



Effect of Creatine Supplementation on the Muscular Inflammation and Muscle Atrophy in High-Fat High-Fructose Fed Obese Growing Rats

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Abstract

Objective: This study aimed to investigate effects of creatine supplementation on high-fat high-fructose fed obese rats. Twenty-four six-week-old male Sprague-Dawley rats were randomly divided into three groups ($n=8$ each): control sedentary group (CD), high-fat high-fructose diet group (HD), and creatine-supplemented high-fat high-fructose diet group (CHD).

Methodology: The CD group was supplemented with AIN-76G diet, and a high-fat high-fructose diet for HD and CHD groups was substituted with 5% corn oil fat to 35% lard. The CHD group was supplemented with 2% creatine. The total soleus muscle protein was extracted and prepared according to the procedure mentioned by Baghirova et al. for muscle cytokine assay and western blotting.

Result: After eight weeks of the experimental period, weights of the soleus muscle in the HD and CHD groups were significantly lower than that in the CD group. In addition, levels of proinflammatory cytokines interleukin (IL)-1 β , IL-6, tumor necrosis factor (TNF)- α in the HD groups were significantly higher than those in the CD group. In contrast, IL-1 β , IL-6 concentrations in the CHD group were significantly lower than those in the HD group and irisin levels of HD and CHD group were significantly lower than those in the CD group. Moreover, levels of muscle protein degradation factors [Muscle RING-finger protein-1 (MuRF1) and atrogin-1] were significantly higher in the HD and CHD groups than those in the CD group.

Conclusion: From these results, it was concluded that creatine supplementation attenuates the inflammation of skeletal muscle via proinflammatory cytokines; however, it has no significant role in preventing muscular atrophy in high-fat high-fructose fed obese rats.

Keywords: Obesity; Creatine; Inflammation; Muscle atrophy; Rat

Abbreviations

5-HT: 5-Hydroxytryptamine; AIN-76G: American Institute of Nutrition-76G; BSA: Bovine serum albumin; BW: Body weight; CD: Control sedentary group; CHD: Creatine-supplemented high-fat high-fructose diet group; FBW: Final body weight; FER: Food efficiency ratio; HD: High-fat high-fructose diet

group; IBW: Initial body weight; IGF-PI3K-AKT-mTOR: Insulin like growth factor- Phosphoinositide 3-kinases- Protein kinase B (PKB)-mammalian target of rapamycin; IL-1 β : Interleukin-1 β ; IL-6: Interleukin-6; LSD: Least significant difference; mRNA: messenger RNA; MuRF1: Muscle RING-finger protein-1; MyoD: Myogenic differentiation; SE: Standard error; SPSS:

Statistical Package for the Social Sciences; TNF- α : Tumor necrosis factor- α .

Introduction

Recently, the incidence rate of obesity has gradually increased worldwide; it is known to cause cardiovascular, musculoskeletal, and physiological issues during the lifespan of an individual [1, 2]. In particular, a high-fat and high-fructose diet induces obesity [3] and results in cardiac mitochondrial dysfunction [4], impaired vascular reactivity [5], insulin resistance in skeletal muscles [6], and non-alcoholic fatty liver disease [7].

With respect to muscle atrophy, sarcopenic obesity further deteriorates abnormal body function, increases mortality, and reduces the quality of life [8]. Additionally, Baek et al. [9] have reported that sarcopenic obesity increases the risk of hyperlipidemia, and insulin resistance and levels of triglycerides in serum have been shown to be significantly correlated based homeostatic model assessment scores. In addition, high-fat diet-fed mice have demonstrated dyslipidemia, reduced muscle strength, reduced myogenin and MyoD protein levels, and increased level of MuRF1 protein in the gastrocnemius muscle [10].

With respect to creatine supplementation, Almeida et al. [11] have reported that five days of short-term creatine addition in rats induces a reduction in levels of inflammatory molecules such as interleukin (IL)-6, cytokine induced neutrophil chemoattractant-1, and toll-like receptor-4, and has a pulmonary protective effect on ischemia-reperfusion injury. Moreover, creatine supplementation has been shown to have antioxidative properties [12, 13]. In contrast, creatine supplementation has a negative effect on the high volume of resistant exercised females, demonstrating muscle soreness and inflammation [14]. Therefore, the effect of creatine supplementation on inflammation is unclear. Another effects of creatine supplementation involve increase of whole-body energetics and expenditure [15]. These effects due to inhibitory role of the accumulation of cytoplasmic triglycerides via inhibition of phosphatidylinositol 3-kinase activation [16].

Obesity is related to increased levels of inflammation in myocytes, which may secrete elevated levels of proinflammatory cytokines and result in muscular inflammation. Collins et al. [17] have communicated increased mRNA levels of muscle atrophy, inflammation, and oxidative stress indices in soleus muscles in a high-fat high-fructose diet rat. Obesity

induces increased mRNA and protein expression levels of IL-6, tumor necrosis factor (TNF)- α , and IL-1 β in the skeletal muscle of mice, suggesting that a high caloric diet aggravates skeletal muscle inflammation [18]. In addition, Collins et al. [3] have demonstrated an obesity-induced increase in the levels of proinflammatory cytokines and inflammatory cells in skeletal muscles of high-fat high-sucrose diet rats. However, effect of creatine supplementation on the obesity-induced inflammation of skeletal muscles in the presence of an obesity induced growing rat is still unclear. Therefore, the present study aimed to investigate the influence of creatine on the inflammatory status and muscle degradation factors of skeletal muscle in high-fat high-fructose diet-fed obese rats.

Materials and Methods

Animals

Twenty-four six-weeks-old male Sprague–Dawley rats were purchased from Samtaco Bio Korea (Hwaseong, Korea). Following a one-week acclimation period, rats were randomly divided into three groups ($n=8$ each): control sedentary group (CD), high-fat high-fructose diet group (HD), and creatine-supplemented high-fat high-fructose diet group (CHD). The rats were provided food and tap water ad libitum for eight weeks. They were housed in individual cages, with controlled temperature ($23 \pm 1^\circ\text{C}$) and humidity ($50 \pm 5\%$) and a 12-h light-dark cycle. All experimental protocols were approved by the Animal Study Committee of Sunmoon University in Asan, Republic of Korea (SM-2018-01-02).

Diets and drinks

Table 1 summarizes the composition of diets of all the three groups. The control diet for the CD group was a mixture of 20% protein, 5% fat, 60% carbohydrates, 5% fiber, 3.5% minerals, and 1.0% vitamin, based on the AIN-76G diet. In the HD group diet, 5% corn oil was substituted with 35% lard. The CHD group was administered 2% creatine. The food efficiency ratio (FER) was calculated as the total weight gain divided by the total food intake during the experimental period. In addition, the CD group was provided ad libitum access to tap water, while the HD and CHD groups were provided ad libitum access to 30% fructose solution. After eight weeks of the experimental procedure, the rats were anesthetized using diethyl ether, after fasting overnight. Blood was drawn from the left ventricle of the heart, and serum was collected by centrifugation of blood samples at 2,000 g for 20 min at 4°C . Skeletal

muscles and other organs were dissected and immediately immersed in liquid nitrogen. Serum and tissue samples were stored at -70°C until analysis

Table 1: Compositions of the diets provided to animals in the three groups (g/kg diet).

Ingredients	CD	HD	CHD
Casein	200	200	200
Starch	500	200	200
Sucrose	150	150	150
Lard	-	350	350
Corn oil	50	-	-
Vit. mix	10	10	10
Mineral mix.	35	35	35
Cellulose	50	50	50
D, L-methionine	3	3	3
Choline chloride	2	2	2
tert-Butylhydroquinone	0.01		
D.L-alpha-tocopherol		1.2	1.2
Creatine	-	-	20

CD, control sedentary group; HD, high-fat high-fructose diet group; CHD, creatine-supplemented high-fat high-fructose diet group

Assay of cytokines

The total soleus muscle protein was extracted and prepared according to the procedure mentioned by Baghirova et al. [19] for cytokine assays and western blotting. The protein concentration was determined using Bradford reagent (Bio-Rad, Hercules, CA, USA). The supernatant of the soleus muscle was used to measure levels of interleukin (IL)-1 β , IL-6, tumor necrosis factor (TNF)- α , and irisin by enzyme-linked immunosorbent assay (R&D Systems, CA, USA).

Western blot assay

Briefly, proteins from prepared soleus muscles were transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA) and blocked for 1 hour at room temperature in 5% bovine serum albumin (BSA), using Tris-buffered saline in Tween-20 (TBST). The primary antibodies used included MuRF1 (Abcam,

Cambridge, MA) or Atrogin-1 (Abcam, Cambridge, MA, USA). Primary antibody reactions were performed for 2 hours at room temperature in 5 % BSA, followed by incubating with a secondary antibody of either horseradish peroxidase-conjugated anti-goat immunoglobulin (Ig)G or anti-rabbit IgG (Santa Cruz, CA, USA) for 1 hour. Target proteins were identified using an enhanced chemiluminescence kit (GE Healthcare, Buckinghamshire, UK). Obtained films were then scanned (Pixma E510 scanner, Canon Inc., Japan) and densitometric analysis of bands was performed using the Quantity One program (Bio-Rad, Hercules, CA, USA).

Statistical analysis

All data were analysed using SPSS software version 18.0 for Windows (SPSS, Chicago, IL, USA). The data are expressed as the mean \pm standard error (SE), and the values were analyzed by one-way analysis of variance,

followed by the least significant difference (LSD) test. Significance was defined as $\alpha=0.05$.

Table 2: Changes in body weight, food intake, FER, and liquid intake.

	CD	HD	CHD
IBW (g)	210.0 \pm 1.59 ^{NS}	210.4 \pm 4.21	211.0 \pm 2.06
FBW (g)	394.5 \pm 9.78 ^a	429.7 \pm 6.92 ^b	415.3 \pm 11.75 ^b
food intake (g/day)	19.2 \pm 0.28 ^a	12.0 \pm 0.32 ^b	11.0 \pm 0.49 ^b
FER	0.11 \pm 0.003 ^a	0.26 \pm 0.009 ^b	0.23 \pm 0.016 ^c
drink intake (ml/day)	26.9 \pm 1.06 ^a	24.3 \pm 0.66 ^b	25.9 \pm 0.46 ^{ab}

Data are expressed as mean \pm standard error (SE). CD, control sedentary group; HD, high-fat high-fructose diet group; CHD, creatine-supplemented high-fat high-fructose diet group; IBW, initial body weight; FBW, final body weight; FER, food efficiency ratio; NS, not significant. Different superscripts indicate significance at $p < 0.05$.

Table 3: Changes in fat and muscle weight.

	CD	HD	CHD
perirenal fat pad (g/100g BW)	1.8 \pm 0.23 ^a	3.0 \pm 0.19 ^b	2.9 \pm 0.21 ^b
epididymal fat pad (g) (g/100g BW)	1.7 \pm 0.15 ^a	2.6 \pm 0.19 ^b	2.2 \pm 0.22 ^{ab}
Soleus muscle (mg/100g BW)	46.7 \pm 1.11 ^a	38.4 \pm 2.22 ^b	39.5 \pm 1.46 ^b

Data are expressed as mean \pm standard error (SE). CD, control sedentary group; HD, high-fat high-fructose diet group; CHD, creatine-supplemented high-fat high-fructose diet group, BW, body weight; different superscripts indicate significance at $p < 0.05$.

Results

As shown in Table 2, the final body weight of rats in the HD group was significantly higher than that of animals in the CD and CHD groups ($p < 0.05$). Food intakes of rats in the HD and CHD groups were significantly reduced than that of the rats in the CD group ($p < 0.05$). However, the FER of HD and CHD rats was significantly higher than that of CD rats ($p < 0.05$). However, the FER of CHD rats was significantly lower than that of HD rats ($p < 0.05$). Finally, the amount of liquid intake of HD rats was significantly lower than that of CD rats ($p < 0.05$).

Table 3 summarizes the weight of fat tissue and soleus muscle of all the three groups. Weights of perirenal fat pad of rats in the HD and CHD group were significantly higher than that of rats in CD group ($p < 0.05$). In

addition, the epididymal fat pad weight of rats in HD group was significantly higher than that of rats in the CD group ($p < 0.05$). Weights soleus muscle of the rats in the HD and CHD groups were significantly lower than that of rats in the CD group ($p < 0.05$).

As shown in Fig. 1, levels of proinflammatory cytokines IL-1 β , IL-6, TNF- α were significantly higher in the soleus muscle of the HD group than those in the CD group ($p < 0.01$, respectively). However, the levels of irisin were significantly lower in the HD and CHD group than that in the CD group ($p < 0.01$). IL-1 β , IL-6 concentrations in the CHD group were significantly lower than those in the HD group ($p < 0.01$, respectively). However, TNF- α and irisin concentrations in the CHD group were not significantly different from those in the HD group.

As shown in Fig. 2, levels of muscle protein degradation factors, MuRF1 and atrogin-1 in the soleus muscle of

the HD and CHD group were significantly higher than those in the CD group ($p < 0.01$).

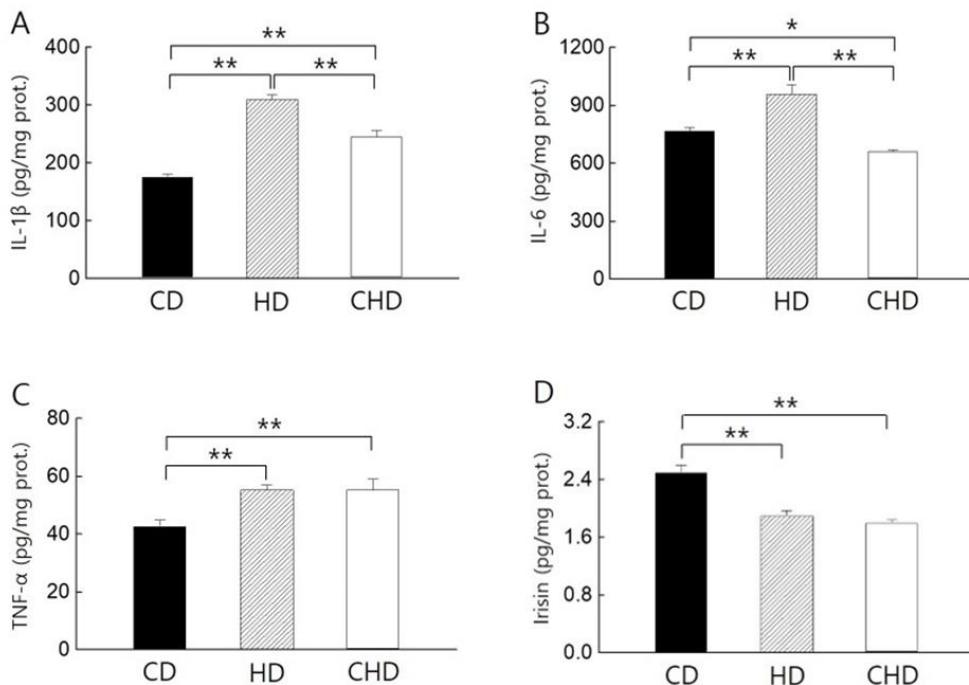


Figure 1: Changes in the levels of cytokines in soleus muscle. Data are expressed as mean \pm standard error (SE). CD, control sedentary group; HD, high-fat high-fructose diet group; CHD, creatine-supplemented high-fat high-fructose diet group; * $p < 0.05$, ** $p < 0.01$.

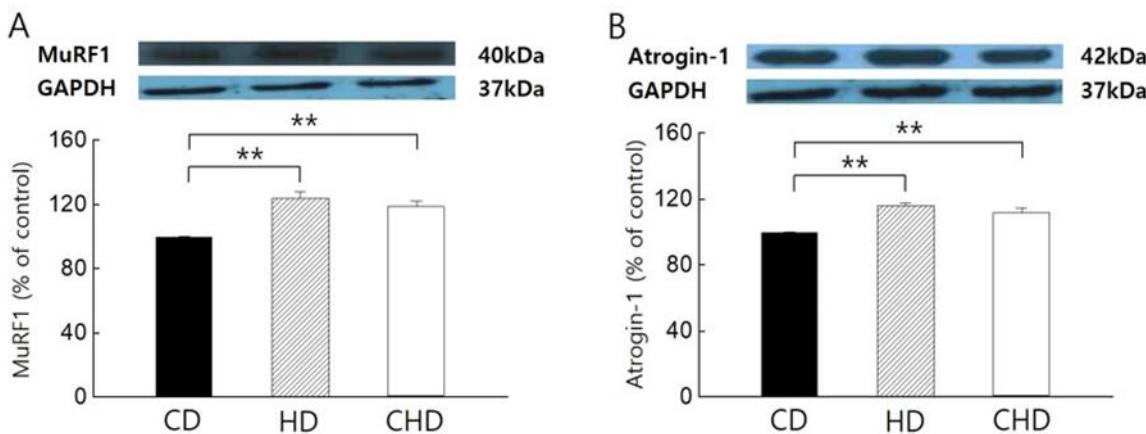


Figure 2: Changes in MuRF1 and atrogin-1 protein expression levels in the soleus muscle. Data are expressed as mean \pm standard error (SE). CD, control sedentary group; HD, high-fat high-fructose diet group; CHD, creatine-supplemented high-fat high-fructose diet group; ** $p < 0.01$.

Discussion

In the present study, we investigated the protective effect of creatine supplementation on obesity-induced inflammation in the skeletal muscle and on skeletal muscle protein degradation factors in rats fed with a high-fat high-fructose diet. Creatine supplementation

alleviated skeletal muscle inflammation in high-fat, high-fructose diet-induced obese rats. However, there was no significant impact on muscle atrophy.

Body weight, FER, and the perirenal fat pad weight in the HD and CHD groups were significantly higher than those in the CD group, which was similar to the trend

for epididymal fat pad for rats in the CHD group; however, the difference was statistically insignificant. In previous studies conducted in humans, creatine supplementation did not change lean body mass over time in young healthy swimmers [20], or body mass in young healthy females who underwent repeated cycle ergometer tests [21]. Additionally, in an animal study, creatine supplementation did not affect body weight or muscle weight in hindlimb suspended rats [22]. Our results also showed no significant differences in body weight and fat tissue weight with creatine supplementation.

Obesity is characterized by chronic low-grade skeletal muscle inflammation [23]. The release of increased pro-inflammatory cytokines, including IL-6, IL-1 β , and TNF- α in the skeletal muscle and liver are triggered by high-fat high-sucrose diet-induced obesity [18, 24]. IL-6 is one of the main deleterious pro-inflammatory cytokine, and severe muscle inflammation and atrophy occur during IL-6 overexpression [24]. In the present study, concentrations of IL-1 β , IL-6, TNF- α were significantly higher in the HD group than those in the CD group. However, IL-1 β , IL-6 concentrations in the CHD group were significantly lower than those in the HD group. In addition, concentrations of irisin were significantly lower in the HD and CHD group than those in the CD group.

A few reports support the use of creatine supplementation as an anti-inflammatory and anti-oxidative agent. Because serotonin [5-HT (5-Hydroxytryptamine)] plays a significant role in the early stages of inflammation in the carrageenan-induced inflammation model [25, 26], the authors utilized the serotonin-induced model of paw edema to demonstrate the effect of creatine supplementation as an anti-inflammatory agent. Compared to the animals in the control group, animals supplemented with creatine showed an alleviated inflammatory response [27]. However, the role of creatine in skeletal muscle inflammation *in vivo* remains unclear.

The present study findings revealed that creatine has a potent anti-inflammatory effect in the skeletal muscle of a high-fat high-fructose diet model of obesity. The skeletal muscle myotubes release various levels of cytokines during obesity [28]. Increased expressions of IL-6, TNF- α , and IL-1 β have been reported in the skeletal muscle of individuals with metabolic disorders induced by obesity in human and animal studies [18, 29, 30]. Our results also demonstrated that, compared to the control diet, high-fat high-fructose diet resulted in increased concentrations of proinflammatory cytokines,

such as IL-6, TNF- α , and IL-1 β , in the skeletal muscle. However, IL-6 and IL-1 β concentration of CHD group were alleviated by creatine supplementation in the CHD group compared to that in the HD group except for TNF- α concentration. In particular, TNF- α appears to stimulate general proteolysis by increasing ubiquitin conjugation to muscle proteins [31]. In the present study, TNF- α concentration of CHD group was not significant difference compared to HD group and this result means that creatine supplementation may did not affect muscle atrophy in growing obese rat.

Park et al. [32] reported that compared to non-sarcopenic elderly women, elderly women diagnosed with pre-sarcopenia or sarcopenia had reduced irisin levels in serum. Our results were consistent with this finding showing that irisin levels of HD and CHD rats that presented with lower muscle weight were significantly lower than those in the CD group. In fact, Reza et al. [33] reported that irisin induced skeletal muscle hypertrophy and attenuated denervation-induced muscle atrophy by IL-6 signaling in rodents and these effects due to activation of muscle satellite cells and elevation of protein synthesis. Our data demonstrated that creatine supplementation for eight weeks did not changed irisin and TNF- α secretion from skeletal muscle and these results in restricted recovery from HD induced muscle atrophy.

Obesity is further characterized by a reduction in muscle mass, accumulation of fat tissue, loss of muscle strength, and physical disability [34]. High caloric diets, including high fat and high carbohydrates, and a sedentary lifestyle represent a public health concern, which can predispose to obesity and insulin resistance [35]. This can lead to skeletal muscle atrophy, due to the degradation of muscle proteins, a reduction in fiber type 1, and an increase in the fiber type 2X [36, 37].

In the present study, creatine supplementation did not affect muscle weight or MuRF1 and atrogin-1 protein expression in the soleus muscle. In a previous study, Ferretti et al. [38] reported that obese rat deteriorated effects of creatine supplementation on skeletal muscle mass by reducing the protein expression of IGF-PI3K-AKT-mTOR pathway. However, they did not study levels of muscle protein degradation factors, MuRF1 and atrogin-1. In the present study, muscle weight and MuRF1 and atrogin-1 protein expression levels in the soleus muscle of the HD and CHD groups were significantly lower than those in the CD group. Therefore, it was suggested that diet-induced obesity suppressed the IGF-PI3K-AKT-mTOR pathway and stimulated the skeletal muscle breakdown proteins,

MuRF1 and atrogin-1 protein. In addition, creatine supplementation did not attenuate skeletal muscle atrophy via irisin and TNF- α secretion and proteolytic proteins such MuRF1 and atrogin-1 protein expression in obese induced growing rats.

Conclusion

From our results, creatine supplementation has a protective role in skeletal muscle inflammation via inhibition of proinflammatory cytokines such as an IL-1 β and IL-6, but no significant role in preventing skeletal muscle atrophy by inhibiting irisin and stimulating TNF- α secretion in diet-induced obesity growing rats.

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Conflict of Interest

The authors declared no conflict of interests.

Data availability statement

The data presented in this study are available upon request from the authors. The data are not publicly available owing to privacy and ethical restrictions.

References

1. Khan SS, Ning H, Wilkins JT, et al. Association of body mass index with lifetime risk of cardiovascular disease and compression of morbidity. *JAMA Cardiol* 2018; 3: 280-287.
2. Collins KH, Herzog W, MacDonald GZ, et al. Obesity, metabolic syndrome, and musculoskeletal disease: Common inflammatory pathways suggest a central role for loss of muscle integrity. *Front Physiol* 2018; 9: 112.
3. Collins KH, Hart DA, Reimer RA, et al. High-fat high-sucrose diet leads to dynamic structural and inflammatory alterations in the rat vastus lateralis muscle. *J Orthop Res* 2016; 34: 2069-2078.
4. Sverdlov AL, Elezaby A, Behring JB, et al. High fat, high sucrose diet causes cardiac mitochondrial dysfunction due in part to oxidative post-translational modification of mitochondrial complex II. *J Mol Cell Cardiol* 2015; 78: 165-173.
5. Sweazey KL, Lekic M, Walker BR. Comparison of mechanisms involved in impaired vascular reactivity between high sucrose and high fat diets in rats. *Nutr Metab (Lond)* 2010; 7: 48.
6. Liu Y, Jifang Yuan J, Xiang L, et al. A high sucrose and high fat diet induced the development of insulin resistance in the skeletal muscle of Bama miniature pigs through the Akt/GLUT4 pathway. *Exp. Anim* 2017; 66: 387-395.
7. Ragab SMM, Elghaffar SKA, El-Metwally TH, et al. Effect of a high fat, high sucrose diet on the promotion of non-alcoholic fatty liver disease in male rats: the ameliorative role of three natural compounds. *Lipids Health Dis* 2015; 14: 83.
8. Stewart ST, Cutler DM, Rosen AB. Forecasting the effects of obesity and smoking on U.S. life expectancy. *N Engl J Med* 2009; 361: 2252-2260.
9. Baek SJ, Nam GE, Han KD, et al. Sarcopenia and sarcopenic obesity and their association with dyslipidemia in Korean elderly men: the 2008-2010 Korea National Health and Nutrition Examination Survey. *J. Endocrinol Invest* 2014; 37: 247-260.
10. Lee K, Jin H, Chei S, et al. Effect of dietary silk peptide on obesity, hyperglycemia, and skeletal muscle regeneration in high-fat diet-fed mice. *Cells* 2020; 9: 377.
11. Almeida FM, Battochio AS, Napoli JP, et al. Creatine supply attenuates ischemia-reperfusion injury in lung transplantation in rats. *Nutrients* 2020; 12: 2765.
12. Sestili P, Martinelli C, Colombo E, et al. Creatine as an antioxidant. *Amino Acids* 2011; 40: 1385-1396.
13. Lawler JM, Barnes WS, Wu G, et al. Direct antioxidant properties of creatine. *Biochem Biophys Res Commun* 2002; 290: 47-52.
14. Hayward S, Wilborn CD, Taylor LW, et al. Effects of a high protein and omega-3-enriched diet with or without creatine supplementation on markers of soreness and inflammation during 5 consecutive days of high-volume resistance exercise in females. *J Sports Sci Med* 2016; 15: 704-714.
15. Chouchani ET, Kazak L, Spiegelman BM. New Advances in Adaptive Thermogenesis: UCP1 and Beyond. *Cell. Metab* 2019; 29: 27-37.
16. Lee N, Kim I, Park S, et al. Creatine inhibits adipogenesis by downregulating insulin-induced activation of the phosphatidylinositol 3-kinase signaling pathway. *Stem Cells Dev* 2015; 24: 983-994.
17. Collins KH, Hart DA, Smith IC, et al. Acute and chronic changes in rat soleus muscle after high-fat high-sucrose diet. *Physiol Rep* 2017; 5: e13270.
18. Shabani M, Sadeghi A, Hosseini H, et al. Resveratrol alleviates obesity-induced skeletal muscle inflammation via decreasing M1 macrophage polarization and increasing the

- regulatory T cell population. *Sci Rep* 2020; 10: 3791.
- 19. Baghirova S, Hughes BG, Hendzel MJ, et al. Sequential fractionation and isolation of subcellular proteins from tissue or cultured cells. *MethodsX*. 2015; 2: 440-445.
 - 20. Thompson CH, Kemp GJ, Sanderson AL, et al. Effect of creatine on aerobic and anaerobic metabolism in skeletal muscle in swimmers. *Br J Sports Med* 1996; 30:222–225.
 - 21. Ledford A, Branch JD. Creatine supplementation does not increase peak power production and work capacity during repetitive wingate testing in women. *J. Strength Cond Res* 1999; 13: 394–399.
 - 22. Marzuca-Nassr GN, Fortes MAS, Guimarães-Ferreira L, et al. Short-term creatine supplementation changes protein metabolism signaling in hindlimb suspension. *Braz J Med Biol Res* 2019; 52: e8391.
 - 23. Xu H. Obesity and metabolic inflammation. *Drug Discov Today Dis* 2013; 1; 10(1-2): 21-25.
 - 24. Park SY, Choi GH, Choi HI, et al. Depletion of mitochondrial DNA causes impaired glucose utilization and insulin resistance in L6 GLUT4myc myocytes. *J Biol Chem* 2005; 280: 9855–9864.
 - 25. Necas J, Bartosikova L. Carrageenan: a review. *Veterinární medicína* 2013; 58:187–205.
 - 26. Eddouks M, Chattopadhyay D, Zeggwagh NA. Animal models as tools to investigate antidiabetic and anti-inflammatory plants. *Evid Based Complement Alternat Med* 2012; 2012: 142087.
 - 27. LeFloc'h N, Melchior D, Obled C. Modifications of protein and amino acid metabolism during inflammation and immune system activation. *Livestock Prod Sci* 2004; 87: 37–45.
 - 28. Wu H, Ballantyne CM. Skeletal muscle inflammation and insulin resistance in obesity. *J Clin Invest* 2017; 127: 43–54.
 - 29. Ciaraldi TP, Ryan AJ, Mudaliar SR, et al. Altered myokine secretion is an intrinsic property of skeletal muscle in type 2 diabetes. *PloS One*. 2016; 11: e0158209.
 - 30. Green CJ, Pedersen M, Pedersen BK, et al. Elevated NF-κB activation is conserved in human myocytes cultured from obese type 2 diabetic patients and attenuated by AMP-activated protein kinase. *Diabetes*. 2011; 60: 2810–2819.
 - 31. Li YP, Lecker SH, Chen Y, et al. TNF-α increases ubiquitin-conjugating activity in skeletal muscle by up-regulating UbcH2/E220k. *FASEB J* 2003; 17: 1048-1057.
 - 32. Park SJ, Ryu SY, Park J, et al. Association of sarcopenia with metabolic syndrome in Korean population using 2009–2010 Korea national health and nutrition examination survey. *Metab Syndr Relat Disord*. 2019; 17: 494–499.
 - 33. Reza MM, Subramaniyam N, Sim CM, et al. Irisin is a pro-myogenic factor that induces skeletal muscle hypertrophy and rescues denervation-induced atrophy. *Nat. Commun* 2017; 8: 1104.
 - 34. Abrigo J, Rivera JC, Aravena J, et al. High fat diet-induced skeletal muscle wasting is decreased by mesenchymal stem cells administration: Implications on oxidative stress, ubiquitin proteasome pathway activation, and myonuclear apoptosis. *Oxid Med Cell Longev* 2016; 2016: 9047821.
 - 35. Mozaffarian D, Katan MB, Ascherio A, et al. Trans fatty acids and cardiovascular disease. *N Engl J Med* 2006; 13; 354: 1601-1613.
 - 36. Blaauw B, Schiaffino S, Reggiani C. Mechanisms modulating skeletal muscle phenotype. *Compr Physiol* 2013; 3: 1645–1687.
 - 37. Roy B, Curtis ME, Fears LS, et al. Molecular mechanisms of obesity-induced osteoporosis and muscle atrophy. *Front Physiol* 2016; 7: 439.
 - 38. Ferretti R, Moura EG, Santos VCD, et al. High-fat diet suppresses the positive effect of creatine supplementation on skeletal muscle function by reducing protein expression of IGF-PI3K-AKT-mTOR pathway. *PLoS One*. 2018; 13: e0199728.
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