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Basic nutritional investigation

Effect of combination of chlorella intake and aerobic exercise training on glycemic control in type 2 diabetic rats

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ABSTRACT

Objectives: Chlorella is a type of unicellular green algae that contains various nutrients. Habitual exercise and chlorella treatment can improve insulin resistance in obese or diabetic animal models. However, the additive effects of combined chlorella intake and aerobic exercise training remain unclear. The aim of this study was to investigate whether a combination of chlorella intake and aerobic exercise training would produce greater effects on improving glycemic control in rats with type 2 diabetes.

Methods: Twenty-wk-old male rats with type 2 diabetes (Otsuka Long-Evans Tokushima Fatty [OLETF] rats) were randomly divided into four groups: sedentary control, aerobic exercise training (treadmill running for 1 h, 25 m/min, 5 d/wk), chlorella intake (0.5% chlorella powder in normal diet), or combination of aerobic exercise training and chlorella intake for 8 wk (n = 7 per group).

Results: Chlorella intake and aerobic exercise training significantly decreased fasting blood glucose, insulin levels, and total glucose area under the curve during the oral glucose tolerance test and increased the insulin sensitivity index concomitant with muscle phosphatidylinositol-3 kinase (PI3K) activity, protein kinase B (Akt) phosphorylation, and glucose transporter 4 (GLUT4) translocation levels. Furthermore, a combination of chlorella intake and aerobic exercise training significantly further improved these effects compared with aerobic exercise training or chlorella intake alone.

Conclusions: These results suggested that chlorella intake combined with aerobic exercise training had more pronounced effects on the improvement of glycemic control via further activation of muscle PI3K/Akt/GLUT4 signaling in rats with type 2 diabetes.

Introduction

Chlorella is a type of unicellular green algae that contains various nutrients such as protein, dietary fiber, minerals, vitamins, and amino acids and has been a popular multicomponent supplement in Asian countries [1]. It has many beneficial effects including improvements in obesity and arteriosclerosis, acceleration of immune function, and better exercise performance [2–6]. In addition, some studies have shown that continuous chlorella intake improved insulin resistance in obese or diabetic animal models [1,7–10]. As a mechanism underlying the chlorella intake-induced improvement in insulin resistance (IR), an increase in expression of glucose transporter 4 (GLUT4) via activation of protein kinase B (Akt) phosphorylation in skeletal muscle is involved [1,10]. Thus, continuous chlorella intake is effective for improving glycemic control in obesity and diabetes via activation of muscle Akt/GLUT4 signaling.

In patients with type 2 diabetes (T2D), exercise and diet therapies are used as a first approach [11–15]. Aerobic exercise training is well known to have beneficial effects on improving IR in patients with T2D [11-13]. Skeletal muscle is a particularly important tissue because it is the largest muscle responsible for insulin-stimulated activation of glucose metabolism [16–18]. As an underlying mechanism, aerobic exercise training activates muscle phosphatidylinositol-3 kinase (PI3K), Akt, and GLUT4 signaling, leading to an improvement in IR in rats with T2D [19,20]. Therefore, we hypothesized that a combination

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of continuous chlorella intake and aerobic exercise training may further improve glycemic control via further activation of muscle PI3K, Akt, and GLUT4 signaling in T2D compared with chlorella intake or aerobic exercise training alone. Indeed, a previous study showed that continuous chlorella intake combined with high-intensity intermittent exercise (HIIE) training further augmented muscle glycolytic and oxidative metabolism in healthy normal rats compared with chlorella intake or HIIE training alone [21]. However, the additive effects of combined chlorella intake and aerobic exercise training on glycemic control in T2D remain unclear.

Therefore, the aim of this study was to investigate whether a combination of continuous chlorella intake and aerobic exercise training would produce beneficial effects by improving glycemic control in rats with T2D. In addition, we investigated whether continuous chlorella intake combined with aerobic exercise training further increased muscle PI3K, Akt, and GLUT4 signaling. To test our hypothesis, Otsuka Long-Evans Tokushima Fatty (OLETF) diabetic rats with continuous chlorella intake, aerobic exercise training, or a combination of both were studied.

Methods

Animals and protocol

Six-wk-old male OLETF rats were obtained from Japan SLC (Shizuoka, Japan). The ethical approval for this study was obtained from the Committee on Animal Care at Ritsumeikan University in Japan. All rats were housed individually in an animal facility under controlled conditions (12/12-h light/dark cycle). After 14 wk, the 20-wk-old OLETF rats were randomly assigned to four groups (n = 7 per group): sedentary control (OLETF-CON), aerobic exercise training (OLETF-EX), chlorella intake (OLETF-CH), or EX+CH (OLETF-EX+CH) combination group. The OLETF-CON and EX groups were given access to water and fed a normal diet (CE-2; CLEA Japan, Tokyo, Japan) ad libitum during the 8-wk experimental period. The OLETF-CH and EX+CH groups were fed the same food containing 0.5% chlorella powder (Sun Chlorella Corp, Kyoto, Japan) [5,21]. Major nutritional components of chlorella powder are shown in Table 1 [1]. In addition, nondiabetic, healthy, and age-matched Long-Evans Tokushima Otsuka (LETO) rats (n = 7) were used as a healthy sedentary control group. Oral glucose tolerance test (OGTT) in all groups was performed 48 h after the last aerobic exercise session under 12-h overnight fasting to avoid the acute effects of the exercise and food intake. Forty-eight hours after the end of the OGTT, all rats were fasted for 12 h, and after measuring the body weight, blood samples were obtained from the abdominal aorta under general anesthesia. After sacrifice, the epididymal fat, gastrocnemius, and soleus muscles were resected quickly, rinsed in ice-cold saline, weighed, frozen in liquid nitrogen, and stored at −80°C until further analysis.

Aerobic exercise training protocol

Before the training experimental period, the EX and EX+CH groups were trained on a rodent treadmill at 10 to 15 m/min for 3 d. The rats in the EX and EX+CH groups ran on the treadmill for 1 h at 25 m/min, without incline, 5 d/wk for 8 wk. The intensity of the exercise was kept constant during the training period, as previously described [20,22].

Table 1
Major nutritional components of chlorella powder

Component	Content (per 100 g of powder)
Calorie	370 kcal
Carbohydrate	7.7 g
Fat	11.2 g
Protein	59.7 g
Ash	6.3 g
Biotin	254 μg
Calcium	410 mg
Carotene	25 000 μg
Chlorophyll	2300 mg
Dietary fiber	9.8 g
Folic acid	3.1 mg
Inositol	388 mg
Iron	140 mg
Magnesium	310 mg
Moisture	5.3 g
Natrium	60 mg
Niacin	34.3 mg
Pantothenic acid	4.13 mg
Phosphorus	1500 mg
Potassium	1000 mg
Zinc	1.9 mg
Vitamin A potency	14 000 IU
Vitamin B_1	1.08 mg
Vitamin B_2	4.93 mg
Vitamin B_6	1.93 mg
Vitamin B_{12}	0.24 mg
Vitamin C	2 mg
Vitamin D	43 600 IU
Vitamin E	4.3 mg
Vitamin K_1	1.31 mg
Amino acid	
Alanine	4.17 g
Arginine	3.12 g
Aspartic acid	4.77 g
Glutamic acid	5.82 g
Glycine	2.97 g
Isoleucine	2.08 g
Leucine	4.45 g
Lysine	2.92 g
Phenylalanine	2.60 g
Valine	3.07 g

Immunoblot analysis

Western blot analysis was performed to assess Akt phosphorylation and GLUT4 translocation levels as previously described [20,23]. Briefly, the gastrocnemius muscle proteins (40 μg) were separated by 10% sodium dodecyl sulfate polyacrylamide gel and transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). The membranes were treated for 1 h with blocking buffer (3% skim milk in phosphate-buffered saline with 0.1% Tween 20 [PBS-T]) and then incubated for 12 h in blocking buffer at 4°C with antibodies (diluted 1:1000 in blocking buffer) against Akt phosphorylated on Ser473

Table 2
Animal characteristics

	LETO (n = 7)	OLETF (N = 28)			
		CON (n = 7)	EX (n = 7)	CH (n = 7)	EX+CH (n = 7)
BW (g)	491.2 ± 9.3	589.1 ± 15.2*	484.3 ± 14†	557.0 ± 12.3†	566.6 ± 11.8†
Epididymal fat mass (g)	7.25 ± 0.43	11.4 ± 0.44*	6.31 ± 0.84†	8.00 ± 1.01†	7.52 ± 0.42†
Gastrocnemius/BW (mg/g BW)	4.70 ± 0.15	3.66 ± 0.14*	4.48 ± 0.17†	3.96 ± 0.01†	3.83 ± 0.15†
Soleus/BW (mg/g BW)	0.45 ± 0.01	0.36 ± 0.02*	0.45 ± 0.02†	0.38 ± 0.02	0.36 ± 0.01
Soleus CS enzyme activity (μmol · g · min^{-1})	18.9 ± 1.4	12.1 ± 0.8*	25.5 ± 1.5†	18.5 ± 1.7†,‡	26.4 ± 0.3†,§
Average dietary intake (g/d)	20.3 ± 0.2	26.7 ± 0.5*	20.4 ± 0.5†	25.8 ± 0.2‡	26.4 ± 0.3‡

BW, body weight; CS, citrate synthase; LETO, healthy-sedentary control group; OLETF-CON, OLETF-sedentary control group; OLETF-EX, OLETF-aerobic exercise training group; OLETF-CH, OLETF-chlorella intake group; OLETF-EX+CH, OLETF-aerobic exercise training and chlorella intake group.
Values are mean ± SE.
* $P < 0.05$ vs LETO.
† $P < 0.05$ vs OLETF-CON.
‡ $P < 0.05$ vs OLETF-EX.
§ $P < 0.05$ vs OLETF-CH.

(#9271, Cell Signaling Technology, Danvers, MA, USA), total Akt (#9272, Cell Signaling Technology), or GLUT4 (#07-1404, Millipore). The membranes were washed three times with PBS-T and then incubated for 1 h at room temperature (22–24°C) with horseradish peroxidase-conjugated secondary antibody and anti-rabbit (Cell Signaling Technology and GE Healthcare Biosciences, Piscataway, NJ, USA) immunoglobulins diluted 1:3000 in blocking buffer. The membranes were then washed three times with PBS-T. Finally, the phosphorylated and total Akt and GLUT4 levels were detected using the Enhanced Chemiluminescence Plus system (GE Healthcare Biosciences), visualized on an ImageQuant LAS 4000 imaging system (GE Healthcare Biosciences). Densitometry was performed using the ImageJ software (ver 1.48; National Institutes of Health, Bethesda, MD, USA).

Preparation of cytosolic and plasma membrane protein fractions

To determine GLUT4 translocation, two different membrane fractions were used as previously described [22,24]. Briefly, the gastrocnemius muscles were homogenized with buffer A (20 mM Tris [pH 7.4], 1 mM EDTA, 0.25 mM EGTA, 1 mM DTT, 50 mM NaF, 25 mM sodium pyrophosphate, 40 mM β-glycerophosphate, and 250 mM sucrose). The resulting homogenates were centrifuged at 400 g for 15 min at 4°C to remove debris. The supernatant was centrifuged again at 50 000 g for 1 h at 4°C. The resulting supernatant was used as the cytosolic protein fraction. Moreover, the pellet from different fractions were solubilized for 1 h at room temperature in buffer B (20 mM Tris [pH 7.4], 1 mM EDTA, 0.25 mM EGTA, 2% Triton X-100, 50 mM NaF, 25 μM sodium pyrophosphate, and 40 mM β-glycerophosphate). The homogenate was centrifuged briefly, and the supernatant was centrifuged for 1 h at 50 000 g before it was used as the plasma membrane fraction. GLUT4 protein expression levels were measured in both the cytosol and membrane fractions by Western blot analysis. GLUT4 translocation was evaluated based on the relative abundance of protein levels in these fractions [22,24].

Measurement of PI3K activity

PI3K activity was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Echelon Biosciences, Salt Lake City, UT, USA), as previously described [20]. Absorption at 450 nm was measured using xMark microplate spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA).

OGTT

After 12-h overnight fasting and under isoflurane anesthesia, glucose (2 g/kg body weight) was dissolved into saline, and rats in all groups received oral administration by using a probe. Blood glucose concentrations were determined using a blood glucose meter (Terumo Corporation, Tokyo, Japan) from tail veins taken at 0 (before glucose injection), 30, 60, and 90 min after oral glucose administration. Total glucose area under the curve (AUC) during the OGTT was calculated using the trapezoidal rule [8,25].

Fasting blood glucose and insulin concentrations

Fasting blood glucose concentrations were assessed three times from the tail vein using a blood glucose meter (Terumo Corporation) after the treatment period under the 12-h overnight fasting conditions. The concentrations of fasting serum insulin were measured using an ELISA kit (Shibayagi, Gunma, Japan), and absorption at 450 nm was measured using xMark microplate spectrophotometer (Bio-Rad Laboratories).

Insulin sensitivity index

As an index of insulin sensitivity, the quantitative insulin sensitivity check index (QUICKI) was calculated from fasting glucose and insulin concentrations [24,26,27]:

$$\text{QUICKI} = 1/[\log(\text{I0}) + \log(\text{G0})]$$

where I0 is the fasting insulin (μU/mL), and G0 is the fasting glucose (mg/dL).

Measurement of citrate synthase enzyme activity

The soleus muscles samples (50 mg) were homogenized with 10 volumes of 250 mM sucrose, 1 mM Tris-HCl (pH 7.4), and 130 mM NaCl on ice using a Teflon homogenizer. The homogenate was centrifuged at 9000 g for 20 min at 0°C, and the pellet was resuspended with homogenate buffer and centrifuged at 600 g for 10 min at 0°C. The supernatant was centrifuged at 8000 g for 15 min at 0°C, and the pellet was resuspended with 250 mM sucrose. To assess the enzyme activity of citrate synthase (CS), 50 μL of each sample was incubated for 2 min at 30°C in a 900 μL incubation mixture containing 100 mM Tris-HCl (pH 8.0), 1 mM 5,5′-dithiobis (2-nitro benzoic acid), and 10 mM acetyl-coenzyme A. The reaction was initiated by the addition of 50 μL of 10 mM oxaloacetate and then was assessed spectrophotometrically at 412 nm for 3 min [20,22].

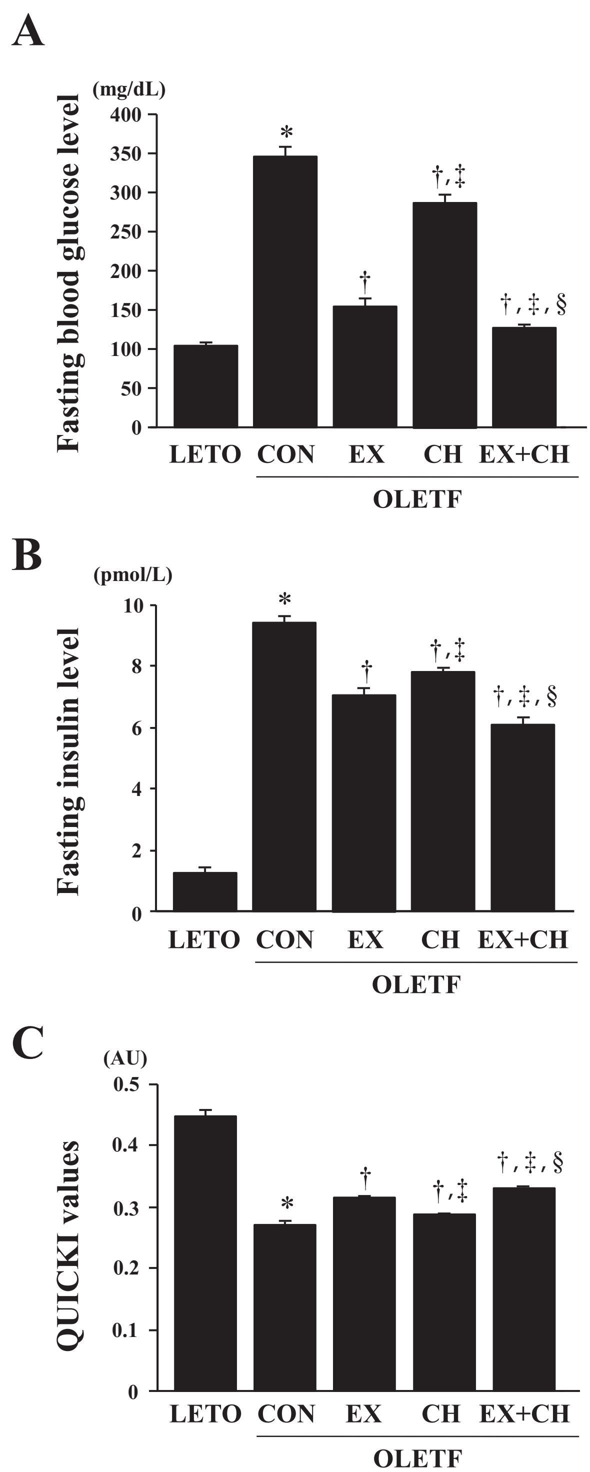

Fig. 1. Effects of 8 wk of aerobic exercise training or chlorella intake on fasting blood glucose (**A**), insulin (**B**) levels, and QUICKI values (**C**). Values are mean ± SE (N = 7 for each group). *$P < 0.05$ vs LETO. †$P < 0.05$ vs OLETF-CON. ‡$P < 0.05$ vs OLETF-EX. §$P < 0.05$ vs OLETF-CH. AU, arbitrary units. LETO, healthy-sedentary control group; OLETF-CON, OLETF-sedentary control group; OLETF-EX, OLETF-aerobic exercise training group; OLETF-CH, OLETF-chlorella intake group; OLETF-EX+CH, OLETF-aerobic exercise training and chlorella intake group; QUICKI, quantitative insulin sensitivity check index.

Statistical analysis

All values were expressed as mean ± SE. Two-way analysis (time × group) of variance (ANOVA) was used to evaluate the change in blood glucose levels during the OGTT. Other data were analyzed using one-way ANOVA. The Bonferroni post hoc comparison test was used to correct for multiple comparisons when the analyses showed significant differences. Relationships between GLUT4 translocation in skeletal muscle and fasting blood glucose (FBG), insulin levels, QUICKI values, or total glucose AUC values during the OGTT in all groups were determined using Pearson's correlation coefficients. $P < 0.05$ was considered significant. All analyses were performed using Stat View 5.0 (SAS Institute, Cary, NC, USA).

Results

Animal characteristics

Body weight and epididymal fat mass in the OLETF-CON group were significantly higher than those in the LETO group ($P < 0.05$; Table 2). Compared with the OLETF-CON group, body weight and epididymal fat mass in the OLETF-EX group were significantly decreased ($P < 0.05$; Table 2). Epididymal fat mass in the OLETF-CH and OLETF-EX+CH groups was significantly decreased compared with that in the OLETF-CON group ($P < 0.05$; Table 2), whereas no significant differences in body weight were observed among the OLETF-CON, OLETF-CH, and OLETF-EX+CH groups (Table 2). Gastrocnemius and soleus muscle masses in the OLETF-CON group were significantly lower than those in the LETO group ($P < 0.05$; Table 2). Gastrocnemius and soleus muscle masses in the OLETF-EX group was significantly increased compared with the OLETF-CON group ($P < 0.05$; Table 2). Compared with the OLETF-CON group, gastrocnemius muscle mass in the OLETF-CH alone and OLETF-EX+CH groups were significantly increased ($P < 0.05$; Table 2), whereas no significant difference in soleus muscle mass was observed among the OLETF-CON, OLETF-CH, and OLETF-EX+CH groups (Table 2). Moreover, soleus muscle CS enzyme activity in the OLETF-CON group was significantly lower than that in the LETO group ($P < 0.05$; Table 2). Soleus muscle CS enzyme activity in the OLETF-EX alone, CH alone, and OLETF-EX+CH groups were significantly increased compared with the OLETF-CON group ($P < 0.05$; Table 2), whereas no significant difference in soleus muscle CS enzyme activity was observed between the OLETF-EX alone and OLETF-EX+CH groups (Table 2). Average dietary intake for 8-wk in the OLETF-CON group was significantly higher than that in the LETO group ($P < 0.05$; Table 2). Compared with the OLETF-CON group, average dietary intake in the OLETF-EX group was significantly decreased ($P < 0.05$; Table 2). However, no significant difference in the average dietary intake was observed among the OLETF-CON, OLETF-CH, and OLETF-EX+CH groups (Table 2).

FBG, insulin concentrations, and insulin sensitivity index

Compared with the LETO group, the OLETF-CON group had significantly higher FBG and insulin levels and lower QUICKI values ($P < 0.05$; Fig. 1 A–C). The OLETF-EX or OLETF-CH alone and OLETF-EX+CH groups had significantly decreased FBG and insulin levels and increased QUICKI values compared with the OLETF-CON group ($P < 0.05$; Fig. 1 A–C). Moreover, the OLETF-EX+CH group showed a further decrease in FBG and insulin levels and increased QUICKI values compared with the OLETF-EX or OLETF-CH alone group ($P < 0.05$; Fig. 1 A–C).

OGTT

Blood glucose levels in all groups were significantly increased by oral glucose administration ($P < 0.05$; Fig. 2A). Blood glucose levels in the OLETF-CON group at 30, 60, and 90 min after the OGTT was significantly higher than in the LETO group ($P < 0.05$; Fig. 2A). Compared with the OLETF-CON group, the blood glucose level in the OLETF-EX alone, OLETF-CH alone, and OLETF-EX+CH groups at 30 and 60 min after the OGTT were significantly decreased ($P < 0.05$; Fig. 2A). Moreover, the OLETF-EX+CH group had further decreased blood glucose levels at 30 min after the OGTT compared with the OLETF-EX or OLETF-CH alone groups ($P < 0.05$; Fig. 2A). Total glucose AUC values during the OGTT in the OLETF-CON was significantly higher than that in the LETO group ($P < 0.05$; Fig. 2B). Compared with the OLETF-CON group, total glucose AUC values during the OGTT in the OLETF-EX or OLETF-CH alone and OLETF-EX

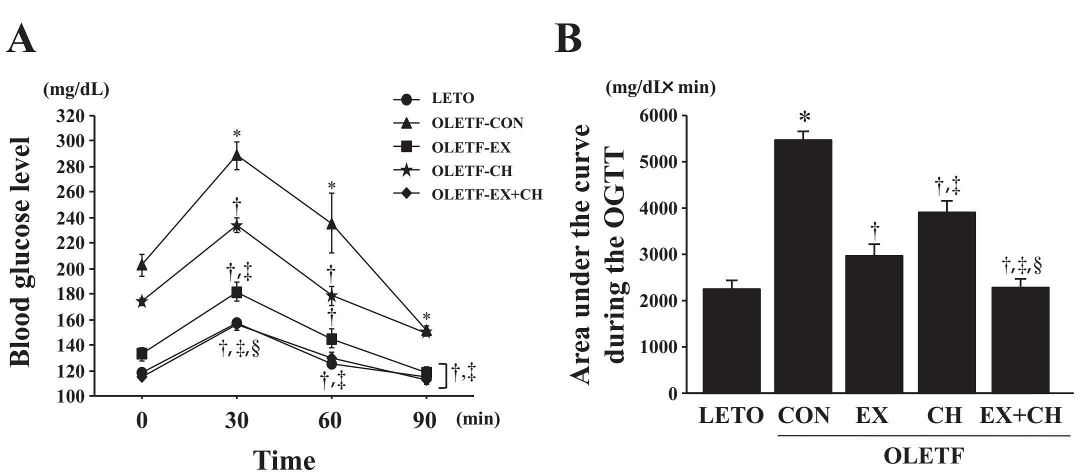

Fig. 2. Effects of 8 wk of aerobic exercise training or chlorella intake on blood glucose level (**A**) and total glucose area under the curve values during the oral glucose tolerance test (**B**) (n = 4 for each group). Values are mean ± SE. *$P < 0.05$ vs LETO. †$P < 0.05$ vs OLETF-CON. ‡$P < 0.05$ vs OLETF-EX. §$P < 0.05$ vs OLETF-CH. LETO, healthy-sedentary control group; OLETF-CON, OLETF-sedentary control group; OLETF-EX, OLETF-aerobic exercise training group; OLETF-CH, OLETF-chlorella intake group; OLETF-EX+CH, OLETF-aerobic exercise training and chlorella intake group.

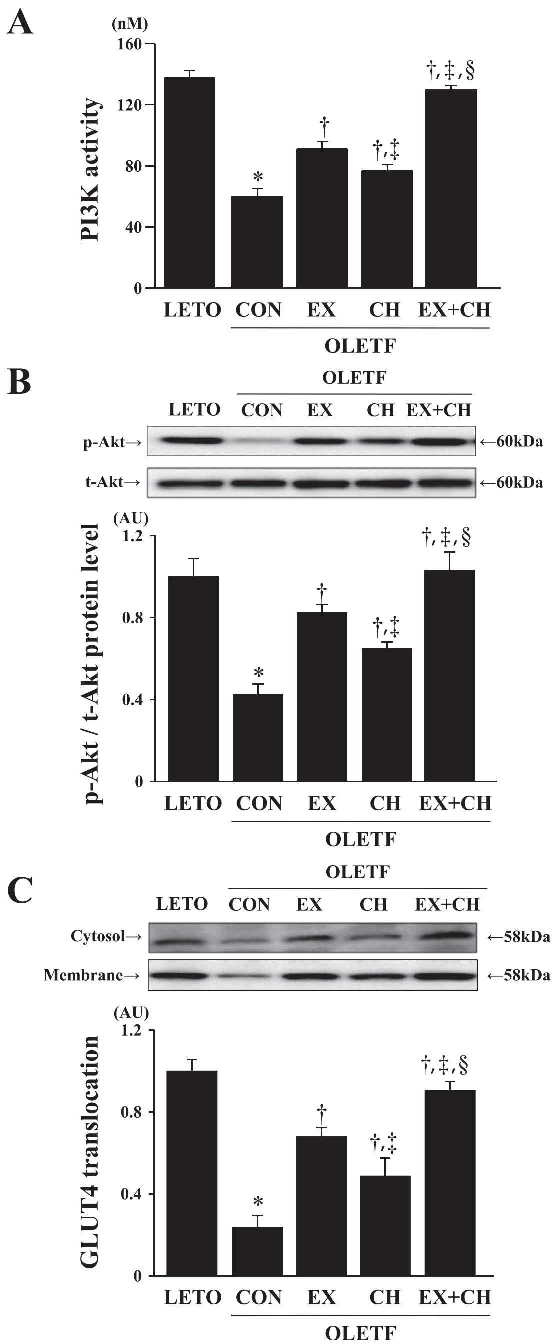

Fig. 3. Effects of 8 wk of aerobic exercise training and/or chlorella intake on PI3K activity (**A**), Akt phosphorylation on Ser473 (**B**) and GLUT4 translocation (**C**) levels in the gastrocnemius muscle. Representative immunoblotting image and histograms of the levels of Akt phosphorylation and GLUT4 in cytosolic and membrane fractions are shown. Akt phosphorylation and GLUT4 translocation levels are represented relative to each expression as fold-changes from the gastrocnemius muscle in the LETO group. Values are mean ± SE (N = 7 for each group). *$P < 0.05$ vs LETO. †$P < 0.05$ vs OLETF-CON. ‡$P < 0.05$ vs. OLETF-EX. §$P < 0.05$ vs OLETF-CH. Akt, protein kinase B; AU, arbitrary units; GLUT4, glucose transporter 4; LETO, healthy-sedentary control group; OLETF-CON, OLETF-sedentary control group; OLETF-EX, OLETF-aerobic exercise training group; OLETF-CH, OLETF-chlorella intake group; OLETF-EX+CH, OLETF-aerobic exercise training and chlorella intake group.

+CH groups were significantly decreased ($P < 0.05$; Fig. 2B). In addition, the OLETF-EX+CH group showed a further decrease in total glucose AUC values during the OGTT compared with the OLETF-EX or OLETF-CH alone groups ($P < 0.05$; Fig. 2B).

PI3K activity, Akt phosphorylation, and GLUT4 translocation in skeletal muscle

Muscle PI3K activity, the levels of Akt phosphorylation, and GLUT4 translocation in the OLETF-CON group were significantly lower than those in the LETO group ($P < 0.05$; Fig. 3 A–C). Compared with the OLETF-CON group, muscle PI3K activity, Akt phosphorylation, and GLUT4 translocation levels in the OLETF-EX alone, OLETF-CH alone, and OLETF-EX+CH groups were significantly increased ($P < 0.05$; Fig. 3 A–C). In addition, the OLETF-EX+CH group had further increased muscle PI3K activity, Akt phosphorylation, and GLUT4 translocation levels compared with the OLETF-EX or OLETF-CH alone groups ($P < 0.05$; Fig. 3 A–C).

Relationships between muscle GLUT4 translocation and glycemic control

GLUT4 translocation in skeletal muscle was negatively correlated with FBG ($r = -0.842$; $P < 0.05$), insulin levels ($r = -0.737$; $P < 0.05$), and total glucose AUC values during the OGTT ($r = -0.852$; $P < 0.05$) and were positively correlated with QUICKI values ($r = 0.733$; $P < 0.05$).

Discussion

The results of this study revealed that a combination of continuous chlorella intake and aerobic exercise training further improved FBG, insulin levels, insulin sensitivity index, and total glucose AUC values during the OGTT in rats with T2D compared with chlorella intake or aerobic exercise training alone. The combined treatment further increased PI3K activity, Akt phosphorylation, and GLUT4 translocation in the skeletal muscle. Furthermore, GLUT4 translocation in the skeletal muscle was negatively associated with FBG, insulin levels, and total glucose AUC values during the OGTT and was positively associated with the insulin sensitivity index. These findings suggest that continuous chlorella intake combined with aerobic exercise training may produce larger beneficial effects on improving glycemic control via the enhancement of muscle glucose uptake in rats with T2D.

Aerobic exercise training is known to improve IR concomitant with activation of PI3K, Akt phosphorylation, and GLUT4 translocation in the skeletal muscle [19,20]. In addition, continuous chlorella intake in obese or diabetic animal models has been shown to restore impairment of muscle Akt phosphorylation [10] and GLUT4 protein expression [1]. Therefore, the molecular mechanism of chlorella intake-induced activation of muscle Akt and GLUT4 signaling may be similar to aerobic exercise training. In the present study, continuous chlorella intake combined with aerobic exercise training further activated muscle PI3K, Akt, and GLUT4 signaling in rats with T2D compared with chlorella intake or aerobic exercise training alone. Thus, a combination of continuous chlorella intake and aerobic exercise training may additively or synergistically activate muscle PI3K, Akt, and GLUT4 signaling, leading to further improvement in glycemic control of T2D.

In the present study, continuous chlorella intake with or without aerobic exercise training improved glycemic control in rats with T2D. However, it is unclear what nutrients in chlorella improved glycemic control. Chlorella is a multicomponent supplement including vitamins, magnesium, and amino acids (e.g., leucine, isoleucine, and arginine) [1]. Leucine [28], isoleucine [29], and arginine [30] treatment enhances GLUT4 protein expression in the skeletal muscle of pigs, C2C12 and L6 myotubes, respectively. Furthermore, vitamin D treatment increases GLUT4 translocation levels in C2C12 myotubes [31]. In addition, in rats with T2D, magnesium supplementation induces an increase in muscle GLUT4 expression level [32]. Thus, leucine, isoleucine, arginine, vitamin D, and magnesium in chlorella may lead to an elevation of muscle GLUT4 expression and translocation.

Hyperglycemia in T2D is a risk factor for coronary artery disease, renal failure, and retinopathy [33–36], and therefore treatment for IR is essential. Although aerobic exercise training improves IR [11–13], it is difficult to carry out habitual aerobic exercise in patients with T2D and obesity [37]. Chlorella intake has safe beneficial effects on the improvement of IR because it has no side effects or toxicities as shown in animal studies [7–10]. In the present study, continuous chlorella intake with aerobic exercise training further improved glycemic control in rats with T2D compared with chlorella intake or aerobic exercise training alone. Therefore, a combination of chlorella intake and aerobic exercise training may be useful as a new nutrition synergistic approach for patients with T2D. Although it is important to examine the combination index (CI) for quantifying synergism, summation, and antagonism of dose–effect relationships [38], this study did not examine the dose effect of chlorella intake and exercise volumes. Therefore, further study is required to examine the CI of dose effects of chlorella intake and exercise volumes.

Conclusion

The findings of this study indicated that a combination of continuous chlorella intake and aerobic exercise training had more pronounced effects on improvements in glycemic control via activation of muscle PI3K, Akt, and GLUT4 signaling in rats with T2D compared with chlorella intake or aerobic exercise training alone.

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