Original article

Obesity promotes oxidative stress and exacerbates blood-brain barrier disruption after high-intensity exercise

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Abstract

Purpose: The purpose of this study was to investigate the effects of obesity and high-intensity acute exercise on oxidant-antioxidant status, neurotrophic factor expression, and blood-brain barrier (BBB) disruption.

Methods: Twenty-four healthy, untrained men (12 non-obese (mean 14.9% body fat) and 12 obese subjects (mean 29.8% body fat)) performed 20 min of continuous submaximal aerobic exercise at 85% maximal oxygen consumption. Blood sampling was performed to examine the oxidant-antioxidant status (reactive oxygen species (ROS) and superoxide dismutase (SOD)), neurotrophic factors (brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF)), and BBB disruption (S100β and neuron-specific enolase) before and after acute exercise.

Results: The obese group showed significantly higher pre-exercise serum ROS levels and significantly lower pre-exercise serum SOD levels than the non-obese group ($p < 0.05$). Serum ROS, SOD, BDNF, NGF, and S100β levels were significantly increased post-exercise compared with pre-exercise levels in both the non-obese and the obese groups ($p < 0.05$). The obese group showed significantly higher serum ROS, BDNF, NGF, and S100β levels post-exercise compared to the non-obese group ($p < 0.05$).

Conclusion: Our study suggests that episodic vigorous exercise can increase oxidative stress and blood neurotrophic factor levels and induce disruption of the BBB. Moreover, high levels of neurotrophic factor in the blood after exercise in the obese group may be due to BBB disruption, and it is assumed that oxidative stress was the main cause of this BBB disruption.

1. Introduction

Cerebral vessels have a structure called the blood-brain barrier (BBB) composed of tight endothelial cell junctions, pericytes, astrocyte end-feet, and a basal lamina. The selective permeability of the BBB restricts the passage of harmful substances that can threaten normal brain function into the brain parenchyma from the extracerebral blood. Thus, the BBB provides a protective function for the brain against rapid changes in blood components.[1,2] On the other hand, a breakdown of the BBB caused by aging or other factors can induce cognitive impairment.[3] In addition, almost all the factors that contribute to the deterioration of the BBB's protective function have been reported to contribute to the pathogenesis of neurologic diseases such as epilepsy, multiple sclerosis, and Alzheimer's disease.[2,4]

The main factors involved in BBB disruption are endoplasmic reticulum stress, glutamate excitotoxicity, and formation of reactive oxygen species (ROS).[5] Increased oxidative stress caused by excessive ROS production and compromised intrinsic antioxidant defense contribute to BBB disruption through several mechanisms, including oxidative damage to cellular molecules, cytoskeletal reorganization, and upregulation of inflammatory mediators.[6] When BBB disruption occurs, high concentrations of S100β and neuron-specific enolase (NSE), brain-specific proteins circulating inside the brain, are observed in the peripheral blood. The concentrations of these proteins have been reported as an index for estimating the extent of the increase in BBB permeability and brain damage.[7,8]

Obesity not only causes diabetes, high blood pressure, and cardiovascular disease but also has recently been reported to

be closely associated with the onset of neurodegenerative disorders.[9,10] A suggested cause of such diseases is an obesity-induced high oxidative stress level in the body.[11] According to Vincent et al.,[12] obesity was accompanied by chronically high oxidative stress levels because of the imbalance between tissue ROS and antioxidants. Olusi[13] also reported that compared to obese subjects with a body mass index (BMI) ≥ 40 kg/m^2, healthy subjects showed significantly lower plasma malondialdehyde concentrations and significantly higher activities of the antioxidant enzymes erythrocyte superoxide dismutase (SOD) and glutathione peroxidase. In addition, it was reported that the brain was less resistant to oxidative stress-induced damage than other tissues because of its relatively lower content of antioxidant enzymes against ROS; therefore, oxidative stress-induced damage to the brain could lead to neuronal apoptosis, triggering the onset of neurodegenerative disorders.[14,15] All these reports suggest a role for obesity in BBB disruption and the pathogenesis of neurodegenerative disorders.

Not only is participation in regular exercise effective for prevention and alleviation of obesity, but it can also reduce oxidative stress levels that have been elevated because of obesity.[16,17] It has also been reported that both regular exercise training and acute exercise were effective for maintaining and enhancing brain function by increasing the expression of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), regardless of the presence of a variety of diseases.[18] However, excessively intense acute exercise can metabolically impair dynamic cerebral autoregulation by overproducing free radicals, leading to the mechanical disruption of the BBB.[19] It is also possible that high oxidative stress levels damage the microtubule cytoskeleton, interfering with vesicular transport and inducing the downregulation of neurotrophic factors.[20] In addition, according to a report by Vincent et al.,[21] the concentrations of plasma hydroperoxides and thiobarbituric acid reactive substances were significantly higher after acute exercise in obese subjects than in non-obese subjects. This suggests that the oxidative stress level of an obese individual can be higher than that of a healthy individual, not only at rest but also immediately after exercise.

Thus, compared to a non-obese individual, in an obese individual it is considered to be a higher risk that acute exercise will decrease the brain protective function of the BBB by increasing the oxidative stress level in the body while also affecting neurotrophic factor expression. However, there have been no reports concerning the effect of obesity and acute high-intensity exercise on oxidative stress levels in the body, neurotrophic factor expression, and BBB disruption. Accordingly, the present study aimed to examine the changes in oxidant-antioxidant status, neurotrophic factor expression, and BBB disruption in non-obese and obese subjects performing high-intensity acute exercise.

2. Methods

2.1. Subjects

Twenty-four healthy untrained men volunteered as subjects for the present study. All subjects met the following criteria before enrollment in the study: (1) no participation in regular physical activity, (2) no chronic health problems or smoking, (3) no history of cardiovascular, metabolic, or respiratory disease, and (4) no consumption of antioxidant supplements within the past 3 months. Subjects attended a brief orientation meeting before data collection, and each subject read and signed a written informed consent statement consistent with Yonsei university guidelines. Subjects were placed into 1 of 2 groups based on BMI and % body fat. Subjects with ≥25% body fat and a BMI ≥25 kg/m^2 were placed into the "obese" group, and those who had <25% body fat and BMI <25 kg/m^2 were placed into the "non-obese" group (Table 1). All study procedures were approved by the National Research Foundation of Korea (NRF-2013S1A5B5A07049580).

2.2. Anthropometric measurements

Anthropometric measures taken 1 week before beginning exercise testing included the measurement of height, body composition, resting blood pressure (BP), and maximal oxygen consumption (VO$_{2max}$). Height and body composition were measured using a stadiometer (seca 213; seca GmbH, Hamburg, Germany) and a bioimpedance analysis device (InBody 220; BioSpace, Seoul, Korea), respectively. BP was measured in a seated position using standard auscultation procedures and a mercury sphygmomanometer (TRIMLINE; PyMaH, Somerville, NJ, USA). VO$_{2max}$ was measured on a treadmill (Q65; Quinton, Seattle, WA, USA) according to the Bruce protocol based on the breath-by-breath method,[22] with each participant wearing a gas analyzer (METAMAX 3B; Cortex, Leipzig, Germany).

2.3. Acute exercise test

The exercise test was carried out by means of a treadmill run of 20 min at an intensity of 85% of the anthropometrically measured VO$_{2max}$. According to the Bruce protocol, with each subject wearing a gas analyzer, VO$_2$ measurement began with exercise onset and continued until each subject's VO$_2$ reached the target value of 85%VO$_{2max}$. At that point, exercise intensity was controlled by adjusting the speed and slope of the treadmill to maintain a VO$_2$ steady state at 85%VO$_{2max}$.

Table 1
Subject characteristics and anthropometric measures (mean ± SD).

Variable	Non-obese ($n = 12$)	Obese ($n = 12$)	p
Age (year)	22.9 ± 2.2	22.9 ± 2.2	1.000
Height (cm)	174.5 ± 3.9	173.2 ± 4.6	0.461
Weight (kg)	65.6 ± 4.2	87.9 ± 10.4	<0.001**
Fat mass (kg)	9.8 ± 2.3	26.1 ± 5.8	<0.001**
BMI (kg/m^2)	21.5 ± 1.6	29.3 ± 3.0	<0.001**
Body fat (%)	14.9 ± 3.2	29.8 ± 3.6	<0.001**
Resting SBP (mmHg)	116.5 ± 4.8	123.1 ± 6.3	0.009*
Resting DBP (mmHg)	74.2 ± 5.6	82.9 ± 7.3	0.003*
VO$_{2max}$ (mL/kg/min)	54.3 ± 3.8	41.8 ± 6.8	<0.001**

*$p < 0.01$, **$p < 0.001$, as determined using the independent t test.
Abbreviations: BMI = body mass index; DBP = diastolic blood pressure; SBP = systolic blood pressure; VO$_{2max}$ = maximal oxygen consumption.

2.4. Blood sampling and analyses

Using a 22-gauge needle and a serum separator tube (Becton Dickinson, Franklin Lakes, NJ, USA), 10 mL of blood was collected from the antecubital vein of each subject immediately pre- and post-exercise. Collected blood samples were centrifuged for 15 min at 3000 rpm and were then stored at −80°C until analysis. The analysis of serum ROS was performed using an OxiSelect *in vitro* ROS/RNS Assay Kit (cat. no. STA-347; Cell Biolabs, San Diego, CA, USA), which is based on the conversion of 2′,7′-dichlorodihydrofluorescein diacetate to 2′,7′-dichlorodihydrofluorescein by ROS; a fluorescence plate reader (LS 55 Luminescence spectrometer; Perkin Elmer, Wellesley, MA, USA) was used to measure the absorbance of fluorescence at 480 nm (excitation) and 530 nm (emission). The analysis of serum SOD was performed using a superoxide dismutase assay kit (cat. no. CM706002; IBL-International, Hamburg, Germany); a microplate reader (GENios; TECAN, Grödig, Austria) was used to measure absorbance at 450 nm. The analyses of serum BDNF and NGF were carried out using a human BDNF enzyme-linked immunosorbent assay (ELISA) kit (cat. no. DBD00; R&D Systems, Minneapolis, MN, USA) and an NGF sandwich ELISA kit (cat. no. CYT304; Chemicon, Temecula, CA, USA), respectively; a microplate reader (EMax; Molecular Devices, Sunnyvale, CA, USA) was used to measure absorbance at 450 nm for quantification. The analyses of serum S100β and neuronspecific enolase (NSE) were performed using an S100B (human) ELISA kit (cat. no. KA0037; Abnova, Jhongli, Taiwan, China) and a human NSE ELISA kit (cat. no. M-0050; Alpha Diagnostic International, San Antonio, TX, USA), respectively; a microplate reader (EMax) was used to measure absorbance at 450 nm for quantification.

2.5. Statistical analyses

Statistical analyses were performed with SPSS statistics for Windows Version 21.0 (IBM Corp., Armonk, NY, USA). Data are presented as the mean ± SD and coefficient of variation, unless otherwise stated. Independent *t* tests were conducted to compare baseline levels of all variables between non-obese and obese groups. Two groups (non-obese and obese) by time point (pre- and post-exercise) repeated measures analysis of variance (ANOVA) was used to examine the effect of exercise on serum ROS, SOD, BDNF, NGF, S100β, and NSE levels. When significant group by time interactions occurred, simple main effects were assessed using independent and paired *t* tests. Levels of significance were set at 0.05.

3. Results

3.1. Subject characteristics

Table 1 shows the subject characteristics. The obese group had significantly greater weight, fat mass, BMI values, % body fat, resting BP, and lower VO_{2max} levels compared with the non-obese group ($p < 0.05$). Baseline variables of age and height were similar between the groups.

3.2. Changes in serum oxidant-antioxidant status

After exercise, repeated-measures ANOVA demonstrated a significant difference across the group by time interaction for serum ROS ($F(1, 11) = 8.346$, $p = 0.015$) and SOD ($F(1, 11) = 4.947$, $p = 0.048$) levels. Serum ROS and SOD levels were significantly increased in post-exercise compared with pre-exercise levels in both the non-obese and the obese groups ($p < 0.05$). In addition, pre- and post-exercise serum ROS levels were significantly higher in the obese group than in the non-obese group ($p < 0.05$). Pre-exercise serum SOD levels were significantly lower in the obese group than in the non-obese group ($p < 0.05$) (Table 2).

3.3. Changes in serum neurotrophic factor levels

After exercise, repeated-measures ANOVA demonstrated a significant difference across the group by time interaction for serum BDNF ($F(1, 11) = 5.973$, $p = 0.033$) and NGF ($F(1, 11) = 8.030$, $p = 0.016$) levels. Serum BDNF and NGF levels were significantly increased in post-exercise compared with pre-exercise levels in both the non-obese and the obese groups ($p < 0.05$). In addition, post-exercise serum BDNF and NGF levels were significantly higher in the obese group than in the non-obese group ($p < 0.05$) (Table 2).

3.4. Changes in serum BBB disruption indices

After exercise, repeated-measures ANOVA demonstrated a significant difference across the group by time interaction for

Table 2

Changes in serum ROS, SOD, BDNF, NGF, S100β, and NSE levels in non-obese and obese groups before and after exercise (mean ± SD).

Variable	Non-obese		Obese	
	Pre	Post	Pre	Post
ROS (mmol)	1.48 ± 0.32	4.75 ± 1.21*	1.93 ± 0.33#	6.05 ± 0.85*,#
SOD (U/mL)	2.65 ± 0.54	3.06 ± 0.71*	2.13 ± 0.66#	3.24 ± 1.00*
BDNF (pg/mL)	27,656.17 ± 5758.55	34,082.00 ± 5420.95*	26,661.25 ± 6217.93	39,387.73 ± 5799.72*,#
NGF (pg/mL)	348.83 ± 67.94	406.05 ± 26.68*	344.88 ± 61.58	449.35 ± 37.43*,#
S100β (ng/L)	52.75 ± 13.79	65.59 ± 18.58*	59.25 ± 16.35	92.47 ± 22.14*,#
NSE (μg/L)	6.23 ± 1.17	6.86 ± 1.36	6.48 ± 1.21	7.70 ± 1.12

* $p < 0.05$, compared with pre-exercise; # $p < 0.05$, compared with non-obese.
Abbreviations: BDNF = brain-derived neurotrophic factor; NGF = nerve growth factor; NSE = neuron-specific enolase; ROS = reactive oxygen species; SOD = superoxide dismutase.

serum S100β levels ($F(1, 11) = 7.422$, $p = 0.020$). Post-exercise serum S100β levels were significantly higher than pre-exercise levels in both the non-obese and the obese groups ($p < 0.05$). In addition, post-exercise serum S100β levels were significantly higher in the obese group than in the non-obese group ($p < 0.05$). In contrast, serum NSE levels were not significantly different among any groups or time points ($F(1, 11) = 1.530$, $p = 0.242$) (Table 2).

4. Discussion

Obesity increases oxidative stress by inducing excessive ROS production through hyperglycemia, excessive blood lipids (free fatty acids), and excessive fat stores in white adipose tissue. Obesity also induces inadequate antioxidant defenses.[23] Regular exercise can reduce the elevated oxidative stress levels caused by obesity.[12] High-intensity acute exercise, however, has been shown to cause either a decrease or an increase in oxidative stress levels.[21] The present study analyzed serum ROS and SOD levels to examine the effect of obesity and high-intensity acute exercise on oxidant-antioxidant status. According to the results, the obese group showed significantly higher baseline ROS levels and significantly lower SOD levels than the non-obese group. This is considered a consequence of a pro-oxidant/antioxidant imbalance promoted by obesity. This is supported by previous studies that have demonstrated an increase in ROS levels, which reflects a high oxidative stress level in the body,[24] when pro-oxidant production prevails over the antioxidant defense system.[25] Additionally, Vincent and Taylor[23] reported an obesity-induced pro-oxidant/antioxidant imbalance with the depletion of enzymatic antioxidants such as SOD.

In addition, compared to non-obese individuals, obese individuals showed significantly higher thiobarbituric acid reactive substance levels, an index of blood oxidative stress,[26,27] but significantly lower activities of such antioxidant enzymes as glutathione peroxidase and SOD.[13,28] On the other hand, the results of the present research show that high-intensity acute exercise increased ROS levels in both the obese and the non-obese groups but that the ROS level was significantly higher in the obese group immediately after exercise. This is assumed to be because of an acceleration of H_2O_2 production, which is a type of ROS generated in obese subjects by a rapid increase in oxygen consumption with vigorous exercise. ROS is generated by the incomplete reduction of oxygen, which is excessively consumed during high-intensity aerobic exercise. Anderson et al.[29] reported that the overconsumption of oxygen in obese subjects is related to mitochondrial H_2O_2 production and the cellular redox state. Fernández-Sánchez et al.[28] suggested that one of the obesity-induced ROS production mechanisms was the increase of oxygen consumption caused by obesity through the increase in mechanical load and myocardial metabolism. The results of the present study are also supported by Vincent et al.,[21] who reported that obese subjects showed higher ROS levels than non-obese subjects after aerobic exercise.

The BBB is a multicellular vascular structure that plays a protective role for the brain by separating the central nervous system from the peripheral blood circulation.[2] The present study analyzed serum S100β and NSE levels to examine the effect of obesity and high-intensity acute exercise on BBB disruption. The results showed that high-intensity acute exercise increased S100β concentrations in both the obese and the non-obese groups but that S100β levels immediately after exercise were significantly higher in the obese group than in the non-obese group. It is believed that this occurs because high-intensity acute exercise induced BBB leakage by increasing ROS production and that BBB disruption in the obese group was accelerated by the additional increase in ROS production owing to obesity. This interpretation is supported by studies showing elevated serum S100β levels in the presence of computed tomography-verified BBB disruption.[30,31] Bailey et al.[19] suggested that the increase of serum S100β levels after aerobic exercise reflects BBB disruption and that this impairment of the dynamic cerebral autoregulation is caused by the overproduction of free radicals generated during high-intensity exercise. In addition, a study by Tucsek et al.[32] suggested that microglial activation, upregulation of proinflammatory cytokines, and high oxidative stress levels can damage the BBB through increased oxidative stress in obese subjects. This increased stress induces a disruption of tight junctions and exacerbates the neuroinflammatory response, despite the presence of protective mechanisms.

With regard to NSE, however, the present research showed no significant differences in NSE concentration. This is in accordance with the preceding report that showed a >80% increase in S100β levels after acute aerobic exercise but no significant difference between pre- and post-exercise NSE levels.[19] This may also be because changes in serum NSE levels are a good index of brain damage but not of BBB disruption. This interpretation is supported by a previous study showing an elevation of serum S100β levels with BBB disruption rather than with neuronal damage.[33] Other studies have proposed that NSE is a peripheral marker for neuronal damage even in the absence of increased BBB disruption.[30,34]

In addition, it is possible that the changes in S100β in this study are caused by damage to the skeletal muscles, because S100β levels increased after intense exercise without a significant increase in serum NSE levels. S100 proteins regulate intracellular processes such as cell growth and motility, cell cycle regulation, and transcription and differentiation in various peripheral tissues,[35] and previous studies have reported that vigorous exercise can induce muscular damage with elevated S100β blood levels from skeletal muscle damage. S100β also stimulates myogenic differentiation and myoblast proliferation, assisting effective muscle regeneration.[36,37] Future studies are needed to examine the significance of S100β as a marker of muscle damage.

Many preceding studies reported that increased neurotrophic factor expression plays a positive role in brain function improvement through involvement in proliferation, migration, survival, and differentiation of neurons as well as in the regulation of synaptic plasticity.[18,38,39] The present study analyzed serum BDNF and NGF levels to examine the effect of obesity and high-intensity acute exercise on neurotrophic factor expression. The results showed that high-intensity acute exercise sig-

nificantly increases BDNF and NGF concentrations in both the obese and the non-obese groups. This supports previous studies that reported a significant increase in peripheral BDNF and NGF levels after submaximal acute exercise.[40,41] Gold et al.[40] reported a significant increase in serum BDNF and NGF concentrations after 30 min of aerobic exercise at 60%VO_{2max}. Slusher et al.[41] also reported that acute aerobic exercise at 75%VO_{2max} could significantly increase BDNF levels in both normal-weight and obese subjects. On the other hand, it has been reported that elevated oxidative stress levels are involved in the downregulation of neurotrophic factors[20] and that high oxidative stress levels have a negative correlation with BDNF levels but a positive correlation with antioxidant capacity.[42,43] Interestingly, however, the results of the present study showed that BDNF and NGF levels immediately after exercise were significantly higher in the obese group, which showed higher ROS levels, than in the non-obese group. It is possible that high oxidative stress in obese subjects is linked to an increase in BBB permeability, resulting in the release of BDNF and NGF from the brain into the blood. Obesity increases the production of H_2O_2 in the mitochondria, leading to oxidative stress and resulting in increased permeability of the BBB with vigorous exercise. This interpretation is supported by reports showing that BDNF and NGF produced in the brain can pass through the BBB[18,44] and also by a study by Goekint et al.[45] that showed that one of the mechanisms responsible for an increase in serum BDNF levels after acute exercise might be an increase in BBB permeability. In addition, because blood BDNF levels are related to central and peripheral neuronal activity,[46,47] we cannot exclude the possibility that increased BDNF levels seen with vigorous exercise in this study were not caused by an increased recruitment of motor units. Additional studies are necessary.

This study has several limitations. Even though our findings suggest that increased serum S100β levels found in the obese group after vigorous exercise reflect BBB disruption, we were not able to examine serum S100β levels in the post-exercise recovery period and do not know if this change was temporary or long lasting. Also, this study evaluated the relationship between high oxidative stress and BBB disruption, but we were unable to evaluate for pathologic changes in the nervous system because this was not an animal study. Furthermore, the study sample size was small; therefore, further studies are necessary to validate these results.

5. Conclusion

Our study suggests that episodic vigorous exercise can induce oxidative stress, increase the levels of blood neurotrophic factors, and induce BBB disruption. It also suggests that increased blood neurotrophic factor levels immediately after exercise in obese subjects are the result of BBB disruption and oxidative stress.

Authors' contributions

HTR participated in study design, subject recruitment, data collection, data processing, data analysis, and drafted the manuscript; SYC participated in data analysis, and drafted the manuscript; WYS conceived of the study, participated in its design and coordination, and helped to draft the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Competing interests

The authors declare that they have no competing interests.

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