Lactate administration induces skeletal muscle synthesis by influencing Akt/mTOR and MuRF1 in non-trained mice but not in trained mice

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Abstract

Background: The perception regarding lactate has changed over the past decades, with some of its physiological roles having been revealed. However, the effects of exogenous lactate on skeletal muscle synthesis remain unclear. Therefore, this study aimed to confirm the effects of long-term and post-exercise lactate administration on skeletal muscle synthesis.

Methods: 32 ICR mice were randomly assigned to non-trained + placebo (Non/Pla), non-trained + lactate (Non/Lac), tranied + placebo (Tr/Pla), or trained + lactate (Tr/Lac) groups. Lactate (3 g/kg) or same volume of saline was immediately administered after exercise training (70% VO_{2max}). Lactate administration and/or exercise training was performed 5 days/week for 5 weeks.

Results: After the experimental period, lactate administration tended to increase skeletal muscle weight and significantly enhanced the gastrocnemius muscle weight. Furthermore, lactate administration increased the mRNA levels of *Akt* ($p = 0.000$) and *mTOR* ($p = 0.009$), decreased protein expression of MuRF1 ($p = 0.001$), and reduced blood IGF1 levels ($p = 0.039$). However, lactate administration after exercise training had no additional effects on almost factors.

Conclusions: These results suggest that long-term lactate administration have a potential to stimulate skeletal muscle synthesis, but lactate administration after exercise training may not be extra beneficial for increasing skeletal muscle because of the lactate administration timing.

Background

Skeletal muscles can be quantitatively altered by the balance between protein synthesis and degradation, which is affected by various factors [1, 2]. Exercises involving muscle contraction and relaxation are widely accepted strategies to increase the amount of skeletal muscle by upregulating protein synthesis [3, 4]. Previous research showed that long-term exercise training induces muscle hypertrophy by increasing protein synthesis signaling such as protein kinase B (Akt)/mammalian target of rapamycin (mTOR) pathway [5, 6]. Furthermore, this signaling can block E3 ubiquitin ligases ((muscle ring-finger protein-1 [MuRF1] and muscle-specific F-box protein [MAFbx1]), which induce atrophy and protein degradation [7–9]. Thus, exercise training has been confirmed to induce skeletal muscle synthesis, but the trigger for exercise-induced muscle synthesis has not been fully elucidated.

During exercise, various metabolic intermediate and bioactive molecules are secreted from skeletal muscles [10, 11]. Among them, lactate had been recognized as a “fatigue-inducing molecule” because early studies reported that lactate induces acidosis and contributes to a decreased exercise capacity [12, 13]. However, recent studies confirmed that acidosis associated with exercise is induced by increased H^+ production from ATP hydrolysis [14]. In addition, lactate is used as an energy resource that can transit between body compartments not only through the Cori cycle between the muscles and liver but also from cell to cell or between intracellular sites by the lactate shuttle (monocarboxylate transporters [MCTs]) [15,

16]. The perception of lactate has been changed by recent research, leading to new possibilities for the role of lactate.

High-intensity exercise, which produces more lactate than low intensity-exercise [17], is acknowledged as a strategy to further increase skeletal muscle synthesis [18, 19]. Similarly, previous studies have demonstrated that exercise training with hypoxia or blood flow restriction, in which lactate production is artificially upregulated [20, 21], increases muscle synthesis and strength over a similar low-intensity exercise in a normal state [22–24]. Thus, there is a possibility that secreted lactate during exercise may affect to increase skeletal muscle synthesis. Moreover, several cell studies on lactate have confirmed the effects of increased lactate concentrations on muscle synthesis. Guo et al. [25] showed that increased lactate concentration in CHO-K1 cells decreased cAMP accumulation, which is an inhibitor of mTOR, and Ohno et al. [26] reported that the higher the concentration of lactate (0 to 20 mM), the larger the myotube diameter in C2C12 cells. Accordingly, blood lactate may partially affect the mechanism of exercise-induced muscle synthesis.

Motivated by the relatively novel physiological roles of lactate, recent research has investigated the effects of exogenous lactate on skeletal muscles. Cerda-Kohler et al. [27] reported that an injection of 3 g/kg of lactate in mice increased blood lactate concentration (approximately 20 mM) after 5 to 15 min and upregulated the phosphorylation of Akt (Thr308 and Ser473) and P70S6K in type2 muscles after 40 min. In addition, our previous study demonstrated that oral administration of 2 g/kg of sodium lactate increased mRNA levels of *IGF receptor*, *Akt* and *mTOR* in the plantaris muscle after 30 to 60 min, but did not affect the degradation factors (*MuRF1* and *MAFbx*) [28]. Previous studies have shown that exogenous lactate administration has positive effects on skeletal muscle synthesis.

These findings suggest that long-term lactate administration induces skeletal muscle synthesis and that lactate administration with exercise training can have additional effects on skeletal muscle synthesis by increasing blood lactate [29]. Nevertheless, only a few studies have investigated the effects of long-term lactate treatment and oral administration, and previous studies have not directly investigated the effect of lactate administration with exercise training on skeletal muscle synthesis. Therefore, the present study had two purposes: (a) to determine the effects of oral lactate administration for 5 weeks on skeletal muscle synthesis and protein synthesis and degradation factors; and (b) to confirm whether lactate administration after exercise training has additional effects on skeletal muscle synthesis.

Methods

Animal care

Ethical approval for this study was obtained from the Konkuk University Institutional Animal Care and Use Committee (No. KU19149). Six-week-old male ICR mice (n = 32) were obtained from Orient Bio (Seongnam, Korea) and assigned to standard cages (4 mice/cage) under controlled conditions (12:12-h

light-dark cycle, 50% humidity, 23 ± 1°C). A standard commercial diet (Orient Bio, Seongnam, Korea) and water were provided ad libitum. Food intake and body weight were measured daily between 9 and 10 a.m.

Study design

Prior to the experiment, the blood lactate concentration of mice (n = 6) after oral lactate administration was measured from the blood obtained from the tail using Lactate Pro2 (Arkray Inc., Kyoto, Japan). Lactate (sodium lactate, 195–05965, Wako Chemical, Osaka, Japan) was orally administered at 3 g/kg [27, 29]. Blood lactate levels significantly increased from 3.03 ± 0.31 mmol/L to 8.22 ± 2.38 mmol/L after 15 min of lactate administration and returned to basal levels (approximately 3.67 ± 1.17 mmol/L) after 3 hours (Table 1). Therefore, exogenous lactate administration increases the blood lactate concentration.

Table 1
Blood lactate concentration after oral administration of lactate.

	Time after lactate administration (min)								
	0	15	30	45	60	90	120	150	180
Lactate (mmol/L)	3.0 ± 0.3	8.2 ± 2.4$^{\$}$	6.7 ± 0.8$^{\$}$	6.5 ± 1.2$^{\$}$	5.7 ± 0.3$^{\$}$	5.7 ± 1.2$^{\$}$	4.9 ± 0.7$^{\$}$	5.2 ± 0.8$^{\$}$	3.7 ± 1.1

Lactate concentration parameters during the resting period after oral administration of lactate. 0 min means before lactate administration and 15, 30, 45, 60, 90, 120, 150, and 180 min means after lactate administration. Results are presented as the mean ± standard deviation. $ $p < 0.05$ vs. 0 min.

In the study, the mice were randomly divided into four groups as follows: non-trained + placebo (Non/Pla; n = 8), non-trained + lactate (Non/Lac; n = 8), trained + placebo (Tr/Pla; n = 8), and Trained + lactate (Tr/Lac; n = 8). Exercise training intensity was set at the “lactate threshold” (approximately 70% maximal oxygen uptake [VO_{2max}]) based on a previous study and was gradually increased to avoid exercise adaptation [30]. Lactate and placebo groups were administered 3 g/kg of sodium lactate and saline solution, respectively. Lactate or saline was administered immediately after each exercise training. Exercise training and lactate administration were conducted 5 days/week from 10 to 11 a.m. Mice were sacrificed by intraperitoneal anesthesia with 10 μL/g of 1.25% avertin 48 h after the last exercise training and lactate administration. Blood and skeletal muscles (soleus, plantaris, gastrocnemius, extensor digitorum longus, and tibialis [EDL]) were collected immediately after sacrifice. The study design is illustrated in Fig. 1.

Blood analysis

Blood samples were immediately obtained and centrifuged at 1,500 × g for 15 min at 4°C before collecting the supernatant (serum). After blood sample preparation, serum concentrations of lactate (KTB1100, Abbkine, Wuhan, China) and glucose (K039-H1, ARBOR ASSAYS, Ann Arbor, MI, US) were measured using a colorimetric kit according to the manufacturer’s protocol and assessed by colorimetric detection at 450 and 560 nm, respectively. Serum concentrations of IGF1 (ab100695, Abcam, Cambridge,

UK) were analyzed using an ELISA kit. Serum IGF1 was detected using a microplate reader at 450 nm absorbance, according to the manufacturer's instructions.

mRNA analysis

Reverse transcription-polymerase chain reaction (RT-PCR) was used to measure the mRNA levels of protein synthesis and degradation factors in the plantaris muscle. Total RNA was extracted using 1 ml TRIzol reagent (79306; Qiagen, Hilden, Germany), and 200 μL chloroform (038-02601, Wako Chemical, Osaka, Japan) was added. After centrifuging the sample at 12,000 × g for 15 min at 4°C, the supernatant was separated in another tube, and 200 μL isopropanol (d2377, DUKSAN, Seoul, Korea) was added before mixing and reacting. To collect RNA pellets, samples were centrifuged at 12,000 × g for 15 min at 4°C. RNA pellets were diluted in 30 μL of DEPC water and heated at 55°C. To synthesize complementary DNA (cDNA) from the extracted total RNA, amfiRivert cDNA Synthesis Platinum Master Mix (R5600; GenDEPOT, Katy, TX, USA) was used following the manufacturer's instructions. cDNA was amplified using amfiEco Taq DNA polymerase (P0701; GenDEPOT, Katy, TX, USA) following the manufacturer's instructions: 1 μL of cDNA with 24 μL of Taq DNA polymerase, 2 × reaction buffer, PCR water, and forward and reverse primers. Detailed primer sequences and qRT-PCR cycling conditions for each gene are presented in Additional file 1. The PCR products were separated using 1% agarose gels composed of 1 × TAE buffer 50 mL, 0.5 g LE agarose, and 1.25 μL Safe-Pinky DNA gel staining solution (S1001-025; GenDEPOT, Katy, TX, USA). PCR product bands were analyzed using Print Graph 2M (ATTO, Sungnam, Korea) and normalized to GAPDH.

Protein analysis

To confirm the protein expression of muscle synthesis and degradation factors, the EDL muscle was analyzed by western blotting. The EDL muscles were homogenized in 250 μL of protein extraction buffer (EzRIPA Lysis kit, ATTO, Tokyo, Japan). Whole lysates were centrifuged at 14,000 × g at 4℃ for 15 min, and the supernatants that were transferred to new tubes underwent protein concentration determination by a BCA assay kit (Thermo Fisher Scientific, Waltham, MA, USA). After samples were denatured by heating at 100°C for 5 min, that total protein was separated by 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). The membranes were blocked for 1 h with 5% skim milk (Difco, Franklin Lakes, NJ, USA) and washed four times for 5 min using phosphate-buffered saline (PBS) with 0.1% Tween 20 (PBS-T). Membranes were incubated overnight (16–24 h) at 4℃ with 3% skim milk and primary antibodies against the following: GAPDH (1:1000, sc-35062, Santa Cruze Biotechnology, USA), IGF receptor (1:1000, #3027, Cell signaling technology, USA), Akt (1:1000, #4691, Cell signaling technology, USA), p-Akt (1:1000, #4058, Cell signaling technology, USA), mTOR (1:1000, #2983, Cell signaling technology, USA), p-mTOR (1:1000, #5536, Cell signaling technology, USA), P70S6K (1:1000, #2708, Cell signaling technology, USA), p-P70S6K (1:1000, #9205, Cell signaling technology, USA), MuRF1 (1:1000, sc-3998608, Santa Cruze Biotechnology, USA), MAFbx (1:1000, sc-166806, Santa Cruze Biotechnology, USA), and FoxO1 (1:1000, #9461, Cell signaling technology, USA). After washing with PBS-T, membranes were incubated with a horseradish peroxidase-conjugated secondary antibody (anti-mouse, sc-516102; Santa Cruz Biotechnology, USA; anti-rabbit, sc-2357, Santa

Cruz Biotechnology, USA) with 3% skim milk for 1 h at room temperature (24–25°C). Immunodetection was performed using an ECL reagent (Amersham Biosciences, Uppsala, Sweden).

Statistical Analysis

All data were analyzed using the IBM SPSS Statistics 25 software. The significant main effects (supplement, Placebo vs. Lactate; and exercise, Non-trained vs. Trained) and interactions were analyzed using a two-way analysis of variance followed by the LSD post hoc test. Significant differences were considered at $p < 0.05$, and all results are shown as mean ± standard deviations.

Results

Effects of long-term and post-exercise lactate administration on body weight, food intake, and skeletal muscle weight

Body weight, food intake, and skeletal muscle weight after 5 weeks are shown in Table 2. After the experiment, body weight did not differ between the groups. However, food intake had main effects (supplement, $p = 0.000$; and exercise, $p = 0.014$) and interactions ($p = 0.002$). Furthermore, body weight of the Non/Lac was significantly lower than that of the Non/Pla ($p = 0.000$), while that of the trained groups was significantly higher than that of the non-trained groups (Non/Pla vs. Tr/Pla, $p = 0.000$; Non/Lac vs. Tr/Lac, $p = 0.000$).

Muscle weight of five different skeletal muscle was measured and individually normalized to body weight. Lactate administration and exercise training tended to increase muscle weight, but the main effects and interactions were observed only in a few skeletal muscles. The soleus muscle only achieved the main effect after exercise ($p = 0.031$). The gastrocnemius muscle achieved the main effect of supplementation ($p = 0.028$) and interaction ($p = 0.000$), and that of Non/Lac and Tr/Pla were significantly higher than that of Non/Pla ($p = 0.000$; and $p = 0.001$ respectively). The plantaris, EDL, and tibialis muscles were not significantly different among the groups.

Table 2
Body weight and skeletal muscle weight after 5 weeks.

	Non-trained		Trained		
	Placebo	Lactate	Placebo	Lactate	
Body weights (g/mouse)	35.93 ± 0.78	34.64 ± 0.93	35.15 ± 0.64	35.47 ± 0.72	Supplement 0.966 Exercise 0.387 Interactions 0.152
Food intake (g/5 week)	121.22 ± 2.1	109.08 ± 2.0*	147.28 ± 3.4#	148.87 ± 2.3#	Supplement 0.000 Exercise 0.014 Interactions 0.002
Soleus (mg/g body weight)	0.44 ± 0.06	0.47 ± 0.06	0.49 ± 0.02#	0.45 ± 0.07	Supplement 0.849 Exercise 0.031 Interactions 0.096
Plantaris (mg/g body weight)	0.99 ± 0.08	1.06 ± 0.07	1.06 ± 0.06	1.07 ± 0.11	Supplement 0.172 Exercise 0. 145 Interactions 0.369
Gastrocnemius (mg/g body weight)	8.40 ± 0.65	9.14 ± 0.35*	8.98 ± 0.34#	8.96 ± 0.9	Supplement 0.028 Exercise 0. 253 Interactions 0.000
Extensor digitorum longus (mg/g body weight)	0.64 ± 0.13	0.71 ± 0.11	0.68 ± 0.07	0.68 ± 0.11	Supplement 0.161 Exercise 0. 506 Interactions 0.532
Tibialis (mg/g body weight)	3.51 ± 0.24	3.58 ± 0.19	3.63 ± 0.19	3.54 ± 0.37	Supplement 0.913 Exercise 0. 689 Interactions 0.411

Muscle weight of five different skeletal muscle was normalized to body weight. Results are presented as the mean ± standard deviation. * $p < 0.05$, placebo vs. lactate; # $p < 0.05$, non-trained vs. trained.

Effects of long-term and post-exercise lactate administration on blood lactate, glucose, and IGF1 levels

The serum levels of lactate, glucose, and IGF1 were measured to determine whether these were affected by long-term and post-exercise lactate administration (Table 3). The serum lactate concentration did not significantly differ between the groups, the serum glucose concentration only had an exercise effect (p = 0.004), and the serum IGF1 concentration had main effects (supplement, p = 0.021; and exercise, p = 0.01) and interactions (p = 0.043); those of Non/Lac were significantly lower than those of Non/Pla (p = 0.039) and Tr/Lac (p = 0.007).

Table 3
Blood concentration of lactate, glucose, and IGF1.

	Non-trained		Trained		
	Placebo	Lactate	Placebo	Lactate	
Lactate (mmol/L)	3.71 ± 0.60	3.29 ± 0.72	3.18 ± 0.29	3.15 ± 0.55	Supplement 0.265 Exercise 0.101 Interaction 0.332
Glucose (mg/dL)	166.8 ± 20.6	155.4 ± 23.5	131.1 ± 32.0#	135.5 ± 21.3	Supplement 0.691 Exercise 0.004 Interaction 0.375
IGF1 (ng/mL)	29.02 ± 4.03	25.17 ± 2.31*	31.34 ± 3.84	29.38 ± 2.94#	Supplement 0.021 Exercise 0. 01 Interaction 0.043

Results are presented as the mean ± standard deviation. * p < 0.05, placebo vs. lactate; # p < 0.05, non-trained vs. trained.

Effects of long-term and post-exercise lactate administration on mRNA of protein synthesis and degradation factors in mice plantaris muscles

RT-PCR analysis was conducted to investigate the mRNA expression of protein synthesis and degradation factors due to long-term and post-exercise lactate administration. The mRNA levels of *IGF receptor*, *Akt*, *mTOR*, and *P70S6K* were measured to confirm protein synthesis (Figs. 2B-E). *IGF receptor* only achieved the main effect of exercise (p = 0.000). Meanwhile, the mRNA of *Akt* had the main effect of supplementation (p = 0.001) and interactions (p = 0.025); that in Non/Lac were significantly upregulated compared with that in Non/Pla (p = 0.000). Furthermore, *mTOR* only achieved the main effect of supplementation (p = 0.009), and that of Non/Lac was significantly upregulated compared with that of Non/Pla (p = 0.043). Although the mRNA level of *mTOR* was increased in Non/Pla, the *P70S6K* of mTOR downregulation was not significantly different between the groups.

The current study measured the mRNA levels of *MuRF1*, *MAFbx*, and *FoxO1* to confirm changes in protein degradation factors after long-term and post-exercise lactate administration (Figs. 2F-H), which revealed that the mRNA levels of protein degradation factors were not affected.

Effects of long-term and post-exercise lactate administration on protein expression of protein synthesis and degradation factors in mice EDL muscle

To elucidate the effects of long-term and post-exercise lactate administration on protein expression of protein synthesis (Figs. 3B-E) and degradation (Figs. 3G-I) factors, western blotting was conducted. Unexpectedly, long-term and post-exercise lactate administration did not significantly affect protein expression of the synthesis factors of the IGF receptor, Akt phosphorylation, and mTOR phosphorylation. In addition, P70S6K phosphorylation was the only main effect of exercise ($p = 0.025$).

Regarding the protein expression of skeletal muscle degradation factors, MAFbx and FoxO1 were not affected by long-term and post-exercise lactate administration. However, the MuRF1 expression achieved the main effects (supplementation, $p = 0.003$; and exercise, $p = 0.000$) and interactions ($p = 0.035$), and that in Non/Lac and Tr/Pla were significantly downregulated compared with that in Non/Pla ($p = 0.001$ and $p = 0.000$, respectively).

Discussion

While the effects of exogenous lactate administration have been investigated, those of oral lactate administration on muscle synthesis have not been elucidated. The present study investigated the effects of long-term lactate administration on skeletal muscle synthesis and the additional effects of lactate administration after exercise training. The main findings were that long-term lactate administration increased skeletal muscle weight and influenced the expression of protein synthesis and degradation factors. However, lactate administration after exercise training did not have any additional effects on skeletal muscle synthesis.

This study showed that lactate administration decreased the amount of food intake by 11.1% and tended to decrease body weight. Appetite is regulated by hormones, such as leptin and ghrelin [31]. Previous studies have reported that lactate inhibits ghrelin secretion by binding ghrelin-producing cells [32] and that central administration of lactate decreases energy intake in rodents [33]. Therefore, the study suggests that oral lactate administration can also reduce food intake by inhibiting ghrelin secretion.

An important feature of long-term exercise training is that it induces skeletal muscle synthesis [3]. Specifically, high-intensity exercise increases muscle synthesis and lactate concentration to a greater extent than low-intensity exercise [17]. This suggests that an increase in blood lactate concentration may be linked to exercise-induced muscle synthesis. Previous studies have reported that lactate activates cell proliferation and increases the myotube diameter and length, myonuclei number, and protein content in cells [26, 34, 35]. Moreover, administration of 1 g/kg of lactate increased the blood lactate concentration by 4.1 ± 0.3 mmol/L, and that lactate administration 5 days/week increased the weight of the tibialis

anterior muscle after 2 weeks in mice [26]. Thus, we hypothesized that long-term lactate administration would increase skeletal muscle weight. As expected, the pilot results showed that acute lactate administration increased the blood lactate concentration. Additionally, the current study demonstrated that skeletal muscle weight tended to increase in the Non/Lac when normalized to body weight and that the weight of the gastrocnemius muscle significantly increased when normalized to body weight. These findings suggest that increasing blood lactate levels may positively affect skeletal muscle synthesis as part of exercise-induced muscle synthesis.

The Akt/mTOR pathway and muscle-specific E3 ubiquitin ligases regulate muscle synthesis and degradation and play important roles in exercise-induced muscle synthesis [36, 37]. Previous studies primarily focused on the effects of acute lactate intake on protein synthesis and showed that exogenous lactate intake can alter the protein balance by stimulating the *Akt/mTOR* pathway in type 2 skeletal muscles [27, 28]. The present study investigated the effects of oral administration of lactate on protein synthesis and confirmed that the mRNA levels of *Akt* and *mTOR* in the plantaris muscle were increased. However, long-term lactate administration did not affect protein expression in the Akt/mTOR pathway in the EDL muscle. Nevertheless, the current results showed that the weight of skeletal muscles increased after long-term lactate administration, which was expected as the concentration of muscle specific E3 ubiquitin ligases decreased. MuRF1, an E3 ubiquitin ligase expressed in skeletal muscle, regulates proteolysis [38]; long-term exercise training attenuates protein degradation by downregulating MuRF1 expression in skeletal muscle [39, 40]. Interestingly, this study showed that MuRF1 expression was downregulated by long-term lactate administration, resulting in increased skeletal muscle weight. These findings are different from those of previous studies and suggest that long-term lactate administration may have a different effect on protein balance when compared with acute administration. The present study confirms that long-term lactate administration can positively affect skeletal muscle weight and the expression of protein synthesis and degradation factors.

IGF1 is generally acknowledged as somatomedin C, which activates cell growth and proliferation [36, 41]. However, several studies have recommended that the IGF1 concentration does not simply influence muscle synthesis; rather, it requires interaction with IGF receptors, physical activity, bio parameters, or other factors [42, 43]. This study confirmed that long-term lactate administration tended to increase skeletal muscle mass despite decreasing levels of circulating IGF1. Similarly, our previous study in rats confirmed that the level of factors for skeletal muscle synthesis increased even though lactate levels were reduced by serum IGF1 [28]. Thus, we suggest that the potential for increasing skeletal muscle synthesis caused by lactate does not depend on IGF1, and that lactate may affect other muscle synthesis pathways. Tsukamoto et al. [44] proposed that lactate inhibits Sir2, which is a negative regulator of MyoD, by decreasing the NAD^+/NADH ratio. Oishi et al. [35] argued that calcium satellite anabolism is mediated by calcium signals and that lactate may stimulate the calcium/calmodulin-activated serine-threonine phosphatases calcineurin and myogenin. However, since this study did not elucidate the mechanisms of skeletal muscle synthesis caused by lactate, further molecular mechanistic studies on the effects of lactate administration on skeletal muscle are needed.

Oishi et al. [35] confirmed that administration of lactate and caffeine compounds combined with exercise training for 4 weeks increased the weight of the gastrocnemius and tibialis anterior muscles, enhanced the total DNA content, and upregulated anabolic signals in rats. Hashimoto et al. [45] showed that lactate-based compounds increased skeletal muscle weight of the plantaris and gastrocnemius muscles in obese rats when combined with voluntary running exercises. However, although the present study showed that lactate affects skeletal muscle weight and protein synthesis and degradation factors, its administration after exercise training did not result in additional effects.

Several studies reported that most (75–80%) of the lactate produced from exercise is preferentially used and stored as an energy source by skeletal muscles during exercise, post-exercise, and rest under aerobic conditions [46, 47]. In addition, Brooks et al. [48] reported that the lactate shuttle, which is activated by exercise training, transports lactate to the skeletal muscles, heart, and liver for energy production and gluconeogenesis. The lactate shuttle, also known as MCT isoforms 1 to 4, transports lactate to “cell-cell” and “intracellular shuttles” and is activated by the external environment, specifically exercise [49]. Coles et al. [50] showed that the MCT1 and 4 proteins and mRNA expression were immediately enhanced after acute exercise (2 hours, 21 m/min, 15% grade) in rat skeletal muscles. Enoki et al. [51] also reported that long-term exercise training (20–25 m/min, 30 min/day, 7 days/week, 3 weeks) increased levels of MCT1 and 4 in rat skeletal muscles. In summary, lactate is immediately transported and eliminated by exercise activated MCTs. In our experiment, lactate was administered immediately after every episode of exercise training. Hence, we supposed that MCTs were activated by exercise training, and that lactate administered immediately post-exercise was preferentially used as an energy source for post-exercise recovery rather than for skeletal muscle synthesis. However, a limitation of this study was that we did not confirm MCT activation in the skeletal muscles. Therefore, a more detailed study investigating the activation of MCTs is needed to confirm the effects of lactate on molecular signaling in skeletal muscle synthesis. Nevertheless, since lactate tended to increase skeletal muscle weight, positive results of skeletal muscle synthesis may be observed if lactate is administered before exercise training to reduce the effect of MCTs.

Conclusion

In summary, the present study confirmed that (a) lactate can increase skeletal muscle mass and has positive effects on protein synthesis (mRNA levels of *Akt* and *mTOR*) and degradation (MuRF1 protein expression); however, (b) the effects of lactate on skeletal muscles tend to disappear after exercise training. Although the study did not elucidate the exact mechanisms of long-term and post-exercise lactate administration, a notable finding was that lactate has the potential to positively regulate skeletal muscle synthesis. Therefore, this study provides new insights into the effects of oral administration of exogenous lactate on skeletal muscle synthesis.

Declarations

Ethics approval and consent to participate

Ethical approval for this study was obtained from the Konkuk University Institutional Animal Care and Use Committee (No. KU19149).

Consent for publication

Not applicable.

Availability of data and materials

All availability of data and materials included in this article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

S.K., J.K., H.P., and K.L. conceived and designed the experiment. S.K., and I.J. performed the experiment. S.K., J.K., D.H., and H.P. analyzed data. S.K., D.H., and I.J. prepared figures and tables. S.K. wrote original draft. All authors contributed to manuscript revision.

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Figures

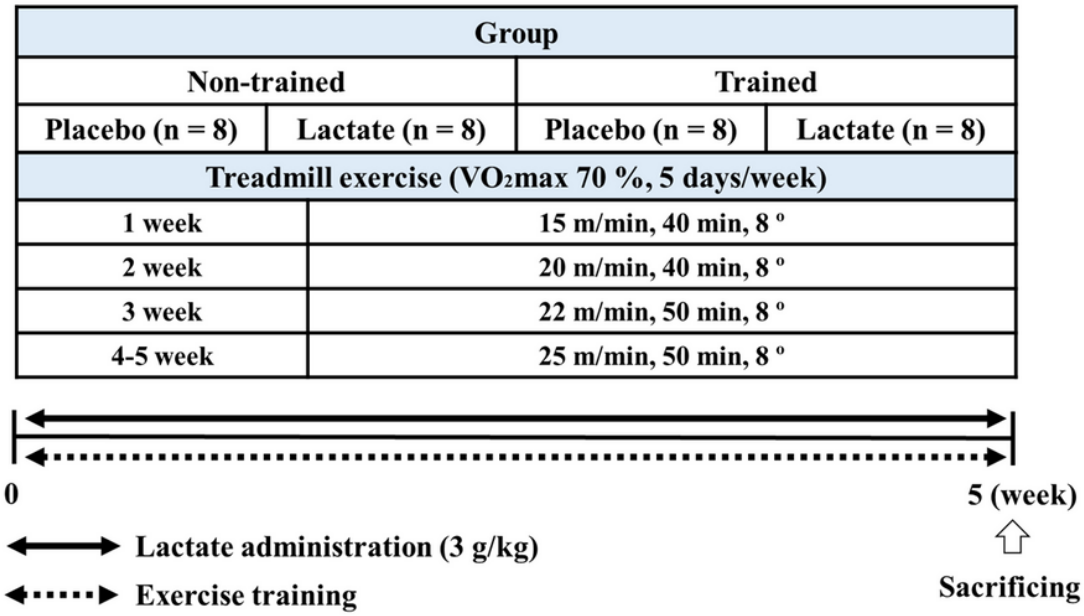

Figure 1

Study design. Study design of long-term and post-exercise lactate administration.

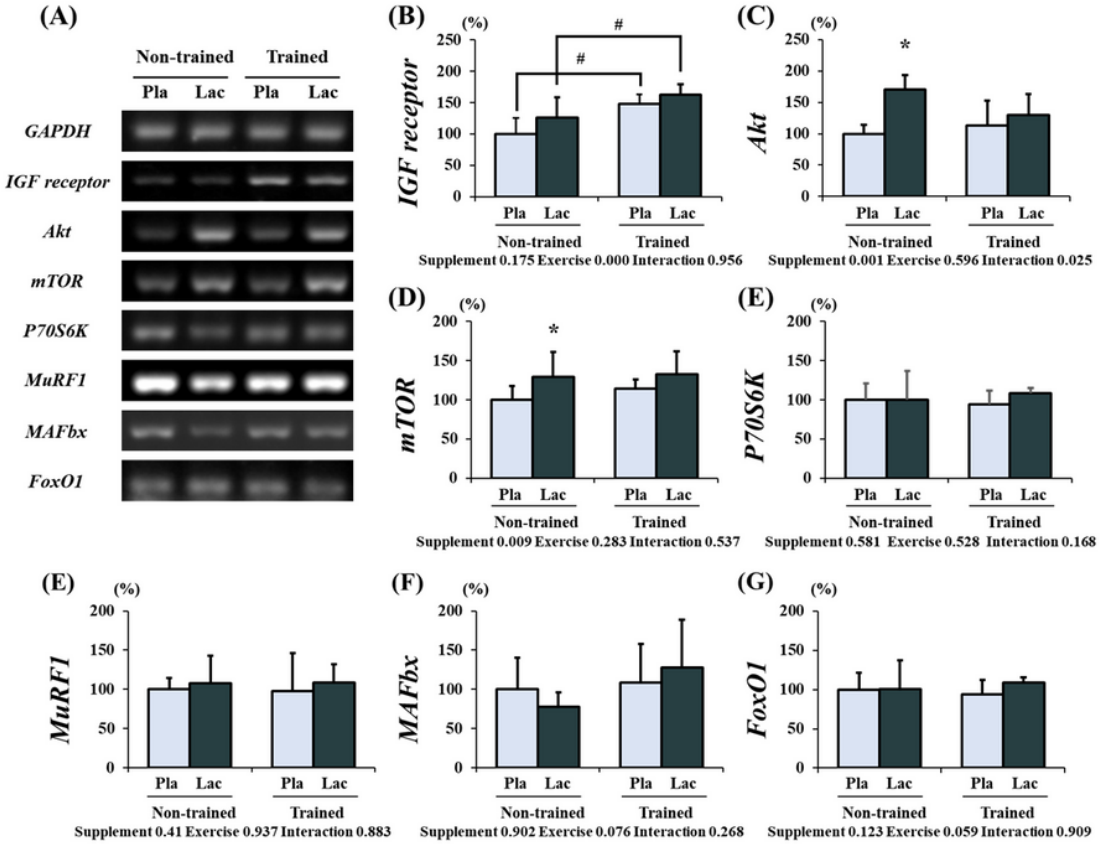

Figure 2

mRNA expression of protein synthesis and degradation factors in the plantaris muscle. (A) Polymerase chain reaction band. (B) *IGF receptor*, (C) *Akt*, (D) *mTOR*, (E) *P70S6K*, (F) *MuRF1*, (G) *MAFbx*, and (H) *FoxO1* expression levels were normalized by GAPDH. Results are presented as means ± standard deviation. * $p < 0.05$, placebo vs. lactate; $^{\#}$ $p < 0.05$, non-trained vs. trained. Pla: placebo (saline administration); Lac: lactate administration.

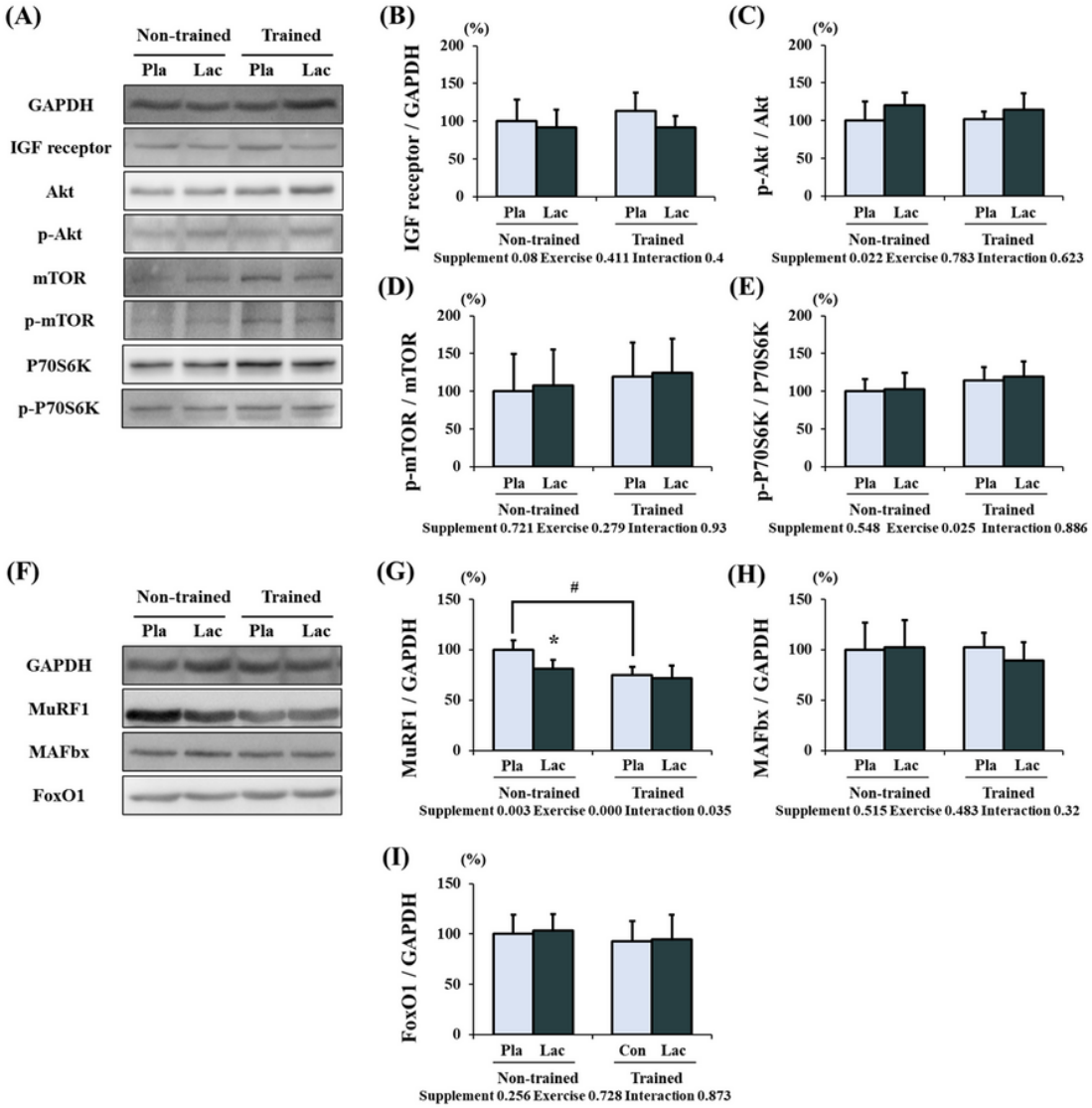

Figure 3

Protein expression of protein synthesis and degradation factors in EDL muscle. (A) and (F) present the western band of protein synthesis and degradation factors, respectively. (B) IGF receptor, (C) Akt, (D) mTOR, (E) P70S6K, (F) MuRF1, (G) MAFbx, and (H) FoxO1 represent protein expression. Results are presented as means ± standard deviation. * $p < 0.05$, placebo vs. lactate; $^{\#}$ $p < 0.05$, non-trained vs. trained. Pla: placebo (saline administration); Lac: lactate (lactate administration).

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