Research Article

Crosstalk between tight junction genes and muscle strength: Applying supplement and resistance training to old male wistar rats

Majid Mohabbat[1], Hamid Arazi[1*]

Abstract

Aim of this study was to determine the relations among the tight junction (TJs) genes, muscle strength and cross-sectional area (CSA) influenced by resistance training with or without specific supplement (a combination of lactobacillus and bifidobacterium probiotics, leucine amino acid and Vitamin-D). For this purpose, 25 male wistar rats in two age groups (3 months in young control and 16-24 months in four other groups) randomly divided in 5 equal groups (old and young control, resistance training, supplement and resistance training plus supplement). After 8 weeks of resistance training trice a week and oral gavage of supplement 5 times per week there were no any relation between grip strength and muscle CSA with zonula occludens-1 (ZO-1) and occludin (Occ) genes. But result of one-way ANOVA revealed that there were significantly differences among study groups in TJs genes, muscle strength and CSA ($P \leq 0.05$). Our finding showed that resistance training along with supplement can increase the level of ZO-1 (P=0.011), and Occ genes (P=0.023) expression. Indeed, resistance training plus supplement had synergistic effect on muscle CSA and grip strength (P=0.001) that can be comparable with young group. In addition, supplement alone appears that doesn't have beneficial impact on physical function but surprisingly our finding shows strong inverse correlation between Occ and grip strength (p=0.015, r= -1.0) in supplement group which implies that although supplement alone can't improve physical function but can maintain intestinal barrier function.

Key Words: **Tight junction, Muscle enhancement, Strength training, Wistar rat, Hypertrophy, Probiotic supplementation**

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Introduction

Aging is accompanied with several diseases and chronic low-grade systemic inflammation. Decrease in muscle mass, function and strength was shown by progress in age and start from 4th to 5th decade of life and called sarcopenia (Walston., 2012). Sarcopenia has a multifactorial cause but it is clear that inflammation and detrimental metabolites in gut plays important role in this scenario (Dalle, Rossmeislova, & Koppo., 2017). It was shown that aging is associated with change in gut microbiome known as dysbiosis. It has been suggested that barrier function of epithelial cells regulate both by microbial metabolite and nutrition. In this context, LPS, indoxyl sulfate (IS) and short chain fatty acid (SCFA) play important role in which LPS and IS cause disassembling of TJs proteins (Sheth, Delos Santos, Seth, LaRusso, & Rao., 2007) but SCFA such as butyrate and propionate can upregulate gene expression of TJS protein (i.e. ZO-1 and Occludin) (Ma, Piao, Mahfuz, Long, & Wang., 2022).

Tight junctions (TJs) are essential components of epithelial tissues that connect neighboring cells in order to provide protective barrier. It is well known that leaky gut by increasing intestinal mucosal paracellular permeability in response to physiologic stressors such as anxiety, intensive exercise or dietary components such as emulsifiers enhancing entry of pathogenic bacteria and bacterial toxins into the systemic circulation, and provoke systemic inflammation that can trigger numerous diseases (Martínez-Arnau, Fonfría-Vivas, & Cauli., 2019). TJs are composed of several transmembrane and intracellular molecules including of zonula occludens (ZO), occludin (Occ) and claudin (Cln). Among this proteins, ZO-1 is essential in coordinating TJs formation and cell polarization as it establishes links between most transmembrane TJs proteins (Allam-Ndoul, Castonguay-Paradis, & Veilleux., 2020). This structure has different physiological function that can regulate proliferation, differentiation, migration, cell polarity and diffusion (Bhat et al., 2019). Indeed, it suffer to damage by different stimuli such and free radicals, pathobionts and proinflammatory cytokines.

In recent years, there is a huge attention to gut microbiome composition changes and its relation to different body organs. It has been shown that alteration in microbiota during different stages of life can modulate physiological function of muscle that known as gut-muscle axis (Przewłócka, Folwarski, Kaźmierczak-Siedlecka, Skonieczna-Żydecka, & Kaczor., 2020). A study by Chen and their colleagues found that gut microbiota alterations may contribute to muscle atrophy throw metabolites and inflammation such as increase in Indoxyl sulfate, LPS and decrease in SCFA (Chen et al., 2023). Other studies have illustrated that alteration in TJs protein such as ZO-1 and Occ also can interfere with body function through increase in gut permeability that allowed to increase level of inflammation and subsequent ROS production (Meyer, Schwesinger, Ye, Denker, & Nigam, 2001; Rao., 2008). Different exercise and nutritional strategies have been conducted to modulate gut microbiota for this purpose such as probiotics, prebiotics and polyphenols administration, aerobic exercise and resistance exercise regime, fecal microbiota transplantation and so on. Studies that used probiotics specially lactobacillus and bifidobacterium reported a positive effect on muscle mass and function which has been attributed to the enrichment of intestinal microbiome content and increased production of SCFA (Al-Sadi et al., 2021; Blackwood et al., 2017). Vitamin D and leucine supplement are another nutritional regime that trigger progress in muscle mass and function in different research. Vitamin D can suppress the activity of atrophy-related transcription factors and upregulate protein synthesis by stimulate or mechanistic mammalian target of rapamycin complex 1 (Uchitomi, Oyabu, & Kamei., 2020). Leucine supplementation also was shown that has beneficial effect on muscle mass and strength in older adult both in combination with resistance training or alone (Martínez-Arnau et al., 2019; Trabal et al., 2015). It seems that combination of these three substances have great impact on muscle function and mass especially in old population and may insert better advantages along with resistance training, but according to our research for finding relation between TJs genes and muscle strength there is no study that investigate this relation to date. Therefore, in this study we aim to clarify the relation between tight junction genes ZO-1 and Occ with muscle strength that can manifest as grip strength in this setting.

Finally, it is unknown whether the change in gene expression or the structure of the TJs genes is responsible for this phenomenon or not, therefore it is necessary to investigate this issue and its relationship with muscle function. Is the change in muscle strength concurrent with the change in gene expression of tight junction and can this change be modified by using exercise and a combination of the mentioned substance in form of a supplement?

Materials and Methods

Study design

This study was conducted in line with the PREPARE guideline and it was done in accordance with the ARRIVE principle. Following acceptance of study design and proposal by scientific group of university of Guilan, number of 25 rats were purchased form the RAZI institute of Iran in two age groups and were placed in the animal laboratory under controlled conditions with 12 hours of light and 12 hours of darkness (starting light at 7 in the morning and turning off at 7 in the evening), temperature (23 ±2 C), and humidity (about 40%) with free access to food and water (ad libitum). After that rats subjected to a number-matched groups and performed the same training program (three days per week) in training groups and was inactive in control groups. After training protocol, some groups had oral gavage of supplementation (1ml of combination of vitamin D, probiotics and leucine amino acid) five times per week in 1 to 2 hours post exercise. During the period of training, rats regularly suffered weight control each week. 2 days after the end of the training regime, grip strength was assessed and all animals were put in a Co_2 chamber for safe and painless Anastasia and after that were sacrificed for the following data collection (weight, blood sample and gastrocnemius muscle tissue). Collected samples were used to analyze with PCR-Real time techniques.

Participants

Twenty-five (5 per group) male Wistar rats between the ages of 3 months (young) and 16-24 months (old) were brought from RAZI institute to engage in this study at the start of the experiment and randomly (by using computerized randomization technique) were separated into five experimental groups; an old control group that continued to normal life cycle (OC; n = 5), a young control group that was the same as the old control group in study period (YC; n = 5), old resistance training group (OR; n = 5) that trained using ladder climbing protocol, old resistance training plus supplement group (ORS; n = 5), that used supplement in addition to resistance protocol, or old supplement group (OS; n = 5), that use the supplement and were untrained.

Training protocol and implementation process

Experimental protocols were designed to minimize suffering and the number of animals used in the study. At the start of the experiment for familiarization, rats were adapted to an RT protocol of climbing a vertical ladder (1.1 m; 0.18 m, 2-cm grid, 80º incline) for three non-consecutive days (48-hour rest intervals) with no weight on the load apparatus. The load apparatus was fixed to the tail by wrapping the proximal portion with a self-adhesive foam strip. The rats were placed at the bottom of the ladder and familiarized with climbing. No pinching was used to stimulate initiate climbing and rats voluntarily started climbing and pulling them just used. the rats rest for 2 minutes at

the top of the ladder. This procedure was repeated until they would voluntarily climb the ladder for three consecutive turns without any stimulus.

Assessment of maximum carrying capacity (MCC)

Two days after the familiarization, each rat was evaluated to determine its maximum carrying load, which consisted of 4-9 ladder climbs with progressively heavier loads. The initial climb was performed with 75% of the animal's body weight. Upon successful completion of this load, an additional 30g weight was added to the load apparatus. The highest load that the animal successfully carried through the entire length of the ladder was considered the maximal carrying capacity for that training session. Failure was determined when the animal could not progress up the ladder after three successive stimuli to the tail. Resistance training (RT) was conducted three times per week (Saturdays, Mondays, and Wednesdays) for 8 weeks. Ladder climbing permitted to complete 8-12 dynamic movements in each climbing. The climbs comprised carrying progressive loads of 65, 85, 95, and 100% of the maximum carrying capacity of each rat. RT sessions consisted of 5-8 movements per climb over 8-14 seconds. While a rat completed climbing with 100% of its carrying capacity, a 30g additional load was added until a new MCC was determined. The resting period between each climb was 2 minutes. The RT protocol was adapted from Hornberger and Farrar (Hornberger Jr & Farrar., 2004). Training sessions were conducted in the morning between 10-12 A.M. Rats in supplement and resistance training plus supplement groups received 1 ml of supplement by oral gavage 1 to 2 hours post exercise sessions and two other days (Fridays and Thursdays). Supplement comprised of lactobacillus plantarum; 2×1010 (CFU/kg/day) (Lee et al., 2021) and bifidobacterium bifidum; 5×106 (CFU/rat/day) (Khailova et al., 2009), Vitamin D; 621.7 IU/Kg (Bass et al., 2020), and leucine Amino acid 0.135 gr/kg/day (Nicastro et al., 2012). It should be noted that 3 samples of each group by computer generated randomizer for further genome and enzymatic analysis.

Gene expression

RNA extraction from muscle tissue sample: 1cm2 of Jejunum tissue from all rats of each group (n=3/group) were taken and was used for this analysis. Initially, the samples were macerated by hand using liquid nitrogen with a pistol and homogenized in TRIZOL reagent (Invitrogen Corporation, California, USA) with a PowerGen 125 homogenizer (Fisher Scientific, Pittsburgh, USA). Subsequently, total RNA was extracted according to the TRIZOL manufacturer's protocol. The RNA pellet was resuspended in nuclease-free water and stored at -80 °C. The Nanodrop 2000 (Thermo Scientific, Wilmington, USA) was used to measure the RNA concentration and purity (ratios 260/280 nm and 260/230 nm). Primers were designed based on the information of ZO1 and Occ genes in the NCBI gene bank. Primer sequences for ZO-1 and Occ genes was shown in table 1. Glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH) was used as a housekeeping gene. Expression level of the desired gene was calculated with the formula $2^{-\Delta\Delta CT}$ in the following steps. First, the threshold cycle of the desired gene of each sample was subtracted from the threshold cycle of the housekeeping gene of the same sample (ΔCt= Ct Target-Ct Housekeeping). In the next step, we subtract the ΔCt of each sample from the sample that required to be compared, and multiply the negative number achieved to the power of two and obtain the relative expression of ZO-1 and Occ genes. ($\Delta\Delta Ct=\Delta Ct_{Target}-\Delta Ct_{Reference}$) $E= 2^{-\Delta\Delta Ct}$. Table 1 shows the primer sequences of ZO-1 and Occ along with housekeeping gene" GAPDH".

Table 1. Primer sequences for Zo1 and Occ genes.

Gene	Primer sequence 5'-3'
ZO-1	F: GGTTGGTATGGTGCCCTGAA R: CCCGCCTTCTGTATCTGTGT
Occ	F: TAGCCATTGTCCTGGGGTTC R: CGGTCCATCTTTCTTCGGGT
GAPDH	F: AGGTCGGTGTGAACGGATTTG R: TGTAGACCATGTAGTTGAGGTCA

Grip strength

A modified forelimb grip strength test was used to evaluate grip strength. The tail of a rodent that grips a bar connected to a monitoring device pulled horizontally by the examiner and the maximal value is recorded as the forelimb grip strength in three trials (Takeshita et al., 2017).

Hematoxylin and eosin staining of muscle tissue

Extracted muscle slide (10 µm-thick sections of gastrocnemius muscle from each sample) remove from freezer to room temperature. After that incubated with hematoxylin solution in a staining jar for 10 min to stain the nuclei. Then transferred to a staining jar with running water (tap water is fine) till the water is clear. After this stage, we transferred the slides to a staining jar with eosin solution for 3 min. After that slides put in staining jars with 70% ethanol for 20 sec, 90% ethanol for 20 sec, 100% ethanol for 1 min and xylene for 3 min respectively. Then bring the slides out from xylene and placed the slides in a fume hood till the slides were dried. Slides mounted with xylene-based mounting media and covered with cover slides. Clips were used to press the slides to squeeze bubbles then slides stored at room temperature. Finally, Hematoxylin and Eosin-stained images were captured with a 14.0 MP digital microscope camera which is attached via a c-mount to the side port of a Leica DMI 6000B microscope (Wang., 2017).

Statistical analysis

In order to determine relation between TJs genes and grip strength Pearson correlation test was used. To find out significance of difference between the variables of the research groups, one-way analysis of variance (ANOVA) and post-hoc Tukey's test were used. Mean and standard deviation were used for descriptive data reporting. After collecting the needed information, all data was analyzed using SPSS version 27 statistical software at a significance level of $p \leq 0.05$

Results

Lung histology

Correlations among ZO-1, Occ, muscle CSA and grip strength of study groups were shown in table 2. Based on the presented result, no significant correlation was observed between couple variables of study in different groups except for Occ and grip strength in supplement group (p=0.015). in this case, complete inverse relationship was observed. Also, quantitative values of variables after applying the interventions for a period of eight weeks are display in the form of mean and standard deviation in table 3.

In section of differences between study groups, it was obvious that both training and supplement intervention could affect level of gene expression than control (old control) group. According to the result of one-way ANOVA test there are significant differences between the different study groups in the expression of ZO-1, Occ, CSA and grip strength (p=<0.0001). The results of Tukey's post-hoc test showed that resistance training and supplementation alone didn't affect normalized values of ZO-1 gene expression in aged male wistar rats (P>0.05), while the

Table 2. Correlations among ZO-1, Occ, muscle CSA and grip strength in different groups

Old control group				
		Zo1	CSA	Grip strength
Zo1	Pearson Correlation		.67	-.86
	Sig. (2-tailed)		.53	.33
Occ	Pearson Correlation	.92	.90	-.99
	Sig. (2-tailed)	.25	.27	.08
Young control group				
		Zo1	CSA	Grip strength
Zo1	Pearson Correlation		.73	-.55
	Sig. (2-tailed)		.47	.62
Occ	Pearson Correlation	.61	-.08	.32
	Sig. (2-tailed)	.58	.94	.79
Supplement group				
		Zo1	CSA	Grip strength
Zo1	Pearson Correlation		.65	-.57
	Sig. (2-tailed)		.54	.61
Occ	Pearson Correlation	.55	.99	-1.00*
	Sig. (2-tailed)	.62	.08	.01
Resistance training group				
		Zo1	CSA	Grip strength
Zo1	Pearson Correlation		-.20	-.01
	Sig. (2-tailed)		.87	.99
Occ	Pearson Correlation	.27	.88	-.96
	Sig. (2-tailed)	.82	.30	.167
Resistance plus supplement group				
		Zo1	CSA	Grip strength
Zo1	Pearson Correlation		.38	.64
	Sig. (2-tailed)		.74	.55
Occ	Pearson Correlation	.08	-.88	.81
	Sig. (2-tailed)	.94	.30	.39
N		3	3	3

*. Correlation is significant at the 0.05 level (2-tailed).

ZO1; stands for Zonula Occludents-1, CSA; stands for cross sectional area, Occ; stands for occludin.

Table 3. Study variables in different groups (M±SD)

Variable	Old control	Young control	Supplement	Resistance	Resistance Plus Supplement
ZO-1 (NGE)	0.001912±0.001599	0.7012±0.2611	0.006986±0.002844	0.06472±0.04753	0.4196±0.05126
Occ (NGE)	0.0004591±0.0001233	0.276±0.1107	0.001611±0.0006639	0.01606±0.001834	0.1622±0.01817
CSA (µm)	79.74±7.11	132.1±8.057	102.2±10.50	118.2±8.579	123.1±6.131
Grip strength (gr)	369.4±67.56	551.1±67.60	407.4±66.47	568.4±44.51	670.9±37.95

NGE; stands for normalized gene expression, CSA; stands for cross sectional area, ZO-1; stands for zonula occludens-1, Occ; stands for occludin. Data was shown in mean and standard deviation.

combination of resistance training with supplementation caused a significant (P=0.011), increase in ZO-1 gene expression. In addition, it is worth noting that after the intervention period, there was no difference between the young control group and resistance training with supplementation (Figure 1A).

Similar results were observed regarding the effect of exercise and supplementation on the expression of Occ gene, so Tukey's post-hoc test revealed that there was no difference between the groups of resistance training and supplementation alone with elderly control group (P>0.05). While the combination of resistance training with supplementation (P=0.023), caused a significant rise in Occ gene expression. In addition, no significant difference was observed between the young control group and resistance training with supplement group (P=0.168) (figure 1 B).

In relation to grip strength, result showed that after eight weeks of training and supplementation, there was a significant difference between the study groups. Tukey's post hoc test result revealed that the supplement alone has no effect on grip strength. While resistance training and resistance training with supplement have a significant effect on grip strength (P=0.001). Also, the results indicate greater grip strength in the elderly group of resistance training plus supplement compared to the young control group (P=0.005) and no significant difference was observed between old resistance group and young control group (P=0.980) (figure 2 A).

Cross-sectional analysis of the gastrocnemius muscle showed that intervention groups had greater CSA in comparison with old control group (P=0.001), and it is interesting to note that after 8 weeks of exercise intervention, there was no significant difference (P=0.344), between the cross-sectional area of the muscle between the resistance training group with supplement and the young control group. It was clear that resistance training is more efficient than supplement alone (P=0.019) and combination of those are better than one alone (figure 2b). Result of Hematoxylin and eosin staining of gastrocnemius muscle between study groups also was shown in figure 3.

Discussion

Result of current study showed that there wasn't any relation between TJs genes, grip strength and muscle CSA in study groups except for supplement group. Regarding to gut-muscle axis, it is well known that there is strong crosstalk among muscle and gut. In this regard, Chen and them collogues reported that Lactobacillus casei Shirota (LcS), cause increase in grip strength,

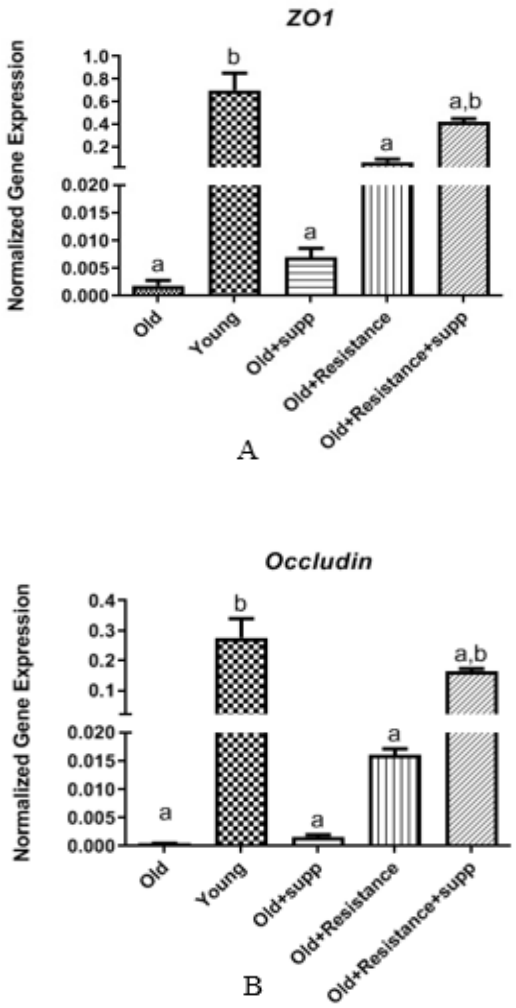

Figure 1. Level of gene expression in different groups; A shows the quantitative value of ZO-1 gene expression between different groups and B shows the quantitative value of Occ gene expression in different groups. ZO-1; stands for zonula occludens-1 and Occ stands for occludin

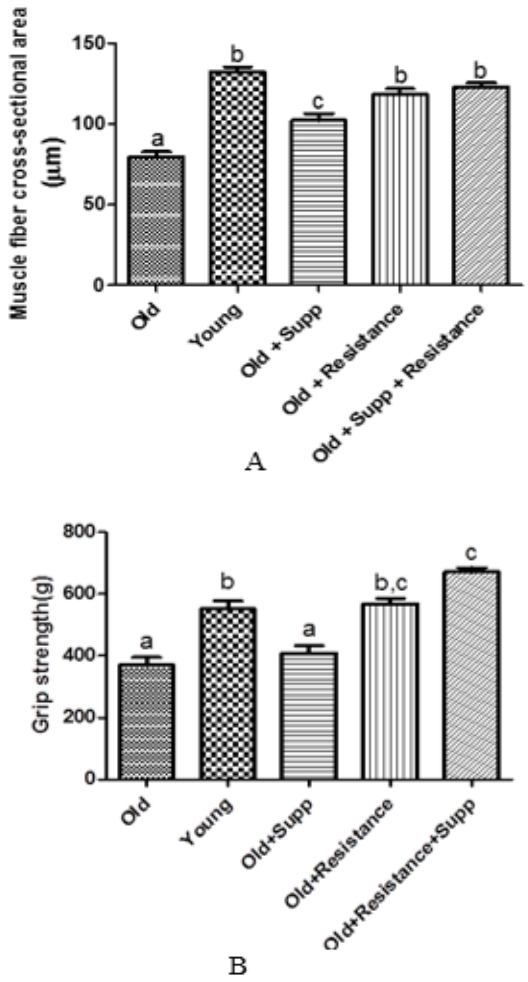

Figure 2. A shows gastrocnemius muscle fiber CSA and B shows grip strength among old and young control groups, old plus supplement group, old resistance training group and old resistance training plus supplement group respectively.

muscle mass and function in the senescence accelerated mouse-prone 8 (SAMP8) by increase in SCFA and decrease in pro-inflammatory cytokines (Chen et al., 2022). In another study, association of sarcopenia and gut microbiota was investigated and result clarified that some dietary fiber can play role in gut muscle axis and lead to a better physical function and grip strength. In large body of scientific literature, it can be seen that some strains of gut bacteria have important role in muscle energy metabolism by their by-product (Zhao et al., 2021) but as mentioned before, there is a debt about relation between physical barrier of intestine as permeable pore and muscle function. In a study on ZO-1 CM-specific knockout mice it determined that atrial mass was increased in this group rather than control group and also indicated high-grade atrioventricular block comparing to control hearts. Author also reported that ZO-1 has unique function in AV node tissue and electrical conduction of heart (Zhang et al., 2020). Hence this finding is related to heart ZO-1 but challenging for our current knowledge of tight junction structure.

Our result showed that there is inverse correlation between Occ and grip strength. It may because antioxidant features of supplement and discuss in part by this fact that probiotics can prevent the accumulation of free radicals, and since no physical activity was done in this group, the supplement only led to the enrichment of the intestinal microbiota and did not have a positive effect on strength. Since this effect has not been observed in other groups and is not related to the muscle CSA, more studies are needed to clarify it. In consistence with this result, Lim and their colleagues (2018) investigate effect of 8 weeks' leucine supplementation and resistance training by different dose on MyoD, myogenin, IGF1 protein level and muscle CSA. All variables increase in group of exercise with leucine supplementa-

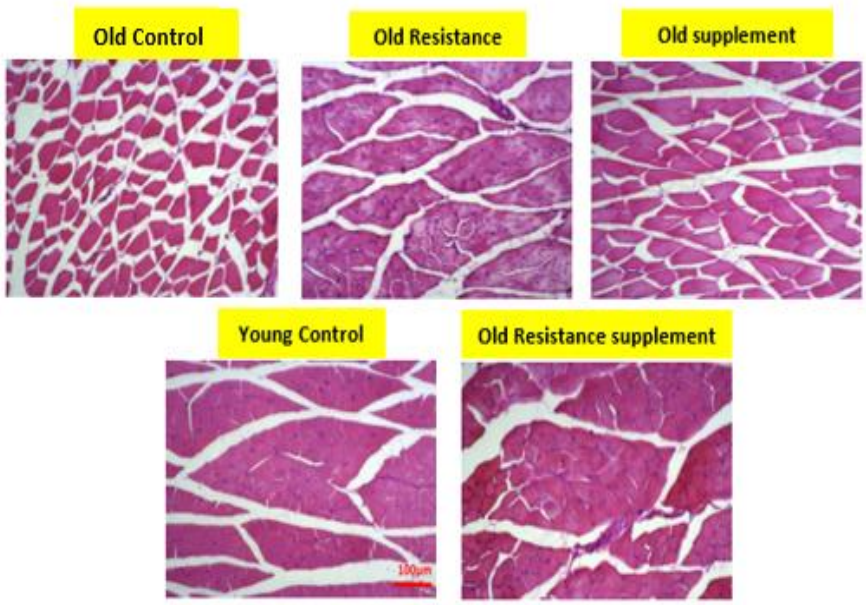

Table 3. Cut portion of CSA post intervention among different study groups. Changes related to the number of myofibers were counted by using Image J software and illustrated with 100 um magnification.

-tion but group that only use leucine supplementation didn't show any increase in muscle hypertrophy and satellite cell activity, regardless of the dose (Lim et al., 2018). In another study by DE Andrade and colleagues (2020) it confirmed that high-dose leucine supplementation didn't enhance gains in muscle strength and mass after 12 weeks of resistance training program in young resistance-trained males. They applied 10 gr of leucine for 2 meals in day vastus lateralis CSA also didn't show any change in this study (DE Andrade et al., 2020). These finding is in contrast with the finding of Ju et al (2023), who administrated 6 gr of leucine along with vitamin D and calcium enrich supplement on hemodialysis patients. It because that their applied resistance training to their protocol (Ju et al., 2023). It is obvious that use of resistance training together with leucine lead to the improvement of the mentioned factors, and leucine alone has no effect on grip strength, therefore supplement group in this study didn't improve grip strength but can sustain Occ gene expression. these controversies may in part explained by their human participant, exercise intervention, dose of leucine, and their health state.

Loss of Occ has been shown to be an important pathogenic factor contributing to the increasing inflammation of intestine. It has been made clear that Occ play important role in macromolecule passage among epithelial cells (Al-Sadi et al., 2011) and autophagy play important role in enhance intestinal paracellular TJs barrier by increasing expression of Occ (Saha et al., 2022). Based on the results of Saha et al and AL-Sedi's studies, it is reasonable to assume that there is a relationship between the physical structure and genes of the intestinal barrier and muscle, because the weakness of this structure and the decrease in the expression of these genes can increase the expression of reactive oxygen species (ROS) through inflammation and intensify the process of sarcopenia (Damiano et al., 2019). However, in part these relations is negative as shown by our research but it should be note that although probiotics and vitamin D plus leucine are good component for TJs gene and structure but in order to obtain the minimum result in the field of grip strength, resistance training is required.

According to our result in ZO-1 and Occ gene expression, resistance training plus supplementation increase level of barrier function of intestinal epithelium but nor resistance training neither supplementation can't increase these levels in comparison with old control group alone. In relation to exercise and tight junction, Zuhl et al (2014), reported in their review study that long-term and high-intensity exercise caused an increase in phosphorylation that subsequently lead to dysfunction of tight junction and increase in permeability. They suggested that prolonged and high intensity exercise can alter the intestinal TJs barrier by increasing core temperature and change in intestinal blood flow (Zuhl et al., 2014). On one hand, our result is in contrast with this study and can attributed this phenomenon to applying supplement to our st-udy. On another hand, although exercise intervention in this study was high intensity but not prolonged and the type of intervention exercise in Zuhl is unclear. In another study, Shin and their colleagues (2020) investigate the effect of 4 weeks aerobic training on TJs genes. In consistence with our result, their finding showed that occludin and claudin gene expression increase as result of treadmill running but ZO-1 didn't change after training course. This study also carried out on old mouse (Shin et al., 2020). It seems that the beneficial effect of the present study on the expression of the aforementioned genes is due to the combination of probiotics and vitamin D. In this regard, West et al (2011). studied the effect of Lactobacillus probiotic supplementation on gastrointestinal symptoms in cyclists. Result indicated that severity and duration of gastrointestinal symptoms reduced by supplementation (West et al., 2011). It is well established that vitamin D is an important component in preventing muscle weakness, and as a component of used supplement in our study it may insert key effect. In this case, Stio and colleagues (2016) found that vitamin D administration can alleviate Ulcerative Colitis (UC) that characterized by epithelial barrier dysfunction. Vitamin D play this role by downregulation of claudin 1 and 2 and upregulating claudin 4 and 7 (Stio et al., 2016). Finally, it can be obviously seen that combination of leucine amino acid, vitamin D, lactobacillus and bifidobacterium probiotics and insert a synergistic effect on TJs gene expression which may be caused by decreasing level of pro-inflammatory cytokines as shown by Zhao et al (2023). They administrated leucine amino acid for 8 weeks and observed that level of antioxidants mRNA expressions and translational levels of ZO-1 and Occ increased linearly (Zhao et al., 2023). Their result suggest that dietary leucine can improve intestinal barrier function although this study was conducted on fish but can enlighten the way. Since the study with features of current study has not been done so far, it is difficult to draw conclusions from the above studies and it is necessary to conduct future studies.

In relation to the effect of resistance training and supplementation on grip strength and muscle CSA our result is in consistent with finding of Prokopidis et al (2022), Hsu et al (2023), and in contrast with Nicastro et al (2011). In a systematic review Prokopidis and their colleagues reported that global muscle strength and muscle mass increase in response to probiotics consumption in human subjects but doesn't affect total lean mass (Prokopidis et al., 2023). Also, Hsu et al examined combined effect of quercetin and leucine on grip strength and muscle mass and their showed that this combination additively increase muscle protein synthesis and grip strength while decrease activation of muscle proteolysis pathway (Hsu et al., 2023). Nicastro and their colleagues investigated role of leucine and resistance training in atrophy induced by dexamethasone. CSA of plantaris muscle didn't change after intervention period (Prokopidis et al., 2023). It has been shown that different stimuli such as resistance training and

leucine amino acid uses different sites to stimulate mTOR activity and induce increased muscle protein synthesis (Deldicque et al., 2005). Therefore, as shown in the study of Bollen et al (2022) vitamin D can activate their receptors that induce IGF-1 expression, and applied hypertrophic effects on muscle or has supportive effects on muscle function. Also, it was indicated that, IGF-1 signaling upregulated during resistance exercise, and as a result affect the IGF-1–Akt–FOXO pathway (Dzik et al., 2019). Also, mechanism of probiotic action on muscle mass may be partly by the enrichment of metabolites such as SCFA, reduction of inflammatory factors such as indoxyl sulfate and cytokines. Because, these factors end up activating the proteolytic pathway and leading to catabolism through inflammation and subsequent high level of free radicals (Lee et al., 2021; Damiano et al., 2019). Finally, it may be interesting to express that combination of mentioned pathway may have surplus effect than one alone on muscle function which is conducted by different stimulus. This needs further investigation. These controversies may result from applying dexamethasone and short period of resistance training. It has been confirmed that antibiotic can alter gut microbiota so it is rational that it may affect gut muscle relationship and cause dysbiosis (Francino et al., 2016). On the other hand, minimum number of sessions that need for increase in muscle CSA has not been regarded in mentioned study. As possible mechanisms underlying the effectiveness of present study, it is possible to mention the combination of multi-factors supplement used. In this case, it has been proven that vitamin D can increase muscle strength and function by increase gene expression of VDR (Bollen et al., 2022) and probiotics can enrich gut microbiome and as previously confirmed by different research reinforced gut muscle axis and SCAF production. On the other hand, leucine can trigger muscle protein synthesis in different age groups. Indeed, it is clear that adding resistance training to these components can lead to improvement of all components efficiency and applies synergistic effect as shown by our finding in resistance training plus supplement group.

Conclusion

Based on our finding, resistance training plus supplement could have additively effect on muscle CSA and grip strength in old Wistar rats. Combination of vitamin D, probiotics and leucine amino acid plus exercise are more effective for TJs gene expression and it possibly because of vitamin D and probiotics functions. It is suggested to conduct a similar study on human subject in the future to clarify more details.

What is already known on this subject?

Gut-muscle axis is a well-known issue and it is clear that there is strong relation between gut microbiome, muscle function and mass. Each component that can increase SCFA maybe will alleviate inflammation and will decrease level of pro-inflammatory cytokines.

What this study adds?

In this study, it was found that although there is a close relationship between intestinal bacterial content and muscle, this relationship is not established between the physical structure of intestinal tight junction and muscle function. There is weak and negative relation between Occ and grip strength that maybe produced by supplement. However, the combination of exercise and supplementation likely be an effective solution to prevent the loss of muscle mass associated with aging and the reduction of the expression of tight junction genes.

Organ Cross-Talk Tips:

- There is strong inverse correlation between Occ and grip strength which is supported by supplement.
- Combination of mentioned supplement and resistance training may be an effective way to increase TJs gene expression.

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None.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Animals had free access to standard food and water. All stages of keeping and slaughtering rats were carried out according to the rules of the Animal Ethics Committee of Shahid Beheshti University, Tehran, Iran (ethical code: IR.SBU.REC.1402.052).

Informed consent Animal study.

Author contributions

Conceptualization: H.A., M.M.; Methodology: H.A., M.M.; Software: M.M.; Validation: M.M., Formal analysis; Investigation: H.A, M.M.; Resources: H.A.; Data curation: H.A., M.M.; Writing - original draft: M.M.; Writing – review & editing: H.A.; Visualization: M.M., H.A.; Supervision: H.A. Project administration: M.M.; Funding acquisition: H.A.

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