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Effects of dietary carbohydrate and energy intake on LAT1 protein expression in rat skeletal muscle

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Running Title: Diet and skeletal muscle LAT1 protein expression

Abstract

Glucose has been reported to reduce the expression of L-type amino acid transporter 1 (LAT1) protein in C2C12 myocytes. We therefore hypothesized that increased dietary carbohydrate and energy intake would reduce LAT1 protein expression in rodent skeletal muscle. Here, we tested this hypothesis. In Experiment 1 to examine the effects of dietary carbohydrate intake, male Sprague Dawley (SD) rats were divided into low-carbohydrate (low-CHO) and high-carbohydrate (high-CHO) diet groups. Each group was fed a low-CHO (20% carbohydrate) or high-CHO (70% carbohydrate) diet, respectively. Total energy intakes of both groups were matched by pair feeding. In Experiment 2 to examine the effects of dietary energy intake, rats were divided into low-Energy diet (fed 68% of ad libitum energy intake) and high-Energy diet (ad libitum) groups. After 7 days of dietary manipulation, the lower leg muscles on one side were percutaneously stimulated and subjected to one acute bout of resistance exercise. The contralateral leg muscle served as an internal control. We collected gastrocnemius muscle 6 h after contraction. In both Experiments 1 and 2, when results were analyzed by two-way analysis of variance, no main effect of diet on LAT1 protein concentration was observed. There was also no main effect of resistance exercise, or no interaction between diet and exercise. These results do not support our hypothesis that increased dietary carbohydrate and energy intake reduce LAT1 protein expression in rodent skeletal muscle. Furthermore, diet may not affect the effects of resistance exercise on LAT1 protein expression.

Keywords

L-type amino acid transporter 1, leucine, gastrocnemius muscle, dietary carbohydrate ratio, total energy intake

標題

糖質ならびにエネルギー摂取量がラット骨格筋の LAT1 タンパク質発現に及ぼす影響

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概要

グルコースは、C2C12 筋細胞における L 型アミノ酸トランスポーター1（LAT1）タンパク質の発現を低下させることが報告されている。そこで我々は、食事からの糖質とエネルギーの摂取量が増加すれば、ラット骨格筋における LAT1 タンパク質発現が減少するという仮説を立てて検証した。実験 1 では、糖質摂取が LAT1 タンパク質発現に与える影響について調べるために、SD 系雄性ラットを低糖質食群と高糖質食群に振り分けた。各群にはそれぞれ

低糖質食（糖質 20%）または高糖質食（糖質 70%）を与えた。両群の総エネルギー摂取量はペアフィーディング法で一致させた。実験 2 においては、エネルギー摂取量の違いが LAT1 タンパク質発現に与える影響について調べるため、ラットを低エネルギー食群（自由摂取エネルギー量の 68%を給餌）と、高エネルギー食群（自由摂取）に分けた。7 日間の食事介入後、片側の下腿筋を経皮的に刺激し、1 回の急性レジスタンス運動を行った。対側の下腿筋はコントロール脚として使用した。筋収縮の 6 時間後に腓腹筋を摘出した。実験 1 と 2 の結果を二元配置の分散分析で解析したところ、LAT1 タンパク質濃度に対する食餌の主効果ならびに、レジスタンス運動の主効果は見られず、交互作用も見られなかった。これらの結果は、食餌からの糖質およびエネルギーの摂取量増加は、骨格筋における LAT1 タンパク質発現を減少させるという我々の仮説を支持するものではなかった。さらに、食餌はレジスタンス運動が LAT1 タンパク質発現量に与える影響には関与しない可能性がある。

Introduction

Being the main components of lean body mass and the protein reservoir in the human body, skeletal muscles not only control locomotion but they are fundamental for breathing, eating, energy expenditure, as well as for glucose, amino acids, and lipids homeostasis and for maintaining a high quality of life[1]. Loss of muscle mass and function has been linked to the risk of metabolic diseases, poor quality of life, increased morbidity, all-cause mortality, and frailty[2,3,4]. Muscle mass is regulated by the balance between muscle protein synthesis and protein breakdown rates. Therefore, muscle hypertrophy could theoretically be caused by increased muscle protein synthesis, decreased degradation, or both. However, among these factors, an increase in protein synthesis may be required to increase skeletal muscle mass[5,6].

The essential amino acids, in particular leucine, stimulate muscle protein synthesis, which is generally dependent on their delivery to intracellular sensors and effector molecules associated with the mTORC1 activity [7,8,9,10,11]. The influx of essential amino acids into skeletal muscle is mediated by amino acid transporters ubiquitously expressed in the plasma membrane of many cell types. The L-type amino acid transporter 1 (LAT1) is the most highly expressed large neutral amino acid transporter in skeletal muscle [12], although others such as LAT2, LAT3, and LAT4 may be present and able to transport essential amino acids [13,14]. Thus, LAT1 expression levels in skeletal muscle may directly contribute to muscle protein synthesis via amino acid transport and regulate muscle mass.

LAT1 primarily transports leucine into the sarcoplasma while co-transporting glutamine out of the cell, whereas the sodium-coupled neutral amino acid transporter 2 (SNAT2) co-transports both sodium and glutamate into the sarcoplasma [15]. These two

transport proteins work in tandem such that SNAT2 brings in glutamine for LAT1 to pump back out of the cell while concomitantly transporting leucine into the cell.

Amino acid transporter expression in skeletal muscle may be dynamic and acutely responsive to dietary amino acids intake and resistance exercise. For example, amino acid ingestion stimulates an increase in LAT1 and SNAT2 gene expression, an event which is followed by increases in protein expression 3 h post-ingestion [16]. These changes have also been reported in response to resistance exercise, whereby overnight resistance-braked running wheel exercise increases LAT1 protein expression mouse skeletal muscle [17]. Gene expression of LAT1 and SNAT2 has also been reported to increase in human muscle at 6 hours after resistance exercise, followed by increased protein expression over a 24-hour recovery period [18].

On the other hand, it was reported that in retinal capillary endothelial cells, LAT1 gene expression decreases with increasing glucose concentration in the medium [19]. The same result was reported in C2C12 myocytes [20]. Thus, glucose down-regulates LAT1 protein expression. However, it is unclear whether dietary carbohydrate intake affects LAT1 protein expression in rodent skeletal muscle, and whether increased dietary carbohydrate suppresses the exercise effect on LAT1.

In the Experiment 1 of the present study, we examined the effect of dietary carbohydrate ratio on LAT1 protein expression in rat skeletal muscle under the same energy intake. We also examined whether the effect of resistance exercise on muscle LAT1 protein expression is affected by dietary carbohydrate ratio. In addition, it might be possible that energy intake, rather than dietary carbohydrate intake per se, affects the expression of LAT1 protein. Therefore, in the Experiment 2 of this study, we also examined the effect of dietary energy intake.

Materials and Methods

Experiment 1 protocol. This experiment examined the effect of the amount of carbohydrate intake on LAT1 expression in skeletal muscle under conditions of equal energy intake. 10-week-old Sprague Dawley (SD) rats, purchased from Kyudo company (Saga, Japan). The rats were housed under 12h light/dark cycle at 22.0 ± 2.0℃ and 50% ± 10% humidity. After preliminary housing for 1 week, they were randomly divided into two groups: fed a low-carbohydrate diet (Protein : Fat : Carbohydrate balance = 2 : 6 : 2) group (low-CHO) and fed a high-carbohydrate diet (Protein : Fat : Carbohydrate balance = 2 : 1 : 7) group (high-CHO) and fed each diet for 7 days. Carbohydrate ratios for low- and high-CHO diets followed previous studies [21]. The diet composition is shown in Table 1. The experimental period, energy intake was standardized for both dietary conditions by pair-feeding. Both groups were allowed to drink freely, and amount of food intake was measured daily.

Experiment 2 protocol. This experiment examined the effect of the amount of energy intake on LAT1 expression in skeletal muscle. 9-week-old male SD rat, purchased from Kyudo company. The rats were housed under the same conditions as in Experiment 1. After preliminary housing for 1 week, they were randomly divided into two groups: a low-Energy group and high-Energy group and fed a high-fat diet (Protein : Fat : Carbohydrate balance = 2 : 6 : 2) for 7 days. The diet composition is shown in Table 1. The low-Energy group was pair-fed with rats fed a high-carbohydrate diet (Protein : Fat : Carbohydrate balance = 2 : 1 : 7) at the same week of age to standardize energy intake, while the high-Energy group was fed ad libitum. Both groups were allowed to drink freely, and amount of food intake was measured daily. When rats are fed ad

libitum on a high-fat diet, their energy intake is well known to exceed their energy requirements. Our approach therefore resulted in overfeeding in the high-Energy group, whereas the low-Energy groups rats consumed an amount equivalent to their energy requirements. It allowed us to examine the effects of overfeeding, even though both group rats ate the same type of chow. The study was approved by the Animal Care and Use Committee of Fukuoka University and was conducted (No. 2113104).

Resistance exercise. In both Experiment 1 and 2, after dietary intervention, all rats underwent muscle contraction using transcutaneous electrical stimulation under anesthesia (4%–5% sevoflurane). Electrical stimulation was applied to the right leg (exercise leg), and the left leg was used as a control (non-exercise leg). Electrical stimulation was set at a frequency of 100Hz and a voltage of 30V, and muscle contractions of 3 seconds were repeated 10 times. This was considered as one set, and a total of 5 sets with a 3-minute interval in between. This acute resistance exercise protocol has been reported to increase the rate of muscle protein synthesis [22]. It has also been reported that continuous application of this protocol induces muscle hypertrophy [22]. After resistance exercise, rats were monitored until they awoke from anesthesia.

Sampling. After 6 h of resistance exercise, the rats were weighed, and their gastrocnemius muscles were collected under anesthesia (4%–5% sevoflurane). Blood samples were also collected from aorta. EDTA-treated blood samples were centrifuged at 1000g for 10 min to collect plasma. All the samples were stored at -80℃ until used for analysis.

Measurement of plasma glucose level.

Plasma glucose levels was measured using a glucose C Ⅱ-test (Fujifilm, Tokyo, Japan). The concentration of glucose in the plasma was measured according to the standard protocol of the kit. Absorbances were measured using a GloMax Discover System (Promega Corporation, WI, USA).

Western blotting. Frozen gastrocnemius samples were homogenized in RIPA buffer (Fujifilm, Tokyo) with protease inhibitor (Roche, Basel, Switzerland) and phosphatase inhibitor (Roche), and the total protein concentration of each was measured using a BCA protein assay kit (Thermo Fisher Scientific, MA, USA). Protein lysate was mixed with Laemmli sample loading buffer (BioRad, CA, USA) and heated at 90℃ for 10 minutes. The aliquots containing equal amounts of protein were separated by SDS-PAGE and the proteins were transferred to PVDF membranes. The membranes were blocked using 3% skim milk in TBS-Tween 20 for 60 minutes at room temperature and then incubated with LAT1 antibody (SantaCruz #sc-374232, TX, USA) for overnight at 4℃. The membrane was then incubated with the secondary antibody (Vector Laboratories, CA, USA) for 90 min at room temperature. The LAT1 protein was visualized using Immnobilo Forte Western HRP (Millipore, MA, USA). An Amersham imager 600 (GE Healthcare Life Science, MA, USA) was used for imaging, and the images were quantified by image Lab software (BioRad). Membranes were stained with Ponceau-S (Sigma-Aldrich, MO, USA) to confirm equal amounts of protein loading.

Statistical analysis. Data are expressed as mean ± standard error (SEM). GraphPad prism 10 (GraphPad, CA, USA) was used for the statistical analysis. Two-way ANOVA

was used for comparisons between the four groups, and an unpaired t-test was used for comparisons between the two groups. $P < 0.05$ was considered to represent statistical significance.

Results

Experiment 1. The amount of carbohydrate intake does not affect LAT1 expression in skeletal muscle under conditions of the same energy intake. The total energy, protein, fat, carbohydrate intake, body weight, and plasma glucose are shown in Table 2. There were no changes in total energy intake and body weight of the high-CHO group and low-CHO group during the 7-day dietary intervention period, confirming that pair-feeding was performed appropriately. As we assumed, the high-CHO group consumed approximately 3.5 times as much carbohydrate as the low-CHO group. There was no change in plasma glucose between low-CHO and high-CHO groups.

The protein expression level of LAT 1 in gastrocnemius are shown in Figure 1. No main effect of diet on the LAT1 protein expression was observed in gastrocnemius. Equally, there was no main effect of resistance exercise, nor were their interaction observed. These results suggest that dietary carbohydrate ratio does not affect LAT1 protein expression under conditions of the same energy intake.

The change in LAT1 protein expression level due to resistance exercise was calculated for each animal by subtracting the value of the contralateral control leg muscle from the value of the exercised leg muscle (Figure 2). Resistance exercise increased LAT1 protein expression by 51% and 22% in the low- and high-CHO groups, respectively. Although this value was not statistically significant, it tended to be higher in the low-CHO group compared to the high-CHO group ($p = 0.12$, effect size=0.91).

244

245 ***Experiment 2***. The amount of energy intake does not affect LAT1 expression in skeletal
246 muscle under conditions of the same type of chow. The total energy, protein, fat,
247 carbohydrate intake, body weight was shown in Table 3. As we assumed, total energy
248 intake were increased in the high-Energy group compared to the low-Energy group
249 during the 7-day dietary intervention period. The high-Energy group consumed an
250 average of approximately 27 kcal/day more energy than the low-Energy group.
251 The protein expression level of LAT 1 in gastrocnemius are shown in Figure
252 3. No main effect of diet on the LAT1 protein expression was observed in
253 gastrocnemius. Equally, there was no main effect of resistance exercise, nor were their
254 interaction observed. These results suggest that the amount of energy intake does not
255 affect LAT1 expression level in skeletal muscle.
256 As, in the Experiment 1, the change in LAT1 protein expression level due to
257 resistance exercise was calculated (Figure 4). Resistance exercise increased LAT1
258 protein expression by 12% and 43% in the low- and high-Energy groups, respectively.
259 However, no significant differences were observed in the change in LAT1 protein
260 expression due to resistance exercise between the low- and high-Energy group ($p=0.35$,
261 effect size=0.62).
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Discussion

In C2C12 myocytes, high concentration of glucose in the culture medium was reported to reduce the LAT1 mRNA expression [20] . Findings from this previous study suggest the possibility that increased muscle glucose uptake decreases LAT1 gene expression. In the experiment 1 of our present study, plasma glucose levels were not significantly different between low-CHO and high-CHO group rats. Even though high carbohydrate intake may cause a temporary increase in blood glucose levels, increased insulin secretion from the pancreas increases muscle glucose uptake and blood glucose levels falls to normal levels. Thus, blood glucose level may remain constant regardless of dietary carbohydrate intake. Therefore, the absence of differences in plasma glucose level does not necessarily mean that muscle glucose uptake is the same between groups. In other words, glucose uptake may be higher in the muscles of high-CHO rats than in the muscles of low-CHO rats. In support of this idea, higher muscle glycogen levels were observed in the high-CHO group rats than in the low-CHO group rats in another experiment conducted under the same dietary conditions as the present study (Kawanaka et al. unpublished data). However, in the experiment 1 of our present study, no effect of dietary carbohydrate ratio on LAT1 protein expression was observed, suggesting that increased glucose uptake may not reduce LAT1 protein expression in skeletal muscle. Furthermore, in the experiment 1, LAT1 protein expression tended to be higher in contralateral control leg of high-CHO rats than in low-CHO rats. Since there is a possibility of type 2 error, we cannot completely rule out the possibility that increased dietary carbohydrate intake increases muscle LAT1 expression.

In the experiment 1 of our present study, the two groups, i.e., the high-CHO and low-CHO diet groups, were compared by pair feeding to ensure the same energy intake. Taking this into account, previous study showing that high glucose in the culture medium reduce LAT1 mRNA expression in myocytes [20] suggest another possibility that the addition of energy, rather than increased glucose uptake, reduces LAT1 gene expression. However, this possibility is also unlikely. This is because, in the experiment 2 of our present study, LAT1 protein expression in the high-Energy group was not different from the low-Energy group, despite higher energy intake.

The reason for the discrepancy between the previous study and the present study is unclear. However, the effects of in vivo dietary manipulation on animal skeletal muscle may be very different from the effects of media manipulation on C2C12 cells. In C2C12 myocytes, pharmacological AMPK activation by AICAR increases LAT1 mRNA levels [20]. Thus, AMPK may up-regulate LAT1 gene expression. On the other hand, high concentrations of glucose in the medium decrease AMPK activity in C2C12 cells [20]. Thus, glucose may down-regulate LAT1 gene expression via inactivation of AMPK. Although glucose concentration in the culture medium affected AMPK activity in C2C12 cells, dietary carbohydrate and energy intake may not have affected AMPK activity in rat skeletal muscle. And this may be the reason why we could not observe changes in LAT1 protein expression. AMPK activity was not measured in this study. Therefore, further studies measuring AMPK activity are required to examine this possibility.

In the previous study, LAT1 mRNA expression was highest in C2C12 myocytes cultured in 0 mM glucose medium, and low concentrations of glucose (1.4 mM) reduced LAT1 mRNA expression to its lowest level. Further increases in glucose

concentration had no further effect. Thus, the effect of carbohydrate on LAT1 gene expression might be maximal at very small amounts of glucose. Therefore, we cannot rule out the possibility that even very small amounts of carbohydrate intake may reduce the LAT1 protein expression in skeletal muscle compared to no carbohydrate intake. The energy percentage of carbohydrate in our low-CHO diet is 20%, which is higher than the 5% energy percentage of carbohydrate in very low carbohydrate diet such as ketogenic diet. We cannot rule out the possibility that our low-CHO diet reduced the expression of LAT1 protein compared to the carbohydrate-free or ketogenic diet. This is a limitation of our study and requires further investigation.

In the Experiment 2, the high-Energy group rats were fed ad libitum on a high-fat diet (Protein : Fat : Carbohydrate balance =2 : 6 : 2). When rats were fed high-fat-diet, their energy intake is well known to exceed their energy requirements. Our approach therefore resulted in overfeeding in the high-Energy group. On the other hand, energy intake of the low-Energy group rats was matched by pair feeding to rats fed a high-carbohydrate diet (Protein : Fat : Carbohydrate balance = 2 : 1 : 7), even though they were on a high-fat diet. Thus, the energy intake of the low-Energy group rats was adjusted to their energy requirement level. Muscle LAT1 protein expression was not different between low- and high-Energy group rats, suggesting that overfeeding does not have any effect on muscle LAT1 protein expression. However, the effects of decreased energy intake below energy requirement, i.e., caloric restriction, were unclear. This requires further investigation.

In the present study, we applied an in situ electrical stimulation-induced resistance exercise model in which current was applied to the leg muscles on one side to induce involuntary muscle contraction. Because the contralateral leg muscle was used as

a control, the increase in LAT1 protein due to exercise could be calculated for each animal by subtracting the value of the contralateral control leg muscle from the value of the exercised leg muscle. And although this value was not statistically significant, it tended to be higher in the low-CHO group than in the high-CHO group. In other words, the higher the dietary carbohydrate ratio, the lower the exercise effect on LAT1 protein expression may be. Acute resistance exercise has been reported to increase LAT1 protein expression in skeletal muscle [17, 18]. The exercise-induced increase in LAT1 expression presumably increases leucine entry into muscle cells and promotes muscle protein synthesis. This may be one mechanism by which exercise induces muscle hypertrophy. If a high-CHO diet exacerbates exercise-induced increase in LAT1 protein, then a diet with a high carbohydrate ratio might adversely affect the effects of exercise in promoting muscle protein synthesis and muscle mass. However, this idea warrants caution in the interpretation. This is because when the results of Experiment 1 were analyzed by two-way analysis of variance, there was no main effect of resistance exercise or no interaction between dietary carbohydrate ratio and exercise. Future studies will be needed to determine the effect of dietary carbohydrate ratio on exercise effect on LAT1 protein expression.

In conclusion, during the 7-day dietary manipulation period, changing the dietary carbohydrate ratio under the same energy intake did not significantly affect the expression of LAT1 protein in the rat gastrocnemius muscle. In addition, changing dietary energy intake did not affect muscle LAT1 protein expression. These results do not support our hypothesis that increased dietary carbohydrate or energy intake decreases LAT1 protein expression in skeletal muscle.

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Authors' contribution statement

A. Yokogawa, K. Tanaka, I. Miura, S. Watanabe, K. Kido, and K. Kawanaka conceived and designed the study; A. Yokogawa, K. Tanaka, I. Miura, Y. Iwao, S. Watanabe, D. Takakura, K. Kido, and K. Kawanaka performed the experiments; A. Yokogawa, T. Tanaka, I. Miura, and K. Kawanaka analyzed the data; all authors interpreted the results; T. Tanaka and I. Miura prepared the figures; I. Miura and K. Kawanaka drafted the manuscript; all authors approved the final version of the manuscript.

Conflict of interest

The author(s) declare that there are no conflicts of interest.

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464 **Table 1. The composition of food used in this study**

	High-Carbohydrate		Low-Carbohydrate (high-fat diet)	
	gm%	**kcal%**	**gm%**	**kcal%**
Protein	**19.2**	**20**	**26**	**20**
Carbohydrate	**67.3**	**70**	**26**	**20**
Fat	**4.3**	**10**	**35**	**60**
Total		**100**		**100**
kcal/gm	**3.85**		**5.24**	
	gm	**kcal**	**gm**	**kcal**
Casein, 30 Mesh	**200**	**800**	**200**	**800**
L-Cystine	**3**	**12**	**3**	**12**
Corn Starch	**506.2**	**2024.8**	**0**	**0**
Maltodextrin 10	**125**	**500**	**125**	**500**
Sucrose	**68.8**	**275.2**	**68.8**	**275**
Cellulose, BW200	**50**	**0**	**50**	**0**
Soybean Oil	**25**	**225**	**25**	**225**
Lard	**20**	**180**	**245**	**2205**
Mineral Mix S 10026	**10**	**0**	**10**	**0**
DiCalcium Phosphate	**13**	**0**	**13**	**0**
Calcium Carbonate	**5.5**	**0**	**5.5**	**0**
Potassium Citrate, 1 H2O	**16.5**	**0**	**16.5**	**0**
Vitamin Mix V10001	**10**	**40**	**10**	**40**
Choline Bitartrate	**2**	**0**	**2**	**0**
FD&C Yellow Dye #5	**0.04**	**0**		
FD&C Blue Dye #1	**0.01**	**0**	**0.05**	**0**
Total	**1055.05**	**4057**	**773.85**	**4057**

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Table 2. The effect of dietary carbohydrate ratio on body weight, total energy intake, total protein intake, total fat intake, total carbohydrate intake

	high-CHO	low-CHO
Body weight (g)	416.3 ± 4.8	409.7 ± 4.3
Total energy intake (kcal/week)	530.0 ± 13.3	530.0 ± 13.3
Total protein intake (kcal/week)	106.0 ± 2.7	106.0 ± 2.7
Total fat intake (kcal/week)	53.0 ± 1.3	318.0 ± 8.0 *
Total carbohydrate intake (kcal/week)	371.0 ± 9.3	106.0 ± 2.7 *
Plasma glucose (mg/dl)	125.1 ± 6.8	124.9 ± 8.8

466 Values are means ± SEM (n = 9/group). *p＜0.05 vs. high-CHO

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Table 3. The effect of energy surplus intake on body weight, total energy intake, total protein intake, total fat intake, total carbohydrate intake

	low-Energy	high-Energy
Body weight (g)	394.5 ± 6.7	425.3 ± 6.8 *
Total energy intake (kcal/week)	607.1 ± 10.1	799.5 ± 16.1 *
Total protein intake (kcal/week)	121.4 ± 2.0	159.9 ± 3.2 *
Total fat intake (kcal/week)	364.3 ± 6.0	479.7 ± 9.7 *
Total carbohydrate intake (kcal/week)	121.4 ± 2.0	159.9 ± 3.2 *

468 Values are means ± SEM (n = 6/group). *p＜0.05 vs. low-Energy

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Figure

Experiment 1.

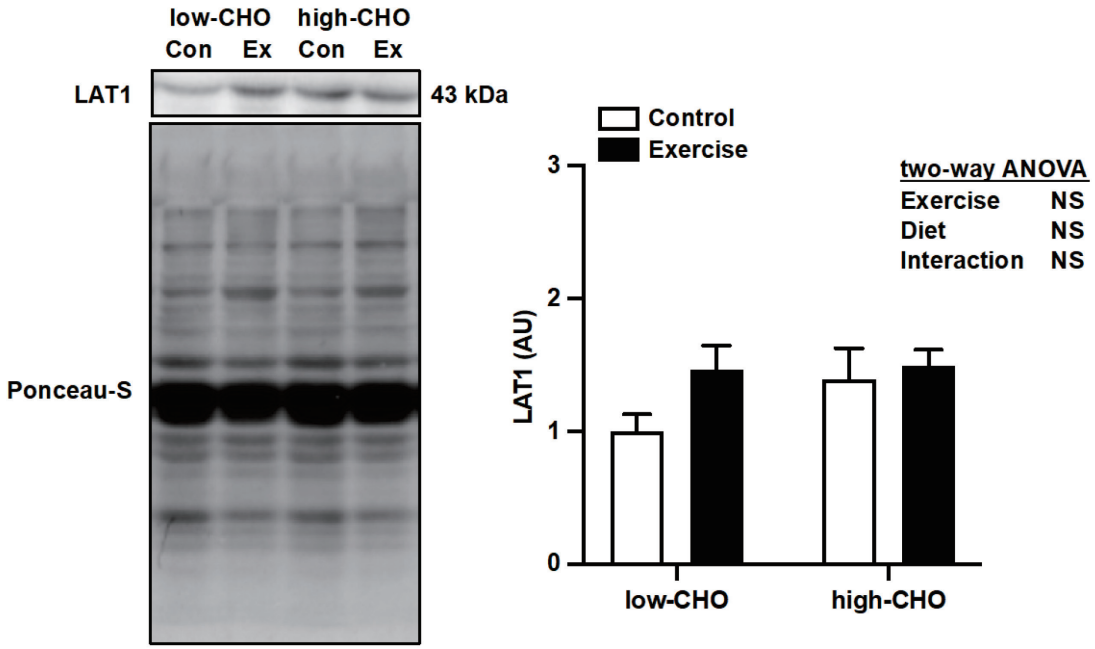


Figure 1

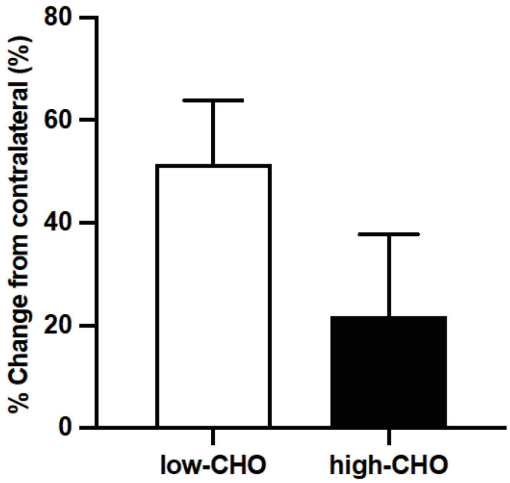
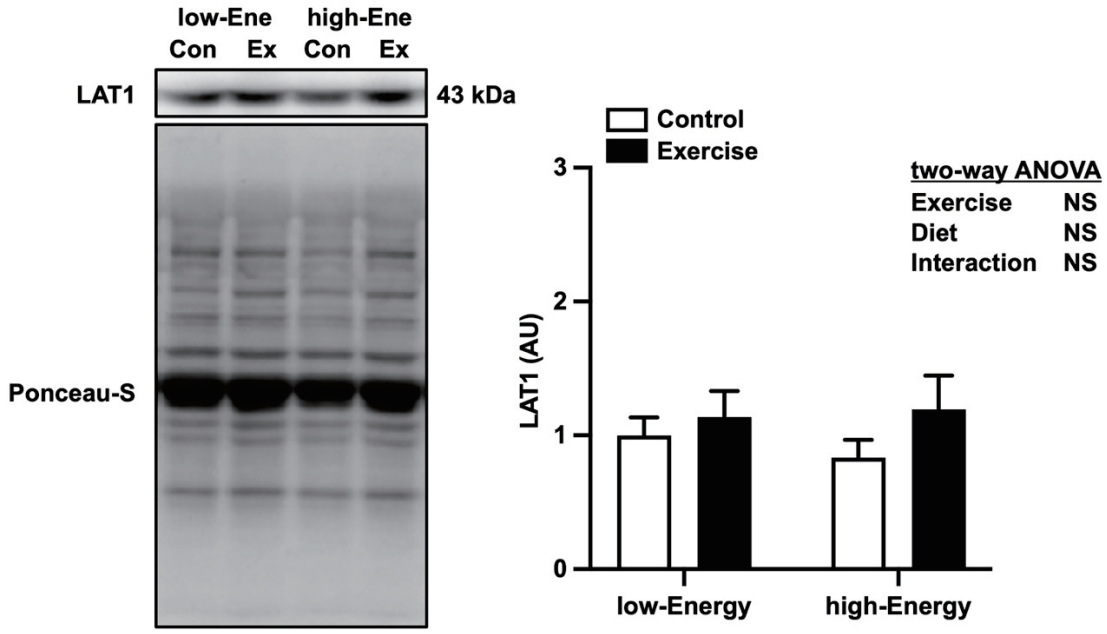


Figure 2

490 ***Experiment 2***

491

492



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494 Figure 3

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496

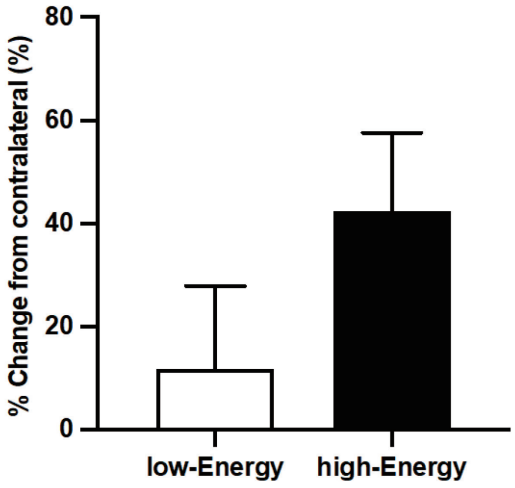
% Change from contralateral (%)
80
60
40
20
0
low-Energy
high-Energy

Figure 4

Figure Legends

Figure 1. Effect of carbohydrate intake on LAT1 protein expression.

Con, control (non-exercise leg); Ex, exercise (exercise leg); AU, arbitrary units. Values are expressed as mean ± SEM (n = 7-9/group). Two-way ANOVA was used for statistical processing.

Figure 2. Effect of carbohydrate intake on the rate of change in LAT1 protein expression by resistance exercise.

AU, arbitrary units. Values are expressed as mean ± SEM (n = 7-9/group).

Figure 3. Effect of different energy intake on LAT1 protein expression.

Con, control (non-exercise leg); Ex, exercise (exercise leg); low-Ene, low-Energy; high-Ene, high-Energy; AU, arbitrary units. Values are expressed as mean ± SEM (n = 6/group). Two-way ANOVA was used for statistical processing.

Figure 4. Effect of different energy intake on the rate of change in LAT1 protein expression by resistance exercise.

AU, arbitrary units. Values are expressed as mean ± SEM (n = 6/group).