Nutritional Sciences 8(3), August, 2005, pp.189-195

Effects of Pinitol Supplementation and Strength Training on Anaerobic Performance and Status of Energy Substrates in Healthy Young Men*

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To assess the effect of pinitol supplementation and strength training for two weeks on the anaerobic capacity during and after exercise, and improvement of glucose metabolism during the recovery period of muscular fatigue with repeated acute bouts of cycling exercise, a total of 24 healthy young men were recruited and randomly and equally divided into three groups; pinitol supplementation group (PSG), placebo group (PLG), and control group (CON). Using a randomized double-blinded design, subjects in PSG were provided pinitol supplement, consumed orally 1.2 g/day, and participated in the resistance exercise program and cycling exercise for two weeks. Subjects in PLG underwent the same protocol as those in PSG but consumed the same amount of placebo. No supplementation and exercise program was given to CON. Before and after the intervention, all subjects were tested for their anaerobic capacities evaluated by Wingate test twice separated by 30 min. During the test, peak anaerobic power (PP), mean anaerobic power, total work, and fatigue index were evaluated. During resting and recovery, blood samples were drawn and plasma pinitol, myo-inositol, chiro-inositol, insulin, free fatty acid, glucose, and lactate levels were analyzed. After two weeks, PP and relative PP of the second biking were improved from the first biking in PSG only ($p<0.05$). No changes were found in all other variables of Wingate test in all groups. No statistical differences between groups and pre- and post-intervention were observed in concentrations of pinitol, myo-inositol, and chiro-inositol, but pinitol concentration was higher during recovery compared to the baseline in all groups and testings ($p<0.05$). Lactate level during recovery was higher than the resting level, but no other blood parameters were significantly changed. In conclusion, two weeks of pinitol supplementation in conjunction with short duration of anaerobic training in healthy young men did not induce any obvious benefits in terms of anaerobic capacity and energy metabolism. Individual and/or population susceptibility may be one factor responsible for adopting pinitol supplementation.

Key Words: Glucose metabolism, D-chiro-inositol, Insulin, Anaerobic capacity

Received July 5, 2005; Revised August 5, 2005; Accepted August 11, 2005

INTRODUCTION

D-chiroinositol, found in trace amounts in tissues and body fluids,[1)] is an important inositol phosphoglycan mediator and participates in the insulin signalling pathways stimulating glucose transport.[2)] It has been suggested that D-chiroinositol have insulin-like effects[3-5)] resulted in lowering postprandial plasma glucose levels without inducing hyperinsulinemia in monkeys,[6)] and reducing plasma glucose in streptozotocin-induced diabetic rats and normal rats.[7)] Thus, it was considered as an useful agent for improving glucose metabolism. Pinitol (3-O-methyl-D-chiroinositol), a methylated derivative of D-chiroinositol, is primarily found in legumes and citrus fruits and contributes as a major metabolic source,[8)] and pinitol containing meals might increase both pinitol and D-chiroinositol levels in the body.

Insulin facilitates muscle glycogen synthesis through its action on both glucose transport and glycogen synthase activity.[9)] During exercise, intramuscular glycogen store is the major source of energy supply for skeletal muscle contractions. Hence, the reduction of muscle glycogen availability limits exercise performance.[10)] Following exercises, the resynthesis of muscle

* This study was financially supported by SolGent Co., Ltd. (Daejeon, South Korea) & Amicogen Co., Ltd. (Jinju, South Korea). The authors would like to express sincere thanks to all participants for their enthusiasm and endeavor.
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glycogen can be accelerated mainly due to increased muscle perfusion and glucose transport capacity to the cell membrane. If carbohydrate becomes sufficient, the rate of glycogen restoration is even greater than the normal resting level resulting supercompensation of glycogen build-up. In this process, metabolic action of insulin is enhanced in the previously glycogen depleted muscles.[11] On the other hand, immediately after exercise, insulin action may be impaired possibly due to elevated concentration of catecholamines and free fatty acids.[12,13] In addition, an eccentric exercise elicited a prolonged decrease in insulin action possibly caused by altered protein expression and function.[14,15] It is also well established that acute physical activity and endurance exercise training lead to enhancements of insulin-mediated glucose metabolism in healthy individuals.[16]

Therefore, it could be hypothesized that pinitol supplementation would increase glucose metabolism during recovery from repeated acute bouts of cycling exercise inducing muscular fatigue. This may be possible since a prolonged decrease of insulin action due to muscular exhaustion would be attenuated by the enhanced insulin like effects of pinitol. We employed a randomized double-blinded experimental design to test this hypothesis, in which we provided either pinitol or placebo supplementation for 2 weeks and trained healthy young men for muscular strength.

METHODS

1. Subjects

A total of 24, non-athletic, healthy young collegiate men were recruited as subjects. Before participation, their medical history forms were evaluated and those who had orthopedic and any metabolic diseases were excluded. The investigators explained the purpose of the experiment and possible risks involved. All subjects participated voluntarily and signed informed consent form approved by the institutional review board. During participation, they were asked not to involve vigorous physical activity other than prescribed by the investigators and to maintain their usual diets.

2. Study Design

This study was constructed by four phases; 1) preliminary testing, 2) pre-testing, 3) experimental intervention, and 4) post-testing. During the preliminary testing, morphological characteristics and cardiorespiratory fitness were examined. Then, subject were randomly and equally divided into three groups; pinitol supplementation group (PSG), placebo group (PLG), and control group (CON). Their physical characteristics were shown in Table 1. During pre-testing phase, each subject underwent an anaerobic capacity test. Following the pre-testing phase, subjects in PSG were given pinitol while PLG were given placebo and both participated in a 2-week resistance exercise program and cycling exercise. Supplementation was provided by the double- blind method, in which investigators and subjects were not informed until the experiment was completed. Subjects in CON were controlled not having supplements and systemic and structural exercise program. After two weeks, the post-testing was conducted identical to the pre-testing.

Table 1. Physical Characteristics of the Subjects

	Pinitol Suppl. Group (n=8)	Placebo Group (n=8)	Control Group (n=8)
Age (yrs)	22.0±2.3	23.8±2.5	21.3±1.9
Height (cm)	176.1±4.0	174.2±6.0	177.0±4.7
Weight (kg)	71.6±9.4	68.7±5.3	70.5±6.2
BMI (kg/m^2)	23.0±2.2	22.7±2.3	22.5±1.6
HRresting[1] ($beat \cdot min^{-1}$)	77.7±16.2	76.0±13.6	82.9±9.8
HRmax[2] ($beat \cdot min^{-1}$)	189.6±2.5	189.3±4.9	191.1±3.5
VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$)	52.4±8.6	52.0±4.8	53.3±4.6
MAP[3] (mmHg)	92.0±7.5	91.3±7.6	93.8±6.9
Body Fat (%)	10.8±4.1	13.6±8.3	12.2±4.7
Fat Free Mass (kg)	61.7±4.0	59.4±5.2	62.1±8.3

Values are M±S.D.
1) seated resting heart rate, 2) maximal heart rate, 3) mean arterial pressure.

3. Preliminary Testing

During the preliminary testing, each subject's height (Jenix, Korea) and weight (CAS 150A, Korea) were measured and his body mass index (BMI; kg/m^2) was calculated. Resting heart rate (HR) while in seated position was continuously monitored (CEO0537, Polar Electro, Finland) and recorded. Blood pressure (BP) was measured manually (Standby, Baumano meter, USA). The mean arterial blood pressure (MAP) was calculated as [(systolic BP-diastolic BP)÷3]+diastolic BP. Subject's body fat (in %) and fat free mass (in kg) were estimated

by a bioelectrical impedance method (GIF-891DX, Gilwoo, Korea).

All subjects underwent the cardiac stress test on a motor driven treadmill (Q65, Quinton Instrument, Co., USA) with incremental workload up to their volitional exertion using a modified Bruce protocol. After warming up, they began exercise with 1.7 mph with 10% grade for 3 min. The grade and speed were increased 2% and 0.8 mph every 3 min. During the testing, their expiratory gases were analyzed for maximal oxygen consumption (Qmc, Quinton Instrument Inc., USA), and HR was recorded.

4. Experimental Procedures and Measurements

At least five days after the preliminary testing, the anaerobic capacity was evaluated by the Wingate test.[17] In this testing, each subject performed a 30-sec maximal cycling exercise bout on a belt resisted stationary bike ergometer (model 668, Monark, Sweden). Before the testing, a subject sat on the ergometer and warmed up for 5 min with a minimum resistance. After warming up period, the resistance of the bike was increased within 15 sec to a target tension which was predetermined according to each subject's body weight (body weight times 0.09). Once the target workload was reached, subjects were encouraged to pedal as fast as they could and to keep the rate for 30 sec. After the termination of 30-sec maximal pedalling, they pedalled 2-3 min with a minimum resistance. After the cessation of the exercise bout, they rested on a comfortable chair for 30 min. After the resting, they exercised again for the second Wingate test. The workload for PSG, PLG, and CON was 6.3±0.6, 6.1±0.4, and 6.4±0.5 kg, respectively.

During the exercise, the pedalling frequency was counted and recorded every five seconds. The peak anaerobic power (PP in Watt) was calculated as [workload× 10 (maximal pedalling frequency×6)]÷5. The relative peak anaerobic power (RPP in $W \cdot kg^{-1}$) was calculated as PP÷body weight. The total work (TW in J) and relative total work (RTW in $J \cdot kg^{-1}$) were calculated as workload ×10 (total pedalling frequency×6), and TW÷body weight, respectively. The mean anaerobic power (MP in Watt) and relative mean anaerobic power (RMP) were estimated using the equations of TW÷30, and AP÷body weight, respectively. The fatigue index (FI) in percent was estimated as [(maximal pedalling frequency-minimal pedalling frequency)÷maximal pedalling frequency]×100.

Before the first and 30 min after the second Wingate test, 8 mL of resting blood samples were obtained in EDTA contained bottles from the antecubital veins. The samples were analyzed for hematocrit in duplicate (Hct, HA-200, Hanil Science Industrial, Korea), hemoglobin (Hb, HemoCue HB 201^{+}, Hemocue AB, Sweden), glucose, lactate (DT 60 Ⅱ, Johnson & Johnson Clinical Diagnostics, USA), free fatty acid (FFA, enzymatic analysis, Sicdia Nefazyme, Eiken; model 7150, Hitachi, Japan), and insulin (radioimmunoassay method, Coat-A-count Insulin, DPC, USA; Cobra 5010 Quantum, Packard, USA) levels.

D-pinitols, myo-inositols and chiro-inositols of blood samples were measured by a Dionex high performance liquid chromatography Gradient Pump Module GP-50 System (Dionex Corp. 01020506, Austin, TX, USA). All specimens were then quantified by a Dionex Pulsed Electrochemical Detector ED50 (Dionex Corp. 00120946, Austin, TX, USA). Buffered solutions of 60 mM NaOH and ion-free distilled water were used as catalyzers and eluted for 1 hour at the speed of 0.4 mL/minute. D-pinitols, myo-inositols and chiro-inositols were mixed at a density of 0.018 mg/mL each and 25 μL of them were injected and used as experimental standards. Chromatograms were recorded by a Donam dsCHROM chromatogram analysis Data System Integrator (Donam Instrument, DS2000, Seoul, Korea) and under this condition, D-pinitols showed about 7 minutes retention time, myo-inositol showed about 8 minutes and chiro-inositols showed about 10 minutes retention time. D-pinitols, myo-inositols and chiro-inositols levels of the specimens were measured against the standard materials and assessed and recorded by their measured areas. High performance liquid chromatography was done by Amicogen Biotech Research.

The room temperature and the relative humidity of the laboratory was maintained at 18~22 ℃ and 40~50%, respectively. No subjects failed to complete all the required procedures.

5. Pinitol Supplementation

For the study, either pinitol (Amicogen Co., Ltd. Korea) or placebo (whey protein; Amicogen Co., Ltd. Korea) was provided. The taste and color were virtually indistinguishable. Subjects were instructed to consume powder type substance after mixing with tap water or other eatable liquid upon their preferences. A volume of 10 cc by a measuring spoon was mixed and consumed in each time, twice a day, in the morning and evening. Thus they consumed 20 cc of substance every day for 2 weeks (1.2 g/day for each of pinitol or placebo). Although some of the subjects complained about smell and taste, consumption of known powders was well

tolerated by all subjects in general and any digestive distraction was not reported. No other dietary regimen was performed.

6. Strength Training Program

The training program was mainly focused on strengthening the lower body including cycling (Horizon Fitness, USA), leg curl, leg extension, leg press, and squat (MedX, USA). For the cycling exercise, subjects pedalled for 30 sec duration as fast as they could as in the Wingate test for 5 times per session. For the leg strength training, they performed each mode of exercise at 80% of one repetition maximum (1 RM in kg) for 12 repetitions. The 1 RM was predicted as W_0+W_1, where, W_0=slightly heavy weight felt by each subject after sufficient warming up, approximately 7-8 repetitions possible at the weight, and $W_1= W_0\times0.025\times R$, where, R=repetition. The individualized exercise workload by groups and modes are shown in Table 2. Each exercise mode was repeated three times in each session. Each session was lasted for 60 min and subjects exercised every other day for two weeks.

7. Statistical Analyses

The collected data were reduced and expressed in mean±standard deviation. For the statistical analyses, SPSS version 11.0 for Windows was used. To find any differences among groups, one-way and two-way ANOVA with repeated measures were employed. When a significant *F*-ratio was found, subsequent post-hoc test was introduced. In cases, paired *t*-test was also utilized. The statistical significance was considered when $p<0.05$.

RESULTS

1. Group Characteristics and Morphology

At baseline, there were no significant differences in the age, height, weight, BMI, BP, MAP, resting and maximal HR, maximal aerobic capacity, %body fat, and fat free mass among groups. Other than the variables measured, physical conditions of the subject pool were considered to be similar since they were college students majoring in physical education who participated in regular physical activities. However, the workload encountered for leg curl and extension during the strength training was higher for PSG than for PLG (Table 2).

Table 2. Strength Training Workload by Groups and Exercise Modes

	Pinitol Suppl. Group (n=8)	Placebo Group (n=8)
Leg curl (lbs)	196±26^{PL}	172±23
Leg extension (lbs)	199±26^{PL}	172±23
Leg press (lbs)	74±7	83±12
Squat (lbs)	69±9	68±13

Values are M±S.D.
PL: significantly different from the placebo group at $p<0.05$.

2. Physical Performances

Wingate test results were shown in Table 3. The two-way ANOVA revealed no interactions among all comparing means. However, when the mechanical work capacity was evaluated separately by groups (measuring time periods×testings), significant *F*-ratios were found.

During pre-testing, subjects in PLG and CON did not show any differences between the first and the second biking in PP and RPP, while the values of the second biking in PP and RPP were higher than those of the first one in TW, RTW, MP, and RMP. On the other hand, in all parameters, the values of the second biking were higher than those of the first one in PSG.

Compared to pre-testing, the values of post-testing were higher in both the first and second biking in PLG and CON. However, for PSG, the differences between pre- and post-testing in most of the parameters were noticed only for the first biking, but not for the second biking. In particular, PP and RPP were improved significantly from the first biking after two weeks of strength training in PSG ($p<0.05$) but the performance level was similar after two weeks of the experimental period between the first and the second biking in PLG and CON ($p>0.05$). No changes in TW, RTW, MP, and RMP were found between the first and the second biking in all three groups ($p>0.05$). It should be noticed that the values of the second biking of pre-testing in PSG were higher than other two groups in all parameters. No significant changes were found in FI ($p>0.05$).

3. Blood Parameters

Analyses for plasma pinitol concentrations and the blood parameters were shown in Table 3 and 4, respectively. No differences between groups and testings were found in concentrations of pinitol, myo-inositol, and chiro-inositol. But, in all groups and all testings, pinitol concentration was higher during recovery compared to the baseline ($p<0.05$). When analyzing groups, time periods, and testings together, no significant interactions

Table 3. Changes of Mechanical Work Parameters during Wingate Test by Groups and Testing Periods

		Pinitol Suppl. Group		Placebo Group		Control Group	
	Testing	1st biking	2nd biking	1st biking	2nd biking	1st biking	2nd biking
Peak power (W)	Pre	689±66*	863±189	647±112	712±120PS	714±99	757±119PS
	Post	779±88*†	903±152	741±75†	825±113†	806±62†	866±108†
Relative peak power ($W \cdot kg^{-1}$)	Pre	9.7±1.2*	12.0±1.6	9.5±1.9	10.4±1.4PS	10.1±1.2	10.8±1.9PS
	Post	10.9±0.7*	12.6±1.3	10.8±0.8†	12.0±1.3†	11.5±1.0†	12.3±1.1†
Total work (J)	Pre	15,648±1,523*	18,785±2,722	14,272±2,112*	16,250±2,011PS	14,902±1,485*	16,522±2,039PS
	Post	18,213±2,595†	19,005±2,605	17,931±1,777†	17,906±1,930†PS	18,227±2,416†	18,477±1,950†
Relative total work ($J \cdot kg^{-1}$)	Pre	220±12*	263±17	208±28*	236±19PS	213±30*	236±38PS
	Post	254±14†	266±13	261±15†	260±12†	260±32†	263±27†
Mean power (W)	Pre	522±51*	626±91	476±70*	541±67PS	497±50*	551±68PS
	Post	607±87†	634±87	598±59†	597±64†	607±81†	616±65†
Relative mean power ($W \cdot kg^{-1}$)	Pre	7.3±0.4*	8.8±0.6	6.9±0.9*	7.9±0.6PS	7.1±1.0*	7.9±1.3PS
	Post	8.5±0.5†	8.9±0.4	8.7±0.5†	8.7±0.4†	8.7±1.1†	8.8±0.9†
Fatigue index (%)	Pre	46±11	48±11	46±15	39±14	52±9	47±10
	Post	51±8	48±7	49±10	45±6	57±10	48±11

Values are M±S.D.
*: significantly different from the 2nd biking; †: significantly different from the pre-testing; PS: significantly different from the pinitol supplement group at $p<0.05$.

Table 4. Plasma Pinitol Concentrations during the Experiment by Groups and Testing Periods

		Pinitol Suppl. Group		Placebo Group		Control Group	
	Testings	Resting	Recovery	Resting	Recovery	Resting	Recovery
Pinitol (μg/mL)	Pre	4.2±4.9*	17.7±10.8	5.8±7.5*	16.0±11.4	7.0±6.6*	11.6±6.8
	Post	6.3±4.8*	24.2±19.6	7.3±7.1*	20.8±14.9	6.0±4.3*	13.3±6.5
Myo-inositol (μg/mL)	Pre	5.1±3.7	4.2±2.9	6.9±4.5	6.1±4.2	4.2±1.5	4.5±1.8
	Post	5.0±2.6	5.2±3.6	7.5±3.8	6.4±3.7	4.3±1.8	4.2±1.9
Chiro-inositol (μg/mL)	Pre	29.6±13.9	28.4±12.8	24.9±13.3	25.5±11.5	24.5±8.8	24.4±10.6
	Post	29.0±8.3	31.0±14.2	26.8±10.9	26.1±8.1	21.8±4.5	21.9±4.2

Values are M±S.D., *: significantly different from the recovery value

Table 5. Responses of Insulin and Free Fatty Acid during the Experiment by Groups and Testing Periods

		Pinitol Suppl. Group		Placebo Group		Control Group	
	Testings	Resting	Recovery	Resting	Recovery	Resting	Recovery
Insulin (μIU/mL)	Pre	11.8±8.1	13.0±13.3	14.9±14.0	6.8±5.4	20.0±23.3	14.3±19.4
	Post	14.6±14.6	10.4±7.7	12.2±10.5	11.2±10.1	9.4±9.3	5.5±2.9
Free Fatty Acid (μEq/L)	Pre	143±45	169±97	171±120	132±49	234±158	138±45
	Post	233±125	215±137	162±109	131±55	252±225	136±34
Glucose (mg/dL)	Pre	114±3	119±9	122±10	113±18	119±12	117±5
	Post	119±12	125±10	112±13	114±8	125±15	122±11
Lactate (mmol/L)	Pre	3.3±0.7*	5.5±1.7	3.0±0.4*	6.2±2.5	2.9±0.4*	6.5±2.6
	Post	3.1±0.4*	6.8±2.1	4.1±1.7*	7.0±1.7	3.0±0.7*	7.6±2.0

Values are M±S.D.
*: significantly different from the recovery value

were found in insulin, free fatty acid, and glucose ($p>0.05$). Lactate levels during recovery were increased significantly from the resting levels in all cases ($p<0.05$).

DISCUSSION

In the present study, the metabolic effect of dietary pinitol in combination with strength training was investigated using healthy young human subjects. Up to date, studies were conducted examining the effect of pinitol for insulin resistance and diabetic subjects.[7,18] In our knowledge, this was the first study evaluating the possibilities whether pinitol might be a candidate for an ergogenic substance for healthy human. At first, we hypothesized that dietary ingestion of pinitol and

subsequent resistance exercise training might enhance the rate of recovery and energy substance utilization. However, we could not clearly demonstrate that pinitol supplementation and resistance training improved physical performance and any metabolic advantages.

It has been noted that exercise increases muscle glucose uptake. Also, exercise may increase the sensitivity to insulin, so glucose is transported into the muscle cells more easily for the same level of insulin.[19] Hence insulin drop normally occurring during intense exercise can accommodate maintaining normal blood glucose.

Although we did not demonstrate a wide range of beneficial effect of pinitol on diverse parameters of anaerobic physical performance, we found that the pinitol supplemented group improved their peak power and the weight-normalized relative peak power while others did not. But it should be carefully interpreted since the peak power of the second biking was higher than that of the first biking within the group as well as higher than other two groups. Also, the pinitol supplemented group exercised at heavier load during the resistance training. Improvement of peak power after the first biking may be the important finding of this study. However, it is not clear how this could be possible.

Using human subjects, recent studies reported no significant effects of pinitol on glucose metabolism. Campbell *et al.*[20] assessed the effect of 6-weeks of oral pinitol supplementation (2 g pinitol/day) on insulin-mediated glucose metabolism in older, nondiabetic subjects (66±8 yrs). They found that the intervention did not influence oral or intravenous glucose tolerances, the insulin and C-peptide responses to these challenges, or insulin sensitivities. Davis *et al.*[18] also reported that 4-weeks of pinitol treatment in obese, mild diabetic old subjects with a dose of 20 $m \cdot kg^{-1} \cdot day^{-1}$ did not alter baseline glucose production and insulin-mediated glucose disposal. The results of the present study supported these observations.

The plasma concentrations of inositols in our study showed no marked changes by the treatments. When pinitol was consumed, the plasma pinitol and D-chiro-inositol level were increased 48 and 14 folds, respectively.[18] In contrast, the concentrations of myo-inositol, D-chiro-inositol, and pinitol were not different between pinitol supplemented and control groups as well as between the baseline and the final week of treatment.[20] This discrepancy may be due to blood sampling time. Davis *et al.*[18] collected their blood samples just after taking the final dose of pinitol supplementation while Campbell *et al.*[20] did 12-h overnight fasting before the tests. In the present study, we encouraged our subjects not to eat at least 5 hours prior testings, preferably overnight fasting. It has been suggested that the oral ingestion of pinitol may be cleared from the blood stream within a relatively short period time.[20]

The individual susceptibility for pinitol supplementation and subjects status have to be considered. Campbell *et al.*[20] suggested that pinitol might influence plasma glucose most consistently in induced diabetic states, with more variable results obtained in basal and naturally occurring hyperglycemic states. The beneficial effects of pinitol ingestion were noticed only and mainly in those who had experienced major disturbances of glucose metabolism,[21] but not in healthy or mild-diabetic subjects.[18,20,22,23] Nonetheless, the mechanism by which pinitol might influence plasma glucose is not known. The present study recruited healthy young men who were physically conditioned before participating in this study. It was postulated that pinitol supplementation may not provide any additional benefit for this group of subjects if they were already in good physical conditions. However, this postulation can not be confirmed since no studies are available for direct comparison.

One interesting observation was the elevation of plasma pinitol concentration during recovery phase after intensive anaerobic activity in all groups. But this was not the case for myo-inositol and chiro-inositol. No previous studies reported an acute exercise effect of blood pinitol and inositol levels. When we further analyzed plasma volume changes, pinitol supplement group was significantly reduced by 4.0±7.6% and 7.5±3.5% from the baseline to the recovery phase, before and after the training period, respectively. But the magnitude of reduction of plasma volume in other two groups were not different before and after the training. Thus, it is unlikely that plasma volume shift may contribute the changes of pinitol concentration after the exercise bout.

In conclusion, two weeks of pinitol supplementation in conjunction with short duration of anaerobic training in healthy young men did not induce any obvious benefits in terms of anaerobic capacity and energy metabolism. Further research using diverse subject populations may be warranted.

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