy of the "control" condition.

Limitations are having a *selected population* of volunteering, obese, *female* hospital workers of Caribbean-Black ancestry not allowing generalization to other populations with different BMI standards and metabolic metrics. However, regardless of geographic heritage our subjects are reproductive-age obese, relatively low socio-economic status (SES) women, representative of a large stratum of prevalently obese populations. Nevertheless, we must emphasize that our findings are limited to women, given well-documented sex differences in the proteomics. This relatively small exploratory study of a convenience sample of volunteers will require replication in larger more diverse populations and in men.

Our *variances* are substantial, reflecting "real-world" clinical research in our environment with significant confounders, yet achieve statistical significance with trends consistent with exercise studies in very different populations and designs (from trained Caucasian male athletes to exhaustive sub-maximal acute studies). We were unable to coordinate pre- and post-exercise blood sampling with our subjects' *follicular phase*, which likely also has contributed to the variance. Time of day was variable for practical reasons. We did not detect any seasonality; the exercise was performed in a temperature-controlled gait laboratory, but we did not measure body temperature.

By design we omitted metrics of diet (macronutrient distribution, 3-day diaries), appetite (hunger ratings, taste preference), size and body composition (weight, BMI, lean body mass) and physical performance (O_2 consumption, lactate production) to avoid imposing biases and leaving these metrics to future studies. There might have been changes in "dietary factors" although we doubt that such changes would occur spontaneously within 11 weeks and with only two 30-minute bouts/week walking 4 miles per hour [60].

We gave no dietary instructions or recommendations and when asked by subjects told them to "continue as usual", although as mentioned some volitionally restricted their intake toward the end of the study and after completion.

This is not a conventional exercise study designed to improve physical fitness/performance; we did not collect such data. It is highly unlikely that 30 min of walking at a comfortable pace without exertion twice weekly for 10–12 weeks would measurably change VO₂ max (a max performance test), just as it would not measurably change body composition, such as decreasing body fat or increasing lean body mass sufficiently to explain improved glucose disposal or decreased plasma triglycerides.

We have not included repeat post-study questionnaires given before to assess stress, sleep, mood, quality of life etc. owing to the short duration of this pilot study seriously affecting re-test reliability of instruments with significant variances requiring larger populations to detect significant differences. However, as mentioned earlier all subjects spontaneously expressed their enjoyment of this exercise, and have since volunteered for more studies.

The unique nature of this study, using a novel, thus uncommon, FDA approved publicly available device by unsolicited volunteering, self-selected hospital employees from a special inner-city population may be construed as reflecting "selection bias". Therefore, we have taken great pains to emphasize the pilot nature of this exploratory hypothesis-generating study requiring replication in other more diverse populations and environments.

Conclusions

We conclude that weight-supported lower-body positive pressure enables low intensity walking in subjects otherwise not inclined to exercise who hereby experience beneficial cardio- and neurometabolic effects without pain. This level of energy expenditure, in the sedentary range, is unlikely to trigger known counter-regulatory mechanisms induced by glycogen depletion common to moderate-high intensity exertion of longer duration recommended in public health guidelines for weight loss and prevention of diabetes and co-morbidities. Amelioration of the dysautonomia of allostatic load is an explanatory candidate mechanism, supported by numerous pre-clinical and clinical studies of effects of exercise and lower-body pressure stimulating the parasympathetic nervous system, modulating cardiac autonomic function [61], central neurogenesis and cognitive function [62, 63] associated with mood.

Supporting information

S1 Table. Characterization of 16 Caribbean-Black women.
(DOCX)

S2 Table. Pre- post- changes in selected gluco-regulatory, lipid and inflammatory molecules.
(DOCX)

S1 Methods. Daily energy expenditure.
(DOCX)

S2 Methods. Blood chemistry.
(DOCX)

S1 Fig. Lower-body positive pressure treadmill (AlterG).
(DOCX)

S2 Fig. A. Fasting s-triglyceride and B. p-ghrelin pre- and post- weight supported exercise.
(TIF)

S1 Dataset.
(ZIP)

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References

1. https://disabilitycompendium.org/sites/default/files/useruploads/2016_ AnnualReport.pdf, accessed 11/22/2018.

2. Kritchevsky SB, Lovato L, Handing EP, Blair S, Botoseneanu A, Guralnik JM et al. Exercise's effect on mobility disability in older adults with and without obesity: The LIFE study randomized clinical trial.Obesity 2017; 25(7):1199–1205. https://doi.org/10.1002/oby.21860 PMID: 28653499

3. Heymsfield SB, Wadden TA. Mechanisms, Pathophysiology, and Management of Obesity. N Engl J Med. 2017; 376(15):1492. https://doi.org/10.1056/NEJMc1701944 PMID: 28402780

4. Schwartz MW, Seeley RJ, Zeltser LM, Drewnowski A, Ravussin E, Redman LM et al. Obesity Pathogenesis: An Endocrine Society Scientific Statement. Endocr Rev. 2017 Aug 1; 38(4):267–296. https://doi.org/10.1210/er.2017-00111 PMID: 28898979

5. MacLean PS, Wing RR, Davidson T, Epstein L, Goodpaster B, Hall KD et al. NIH working group report: Innovative research to improve maintenance of weight loss. Obesity (Silver Spring). 2015 Jan; 23(1):7–15. https://doi.org/10.1002/oby.20967 PMID: 25469998

6. Freedhoff Y, Hall KD. Weight loss diet studies: we need help not hype. Lancet. 2016 Aug 27; 388 (10047):849–51. https://doi.org/10.1016/S0140-6736(16)31338-1 PMID: 27597452

7. Simonson S, Shimon J, Long E, Lester B. Effects of a walking program using the Alter-G antigravity treadmill system on an extremely obese female: A case study. Clin Kinesiol. 2011; 65:29–38.

8. Pedersen BK, Saltin B. Exercise as medicine—evidence for prescribing exercise as therapy in 26 different chronic diseases. Scand J Med Sci Sports 2015; 25 Suppl 3:1–72.

9. Carter-Nolan PL, Adams-Campbell LL, Williams J. Recruitment strategies for black women at risk for noninsulin-dependent diabetes mellitus into exercise protocols: a qualitative assessment. J Natl Med Assoc. 1996; 88 (9):558–62. PMID: 8855646

10. Recommended levels of physical activity for adults aged 18–64 years. http://www.who.int/dietphysicalactivity/factsheet_adults/en/, accessed 11/22/2018

11. Mayer J, Thomas DW. Regulation of food intake and obesity. Science 1967; 156: 328–37. PMID: 4886532

12. Beaulieu K, Hopkins M, Blundell J, Finlayson G. Homeostatic and non-homeostatic appetite control along the spectrum of physical activity levels: An updated perspective. Physiol Behav. 2018 Aug 1; 192:23–29. https://doi.org/10.1016/j.physbeh.2017.12.032 PMID: 29289613

13. Shook RP, Hand GA, Drenowatz C, Hebert JR, Paluch AE, Blundell JE, et al. Low levels of physical activity are associated with dysregulation of energy intake and fat mass gain over 1 year. Am J Clin Nutr. 2015; 102(6):1332–8. https://doi.org/10.3945/ajcn.115.115360 PMID: 26561620

14. Dulloo AG, Miles-Chan JL, Schutz Y. Collateral fattening in body composition autoregulation: its determinants and significance for obesity predisposition. Eur J Clin Nutr. 2018; 72(5):657–664. https://doi.org/10.1038/s41430-018-0138-6 PMID: 29559726

15. Pontzer H, Durazo-Arvizu R, Dugas LR, Plange-Rhule J, Bovet P, Forrester TE, et al. Constrained Total Energy Expenditure and Metabolic Adaptation to Physical Activity in Adult Humans. Curr Biol. 2016 Feb 8; 26(3):410–7. https://doi.org/10.1016/j.cub.2015.12.046 PMID: 26832439

16. Rasgon NL, McEwen BS. Insulin resistance–a missing link no more. Mol Psychiatry 2016; 21 (12):1648–52. https://doi.org/10.1038/mp.2016.162 PMID: 27698431

17. Zschucke E, Renneberg B, Dimeo F, Wüstenberg T, Ströhle A. The stress-buffering effect of acute exercise: Evidence for HPA axis negative feedback. Psychoneuroendocrinology 2015; 51:414–25. https://doi.org/10.1016/j.psyneuen.2014.10.019 PMID: 25462913

18. Poulimeneas D, Yannakoulia M, Anastasiou CA, Scarmeas N. Weight Loss Maintenance: Have We Missed the Brain? Brain Sci. 2018 Sep 11; 8 (9). pii: E174. https://doi.org/10.3390/brainsci8090174] PMID: 30208568

19. Bailey DP, Broom DR, Chrismas BC, Taylor L, Flynn E, Hough J. Breaking up prolonged sitting time with walking does not affect appetite or gut hormone concentrations but does induce an energy deficit and suppresses postprandial glycaemia in sedentary adults. Appl Physiol Nutr Metab. 2016 Mar; 41 (3):324–31. https://doi.org/10.1139/apnm-2015-0462 PMID: 26872294

20. Batacan RB Jr, Duncan MJ, Dalbo VJ, Tucker PS, Fenning AS. Effects of light intensity activity on CVD risk factors: A systematic review of intervention studies. Biomed Res Int. 2015; https://doi.org/10.1155/2015/596367 PMID: 26543862

21. Duvivier BMFM, Schaper NC, Koster A, van Kan L, Peters HPF, Adam JJ, et al. Benefits of substituting sitting with standing and walking in free-living conditions for cardiometabolic risk markers, cognition and mood in overweight adults. Front Physiol. 2017 Jun 8; 8:353. https://doi.org/10.3389/fphys.2017.00353 PMID: 28642713

22. Dohrn I-M, Kwak L, Oja P, Sjöström M, Hagströmer M. Replacing sedentary time with physical activity: a 15-year follow-up of mortality in a national cohort. Clinical Epidemiology. 2018; 10:179–186. https://doi.org/10.2147/CLEP.S151613 PMID: 29416378

23. Ross R, Hill JO, Latimer A, Day AG. Evaluating a small change approach to preventing long term weight gain in overweight and obese adults—Study rationale, design, and methods. Contemp Clin Trials. 2016; 47:275–81. https://doi.org/10.1016/j.cct.2016.02.001 PMID: 26855121

24. http://www.alterg.com/products/anti-gravity-treadmills/m320-f320/professional-pt accessed 6/1/2017

25. Cutuk A, Groppo ER, Quigley EJ, White KW, Pedowitz RA, Horgens AR. Ambulation in simulated fractional gravity using lower body positive pressure: cardiovascular safety and gait analyses. J Appl Physiol (1985) 2006; 101:771–77. https://doi.org/10.1152/japplphysiol.00644.2005 PMID: 16777997

26. Ainsworth BE, Haskell WL, Hermann SD, Meckes N, Bassett DR Jr, Tudor-Locke C, et al. 2011 Compendium of Physical Activities: a second update of codes and MET values. Med Sci Sports Exerc. 2011; 43:1575–81. https://doi.org/10.1249/MSS.0b013e31821ece12 PMID: 21681120

27. Figueroa MA, Wicke J, Manning J, Escamilla P, Santillo N, Wolkstein J, et al. Validation of ACSM metabolic equations in an anti-gravity environment: A pilot study. Int J Appl Sci Technol. 2012; 2:204–10.

28. Riebe D (2014) General principles of exercise prescription. ACSM's Guidelines for Exercise Testing and Prescription. In Pescatello LS, Editor. Lippincott, Williams & Wilkins, Baltimore MD. 9th edition. Chapter 7: pp161–90.

29. Baker PR, Francis DP, Soares J, Weightman AL, Foster C. Community wide interventions for increasing physical activity. Cochrane Database Syst Rev. 2015; https://doi.org/10.1002/14651858.CD008366.pub3 PMID: 25556970

30. Proudler AJ, Godsland IF, Bruce R, Seed M, Wynn V. Lipid and carbohydrate metabolic risk markers for coronary heart disease and blood pressure in healthy non-obese premenopausal women of different racial origins in the United Kingdom. Metabolism 1996; 45: 328–33. PMID: 8606639

31. Lin SX, Carnethon M, Szklo M, Bertoni A. Racial/ethnic differences in the association of triglycerides with other metabolic syndrome components: the Multi-Ethnic Study of Atherosclerosis. Metab Syndr Relat Disord. 2011; 9: 35–40. https://doi.org/10.1089/met.2010.0050 PMID: 20958206

32. Nordestgaard BG, Varbo A. Triglycerides and cardiovascular disease. Lancet 2014; 384:626–35. https://doi.org/10.1016/S0140-6736(14)61177-6 PMID: 25131982

33. Kral JG, Lundholm K, Björntorp P, Sjöström L, Scherstén T. Hepatic lipid metabolism in severe human obesity. Metabolism 1977; 26:1025–31. PMID: 895533

34. Björntorp P, De Jounge K, Sjöström L, Sullivan L. The effect of physical training on insulin production in obesity. Metabolism 1970; 19 (8):631–8. PMID: 5429278

35. Mudaliar U, Zabetian A, Goodman M, Echouffo-Tcheuqui JB, Albright AL, Gregg EW, et al. Cardiometabolic Risk Factor Changes Observed in Diabetes Prevention Programs in US Settings: A Systematic Review and Meta-analysis. PLoS Med. 2016; https://doi.org/10.1371/journal.pmed.1002095 PMID: 27459705

36. Ropelle ER. IL-6 and IL-10 anti-inflammatory activity links exercise to hypothalamic insulin and leptin sensitivity through IKKbeta and ER stress inhibition. PLoS Biol 2010 Aug 24; 8(8). pii: e1000465.

37. Solomon TP, Malin SK, Karstoft K, Kashyap SR, Haus JM, Kirwan JP. Pancreatic β-cell function is a stronger predictor of changes in glycemic control after an aerobic exercise intervention than insulin sensitivity. J Clin Endocrinol Metab. 2013; 98:4176–86. https://doi.org/10.1210/jc.2013-2232 PMID: 23966244

38. Ellingsgaard H, Hauselmann I, Schuler B, Habib AM, Baggio LL, Meier DT, et al. Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. Nat Med. 2011; 17:1481–89. https://doi.org/10.1038/nm.2513 PMID: 22037645

39. Koehl M, Meerlo P, Gonzales D, Rontal A, Turek FW, Abrous DN. Exercise-induced promotion of hippocampal cell proliferation requires beta-endorphin. FASEB J. 2008; 22(7):2253–62. https://doi.org/10.1096/fj.07-099101 PMID: 18263701

40. Malkova D, McLaughlin R, Manthou E, Wallace AM, Nimmo MA. Effect of moderate-intensity exercise session on preprandial and postprandial responses of circulating ghrelin and appetite. Horm Metab Res. 2008; 40:410–415. https://doi.org/10.1055/s-2008-1058100 PMID: 18401836

41. Larson-Meyer DE, Palm S, Bansal A, Austin KJ, Hart AM, Alexander BM. Influence of running and walking on hormonal regulators of appetite in women. J Obes. 2012; 2012:1–15.

42. Coplan JD, Syed S, Perera TD, Fulton SL, Banerji MA, Dwork AJ, et al. Glucagon-like peptide-1 as predictor of body mass index and dentate gyrus neurogenesis: neuroplasticity and the metabolic milieu. Neural Plast. 2014; https://doi.org/10.1155/2014/917981 PMID: 25506432

43. Herisson FM, Waas JR, Fredriksson R, Schiöth HB, Levine AS, Olszewski PK. Oxytocin acting in the nucleus accumbens core decreases food intake. J Neuroendocrinol. 2016; 28(4). https://doi.org/10.1111/jne.12381 PMID: 27114001

44. Ito N, Yabe T, Gamo Y, Nagai T, Oikawa T, Yamada H, et al. I.c.v. administration of orexin-A induces an antidepressive-like effect through hippocampal cell proliferation. Neuroscience 2008; 157:720–32. https://doi.org/10.1016/j.neuroscience.2008.09.042 PMID: 18952152

45. Blevins JE, Ho JM. Role of oxytocin signaling in the regulation of body weight. Rev Endocr Metab Disord. 2013; 14:311–29. https://doi.org/10.1007/s11154-013-9260-x PMID: 24065622

46. Perello M, Raingo J. Leptin activates oxytocin neurons of the hypothalamic paraventricular nucleus in both control and diet-induced obese rodents. PLoS One 2013; 8(3):e59625. https://doi.org/10.1371/journal.pone.0059625 PMID: 23527232

47. Wildmann J, Krüger A, Schmole M, Niemann J, Matthaei H. Increase of circulating beta-endorphin-like immunoreactivity correlates with the change in feeling of pleasantness after running. Life Sci. 1981; 38 (11):997–1003.

48. DeBoer LD, Powers MB, Utschig AC, Otto MW, Smits JAJ. Exploring exercise as an avenue for the treatment of anxiety disorders. Expert Rev Neurother. 2012; 12:1011–22. https://doi.org/10.1586/ern.12.73 PMID: 23002943

49. Herman AM, Ortiz-Gozman J, Kochukov M, Herman L, Quast KB, Patel JM, et al. A cholinergic basal forebrain feeding circuit modulates appetite suppression. Nature 2016; 538(7624):253–56. https://doi.org/10.1038/nature19789 PMID: 27698417

50. Rodriguez D, Silva V, Prestes J, Rica RL, Serra AJ, Bocalini DS, et al. Hypotensive response after water-walking and land-walking exercise sessions in healthy trained and untrained women. Internat J Gen Med. 2011; 4:549–554

51. Afzal A, Fung D, Galligan S, Godwin EM, Kral JG, Salciccioli L, et al. The effect of lower body weight support on arterial wave reflection in healthy adults. J Am Soc Hypertens. 2014; 8:388–93. https://doi.org/10.1016/j.jash.2014.03.004 PMID: 24794204

52. Chastin S, Egerton T, Leask C, Stamatakis E. Meta-analysis of the relationship between breaks in sedentary behavior and cardiometabolic health. Obesity 2015; 23: 1800–10. https://doi.org/10.1002/oby.21180 PMID: 26308477

53. Francois ME, Baldi JC, Manning PJ, Lucas SJ, Hawley JA, Williams MJ, et al. 'Exercise snacks' before meals: a novel strategy to improve glycaemic control in individuals with insulin resistance. Diabetologia 2014; 57:1437–45. https://doi.org/10.1007/s00125-014-3244-6 PMID: 24817675

54. Salciccioli L, Kamran H, Liu Y, Kral JG, Lazar JM. Heart rate decreases during head out of water immersion and with lower body weight support: A measure of vagal reserve? J Strength Cond Res. 2017 Apr; 31(4):e69. https://doi.org/10.1519/JSC.0000000000000562 PMID: 28328721

55. Lee KY, Han JY, Kim JH, Kim DJ, Choi IS. Physiological responses during the lower body positive pressure supported treadmill test. Ann Rehabil Med. 2016; 40(5):915–23. https://doi.org/10.5535/arm.2016.40.5.915 PMID: 27847722

56. Eiken O. Responses to dynamic leg exercise in man as influenced by changes in muscle perfusion pressure. Acta Physiol Scand Suppl. 1987; 566:1–37. PMID: 3480686

57. Rifkind JM, Nagababu E, Dobrosielski DA, Salgado MT, Lima M, Ouyang P, et al. The effect of intermittent pneumatic compression of legs on the levels of nitric oxide related species in blood and on arterial function in the arm. Nitric Oxide 2014; 40:117–22. https://doi.org/10.1016/j.niox.2014.06.007 PMID: 24973574

58. Bos I, De Boever P, Vanparijs J, Pattyn N, Panis LI, Meeusen R. Subclinical effects of aerobic training in urban environment. Med Sci Sports Exerc. 2013; 45:439–47. https://doi.org/10.1249/MSS.0b013e31827767fc PMID: 23073213

59. Brito JP, Montori VM, Davis AM. Metabolic surgery in the treatment algorithm for type 2 diabetes: A Joint statement by international diabetes organizations. JAMA 2017; 317(6): 635–36. https://doi.org/10.1001/jama.2016.20563 PMID: 28196240

60. Whybrow S, Hughes DA, Ritz P, Johnstone AM, Horgan GW, King N, et al. The effect of an incremental increase in exercise on appetite, eating behaviour and energy balance in lean men and women feeding ad libitum. Br J Nutr. 2008 Nov; 100(5):1109–15. https://doi.org/10.1017/S0007114508968240 PMID: 18377694

61. Voulgari C, Pagoni S, Vinik A, Poirier P. Exercise improves cardiac autonomic function in obesity and diabetes. Metabolism 2013; 62(5):609–21. https://doi.org/10.1016/j.metabol.2012.09.005 PMID: 23084034

62. Erickson KI, Voss MW, Prakash RS, Basak C, Szabo A, Chaddock L, et al. Exercise training increases size of hippocampus and improves memory. Proc Natl Acad Sci U S A 2011; 108(7):3017–22. https://doi.org/10.1073/pnas.1015950108 PMID: 21282661

63. Inoue K, Okamoto M, Shibato J, Lee MC, Matsui T, Rakwail R, et al. Long-term mild, rather than intense, exercise enhances adult hippocampal neurogenesis and greatly changes the transcriptomic profile of the hippocampus. PLoS One 2015; Jun 10; 10(6):e0128720. https://doi.org/10.1371/journal.pone.0128720 PMID: 26061528