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Combination of amylopectin and chromium form improves energy storage and reduces muscle fatigue in rats during exhaustive exercise

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Abstract: This study evaluated the effects of amylopectin (A) and chromium (Cr) forms alone or in combination on energy storage, muscle fatigue-related biochemical markers, and expression of peroxisome proliferator-activated receptors (PPAR-γ) and glucose transporter-2 and -4 (GLUT-2 and GLUT-4) in rats during exhaustive exercise. Thirty-five male Wistar rats were divided into 5 groups (n = 7) as follows: 1) exhaustive exercise (E); 2) E+A; 3) E+A+chromium picolinate (CrPic); 4) E+A+chromium histidinate (CrHis), and 5) E+A+CrPic+CrHis. Rats received 0.0316 g of amylopectin and 11.06 µg of elemental Cr kg^{-1} body weight given as CrPic and CrHis per day. The mean duration of exercise differed (55 ± 1.15 vs. 76 ± 1.91) between groups ($P < 0.05$). Blood glucose level was significantly reduced in the E+A+CrPic+CrHis group compared to the E group. The E+A+CrPic+CrHis group was superior in terms of increasing muscle and liver glycogen contents and blood insulin concentration and of decreasing serum lactate concentration ($P < 0.05$ for all). However, A and Cr did not alter GLUT-2, GLUT-4, and PPAR-γ expressions. In conclusion, A in combination with supplemental Cr enhanced energy-yielding nutrient conservation and reduced muscle fatigue in exercised rats. They can also help prevent racehorse performance and disability problems.

Key words: Amylopectin, chromium picolinate, chromium histidinate, exercise, muscle fatigue

1. Introduction

Carbohydrates, the most efficient fuel source and the only source of energy for certain vital cells such as brain cells and blood cells, are stored in the form of glycogen, mostly in the liver and muscles (1,2). During high-intensity exercise or overnight fasting, no quick supply of simple sugars is available in the body. Hence, carbohydrate stores (glycogen) are crucial for supplying the energy necessary for homeostasis (3). Generally, 3–4 g of carbohydrate per minute is utilized during constant exercise. If the exercise continues for more than 2 h, the body carbohydrate stores are exhausted and metabolism will be shaped by fatigue as a reflection of the accumulation of lactate (4,5). Individuals who do not routinely exercise face a fatigue experience known as end-of-exercise muscle fatigue. This situation is especially more evident when a sedentary lifestyle moves to sudden and intense exercise (6). Physical fatigue is caused by energy source depletion and excess reactive free radicals and metabolites (e.g., lactate) (7).

While exhaustive exercise can cause muscle damage and induce fatigue, regular exercise can reduce the risk of chronic diseases such as diabetes mellitus, obesity, and cardiovascular diseases (8–10). Indeed, regular exercise reduces blood triglyceride (TG) and glucose concentration and elevates blood high-density lipoprotein (HDL) cholesterol concentration (11,12), improves insulin sensitivity (13,14), and regulates various biomarkers such as glucose transporters and peroxisome proliferator-activated receptors (PPAR-γ) in the body (15,16). Exercise stimulates PPAR-γ signaling events and upregulates lipid metabolism genes (17), and provides insulin activity by increasing the mobilization of glucose transporters and internalization of glucose (18,19).

Amylopectin (A) and chromium (Cr) are micronutrients commonly used to boost energy by

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athletes (20) and reduce muscle fatigue after exercise. A, one of the two components of starch, is a water-insoluble polysaccharide found in plants. Supplementation of A has been shown to modulate glucose and insulin metabolism (21), and control pancreatic cells growth (22). Cr not only acts as an antioxidant (23), but also involves the metabolism of carbohydrate, fat, and protein (24,25) and enables the activation of insulin and reduces fat deposits in the body (26). Cr is commonly used in the management of glucose and insulin metabolism by diabetic patients (27–29) and for muscle fatigue (30). Moreover, recent studies reported that Cr elevates insulin-stimulated glucose uptake in adipocytes (31) and cultured muscle cells (32). Although numerous studies on the effects of A (21,22) and Cr [CrPic (Cr, 12.43%) and CrHis (Cr, 25.22%)] on various metabolic parameters of exercised rats have been published (27–29), their synergic effects on energy storage and muscle fatigue-related biochemical parameters have not been elucidated. Therefore, the present study was undertaken to investigate the effect of A and Cr (US Patent No. 20170239267A1) supplementation on blood glucose and insulin levels, lactate concentration, lipid profile, glycogen storage, hematological parameters, and liver metabolites in rats during exhaustive exercise.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (8 weeks old, 200–250 g body weight) were obtained from the animal house of the Experimental Research Center of Fırat University. They were housed in polystyrene cages and maintained under controlled standard conditions (12 h light and 12 h dark cycle; temperature: 22 ± 2 °C; relative humidity: 55 ± 5%), with free access to food and water. The study was approved by the Institutional Animal Ethics Committee (Permit Number: 2016/120, 12 October 2016) and performed in accordance with the guidelines for laboratory animal use as described by European Community guidelines (33).

2.2. Experimental design and period

Thirty-five rats were randomly divided into 5 groups of 7 rats each and treated as follows: Group 1 (E), rats fed on standard diet and submitted to exhaustive exercise on treadmill; Group 2 (E+A), exhaustively exercised rats receiving amylopectin; Group 3 (E+A+CrPic), exhaustively exercised rats receiving amylopectin + CrPic; Group 4 (E+A+CrHis), exhaustively exercised rats receiving amylopectin + CrHis; Group 5 (E+A+CrPic+CrHis), exhaustively exercised rats receiving simultaneously amylopectin, CrPic, and CrHis. Rats were treated with 0.0316 g of amylopectin and 11.06 µg of elemental Cr/kg body weight given as CrPic and CrHis per day orally by gavage for 1 week. Group 5 rats received the same amount of elemental Cr but this Cr was half met by CrPic and the other half by CrHis. A and Cr doses were determined on the basis of the studies done by Ziegenfuss et al. (34) and Sahin et al. (35). Ingredients and nutrient compositions of the diet are shown in Table 1. Administration of A, CrPic, and CrHis began 1 week before acute exercise and they were given by gavage daily until exhaustive exercise session. A, CrPic, and CrHis were provided by Nutrition 21 Inc. (Purchase, NY, USA).

Animals were previously adapted on a motorized rodent treadmill with an electric shock grid (Treadmill, MAY-TME 0804, Commat Limited, Ankara, Turkey) for 1 week of 10-min runs at 10 m/min on a 15% grade in the week prior to the experimental exercise. For exhaustive

Table 1. Ingredients and nutrient composition of the diet.

Ingredient	%
Yellow corn	30.22
Barley	10.07
Soybean meal	38.28
Sunflower meal	6.04
Wheat bran	10.08
Molasses	3.02
Limestone	1.51
Salt	0.08
DL-methionine	0.30
Dicalcium phosphate	0.20
Vitamin and mineral premix[1]	0.20
Chemical composition (% of dry matter)	
Crude protein	24.00
Metabolizable energy, kcal/kg[2]	3100
Ether extract	3.40
Crude cellulose	6.90
Ash	8.10
Calcium	1.30
Phosphorus	0.90

[1]The vitamin-mineral premix provides the following (per kg): all-*trans*-retinyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; all-*rac*-a-tocopherol acetate, 12, 5 mg; menadione (menadione sodium bisulfate), 1.1 mg; riboflavin, 4.4 mg; thiamine (thiamine mononitrate), 1.1 mg; vitamin B6, 2.2 mg; niacin, 35 mg; Ca-pantothenate, 10 mg; vitamin B12, 0.02 mg; folic acid, 0.55 mg; *d*-biotin, 0.1 mg; manganese (from manganese oxide), 40 mg; iron (from iron sulfate), 12.5 mg; zinc (from zinc oxide), 25 mg; copper (from copper sulfate), 3.5 mg; iodine (from potassium iodide), 0.3 mg; selenium (from sodium selenite), 0.15 mg; choline chloride, 175 mg.

[2]Metabolizable energy calculated according to 1995 National Research Council guidelines.

exercise, animals ran at 30 m/min on a 15% grade until exhaustion (36).

2.3. Sample collection

After acute exercise, the rats were sacrificed by cervical dislocation under anesthesia and blood, liver, and muscle samples were collected. Blood samples were centrifuged at 5000 rpm at 4 °C for 10 min in a refrigerated centrifuge (Universal 320R, Hettich, Germany) before the separation of sera. After centrifugation, plasma was separated, aliquoted, and stored at –20 °C until biochemical analysis. Liver and muscle tissues were immediately frozen and stored at –80 °C in a freezer.

2.4. Laboratory analyses

Glucose, glycogen, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), as well as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, were determined using an automatic biochemical analyzer (Samsung Labgeo PT10, Suwon, Korea). Hematological parameters were determined using a blood count device (EXIGO-EOS, Spanga, Sweden). Serum lactate levels were analyzed with the ELISA Lactate Kit (Cayman Chemical Co., Ann Arbor, MI, USA) with inter- and intraassay coefficients of variation of 3.1% and 6.5%, respectively. Serum insulin level was evaluated by rat-specific kits (Linco Research Inc., St Charles, MO, USA) via ELISA (BIOTEK-ELX800, Winooski, VT, USA) with inter- and intraassay constants of 3.2% and 5.7%, respectively. The glycogen contents in the tissue samples were analyzed using a commercial glycogen assay kit (Cayman Chemical Co.) with inter- and intraassay coefficients of variation of 3.8% and 7.1%, respectively. Liver triglyceride level was detected with a commercial triglyceride kit as described previously (37).

2.5. Western blot analysis

Briefly, muscle and liver supernatants were homogenized in phosphate-buffered saline (PBS) with a protease inhibitor cocktail and the protein level was determined. The nitrocellulose membranes were washed in PBS and blocked by 1% bovine serum albumin (BSA) for 1 h before the application of the primary antibody. The membranes were incubated overnight at 4 °C with anti-PPAR-γ, anti-GLUT-2, and anti-GLUT-4. The next day, the membranes were incubated with secondary antibodies (diluted at a concentration of 1:1000) conjugated with horseradish peroxidase. The loading of proteins was controlled by a monoclonal mouse antibody versus β-actin (A5316; Sigma-Aldrich, St Louis, MO, USA). Specific binding was detected using diaminobenzidine and H_2O_2 as substrates as described previously (35).

2.6. Statistical analysis

Data are presented as the mean ± standard error of the mean. Data were analyzed by one-way analysis of variance using the PROC GLM procedure of SAS software (version 9). Comparisons among the groups were made with the post hoc Tukey HSD. P-values of 0.05 or less were taken to imply statistical significance.

3. Results

3.1. Body weight, endurance time, and serum parameters

The body weight was statistically unchanged ($P > 0.05$) among the groups. The highest endurance time was found in the E+A+CrHis and E+A+CrPic+CrHis groups, while the lowest value was found in the E group ($P < 0.05$). In addition, the mean endurance time of the E, E+A, E+A+CrPic, E+A+CrHis, and E+A+CrPic+CrHis groups were 55 ± 1.15, 61 ± 1.53, 67 ± 1.73, 74 ± 1.41, and 76 ± 1.91 min, respectively. Endurance time was significantly higher in groups E+A+CrHis and E+A+CrPic+CrHis than E, E+A and E+A+CrPic ($P < 0.05$). It was found that group E+A+CrPic+CrHis had the highest increases ($P < 0.05$). Compared to CrPic, CrHis increased endurance time even more ($P < 0.05$).

There were no significant differences in AST and ALT levels as well as lipid profiles (TC, TG, HDL-C, and LDL-C, $P > 0.05$) (Table 2). Blood glucose concentration decreased in the E+A+CrPic+CrHis group compared to the E group ($P < 0.05$; Table 2). Muscle and liver glycogen content as well as a blood insulin concentration were the highest in the E+A+CrPic+CrHis group and were the lowest in the E group ($P < 0.05$; Table 2). Although there was no change in hepatic TG among the groups, blood lactate concentration was highest in the E group and lowest in the E+A+CrPic+CrHis group (Table 2).

3.2. Hematology

A and Cr forms (CrPic and CrHis) as well as their combinations increased WBC, LYM%, LYM#, and MID# but decreased GR% and P-LCR% compared to the control group (Table 3). The highest values of WBC and MID# were observed in exercised rats supplemented with both A and two Cr sources. Moreover, the most notable change in LYM%, GR%, and LYM# levels was found in exercised rats supplemented with A and Cr as CrPic. On the other hand, simultaneous administration of A and Cr as CrHis was most efficient in lowering P-LCR% level in exercised rats (Table 3). Other hematological parameters were unresponsive to the treatments.

3.3. Expression of regulatory proteins in muscle and liver

Despite trends for higher values in the E+A+CrPic+CrHis group, no significant differences in muscle and liver PPAR-γ expression or liver GLUT-2 and muscle GLUT-4 levels across the treatments were observed (Figures 1 and 2).

4. Discussion

Micronutrients such as A and Cr are efficient in modulating glucose and insulin metabolism (34,37–39), as observed in

Table 2. Effects of amylopectin and chromium form on blood, muscle, and liver metabolites in exercised rats.

	Groups[1]				
Parameters[2]	E	E+A	E+A+CrPic	E+A+CrHis	E+A+CrPic+CrHis
Body weight, g	249.86 ± 11.10	251.29 ± 14.20	255.71 ± 6.24	252.14 ± 5.33	244.00 ± 7.52
Endurance time, min	55.00 ± 1.15^{d}	61.00 ± 1.53^{c}	67.00 ± 1.73^{b}	74.00 ± 1.41^{a}	76.00 ± 1.91^{a}
AST, U/L	364.23 ± 10.54	350.52 ± 3.50	355.61 ± 60.48	365.13 ± 23.47	367.29 ± 11.98
ALT, U/L	99.33 ± 10.09	94.43 ± 14.33	96.86 ± 4.17	94.86 ± 3.71	89.43 ± 3.54
TC, mg/dL	96.67 ± 4.72	90.43 ± 5.07	90.57 ± 5.94	91.57 ± 5.52	90.71 ± 3.48
TG, mg/dL	137.50 ± 3.69	138.57 ± 2.76	132.86 ± 4.92	139.43 ± 14.13	130.43 ± 6.19
HDL-C, mg/dL	24.83 ± 0.60	22.71 ± 0.78	23.43 ± 0.78	23.43 ± 0.48	23.57 ± 0.53
LDL-C, mg/dL	42.33 ± 2.99	38.57 ± 5.53	38.29 ± 4.24	40.43 ± 3.18	34.14 ± 4.26
Glucose, mg/dL	129.50 ± 9.00^{a}	127.40 ± 2.57^{a}	126.29 ± 12.34^{ab}	123.29 ± 3.87^{ab}	96.57 ± 8.45^{b}
Lactate, mmol/L	4.10 ± 0.12^{a}	3.21 ± 0.06^{b}	3.64 ± 0.11^{b}	3.51 ± 0.12^{b}	2.65 ± 0.08^{c}
Insulin, ng/mL	7.93 ± 0.21^{c}	10.23 ± 0.36^{b}	11.34 ± 0.52^{b}	11.63 ± 0.55^{ab}	13.47 ± 0.54^{a}
Muscle glycogen, mg/g	2.49 ± 0.12^{c}	3.67 ± 0.10^{ab}	3.08 ± 0.32^{bc}	3.37 ± 0.41^{abc}	4.23 ± 0.26^{a}
Liver glycogen, mg/g	43.55 ± 2.49^{c}	53.31 ± 1.93^{ab}	51.78 ± 2.47^{bc}	52.54 ± 1.89^{ab}	61.22 ± 1.88^{a}
Liver triglycerides, µmol/g	34.86 ± 1.78	33.29 ± 1.82	34.57 ± 2.15	33.71 ± 1.43	33.29 ± 2.22

[1]Number of rats = 7 per group. E: Exercise; A: amylopectin; CrPic: chromium picolinate; CrHis: chromium histidinate. Mean values with different superscripts within the same rows differ ($P < 0.05$). [2]ALT: Alanine aminotransferase; AST: aspartate aminotransferase: TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

the current study. Indeed, the combination of A and Cr as CrPic elevated blood glucose level while A combined with Cr as CrHis reduced it. Because A significantly reduced the blood glucose level when supplemented alone, its combination with CrPic may exhibit an inhibitory effect. The reduction in blood glucose levels in rats supplemented with both A and Cr as CrHis may be due to the high distribution and absorptive property of CrHis, as reported previously (40,41). Although no previous studies on the combined effects of A and Cr in exercised rats have been found in the literature, Cefalu and Hu (42) reported that CrPic ingestion modulates glucose and insulin levels in type 2 diabetic rats. Our findings suggest that the antifatigue properties of A+Cr might be related to its ability to improve the metabolic control of exercise and energy metabolism.

Leon and Sanchez (43) reported that exercise induced a significant decrease in LDL and TG concentrations, but had no effect on blood total cholesterol and transaminase (AST and ALT) levels. In the present study, we found that transaminase (AST and ALT) levels and lipid profiles (TC, TG, HDL-C, and LDL-C) were statistically unchanged in all groups. These results are similar to previous findings (44). On the contrary, ingestion of Cr as chromium malate has been reported to reduce TC, LDL-C, and TG levels and increase HDL-C concentration in type 2 diabetic rats (45). Moreover, it has been shown that Cr supplementation significantly decreased serum total cholesterol level during exercise (44). In the current study, it is possible that the low level of lipid parameters achieved with exercise masked the ability of A+Cr to exert an antilipidic effect. The nonsignificant change in AST and ALT concentrations in exercised rats supplemented with A+Cr may indicate no toxicity or harmful effects on hepatocytes and therefore confirms the safety of these micronutrients as reported by Anderson et al. (41) and Tuzcu et al. (46).

Hematological parameters are good indicators of the general health condition. Noushand et al. (47) reported that immediately after exercise the hemoglobin, hematocrit, erythrocyte, and leukocyte levels increased significantly while platelet and reticulocyte counts remained unchanged. In the present study, hematological parameters were affected by A+Cr supplementation. A and Cr ingestion significantly increased WBC, LYM%, LYM#, and MID# and decreased GR% and P-LCR% compared to the control group. In a similar study, Toghyani et al. (48) reported that CrPic supplementation significantly increased hemoglobin, MCH, and MCHC levels but there was no significant change in WBC, RBC, PCV, MCV, and thrombocyte levels. Improvement in hematological parameters observed in the current study may be correlated with the high glycogen storage after A+Cr

Table 3. Effects of amylopectin and chromium form on hematological parameters in exercised rats.

Parameters[2]	Groups[1]				
	E	E+A	E+A+CrPic	E+A+CrHis	E+A+CrPic+CrHis
WBC, µL	8.12 ± 2.48^{c}	12 ± 1.66^{ab}	12.31 ± 3.32^{ab}	9.99 ± 2.25^{ab}	13.54 ± 3.37^{a}
LYM, %	53.17 ± 7.39^{c}	63.99 ± 6.05^{ab}	71.03 ± 5.44^{a}	61.69 ± 11.07^{ab}	64.37 ± 6.14^{ab}
MID, %	9.25 ± 7.96	15.34 ± 5.06	15.60 ± 4.63	8.79 ± 4.96	18.41 ± 7.46
GR, %	37.58 ± 6.35^{a}	20.67 ± 8.23^{bc}	13.37 ± 1.92^{c}	29.53 ± 12.68^{ab}	17.21 ± 4.40^{c}
LYM, #	4.33 ± 1.58^{c}	7.69 ± 1.31^{ab}	8.89 ± 2.97^{a}	6.19 ± 1.87^{ab}	8.8 ± 2.62^{a}
MID, #	0.75 ± 0.55^{c}	1.84 ± 0.60^{a}	1.81 ± 0.33^{ab}	0.83 ± 0.42^{bc}	2.51 ± 1.01^{a}
GR, #	3.03 ± 1.04	2.47 ± 1.16	1.61 ± 0.35	2.97 ± 1.64	2.23 ± 0.35
RBC, µL	7.81 ± 0.11	7.64 ± 0.53	7.85 ± 0.31	7.82 ± 0.58	7.97 ± 0.36
Hgb, g/dL	16.98 ± 0.28	16.53 ± 1.15	16.97 ± 0.79	16.26 ± 0.93	16.59 ± 0.93
HCT, %	71.08 ± 1.23	68.76 ± 4.13	71.94 ± 3.17	70.64 ± 3.95	72.17 ± 3.16
MCV, fL	91.22 ± 0.73	90.13 ± 2.28	91.76 ± 1.75	90.54 ± 2.86	90.59 ± 2.56
MCH, pg	21.72 ± 0.30^{a}	21.59 ± 0.50^{ab}	21.57 ± 0.52^{ab}	20.79 ± 0.69^{b}	20.76 ± 0.70^{b}
MCHC, g/dL	23.85 ± 0.23^{a}	23.99 ± 0.37^{a}	23.54 ± 0.14^{a}	22.96 ± 0.46^{b}	22.94 ± 0.44^{b}
RDW-SD, fL	41.8 ± 0.99	41.67 ± 2.81	41.66 ± 2.11	43.27 ± 4.40	41.94 ± 2.11
RDW-CV, %	15.97 ± 0.52	16.14 ± 1.18	15.83 ± 1.02	16.63 ± 1.32	16.13 ± 0.80
PLT, µL	1016.33 ± 251.55	1113.43 ± 160.38	1049.14 ± 204.84	1065.57 ± 82.69	1081.43 ± 169.04
MPV, fL	11.82 ± 0.33	11.51 ± 0.28	11.43 ± 0.55	10.77 ± 1.43	11.46 ± 0.28
PDW, %	17.4 ± 0.53	17.59 ± 0.95	16.63 ± 1.47	15.61 ± 3.37	17.19 ± 1.35
PCT, %	1.20 ± 0.31	1.23 ± 0.21	1.12 ± 0.35	1.24 ± 0.11	1.16 ± 0.17
P-LCR, %	32.67 ± 1.33^{a}	30.04 ± 1.50^{ab}	30.43 ± 2.02^{ab}	24.90 ± 9.96^{b}	29.23 ± 1.91^{ab}

[1]Number of rats = 7 per group. E: Exercise; A: amylopectin; CrPic: chromium picolinate; CrHis: chromium histidinate. Mean values with different superscripts within the same rows differ ($P < 0.05$). [2]WBC: White blood cell; LYM%: percentage of lymphocyte cell count; LYM#: lymphocyte cell count; MID%: percentage of mononuclear cell count; MID#: mononuclear cell count; GR%: percentage of granulocytes cell count; GR#: granulocytes cell count; RBC: red blood cell; Hgb: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; PLT: platelet; PCT: procalcitonin; MPV: mean platelet volume; PDW: platelet distribution width; RDW-SD: red blood cell distribution width; RDW-CV %: percentage of red blood cell distribution width; P-LCR %: percentage of platelet larger cell ratio.

supplementation. For instance, it has been shown that increased lymphocyte levels stimulate glycogen storage in the presence of high glucose concentration (49,50).

During exercise, muscle fatigue can be evaluated by multiple biochemical indicators, such as lactate and glucose levels (51,52). Lactate, a substrate in skeletal muscle, acts as a precursor of gluconeogenesis in muscles and liver after exercise. Indeed, after acute exercise, approximately 75% of the total amount of lactate accumulated in the muscles is used for energy production in the exercising body (53). The normal value of lactate is approximately 2 mmol/L (54). During exercise, its accumulation can negatively affect the central nervous system and cause muscle fatigue (55). On the other hand, the maintenance of blood glucose level during exercise could extend exercise duration and improve performance (56). In the current study, the lactate level of exercised rats in all groups was above the normal value. Similar results (elevated lactate level after exercise) have been reported by Fonseca et al. (57). Remarkably, A and Cr supplementation significantly decreased the serum lactate level of exercised animals. Similarly, Cr supplementation resulted in a reduction of lactate level after exercise, possibly indicating that Cr contributed to a better utilization of plasma glucose and to a better adaptation of animals to physical activity (57). In another similar study, Davis et al. (58) reported that the combination of Cr and carbohydrate exhibited the highest antifatigue activity compared with Cr alone. The lowest value of lactate was noted in rats supplemented simultaneously with A and either Cr form. These results confirm the antifatigue

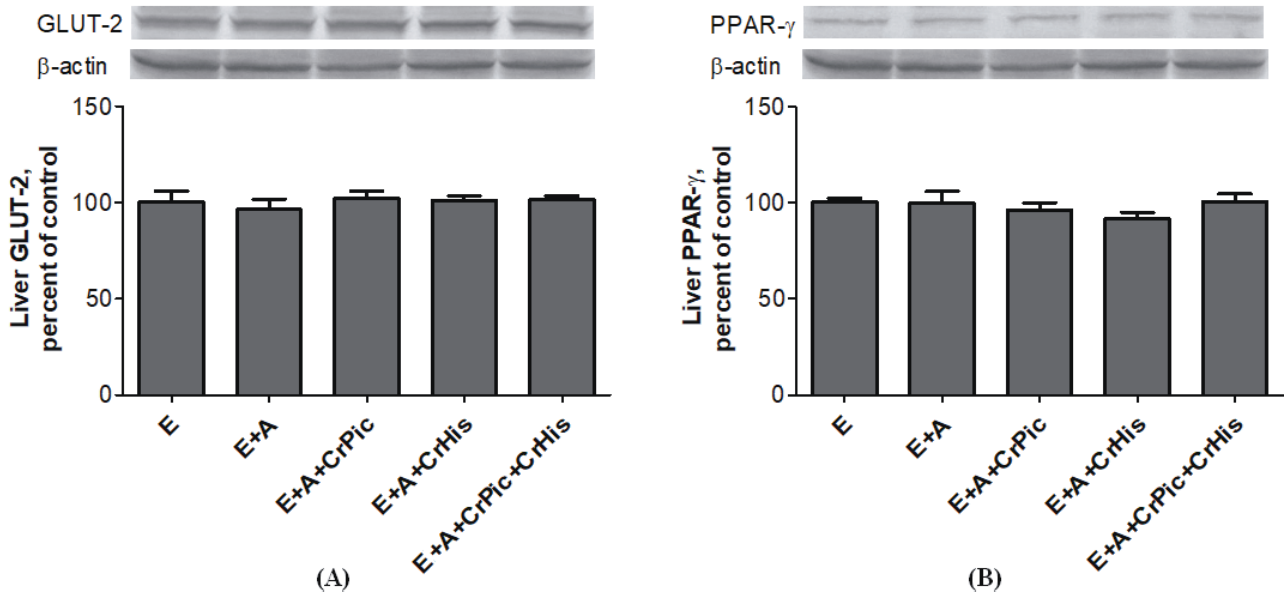

Figure 1. Effects of amylopectin and chromium form on the expression of GLUT-2 (A) and PPAR-γ (B) in the liver of exercised rats. E: Exercise; A: amylopectin; CrPic: chromium picolinate; CrHis: chromium histidinate; GLUT-2: glucose transporter-2; PPAR-γ: peroxisome proliferator-activated receptor gamma. Bars represent the standard error of the mean. Blots were repeated at least 3 times (n = 3) and a representative blot is shown. β-Actin was included to ensure equal protein loading.

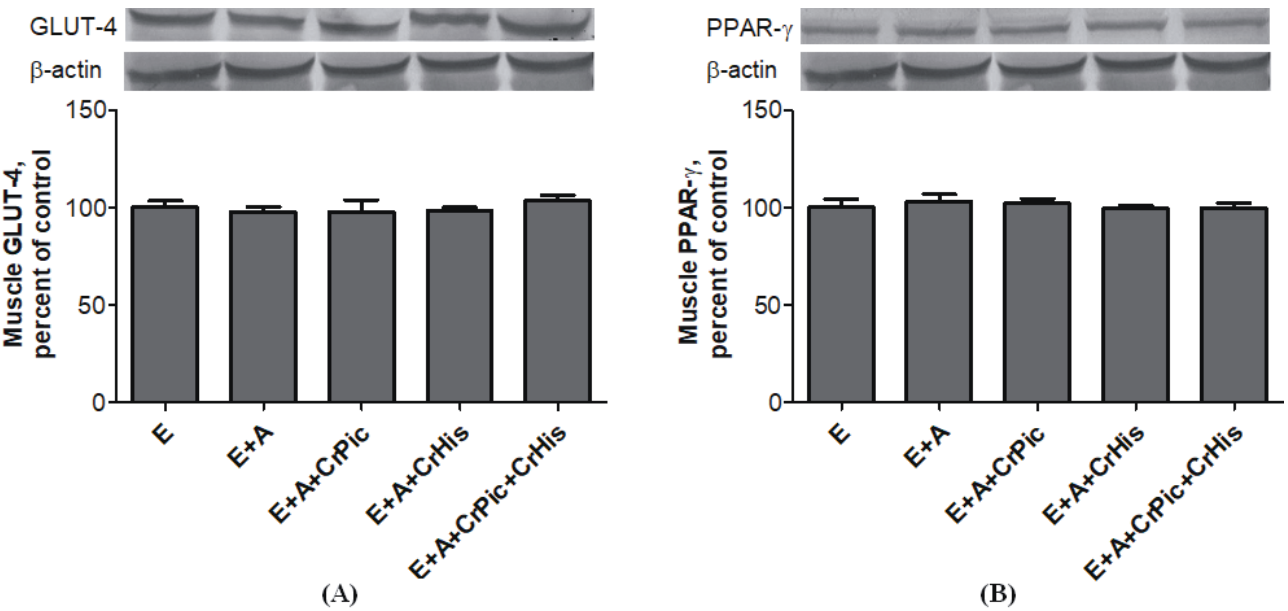

Figure 2. Effects of amylopectin and chromium form on the expression of GLUT-4 (A) and PPAR-γ (B) in the muscle of exercised rats. E: Exercise; A: amylopectin; CrPic: chromium picolinate; CrHis: chromium histidinate; GLUT-4: glucose transporter-4; PPAR-γ: peroxisome proliferator-activated receptor gamma. Bars represent the standard error of the mean. Blots were repeated at least 3 times (n = 3) and a representative blot is shown. β-Actin was included to ensure equal protein loading.

properties of A reported by Zhang et al. (59). The low lactate level in plasma of rats supplemented with A+Cr could be due to low lactate influx from muscles, resulting from high lactate conversion to glycogen. Alternatively, decreased lactate level after A+Cr ingestion could indicate a low redistribution of intracellular glucose and low glucose uptake.

During exhaustive exercise, ATP is consumed continuously by myocytes, which can be generated through oxidative phosphorylation in the muscle (60). Under this

condition, glycogen is converted into glucose to generate ATP (61), resulting in a depletion of glycogen storage in the liver and muscles (62). In the current study, increased glycogen storage in the livers and muscles of exercised rats supplemented with A+Cr may have been mediated by increasing the concentration of ATP, which would be helpful for increasing energy storage. Because A+Cr ingestion improves glycogen storage in exercised rats, this could indicate the greater contribution of glucose uptake to glycogen formation. Similarly, Hsiao et al. (63) reported that supplementation with Hualian No. 4 wild bitter gourd significantly increased glycogen storage and reduced fatigue in exercised mice. On the contrary, Volek et al. (62) showed that Cr supplementation induced a nonsignificant change in muscle glycogen synthesis during recovery from high-intensity exercise. Campbell et al. (64) also reported that the liver glycogen phosphorylase activities in chromium-supplemented rats remained unaffected after 8 weeks of dietary treatment.

Glucose transporters (GLUT-2 and GLUT-4) and PPAR-γ are biomarkers involved in various metabolic processes. Exercise increases GLUT-2 and GLUT-4 expression and insulin-stimulated glycogen storage in muscle (15). On the other hand, PPAR-γ is involved in the regulation of glucose, lipid, and carbohydrate metabolisms (65), and its level can reflect athletic performance (16,66). Numerous studies indicated that exercise increases PPAR-γ expression in the liver (67,68) and skeletal muscle (69). In this study, no significant changes in liver and muscle GLUT-2, GLUT-4, and PPAR-γ expressions were observed, although there was a trend for higher GLUT-4 expression in the muscles of animals treated with both A and Cr (CrPic and CrHis). It has been reported that Cr supplementation is able to improve many aspects of insulin signaling, including GLUT-4 translocation (70,71).

The present study demonstrated that A and Cr as well as their combination improved energy reserves and hematological parameters and reduced muscle fatigue after exercise. However, these were not accompanied by changes in liver transaminases levels, blood lipid profiles, and muscular and hepatic expression of glucose transporters and PPAR-γ in the muscles and livers of exercised rats. A and Cr could be potential agents with antifatigue pharmacological effects. They can also help prevent racehorse performance and disability problems.

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