Original Article

Effects of Exercise and a High-Fat, High-Sucrose Restriction Diet on Metabolic Indicators, Nr4a3, and Mitochondria-Associated Protein Expression in the Gastrocnemius Muscles of Mice with Diet-Induced Obesity

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Background: Exercise and high fat, high sucrose restriction diets are well known treatments for obesity. The aim of this study was to measure the effects of those lifestyle interventions on molecular transducers of exercise, such as Nr4a3, mitochondria-associated proteins, and muscle function.

Methods: We conducted 8 weeks of treadmill exercise and sucrose or fat restriction diets in obese mice. The mice were divided into eight groups: the normal diet (CON) group, normal diet with exercise (CONEX) group, high fat, high sucrose diet (HFHS) group, HFHS with exercise (HFHSEX) group, sucrose restriction (SR) group, SR with exercise (SREX) group, high fat, high sucrose restriction (ND) group, and ND with exercise (NDEX) group.

Results: The 8 weeks of exercise reduced body weight, improved lipid profiles (total cholesterol, triglycerides), and increased hanging time. The combination of exercise and a fat and sucrose restriction diet improved glucose tolerance and increased grip strength. The 8 weeks of intervention did not significantly affect the Nr4a3 protein level. The sucrose and fat restriction diet increased the phosphorylated protein kinase B (pAkt)/Akt ratio, and its level was lower in the HFHS group. Exercise increased the protein expression level of PGC-1α in obese conditions. Moreover, SR led reduced the phosphorylated AMP-activated protein kinase (pAMPK)/AMPK ratio and PGC-1α to the control level.

Conclusion: The 8 weeks of exercise or a sucrose and fat restriction diet improved metabolic indicators and muscle function. SR reduced pAMPK/AMPK and PGC-1α to the control level. Nr4a3 protein expression was not significantly changed by either exercise or a fat and sucrose restriction diet.

Key words: Metabolic diseases, Obesity, Exercise, Sucrose, Fat, Mitochondria, Nr4a3

INTRODUCTION

It is widely known that metabolic diseases, such as type 2 diabetes mellitus (T2DM), obesity, and metabolic syndrome, pose serious risks to public health. To make matters worse, the prevalence of those diseases is projected to increase in the future.[1,2] Among the metabolic diseases, metabolic syndrome is a complex disease that involves central obesity, dyslipidemia, and insulin resistance.[3] Previous research has revealed that obesity and T2DM are major causes of metabolic syndrome.[4]

Although various methods for the treatment of metabolic syndrome and obesity are being discovered and implemented, lifestyle interventions—for example, exercise and high sucrose, high fat restriction diets—are widely used for their potential cost effective-

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ness[5] and few side effects. In addition, exercise has salutary physiological effects, not only for healthy people but also for patients, when its routine is properly prescribed.[6]

In terms of the molecular transducers of exercise, the exercise-responsive transcription factor neuron-derived orphan receptor 1 (Nr4a3 or NOR1) is a ligand-free orphan receptor, and its gene expression is elevated by acute aerobic exercise and beta-adrenergic signaling.[7,8] Functionally, Nr4a3 has a causal relationship with glucose-stimulated insulin secretion in islets[9] and the translocation of GLUT4 in L6 cells, which can affect glucose utilization.[10] An Nr4a3 hyperexpression study showed that glucose transport and protein kinase B (Akt) phosphorylation are increased in C2C12 cells.[11] A study of an Nr4a3 transgenic mouse model reported an increase in oxidative capacity, which is representative of an increase in mitochondrial content and the proteins of the mitochondrial transport chain complexes.[12] However, the effects of exercise or diet-induced reductions in fat and sucrose on Nr4a3 and, by extension, on mitochondrial related proteins (AMP-activated protein kinase [AMPK] and peroxisome proliferator-activated receptor gamma coactivator 1-alpha [PGC-1α]) in obese conditions remain elusive.

In conjunction with Nr4a3, AMPK and PGC-1α are also well-known molecular transducers of exercise. In particular, these proteins are associated with mitochondria and play a role as metabolic sensors that can contribute to the regulation of metabolic homeostasis.[13] In addition, they are the major molecules composing the key pathway of PGC-1α–NRF1–TFAM mitochondrial biogenesis, and their expression is affected by endurance exercise.[14,15] People with T2DM and insulin resistance are reported to have decreased mitochondrial function.[16] Therefore, investigating changes in the expression of AMPK and PGC-1α in various obesity or metabolic conditions is important to improve treatments for metabolic diseases.

The effects of chronic exercise and a sucrose and fat restriction diet on Nr4a3 and mitochondria-associated protein expression in metabolically abnormal, obese conditions is inadequately understood.[17,18] These proteins could be a therapeutic target to cure metabolic diseases. The purpose of this study was to investigate the effect of aerobic exercise and a high-fat, high-sucrose diet (HFHS) on the molecular transducers of exercise, Nr4a3 and the mitochondrial function-associated proteins AMPK and PGC-1α. In addi-

tion, changes in muscle function and metabolic indicators were measured.

METHODS

Animal and diet protocols

Three-week-old male C57Bl/6 mice were purchased from the central lab and raised in the College of Veterinary Medicine at Seoul National University. The mice were housed in a consistent, specific pathogen-free, ad libitum environment (temperature, 22°C±2°C; humidity, 55%–60%; 12 hour-light/dark cycle). The animal experiments were approved by the Animal Care and Use Committee (IACUC No. SNU-170518-9) at Seoul National University. The animals were closely monitored during the breeding period. The normal (Research Diets, New Brunswick, NJ, USA; D12450J) and high fat (Research Diets, D12492), high sucrose (Daejung, Siheung, Korea; Saccharose, 7501-4400) diets were purchased and supplied. The composition of the intervention group diet was 60% fat, 20% protein, and 20% carbohydrate, whereas that of the control group diet was 10% fat, 20% protein, and 70% carbohydrate. Compared with the normal plain water, the high sucrose concentration was 24%, and it was sanitized. Sample preparation was conducted after the 8-week intervention. The mice were fasted for 16 hours and anesthetized by isoflurane. Blood was collected by cardiac puncture using a tube (BD Microtainer, Franklin Lakes, NJ, USA). Whole groups of gastrocnemius muscles were collected at the same time for the biochemical analysis.

Experimental design

As shown in Fig. 1, after a 1-week adaptation period, we supplied a high fat, high sucrose diet to the mice for 8 weeks to induce an obese condition. Next 8 weeks of exercise and diet intervention were conducted. The obese mice (HFHS) were randomly divided into six groups for the intervention period: sucrose restriction (SR; n=11), sucrose restriction+exercise (SREX; n=11), high fat+high sucrose (HFHS; n=16), high fat+high sucrose+exercise (HFHSEX; n=11), high fat, high sucrose restriction group (ND; n=11), and high fat, high sucrose restriction group+exercise (NDEX; n=11). The non-obese control mice are presented as the control (CON; n=6), control+exercise (CONEX; n=6) groups (Table 1).

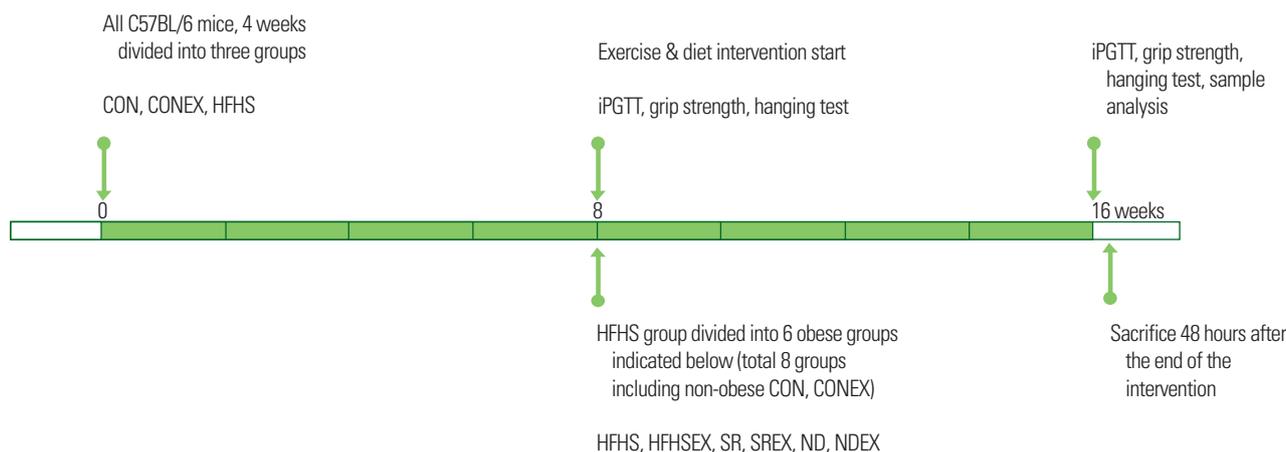Lee JH, et al. Effects of Exercise and Diet on Muscle

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Figure 1. Research design. CON, normal diet group; CONEX, normal diet with exercise group; HFHS, high fat, high sucrose diet; iPGTT, intraperitoneal glucose tolerance test; HFHSEX, high fat, high sucrose with exercise; SR, sucrose restriction; SREX, sucrose restriction with exercise; ND, high fat, high sucrose restriction group; NDEX, high fat, high sucrose restriction with exercise group.

Table 1. Group description

Group	Group explanation		Intervention	Intervention duration
	Diet	Exercise		
Non-obese				
CON	Normal chow	No	None	None
CONEX	Normal chow	Treadmill	Exercise	8 wk
Obese				
SR	High fat	No	Sucrose R	8 wk
SREX	High fat	Treadmill	Sucrose R, Ex	8 wk
HFHS	High fat, high sucrose	No	Fat, sucrose F	8 wk
HFHSEX	High fat, high sucrose	Treadmill	Fat, sucrose F, Ex	8 wk
ND	Normal chow	No	Fat, sucrose R	8 wk
NDEX	Normal chow	Treadmill	Fat, sucrose R, Ex	8 wk

R, restriction; Ex, exercise; F, feeding.

Body weights and the food supply were measured three times per week. After some animals were chosen for the biochemical analysis (n = 6), the remaining mice (n = 5) were used to conduct the hanging time test, grip strength test, and intraperitoneal glucose tolerance test (iPGTT). The gastrocnemius muscles were collected 48 hours after the end of the intervention and stored at −70°C until their use in the biochemical analysis.

Exercise protocol

An Exer 3/6 treadmill (Columbus Instruments, Columbus, OH, USA) was used to exercise the animals. For the first day of adaptation, the speed was 8 m/min for 5 minutes, and on the second day, it was 5 minutes at 8 m/min and 5 minutes at 10 m/min. During the 8-week intervention period, moderate exercise (65%–70% based

on previous studies) was conducted in all exercise groups.[19,20] The speed was increased by an increment of 2 m/min every 2 minutes until the speed of 12 m/min, and then the speed was fixed for 1 hour. At the end of the hour of exercise, the mice were run at a speed of 5 m/min for 5 minutes as cool down phase. The increment of the treadmill was 12°. During each exercise period, the mice were monitored carefully and encouraged to keep running with a soft touch using a brush. To prevent injury to the mice, a sponge was installed at the end of the track.

Hanging time test

The hanging time test was conducted to measure muscle condition.[21] An iron grid 250 mm × 200 mm, with 1 cm × 1 cm holes was used. The mice were put on the grid and made to grasps with all four paws firmly. The grid was then turned upside down and fixed 25 cm above a soft sponge. After the grid was reversed and fixed, we measured the time until the mouse fell from the grid. There were two trials and the summation of the two trial times was used. If the mouse fell off too fast or would not grasp the grid, then they were allowed to take a rest.

Grip strength test

The grip strength test was conducted to measure the strength of the limb muscles.[21] The mice were placed on the grid and made to grasp the grid firmly. The tail of each mouse was then pulled until they let go of the grid. A grip strength meter was used. We per-

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formed three trials for each mouse and used the greatest force recorded overall. The results were normalized by the body weight of each mouse.

Biochemical and serum lipid analysis

To analyze the protein content of the muscles in the mice used in this study, the gastrocnemius muscle was properly collected, and western blot testing was conducted. To extract the protein from the muscle homogenate, a lysis buffer (RIPA buffer; Thermo Fisher Scientific, Waltham, MA , USA; #89900) was used along with a protease (Sigma-Aldrich, Darmstadt, Germany; #4693159001) and a phosphatase (Sigma-Aldrich, #4906845001). To quantify the supernatant, we used a BCA analysis (Pierce BCA protein assay kit, Thermo Scientific). After the extraction was completed, the remaining sample was stored at –20°C in sodium dodecyl sulfate polyacrylamide (SDS) sample buffer.

The separation of the protein was conducted using SDS gel electrophoresis with a 12% acryl amide gel. The protein was transferred to a polyvinylidene fluoride membrane under 100 V for 1 hour. The blocking of the membrane was conducted using 5% skim milk. The primary antibody used in the experiment and the membrane were stored overnight at 4°C. To detect Nr4a3, the exposure time was 20 minutes, and the membrane was covered with a film to prevent drying. The following antibodies were used: phospho-Akt (Ser473) antibody #9271 (Cell Signaling, Danvers, MA, USA; 1:1,000), anti-PGC1 alpha antibody #ab54481 (Abcam, Cambridge, UK; 1:1,000), Akt antibody #9272 (Cell Signaling, 1:1,000), phospho-AMPKα (Thr172) (40H9) rabbit mAb #2535 (Cell Signaling, 1:1,000), AMPKα (D5A2) rabbit mAb #5831 (Cell Signaling, 1:1,000), monoclonal antibody-succinate dehydrogenase complex, subunit A, flavoprotein variant [SDHA] (D6J9M) XP rabbit mAb #11998 (Cell Signaling, 1:1000), anti-NOR1 (Nr4a3) antibody (ab94507) rabbit (Abcam, 1:500), and anti-GAPDH (#2118; Cell Signaling, 1:5,000). To image the protein, Immobilon western chemiluminescent horseradish peroxidase substrate (#WBKLS0500; Millipore, Burlington, MA, USA) and Nano quant (Tecan, Männedorf, Switzerland) were used. Serum lipid (triglyceride [TG] and total cholesterol [TC]) concentrations were measured using kits (Asan Pharm, Seoul, Korea), and the whole process followed the manufacturer's guideline.

Intraperitoneal glucose tolerance test

The iPGTT was conducted after a 16-hour fast. First, 250 mg× mL^{-1} glucose water solution at 2.5 g×kg^{-1} body weight was injected into each mouse intraperitoneally. The blood from the tail vein was used to measure the glucose levels (Accu-Chek Go; Roche Diagnostic, Mannheim, Germany). The time points for testing were 0, 30, 60, and 120 minutes after the glucose injection.

Statistical analysis

For the statistical analysis, Graph Pad Prism 7 (Graph Pad Inc., La Jolla, CA, USA) and IBM SPSS version 23 (IBM Corp., Armonk, NY, USA) were used. Data are presented as mean ± standard error of the mean. The rubric for statistical significance was $P < 0.05$. One-way, two-way analysis of variance (ANOVA) and independent Student t-test were used for comparisons among groups.

RESULTS

Effects of high-fat, high-SR diet and aerobic exercise on metabolic indicators

Body weight changes were noted during the 8 weeks of exercise and dietary intervention in this study. Compared with the HFHS group, the body weights of all groups except the HFHSEX, SR, and SREX groups were significantly lowered beginning 2 weeks after the intervention began ($P < 0.05$). The ND and NDEX groups recovered their weight to control levels, and the exercise group showed a lower body weight than the non-exercise group (Fig. 2A).

In the iPGTT test, the ND and NDEX groups showed significantly lower glucose levels than the HFHS group over time ($P < 0.05$). In addition, the overall glucose level of the SREX group was lower than that of the SR group after 30 minutes, but the ND and HFHS groups did not differ significantly (Fig. 2B, C).

Blood lipid levels were measured to show changes in lipid metabolism during the intervention. The TC level in the HFHS group was significantly higher than that in the control group ($P < 0.05$). The SREX group had significantly lower TC than the SR group, and the NDEX group had lower TC than the ND group ($P < 0.05$). Compared with the NDEX group, the SR, SREX, ND, and NDEX groups showed significantly lower blood TC levels ($P < 0.05$) (Fig. 2D). The TG level in the HFHS group was higher than that of the
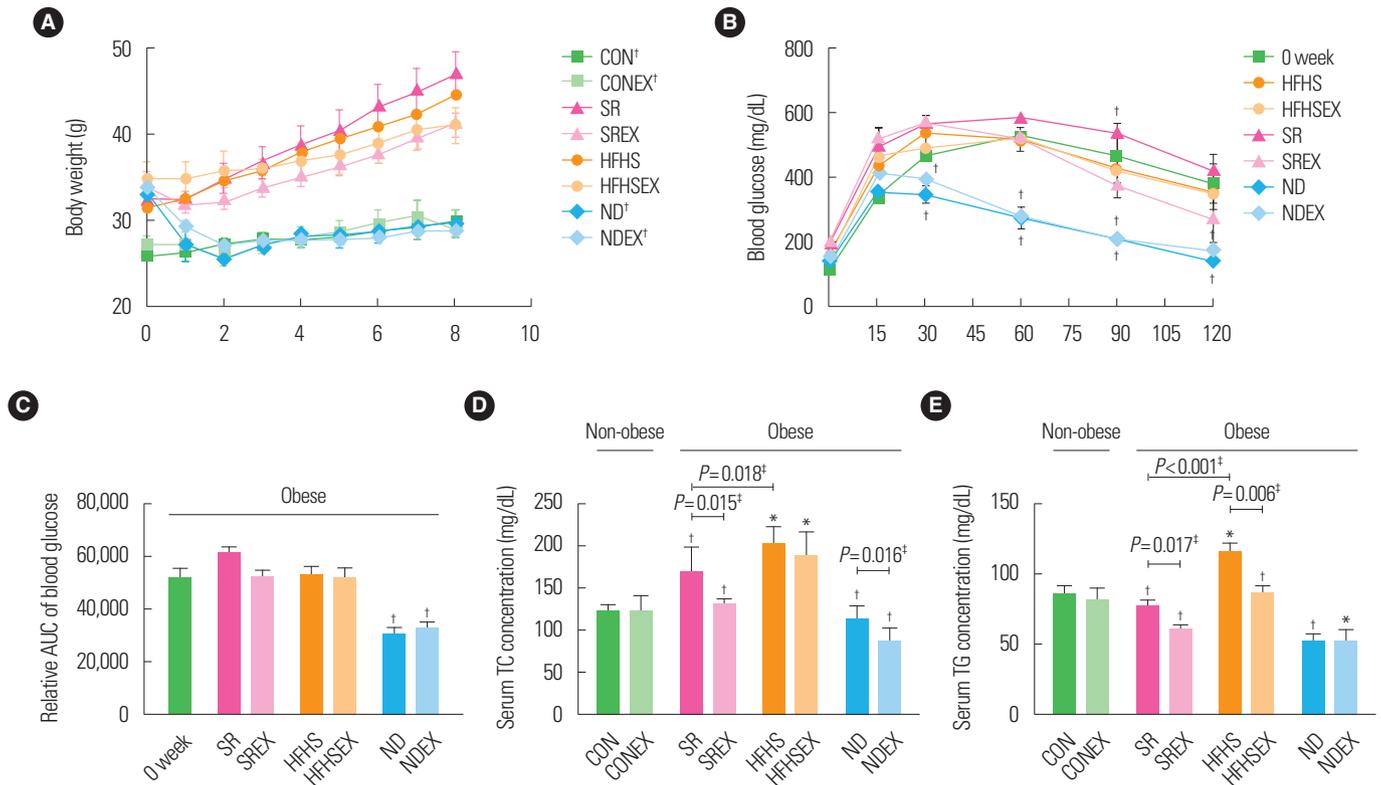
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Figure 2. The effect of exercise and diet restriction on metabolic indicators. (A) Body weight, (B) intraperitoneal glucose tolerance test (iPGTT), (C) iPGTT area under the curve (AUC), (D) Serum total cholesterol (TC) and (E) triglyceride (TG) levels. Values are presented as mean ± standard error of the mean, and the statistical significance level was set at $P<0.05$. *$P<0.05$ vs. CON; †$P<0.05$ vs. HFHS; *,†Statistical analysis was performed using one-way analysis of variance (ANOVA) and ‡independent t-test (indicated as a bar line between two groups). The significance of group differences in blood glucose and body weight were analyzed by two-way ANOVA (†$P<0.05$ vs. HFHS). CON, normal diet group; CONEX, normal diet with exercise group; SR, sucrose restriction group; SREX, sucrose restriction with exercise group; HFHS, high fat, high sucrose group; HFHSEX, high fat, high sucrose with exercise group; ND, high fat, high sucrose restriction group; NDEX, high fat, high sucrose restriction with exercise group; 0 week: starting week of intervention.

control group. Compared with the HFHS group, the SR, SREX, HFHSEX, and ND groups showed significantly lower TG levels ($P<0.05$). An exercise effect was observed in the HFHSEX and SREX groups compared with the HFHS and SR groups (Fig. 2E).

Effects of high-fat, high-SR diet and aerobic exercise on Nr4a3 and mitochondria-associated proteins

Nr4a3 protein expression did not differ significantly among the groups according to the exercise and diet intervention. However, Nr4a3 tended to increase in the HFHSEX group compared with the HFHS group ($P<0.075$) (Fig. 3A, B). The protein analysis in muscle showed that the pAMPK/AMPK ratio was significantly increased in the CONEX group compared with the CON group ($P<0.05$) (Fig. 3A, C). We also found a significant reduction in the SR group compared with the HFHS group ($P<0.05$). No oth-

er significant differences among the groups were found.

In terms of PGC-1α, there was a significant decrease in the SR group compared with the HFHS group ($P<0.05$). The 8-week exercise intervention was significantly effective in the HFHSEX group, as shown in the comparison with the HFHS group results ($P<0.05$) (Fig. 3A, D).

The SDHA protein level was not significantly changed after 8 weeks of exercise and diet restriction intervention. However, compared with the control group, the SDHA levels in all the other groups were significantly increased ($P<0.05$) (Fig. 3A, E).

Effects of high-fat, high-SR diet and aerobic exercise on muscle function

The gastrocnemius muscle wet weight was measured after dissection. The weight was significantly lower in the control group

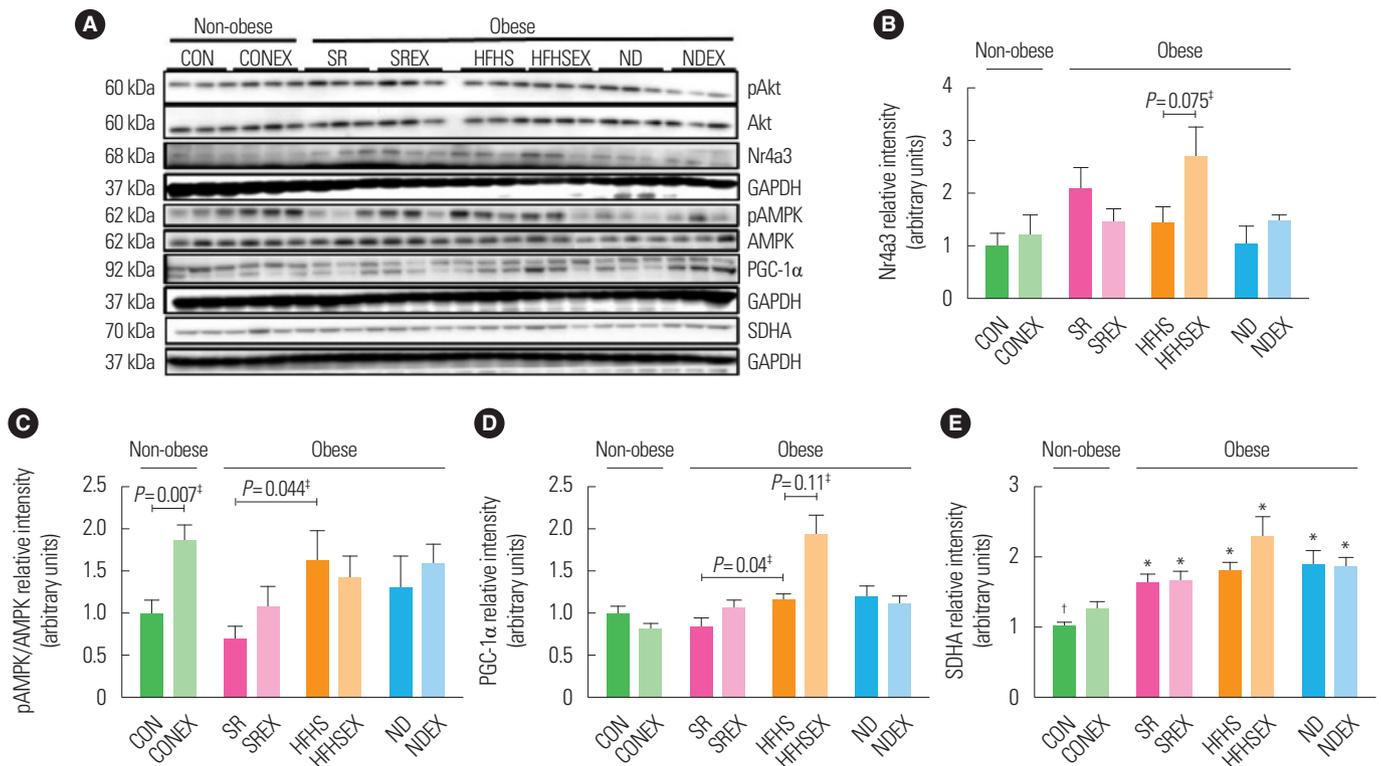Lee JH, et al. Effects of Exercise and Diet on Muscle

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Figure 3. The expression level of Nr4a3 and molecular markers of mitochondria-associated proteins. (A) Immunoblotting of pAkt/Akt, Nr4a3, GAPDH, pAMPK/AMPK, PGC-1α, and SDHA, (B) Nr4a3, (C) ratio of pAMPK/AMPK, (D) muscle PGC-1α protein expression, (E) muscle SDHA protein expression. GAPDH was used as a normalization control. Values are presented as mean ± standard error of mean, and the statistical significance level was set at $P < 0.05$. *$P < 0.05$ vs. CON; †$P < 0.05$ vs. HFHS; *,†Statistical analysis was performed using one-way analysis of variance and ‡independent t-test (indicated as a bar line between groups). CON, normal diet group; CONEX, normal diet with exercise group; SR, sucrose restriction group; SREX, sucrose restriction with exercise group; HFHS, high fat, high sucrose group; HFHSEX, high fat, high sucrose with exercise group; ND, high fat, high sucrose restriction group; NDEX, high fat, high sucrose restriction with exercise group; pAkt, phosphorylated protein kinase B; Nr4a3, neuron-derived orphan receptor 1 (NOR1); GAPDH, glyceraldehyde 3-phosphate dehydrogenase; pAMPK, phosphorylated AMP-activated protein kinase; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; SDHA, succinate dehydrogenase complex, subunit A, flavoprotein variant.

than it was in the HFHS group ($P < 0.05$). There was no difference between the SR and HFHS groups, and the muscle weight in the ND and NDEX groups was significantly higher than in the HFHS group (Fig. 4A).

Because it is one of the major insulin and muscle hypertrophy signaling components, the phosphorylated protein kinase B (pAkt)/Akt ratio was measured. We found that changing HFHS mice to the normal diet (ND group) increased the pAkt/Akt ratio compared with the HFHS group ($P < 0.05$). In addition, the level in the HFHS group was lower than that in the control group (Fig. 4B).

Grip strength is an indicator of muscle quality. Compared with the control (week 0), the SR, HFHS, and HFHSEX groups had significantly decreased grip strength ($P < 0.05$). In addition, compared with the HFHS group, the level of strength in the ND and NDEX groups was increased. Furthermore, we found an exercise

effect in the SREX group compared with the SR group (Fig. 4C).

To measure muscle endurance, the hanging test was performed. The muscle endurance level was increased in the NDEX group compared with the HFHS group. In addition, compared with the control group (week 0), the level was significantly decreased in the SR and HFHS groups. Overall, the exercise groups had greater muscle endurance than the non-exercise groups ($P < 0.05$). In particular, the NDEX group had better endurance than the HFHS group (Fig. 4D).

Influence of exercise, diet type, and their interactions on metabolic indicators, Nr4a3, and mitochondria-associated proteins in the gastrocnemius muscle and serum

To investigate the influence and interaction of exercise (exercise, no exercise) and diet intervention types (control, SR, HFHS, ND)

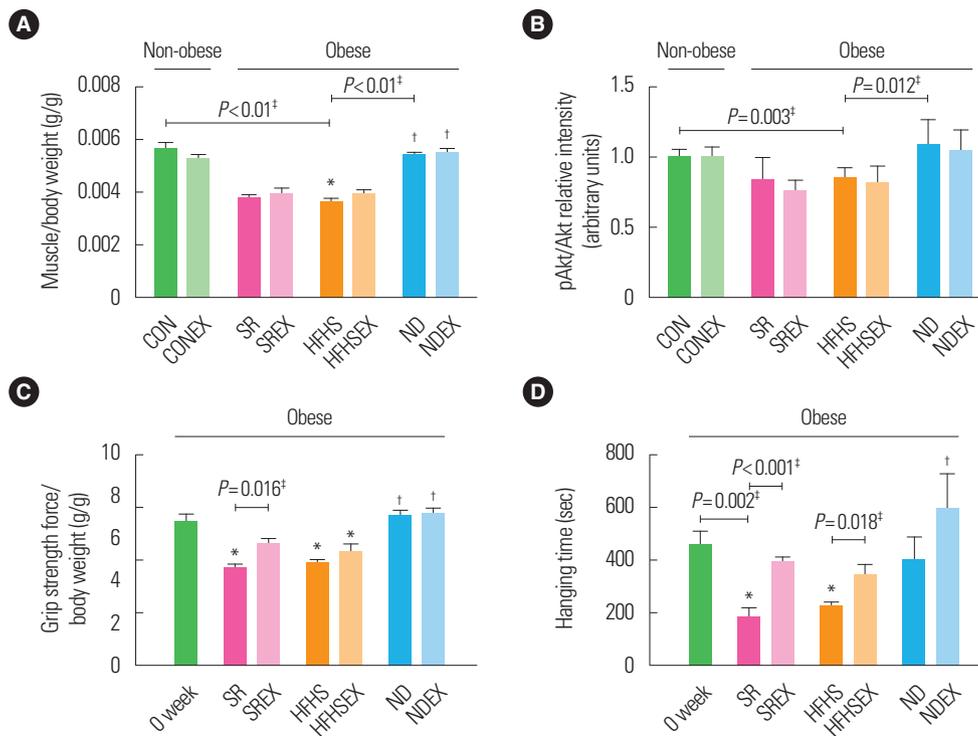Lee JH, et al. Effects of Exercise and Diet on Muscle

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Figure 4. Muscle weight, pAkt/Akt, and muscle function indicators. (A) Gastrocnemius muscle wet weight, (B) pAkt/Akt ratio, (C) grip strength test, (D) hanging time. GAPDH was used as a normalization control. Values are presented as mean ± standard error of mean, and the statistical significance level was set at $P < 0.05$. *$P < 0.05$ vs. CON; †$P < 0.05$ vs. HFHS; *,†Statistical analysis was performed using one way analysis of variance and ‡independent t-test (indicated as a bar line between groups). 0 week, starting week of intervention; CON, normal diet group; CONEX, normal diet with exercise group; SR, sucrose restriction group; SREX, sucrose restriction with exercise group; HFHS, high fat, high sucrose group; HFHSEX, high fat, high sucrose with exercise group; ND, high fat, high sucrose restriction group; NDEX, high fat, high sucrose restriction with exercise group; Akt, protein kinase B.

Table 2. The influence of exercise, diet, and their interactions on metabolic indicators, Nr4a3, and mitochondria-associated proteins in the gastrocnemius muscle and serum

Variable	Group (n)	Exercise		Diet		Exercise & diet	
		F-value	P	F-value	P	F-value	P
Serum TC	6	11.670	0.001*	57.786	0.000*	2.041	0.122
Serum TG	6	7.686	0.008*	21.038	0.000*	2.180	0.105
pAkt/Akt	6	1.408	0.242	13.953	0.000*	0.128	0.943
Nr4a3	6	1.700	0.200	3.273	0.031*	2.419	0.080
pAMPK/AMPK	6	3.355	0.074	2.565	0.068	1.374	0.265
PGC-1α	6	4.463	0.041*	10.968	0.000*	5.924	0.002*
SDHA	6	2.729	0.106	12.478	0.000*	1.058	0.378

Values are presented according to two-way analysis of variance analysis.
*$P < 0.05$.
TC, total cholesterol; TG, triglycerides; pAkt, phosphorylated protein kinase B; Nr4a3, neuron-derived orphan receptor 1 (NOR1); pAMPK, phosphorylated AMP-activated protein kinase; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; SDHA, succinate dehydrogenase complex, subunit A, flavoprotein variant.

on the expression of metabolic indicators, a two-way ANOVA was conducted. We found that the interaction between exercise and diet affected PGC-1α and tended to affect Nr4a3 ($P = 0.080$). In detail, TC and TG differed significantly according to exercise, and TC, TG, pAkt/Akt, NR4A3, PGC-1α, and SDHA differed significantly according to the diet type ($P < 0.05$) (Table 2).

DISCUSSION

A Western diet, which is known to be high in fat and sucrose, is one cause of obesity, T2DM, and metabolic syndrome.[22] We observed that 8 weeks of HFHS diet induced obese conditions in mice. The blood concentrations of TG, TC, and glucose in the HFHS mice were abnormally high, in line with those found in previous studies.[23,24] In addition, reduced Akt phosphorylation, that signifies impaired insulin signaling,[25] was found in the HFHS group. Overall, these abnormal physiological parameters in the mouse model clearly mimic metabolic syndrome.[26] We assessed the gastrocnemius muscle because it can be used in plantar flexion,

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which can play a role as an agonist in treadmill exercise.[27] Furthermore, this muscle is sensitive to an HFHS diet because it contains a high proportion of glycolytic fibers.[28,29]

To confirm the beneficial effects of exercise and the diet restriction intervention, we investigated metabolic indicators, the expression levels of both Nr4a3 and mitochondria-associated proteins, and muscle function parameters. In terms of metabolic indicators, 8 weeks of SR was enough to improve the abnormal blood lipid profile (Fig. 2C, D). We also found diet and exercise effects on the reduction of glucose levels (Fig. 2B). However, the SR group did not exhibit any significant differences from the HFHS group, which suggests that the effects of a high-fat diet could be important.

Because mitochondria are associated with muscle function,[30] we conducted grip strength and hanging time tests. The impaired grip strength and hanging ability of the mice were recovered by both the exercise and diet interventions (Fig. 4C, D). However, a reduction in sucrose alone did not increase the hanging time, presumably because the continuation of the high-fat diet caused a significant increase in body weight. These results are supported by the decreased wet muscle mass found in the HFHS and SR groups (Fig. 4A). Given that the deletion of Akt/PKB can lead to severe muscle atrophy and impaired mTOR activity,[31] the reduced muscle mass is also in line with the decrease of Akt phosphorylation found in the HFHS group and the increase found in the ND group (Fig. 4B).

A recent transcriptomic profiling study confirmed that Nr4a3 is an exercise-responsive gene and that silencing it could lead to a decrease in the maximal oxygen consumption rate and the phosphorylation of the mitochondrial oxidative complex.[17] Many types of research have been conducted to investigate the role and expression patterns of Nr4a3, but most of it analyzed Nr4a3 at the mRNA level after an acute exercise intervention.[8,32] In this study, an 8-week diet and exercise intervention did not change the protein expression of Nr4a3 (Fig. 3B). However, when compared with the HFHS group, there was an increasing tendency in the HFHSEX group ($P = 0.075$). Based on previous research showing that Nr4a3 is an early exercise-responsive molecule,[17] the observed non-significant results could be due to the mice having already adapted to 8 weeks of chronic exercise and a high fat, high SR diet. Additional investigation with different timelines in the model of obese mice is needed to confirm the exact changes in Nr4a3 after an exercise and diet

intervention.

The pAMPK/AMPK ratio showed no exercise effects in the SREX, NDEX, or HFHSEX groups in this study (Fig. 3C). That could suggest that 8 weeks of a high-fat and high-sucrose diet could increase the phosphorylation level of AMPK.[33] Meanwhile, compared with the HFHS group, the SR group, which was deprived of sucrose, showed a pAMPK expression level that had decreased to the level of the control group. That finding could be explained by our lipid analysis, which showed that the TG level also decreased in the SR group, because TG can act as an activator of AMPK.[33] PGC-1α is closely related to mitochondrial function and biosynthesis.[34] The effect of an HFHS diet on PGC-1α remains unclear. Some studies have shown that PGC-1α mRNA decreased after supplying a high-fat diet,[35,36] whereas another study reported that an increase in PGC-1α protein was observed after 4 weeks of an energy-rich diet.[37] In the current study, the control and HFHS groups did not differ significantly (Fig. 3D), partly because the AMPK level in the HFHS group did not differ significantly from the control, and AMPK can stimulate the phosphorylation of PGC-1α.[30] However, further investigations with various durations and compositions of HFHS supplementation should be conducted. We did find an exercise effect in the HFHSEX group compared with the HFHS group, and that beneficial effect in obese conditions could be the result of enhanced mitochondrial biogenesis. In addition, the pAMPK/AMPK ratio in the SR group decreased to be comparable with the control level. The changes in AMPK-PGC-1α reported here support the idea that SR could contribute to mitochondrial homeostasis by controlling mitochondrial biogenesis and cellular metabolic balance[38] in obese conditions.

Compared with the expression level in the control group, the SDHA protein level was increased in all the groups except CONEX (Fig. 3E). Because the elevated groups represent the intervention groups, which were fed an HFHS diet for 8 weeks, this finding is consistent with a prior study in which SDHA levels increased in T2DM and obesity conditions.[39] Another study showed an increase in the SDHA mRNA levels in the EDL muscle in an obese diabetic db/db mouse model.[40]

A limitation of our study is that, although SR changed some parameters, the remaining high fat levels in the diet of the SR group probably made it hard to discern the effect of SR alone. In addition,

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although Akt phosphorylation was measured as an indicator of insulin resistance, it might have been better to analyze the blood level of insulin, glycosylated hemoglobin, or the muscle expression level of GLUT4, along with other proteins downstream of mitochondrial biogenesis (for example, NRF-1 or TFAM), which could have provided a more profound description of metabolic disorder conditions and the effect of our interventions.

In conclusion, we have shown that 8 weeks of exercise was beneficial in reducing body weight, improving the lipid profile, and increasing the level of PGC-1α in obese conditions. The combination of exercise and diet restriction improved glucose tolerance. Eight weeks of intervention did not significantly affect the protein level of Nr4a3. SR decreased the serum lipid level and reduced the elevated pAMPK/AMPK and PGC-1α levels to the control level. Finally, 8 weeks of exercise and diet intervention improved muscle function. These results partially explain the beneficial effects of exercise and high fat and high sucrose restriction diet in obese conditions.

CONFLICTS OF INTEREST

Wook Song is an editorial board member of the journal, but he was not involved in the peer reviewer selection, evaluation, or decision process of this article. Otherwise, no other potential conflicts of interest relevant to this article were reported.

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AUTHOR CONTRIBUTIONS

Study concept and design: JHL, DZ, and WS; acquisition of data: JHL, DZ, SEK, and HES; analysis and interpretation of data: JHL, SEK, DZ, and WS; drafting of the manuscript: JHL, DZ; critical revision of the manuscript: JHL, DZ, SEK, HES, and WS; statistical analysis: JHL, SEK, and DZ; obtained funding: WS; administrative, technical, or material support: WS; and study supervision: WS.

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