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Original Article

Effects of exercise on netrin-1 and TNF-α levels in non-inflammatory conditions

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Abstract

Aim: Exercise has been shown to increase netrin-1 expression and decrease TNF-α response in inflammatory conditions. However, the effect of exercise on netrin-1 and TNF-α levels in non-inflammatory conditions has not yet been explicated. The objective of this study was to investigate the effect of exercise on netrin-1 levels in relation to TNF-α.

Material and Methods: The exercise group (n=6) consisted of 6 male Balb-c mice that were kept in cages with access to a running wheel for 6 weeks. The control group (n = 6) was kept in cages without a running wheel for the same time period. At the end of 6 weeks, both groups were sacrificed. Netrin-1 and TNF-α levels of the muscle, brain, and kidney tissues were measured.

Results: Netrin-1 and TNF-α levels were not different between the two groups in all tissues. Netrin-1 and TNF-α levels were correlated in the brain, muscle, and kidney tissues of the exercise group and the muscle and kidney tissues of the control group.

Conclusion: In non-inflammatory conditions netrin-1 and TNF-α levels are correlated in muscle and kidney tissues but not in brain tissues. Exercise does not affect netrin-1 and TNF-α levels of muscle and kidney tissues but induces a correlation between netrin-1 and TNF-α levels of brain tissue.

Keywords: Exercise; netrin-1; TNF-α; muscle; brain; kidney

INTRODUCTION

Netrin-1 is a glycoprotein which has been shown to guide axon growth during embryonic development. Netrin-1 guides axons by chemoattractive and chemorepulsive signals to their destination to make synapses in a three-dimensional environment. Although chemotactic properties are complex, the netrin receptor deleted in colorectal cancer (DCC) is responsible for chemoattraction and UNC5 is responsible for chemorepulsion and binding of netrin to the receptor (DCC or UNC5) induces changes in the cytoskeleton of the cell (1).

Besides regulatory role of netrin-1 in the establishment of neuronal networks in the embryologic development of the brain, mature adult brain also synthesizes netrin-1 (2). Our knowledge about function of netrin-1 in the developed brain is limited but netrin-1 signaling pathway components are promising therapeutic targets in the treatment of neurologic diseases (2). Netrin-1 has anti-inflammatory and neuroprotective roles in the adult brain. In a number of neurological disease models in which inflammation contributes to the disease like multiple sclerosis, netrin-1 signaling activated anti-inflammatory or neuroprotective signaling pathways (2). Netrin-1 infusion restricted β-amyloid accumulation and improved memory function of mice in an Alzheimer's disease model (3). Recently, Wong et al. (4) reported that neuron originated netrin-1 had a major regulatory role in synaptic transmission and plasticity and was required for spatial memory consolidation.

Following the discovery of netrin-1, the presence of netrin-1 and its receptors have been shown in tissues other than the nervous system. Furthermore, netrin-1 plays a regulatory role in the angiogenesis, inflammation, the immune response, formation and development of tissues (5,6). Immunomodulatory functions of netrin-1 have

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been reported recently (5,7). During acute inflammation netrin-1 is expressed on the endothelial cell surface. It reduces TNF-α response (5), neutrophil migration to the extravascular space, neutrophil and proinflammatory monocyte infiltration to the inflammatory tissue and suppresses COX2 expression (7).

TNF- α is a key cytokine in the regulation of inflammation which was first described as a cytotoxic agent against tumor cells in 1975. Activated macrophages and monocytes are the primary sources of TNF- α production, but it is also produced by neutrophils, lymphocytes, mastocytes, fibroblasts, and smooth muscle cells (8). TNF- α is the first cytokine detected in the blood in response to tissue damage and bacterial products like lipopolysaccharides. It is a chemoattractant for neutrophils and helps neutrophil migration by increasing the expression of adhesion molecules on the endothelial cells (9).

Exercise causes stress at the cellular level and leads to a cytokine response which is different from the inflammatory cytokine response. Although TNF-α is the first cytokine in the cytokine cascade in infections, its levels do not change in response to exercise (10). However, in the presence of inflammation, exercise decreases TNF-α levels and the inflammatory response (11,12) and increases netrin-1 levels (13,14).

Although it is known that exercise increases netrin-1 expression and reduces TNF-α levels in inflammatory conditions (11-14) there is not any available data about the effect of exercise on netrin-1 levels in relation to TNF-α levels in non-inflammatory conditions. The aim of this study was to investigate TNF-α and netrin-1 levels in different tissues of healthy mice and to evaluate the effect of exercise on netrin-1 and TNF-α levels of the muscle, brain, and kidney tissues.

MATERIAL and METHODS

Animals and experimental design

Twelve adult male Balb-c mice were used in this study. The mean weight of the exercise group and control group were 30.58 ± 1.44 g and 29.71 ± 1.37 g respectively. Mice were housed in individual cages in a 12h-light/12h-dark cycle at constant humidity (60%) and room temperature (22±1°C) with ad libitum access to laboratory chow and water. Animal Care Committee of Dokuz Eylul University School of Medicine's approval for the study (35/2018) was provided. Study was carried out in Experimental Research Laboratory of Physiology Department in Dokuz Eylul University. Mice were divided into two groups randomly; exercise (n=6) and control (n=6) groups. After an adaptation period of one week, a running wheel with a diameter of 11.5 cm was placed in the cages of the exercise group where mice had free access to the running wheel for 6 weeks. The control group stayed in cages during the 6-week study period without a running wheel in their cages.

Voluntary wheel running is a common model to investigate the physiological effects of aerobic exercise. Mice run freely inside a plastic wheel. Daily running distance can be calculated by the number of rotations of the wheel, which is counted by a digital counter connected to the wheel (15,16).

At the end of 6 weeks of exercise, both the control and exercise groups were sacrificed under CO2 anesthesia. Left gastrocnemius, left kidney and brain tissues of mice were removed. Gastrocnemius muscles were cut into half longitudinally and medial part of the muscles were used in biochemical evaluations. Kidneys were cut into half coronally and dorsal parts of kidneys were used in biochemical evaluations. Cerebrum of the brain was dissected and was cut into half (left and right cerebrums). Left cerebrums were used in biochemical evaluations. All tissues were stored at −80°C until biochemical analysis.

Biochemical evaluations

Frozen samples of muscle, kidney, and brain tissues (cerebrum) were weighed and homogenized with steel beads using the BioSpec Mini-Beadbeater-16 (BioSpec Products Inc., USA) in 10 volumes of PBS, pH:7.4 and centrifuged at 5000 g for 15 min at 4°C. The supernatants were used for all biochemical analyses.

Measurement of netrin-1 and TNF-α levels in the tissues was performed with mouse-specific ELISA kits in accordance with the kit protocols. Mouse TNF-α ELISA kit (Elabscience®, USA; Catalog No: E-EL-M0049, assay sensitivity: 18.75 pg/mL, detection range 31.25 ~ 2000 pg/mL) and mouse netrin-1 ELISA kit (Bioassay Technology Laboratory, China; Catalog No: E1802Mo, assay sensitivity: 0.38 ng/L, detection range 0.5 ~ 200 ng/L) were used. Protein analysis was performed according to the manufacturer's guide for the BCA protein Assay kit (BCA Protein Assay Kit, PierceTM, USA; Catalog No:23227). All results were calculated with mg protein per tissue. Absorbency changes were measured using a microplate reader (ELx800, BioTek Instruments, Inc., Winooski, VT, USA) at 450 nm for ELISA kits and 560 nm for the protein assay kit.

Statistical Analysis

SPSS software for Windows, version 22.0 (SPSS, Chicago, IL) was used for the statistical analysis. Mann-Whitney U test was used to analyze differences between the groups. Spearman correlation analysis was used to calculate correlations between the tissue levels of netrin-1 and TNF-α. Results were presented as mean ± standard deviation.

RESULTS

Wheel-running distance was recorded at 1 p.m. each day for 6 weeks. Mean running distance for the exercise group in running wheel cages was 2.7 ± 0.8 km/day and running time was 158 ± 6.41 min/day

Netrin-1 and TNF-α levels of the exercise group were not statistically different from the control group in all tissues (Table 1).

Table 1. Mean netrin-1 and TNF-α levels of brain, muscle and kidney tissues of control and exercise groups

	Netrin-1 (pg/mg protein)		TNF-α (pg/mg protein)	
	Control Group	Exercise Group	Control Group	Exercise Group
Tissue				
Brain	1.64 ± 0.28	1.36 ± 0.44	6.89 ± 1.22	6.77 ± 1.25
Muscle	4.52 ± 0.67	3.54 ± 1.29	23.35 ± 6.73	16.33 ± 7.12
Kidney	0.74 ± 0.71	0.72 ± 0.39	4.25 ± 4.47	3.62 ± 2.43

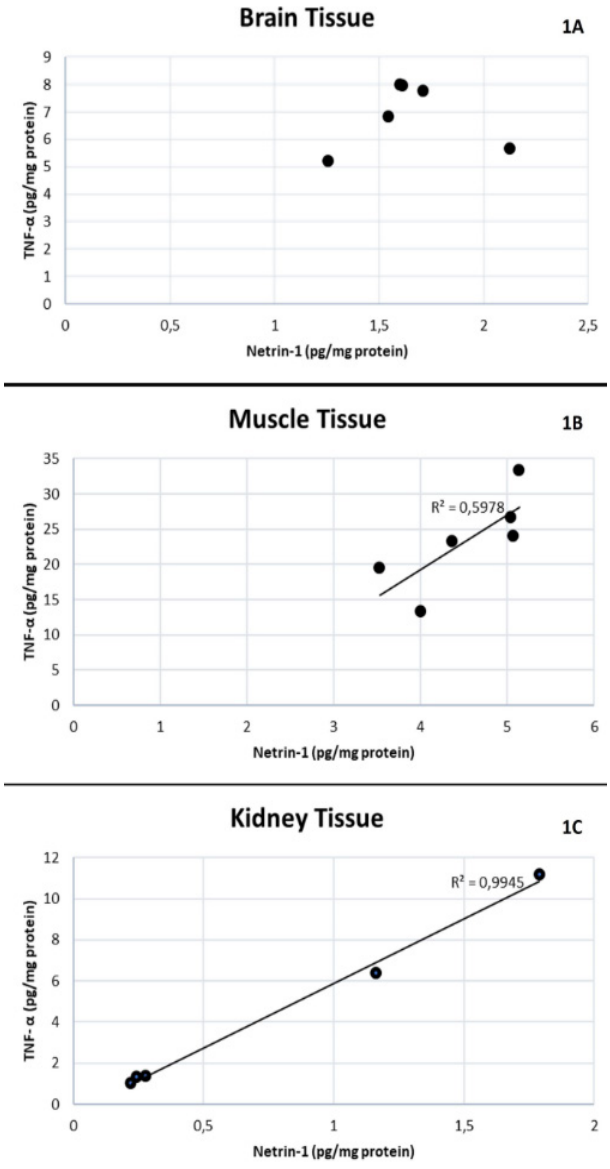

Figure 1. Correlation graphics of control group; A: brain tissue, B: muscle tissue, C: kidney tissue

There was a strong correlation between netrin-1 and TNF-α levels of the brain , muscle and kidney tissues for the exercised group (brain, r= 0.829, p= 0.042; muscle, r= 0.943, p= 0.005, kidney, r= 1.0, p<0.0001, r:correlation coefficient) (Figure 1A-1B-1C) and muscle and kidney tissues of the control group (muscle, r= 0.886; kidney , r= 1.0, p<0.0001) (Figure 2B-2C). Netrin-1 and TNF-α levels of the brain were not correlated in control group (p=0.7) (Figure 2A).

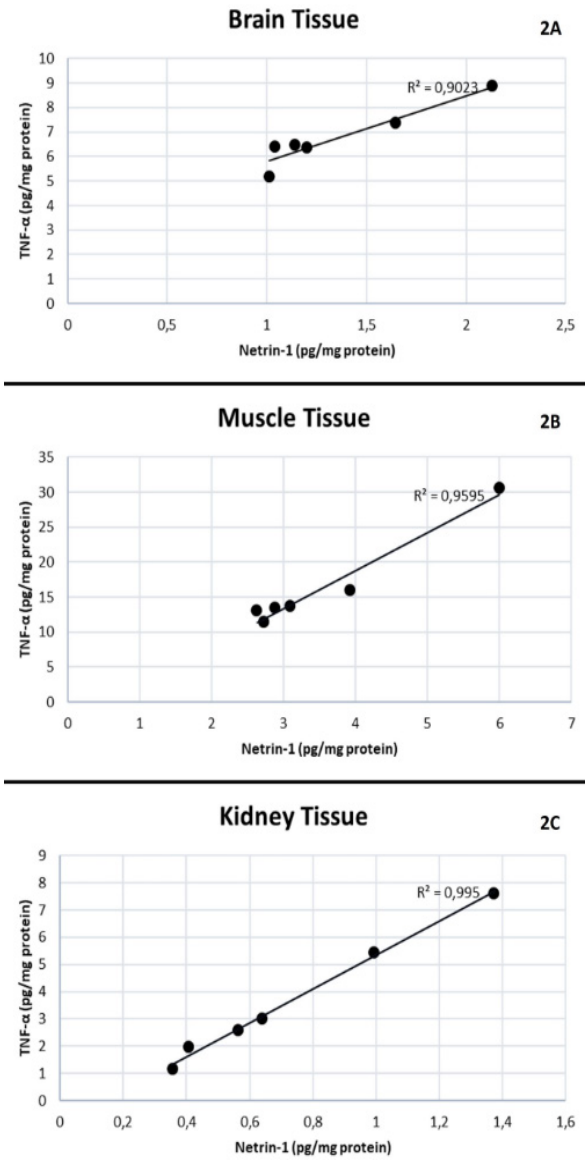

Figure 2. Correlation graphics of exercise group; A: brain tissue, B: muscle tissue, C: kidney tissue.

DISCUSSION

Netrin-1 and TNF-α levels were not affected by regular aerobic exercise in the muscle, brain, and kidney tissues of the healthy mice. Although inflammatory cytokine cascade starts with TNF-α, aerobic exercise induced cytokine cascade typically starts with an increase in IL-6 and followed by increases in IL-1 and IL-10. Both acute and chronic exercise in moderate-intensity did not affect TNF-α levels (10). Compatible with the literature, TNF-α levels did not change with aerobic exercise in this study. Similarly, netrin-1 levels also were not affected by regular aerobic exercise. To our knowledge, this is the first study examining the effects of exercise on netrin-1 levels in healthy mice.

Netrin-1 administration in inflammatory conditions decreased the inflammatory response and protected tissues from excessive damage. Exogenous administration of netrin-1 decreased inflammatory changes in acute inflammatory peritonitis (17) and acute pancreatitis (18). Mutz et al. (7) and Mirakaj et al. (19) reported reduced pulmonary inflammatory changes and reduced TNF-α levels in acute lung injury by the application of netrin-1. Tadagavadi et al. (20) induced ischemia-reperfusion injury within mice kidneys. Ischemia and reperfusion increased TNF-α production and caused kidney injury which was reduced by netrin-1 administration. Also, similar effects were shown in excessive endogen production of netrin-1. Overexpression of netrin-1 by gene transfer promoted cerebral healing following middle cerebral artery occlusion in mice (21) and ameliorated cisplatin nephrotoxicity (22).

Exercise has anti-inflammatory effects, especially in inflammatory conditions. Starkie et al. (12) injected Escherichia Coli endotoxin to healthy volunteers and investigated TNF- α response. Exercise blunted the TNF-α response in comparison with the control subjects. Circulating TNF-α levels were higher than control subjects in inflammatory conditions like diabetes (10), cardiovascular diseases (10,23), rheumatic diseases (11). Regular aerobic exercise reduced high TNF-α levels to the level of control subjects (10,11,23).

Effect of exercise on netrin-1 levels was investigated by two other studies (13-14). Both investigated the effect of exercise in inflammatory conditions, and both reported that exercise increased netrin-1 expression. Daliang et al. (13) induced myocardial infarction in rats by ligation of the coronary artery. One group exercised for 10 weeks following the surgical procedure. Both exercising and non-exercising myocardial infarction groups' TNF-α levels were higher than the controls. TNF-α levels of the exercising myocardial infarction group were significantly lower, and netrin-1 levels were significantly higher than the non-exercising myocardial infarction group. The exercise group developed less myocardial fibrosis, and Daliang et al. (13) concluded that regular exercise following myocardial infarction reduced the development of myocardial fibrosis through increased netrin-1 expression. Liu et al. (14) induced cerebral ischemia via middle cerebral artery occlusion in rats. Rats are forced to run on a treadmill following surgery 30 minutes a day for four weeks. Aerobic exercise enhanced netrin-1 expression in the peri-ischemic brain areas of rats simultaneously with improvements in behavioral tests. They concluded that netrin-1 has a role in exercise's beneficial effect following ischemic brain injury (14).

Although exercise increases netrin-1 expression and reduces TNF-α levels in inflammatory conditions, this was not the case in healthy mice as these levels were not affected. The anti-inflammatory effect of exercise has been well studied. Exercise can induce cortisol and increase IL-6 levels up to 100-fold. Cortisol is a potent anti-inflammatory hormone. IL-6 inhibits TNF-α synthesis in the monocytes, which inhibits TNF-α release from the monocytes (24). However, knowledge of the regulatory mechanisms encompassing the relationship between exercise and netrin-1 remains limited and requires further investigation.

In this study, netrin-1 levels were strongly correlated with TNF-α levels of the muscle and kidney tissues. Netrin-1 has anti-inflammatory effects and reduces TNF-α production (5-7). However, the effects of TNF-α to netrin-1 expression remains contradictory. Yang et al. (25) reported that the primary mouse colon epithelial cells treated with TNF-α increased netrin-1 production after 5 hours in a dose-dependent manner. Similarly, when the blood-brain barrier (BBB) endothelial cells were treated with TNF-α netrin-1 expression increased (26). Conversely, LY et al. (27) treated human umbilical vein epithelial cells with TNF-α and demonstrated a 90% decrease in netrin-1 mRNA levels at 6 hours following stimulation. Since 10 ng/ml netrin-1 dosage was used in both studies, the difference might be due to the tissues being different.

We did not observe a correlation between netrin-1 and the TNF-α levels in the brain tissues of the sedentary mice. Interestingly exercise changed this situation, and we observed a strong positive correlation in the brain tissues of the exercised mice. It has been reported that the main source of netrin-1 in the brain is BBB endothelial cells, and netrin-1 expression in the brain during homeostasis is minimal (26,27). BBB endothelial cells increased netrin-1 production in response to TNF-α. TNF-α altered the expression of tight junction proteins causing decreased and fragmented synthesis within endothelial cells. Netrin-1 reduced this effect of TNF-α on endothelial cells and netrin-1 was required for proper expression of the junctional proteins. Exercise also enhanced BBB function and prevented tight junction damage. Decreased TNF-α response of the macrophages to lipopolysaccharide with aerobic training has been shown before (28). Similarly, exercise could elicit an increased responsiveness of netrin-1 expression to TNF-α, which might be the reason this correlation was found in the exercised group

CONCLUSION

Netrin-1 and TNF-α appear to have a complex interaction. Netrin-1 and TNF-α levels are correlated in muscle and kidney tissues but not in brain tissues. Exercise does not affect netrin-1 and TNF-α levels of muscle and kidney

tissues but induces a correlation between netrin-1 and TNF-α levels of brain tissue. Since little is known about the effects of exercise on these interactions and the role of netrin-1, there is a definite need for further research focusing on netrin-1 and exercise.

Competing interests: The authors declare that they have no competing interest.

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Ethical approval: This study was approved by the Institutional Ethics Committee and conducted in compliance with the ethical principles according to the Declaration of Helsinki.

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