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Effects of calcium pyruvate supplementation during training on body composition, exercise capacity, and metabolic responses to exercise

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ABSTRACT

Objective: We evaluated the effects of calcium pyruvate supplementation during training on body composition and metabolic responses to exercise.

Method: Twenty-three untrained females were matched and assigned to ingest in a double blind and randomized manner either 5 g of calcium pyruvate (PYR) or a placebo (PL) twice daily for 30 d while participating in a supervised exercise program. Prior to and following supplementation, subjects had body composition determined via hydrodensiometry; performed a maximal cardiopulmonary exercise test; and performed a 45-min walk test at 70% of pre-training VO2 max in which fasting pre- and post exercise blood samples determined.

Results: No significant differences were observed between groups in energy intake or training volume. Univariate repeated measures ANOVA revealed that subjects in the PYR group gained less weight (PL 1.2 ± 0.3 , PYR 0.3 ± 0.3) kg, $P = 0.04$), lost more fat (PL 1.1 \pm 0.5; PYR -0.4 ± 0.5 kg, $P = 0.03$), and tended to lose a greater percentage of body fat (PL 1.0 ± 0.7 ; PYR $-0.65 \pm 0.6\%$, $P = 0.07$), with no differences observed in fat-free mass (PL 0.1 ± 0.5 ; PYR 0.7 ± 0.3 kg, $P = 0.29$). However, these changes were not significant when body composition data were analyzed by MANOVA (*P* = 0.16). There was some evidence that PYR may negate some of the beneficial effects of exercise on HDL values. No significant differences were observed between groups in maximal exercise responses or metabolic responses to submaximal walking.

Conclusions: Results indicate that PYR supplementation during training does not significantly affect body composition or exercise performance and may negatively affect some blood lipid levels.

Keywords Pyruvate, Body composition, Lipids, Ergogenic aid, Exercise

INTRODUCTION

Pyruvate is a three-carbon compound that serves as the gateway compound between the glycolysis pathway and the Krebs cycle. During highintensity anaerobic exercise, the pyruvate that is formed from the breakdown of sugars and amino acids is converted into lactate by lactate dehydrogenase. Under aerobic conditions, the pyruvate is shuttled into the mitochondria, where it is converted in acetyl coenzyme A by the pyruvate dehydrogenase complex. Several previous studies have indicated that calcium and/or sodium pyruvate supplementation enhances weight and fat loss and improves exercise capacity primarily in overweight individuals (1) , (2) , (3) and (4). Hence, pyruvate has recently become a

popular weight-loss supplement and proposed ergogenic aid.

However, these claims have been based on a small number of studies primarily emanating from one laboratory. For example, Stanko et al. (1) investigated the effects of pyruvate supplementation on body composition alterations in morbidly obese women who were housed in a metabolic ward for 21 d. Subjects were restricted from performing any exercise while consuming hypocaloric diets ranging from 2.1 to 4.25 MJ/d. Subjects were fed in a double blind and randomized manner with pyruvate or glucose to account for 13% or 20% of daily energy intake (∼16 g/d), respectively. Results indicated that subjects fed pyruvate exhibited greater weight loss and fat loss, with no changes in lean body mass. This research group also reported that large doses of calcium pyruvate (i.e., 22 to 44 g/d for up to 6 wk) resulted in positive changes in body composition (5) and (6). Collectively, these findings suggest that ingestion of 16 to 44 g/d of calcium pyruvate may promote weight loss in overweight populations.

Although these findings support the potential use of calcium pyruvate as a weight-loss dietary supplement, the practicality and affordability of subjects taking large doses of calcium pyruvate in an attempt to manage body composition has been questioned (7). For this reason, several research groups have evaluated the effects of ingesting smaller amounts of calcium pyruvate on weight loss. For example, Kalman et al. (7) reported modest but significant decreases in body weight and body fat in subjects administered 6 g/d of calcium pyruvate for 6 wk in comparison with placebo. Conversely, Stone et al. (8) reported that pyruvate supplementation (0.22 $g \cdot kg^{-1} \cdot$ d^{-1} or about 9 g/d for 5 wk) did not significantly affect body composition or training adaptations in college football players.

Although several studies have indicated that pyruvate supplementation may affect body composition, the mechanisms of action are not fully understood. One theory proposes that pyruvate may influence the manner and efficiency in which ingested foods are used, resulting in enhanced lipolysis and an increased proportion of energy derived from fat (1). In addition, Newsholme (9) proposed that pyruvate may activate a futile cycle, such as the pyruvatephosphoenolpyruvate cycle, resulting in excess energy and fat oxidation. However, more research is needed to understand the effects of calcium pyruvate supplementation on appetite, energy intake, energy metabolism, and body composition before conclusions can be drawn. The purpose of this study was to 1) determine whether calcium pyruvate supplementation affects body composition in moderately overweight, untrained women who were initiating a standard exercise program; 2) evaluate the effects of calcium pyruvate supplementation on metabolic responses to maximal and sustained exercise; and 3) examine the effects of pyruvate supplementation on clinical chemistry profiles.

MATERIALS AND METHODS

Subjects

Eighty-seven women responded to advertisements posted in local newspapers and on the campus of the University of Memphis (Memphis, TN, USA). From this pool, 34 women initially enrolled in the study. Subjects were informed about the experimental procedures, they completed medical history and exercise training forms, and they signed informed consent statements that adhered to guidelines established by the American College of Sports Medicine and the institutional review board at the University of Memphis. Twenty-three healthy, moderately overweight women completed all aspects of the study. Subjects were 33 ± 8 y of age, weighed 71.5 \pm 11 kg, had a body mass index of 27.4 \pm 3 $kg/m²$, and had a maximal oxygen uptake of 34.4 \pm 7 mL · kg⁻¹ · min⁻¹. None of the subjects was involved in a resistance training program for 3 mo before the start of this study. Because data were collected before and after a 30-d exercise and nutrition intervention, no specific effort was made to standardize the start of the study to a given time of subjects' menstrual cycles. However, subjects did not start the study during men-

struation. Most women involved in this study reported taking oral contraceptives. Subjects who did not complete the study withdrew or were withdrawn primarily due to an inability to comply with the study training protocol and/or personal issues.

Experimental design

Subjects were instructed to maintain their normal diets throughout the study and were not allowed to ingest any other nutritional supplements (i.e., vitamins, minerals, proposed ergogenic aids, etc.). Baseline assessments were conducted over a 3-d period and involved participating in two testing sessions. Before reporting to the laboratory for baseline testing, subjects recorded dietary intake (food and liquid) for 4 d. Subjects observed a 4-h fast before reporting to the laboratory on each visit. In the first visit, subjects had body mass and body composition determined, and they performed a maximal cardiopulmonary exercise test on a treadmill. During the second visit, subjects reported to the laboratory after a 4-h fast and donated venous blood samples. Subjects rested for 30 min and then performed a 45-min walk test on a treadmill at a work rate corresponding to 70% of maximal oxygen uptake. Venous blood samples were also obtained immediately after the walk test.

Once baseline tests were conducted, subjects were matched and paired based on body mass, percentage of body fat, body mass index, age, and reported activity levels. In a randomized and double-blind fashion, subjects were then assigned to a pyruvate group or a placebo group. Subjects ingested 5 g of calcium pyruvate (PYR) or 5 g of a placebo (PL) that contained 2.5 g of calcium carbonate, 4 g of maltodextrin, and 1 g of dextrose two times daily (10 g/d) for 30 d. Supplements were prepared in powdered form with similar taste, texture, and appearance and coded in generic packets for double-blind administration. Subjects were instructed to mix the supplement powder into approximately 8 oz of fluid and to ingest the solution two times daily. Supplement packets were administered in two 15-d supply allotments. Empty packets were collected from subjects to determine compliance in taking the supplements. In addition, subjects completed a questionnaire after the study and reported a 94% compliance rate in taking the supplements.

Subjects were then prescribed a 30-d program of resistance training and walking. Resistance training involved performing two sets of 8 to 12 repetitions on eight Nautilus machines (seated leg press, leg extension, leg curl, bench press, shoulder press, lateral pull-down, back extension, and abdominal curl) three times per week. Subjects were instructed to rest approximately 60 s between each set and to complete both sets of each exercise before continuing to the next exercise. Once subjects could complete 12 repetitions of an exercise for both sets, they were instructed to progress to the next machine weight. Subjects also walked for 30 min at a work rate equivalent to 70% of maximal oxygen uptake three times per week. Exercise sessions were monitored by research assistants and all workouts were recorded in training logs.

Sessions after testing were conducted over a 3-d period after 30 d of supplementation and training. All post-testing sessions were conducted in a similar manner as testing before supplementation. Therefore, subjects recorded dietary intake for the final 4 d of the experimental period, had their body compositions determined, performed a maximal cardiopulmonary exercise test, and repeated the 45-min walk test over a 3-d period. Subjects were instructed to consume the same meal and fluids 4 h before each walk test. The only difference between testing sessions was that subjects ingested their assigned supplement mixed in a non-caloric flavored drink 30 min before donating a pre-exercise blood sample.

Procedures

Nutritional records were interpreted and analyzed by a registered dietitian using Food Processor III nutritional analysis software (Nutritional Systems, Salem, OR, USA). Body weight was measured with the subjects dressed in bathing suits and standing on a calibrated electronic scale with a precision of ±0.02 kg (Sterling Scale Company,

Southfield, MI, USA). Body composition was determined by standard hydrostatic weighing procedures (10) and a spring-loaded autopsy scale (Chatillon, New York, NY, USA). Vital capacity was assessed before underwater submersions with a Quinton Q-Plex metabolic cart spirometer (Quinton Instruments, Seattle, WA, USA). Vital capacity was also obtained with a hand-held spirometer (Micro Medical Limited, Kent, UK) during each submersion trial to verify that subject expired all of their air. Subjects performed 8 to 12 consecutive underwater weighing tests until the highest underwater weight could be replicated three times. Body composition was determined by using the average of the best two trials. Residual lung volume was estimated as 28% of vital capacity according to standard procedures (10) and (11). Body density and body composition were then calculated with Siri's equation (10).

The maximal cardiopulmonary exercise and 45-min walk tests were performed on a Quinton Q-55 treadmill attached to a Quinton Q-Plex metabolic measurement cart (Quinton Instruments). Metabolic analyzers were calibrated to certified gases, and the Pneumotach was calibrated by using a 3-L volume syringe before each test according to standard procedures. Bruce's maximal exercise test protocol was used to obtain maximal exercise responses. Subjects received verbal encouragement to exercise to the best of their ability. The test was terminated once the subjects reached volitional exhaustion according to standard termination criteria. Ventilatory anaerobic thresholds were determined in a blind manner according to standardized criteria, namely by the point of non-linear increase in ventilation, a non-linear increase in carbon dioxide production, and an increase in respiratory exchange ratio (RER) as work rate incrementally increased (12).

During the 45-min walk test, subjects exercised at a work rate corresponding to 70% of pretraining maximal oxygen uptake. Breath-bybreath oxygen uptake, RER, and exercise heart rate responses were obtained throughout the walk test and averaged at 3-min intervals for statistical analysis. In addition, rating of perceived exertion was obtained every 5 min during testing.

Blood samples were obtained from an antecubital forearm vein according to standard phlebotomy procedures before and after the walk tests. Venous blood was collected into two 10 mL serum separation tubes and a 5-mL K_3 anticoagulation tube that contained ethylenediaminetetra-acetic acid. Serum separation tubes were centrifuged at 5000 revolutions/min for 10 min with a Biofuge 17R centrifuge (Heraeus Inc., Mannheim, Germany). Serum was then separated from the serum separation tubes, placed in serum storage containers, and refrigerated until analysis. The tube that contained ethylene-diaminetetraacetic acid and one serum vial were shipped overnight in cold containers to Quest Diagnostics (St. Louis, MO, USA) for clinical analysis. A complete clinical chemistry panel (31 items) was run on serum samples by using the Technicon DAX (model 96-0147, Technicon Inc., Terrytown, NY, USA) automated chemistry analyzer according to standard clinical procedures. Cell blood counts with percent differentials were run on whole blood samples by using a Coulter STKS automated analyzer (Coulter Inc., Hialeah, FL, USA) according to standard procedures. The remaining serum vials were frozen at −80°C until the end of the study. Non-esterified fatty acids, glycerol, and β-hydroxybutyrate concentrations were determined by a Milton-Roy DUV spectrophotometer (Milton-Roy Company, Rochester, NY, USA) using kits for non-esterified fatty acids (Wako Diagnostic, Richmond, VA, USA) and for triacylglycerol GPO-trinder and βhydroxybutyrate (Sigma Diagnostic, St. Louis, MO, USA). Test-to-test reliability of performing these assays ranged from 2% to 6% for individual assays, with an average variation of $\pm 3\%$.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) for repeated measures with least significant difference post hoc procedures by using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA). Interactions between groups were also examined by calculating Δ scores (values after

versus before supplementation) and analyzing the Δ data by one-way ANOVA when only one data point was analyzed (e.g., changes in body composition data) or by repeated measures ANOVA when multiple data points were analyzed (e.g., changes in mean oxygen uptake and RER values during the walk test). This procedure yields the same statistical outcomes as observed in raw data analysis and has been used in numerous studies to demonstrate changes observed across groups in a simple and statistically sound manner. Because determination of body composition using hydrostatic weighing techniques assesses three related measurements of body mass, a multivariate ANOVA was run on body composition data to assess the overall effect of the supplementation protocol on body composition variables to account for possible experiment-wise error. Data were considered statistically significant when the probability of type I error was 0.05 or less. Statistical power and 95% confidence intervals (CIs) are also presented on selected variables. Data are presented as means ± standard deviations.

RESULTS

Training volume

No significant differences were observed between groups in total resistance training volume (PL 49 579 ± 5186 kg, PYR 54 409 ± 3995 kg, *P* $= 0.47$) or total exercise time (PL 347 \pm 41 min, PYR 388 ± 27 min, $P = 0.40$).

Dietary variables

Table 1 presents dietary intake data for the PL and PYR groups. No significant differences were observed between groups in energy intake, fat intake, protein intake, or carbohydrate intake. Although not significant, there was some evidence that subjects in the PYR group decreased energy intake after supplementation (PL -8.0 ± 3) $kJ \cdot kg^{-1} \cdot d^{-1}$, PYR -21.5 ± 12 kJ · $kg^{-1} \cdot d^{-1}$, $P =$ 0.30).

Table 1. Pre- and postsupplementation relative energy intake for the PYR and PL groups*

PL: placebo; PYR: calcium pyruvate

*Data are presented as mean + standard deviation. Relative energy intake was measured as kilojoules per kilogram per day.

Body composition

Table 2 presents the body composition data observed between groups, and Fig. 1 presents the changes observed in body composition variables from baseline values (i.e., Δ values). Univariate repeated measures ANOVA showed significant group \times time interactions in body weight ($P =$ 0.04) and body fat $(P = 0.03)$. Changes in percentage of body fat tended to be different $(P =$ 0.07), with no differences observed between groups in fat-free mass $(P = 0.29)$. Similarly, one-way ANOVA performed on body composition Δ values showed significant differences between groups in body mass (PL 1.2 ± 0.3 kg, CI = −0.39–1.0; PYR 0.3 ± 0.3 kg, CI = 0.7–1.8; *P* $= 0.04$) and fat mass (PL 1.1 \pm 0.5 kg, CI = 0.03– 2.2; PYR -0.4 ± 0.5 kg, CI = -1.4 to 0.6; P = 0.03), with some evidence that subjects in the PYR group lost a greater percentage of body fat (PL $1.0 \pm 0.7\%$, CI = 0.45–2.5; PYR -0.65 \pm 0.6%, CI = −1.9 to 0.6; *P* = 0.07). No significant differences were observed in changes in fat-free mass (PL 0.1 ± 0.5 kg, CI = -1.0 to 1.2; PYR 0.7 \pm 0.3 kg, CI = 0.02–1.4; *P* = 0.29). However, four observations should be noted. First, changes in body composition variables were relatively minor and well within the typical test-to-test variability (i.e., 5% to 8% using hydrostatic weighing techniques to assess body composition). Second, the significant interactions observed in body mass and fat mass appeared to have been influenced to a greater degree by changes observed in the PL group rather than in the PYR group. Third, multivariate ANOVA of related body composition variables indicated that there

was no overall effect on body composition variables $(P = 0.16)$. Fourth, univariate body composition data results would not be considered statistically significant if Bonferroni's adjustment to the α level (*P* < 0.05/3 = *P* < 0.017) was applied to account for possible experiment-wise error in analyzing related body composition submass measurements. Therefore, the minor changes observed in body composition variables were not considered statistically significant.

PL: placebo; PYR: calcium pyruvate

*Data are presented as mean ± standard deviation

Figure 1. Changes in TBM, LM, FM, and BF for the placebo group (open bars) and the pyruvate group (solid bars). $* P < 0.05$. *† Difference of P* < 0.10 to *P* > 0.05. *Data are presented as mean ± standard deviation. BF: body fat; FM: fat mass; LM: lean mass; TBM: total body mass.*

No significant or clinically meaningful differences were observed between groups in fasting urea nitrogen, creatinine, ratio of urea nitrogen to creatinine, uric acid, glucose, electrolyte status, total protein, albumin, white blood cells, or types of lymphocytes. There was some evidence that muscle and liver enzymes after training and supplementation were lower in the PYR group (creatine kinase $11 \pm 18\%$ versus $10 \pm 11\%$, $P = 0.97$; lactate dehydrogenase 4 ± 5% versus −9 ± 4%, *P* $= 0.06$; aspartate aminotransferase $16 \pm 7\%$ versus $-1 \pm 7\%$, $P = 0.13$; alanine aminotransferase 23 ± 17% versus −21 ± 9%, *P* = 0.03; γ-glutamyl transaminase 10 ± 12 % versus -7 ± 6 %, $P =$ 0.06). Table 3 presents blood lipid profiles for the PL and PYR groups. There was some evidence that PYR supplementation during training cancelled some of the normally positive effects of exercise on blood lipid profiles (total cholesterol 3 ± 12% versus −2 ± 15%, *P* = 0.38; highdensity lipoprotein $14 \pm 21\%$ versus $-6 \pm 13\%$, *P* $= 0.01$; ratio of total cholesterol to high-density lipoprotein cholesterol $-8 \pm 16\%$ versus $4 \pm 12\%$, $P = 0.06$; low-density lipoprotein $4 \pm 25\%$ versus $-7 \pm 17\%$, $P = 0.25$; very low-density lipoprotein −9 ± 28% versus 19 ± 59%, *P* = 0.17; triacylglycerols $-13 \pm 23\%$ versus $18 \pm 60\%$, $P = 0.13$).

Maximal exercise capacity

Exercise training significantly increased maximal oxygen uptake and time to exhaustion by averages of 10.1% and 14%, respectively. However, no significant differences were observed between groups in the increases in maximal oxygen uptake, ventilatory anaerobic threshold, RER, heart rate, or time to exhaustion.

Walk test

Fig. 2 presents the changes observed from baseline values in oxygen uptake and RER. No significant differences were observed in mean oxygen uptake values to perform the walk (day 0: PL 1.87 ± 0.26 L/min, PYR 1.71 ± 0.19 L/min; day 30: PL 1.63 ± 0.25 L/min, PYR 1.64 ± 0.20 L/min; $P = 0.37$ for group, $P = 0.003$ for time, P

Variable	Group	Day 0	Day 30	P
CHL (mM/L)	PL	4.88 ± 0.66	4.99 ± 0.69	0.38 for group
				0.90 for time
	PYR	4.70 ± 0.98	4.56 ± 1.01	0.35 for group \times time
HDL (mM/L)	PL	1.38 ± 0.43	1.52 ± 0.35	0.03 for group
				0.34 for time
	PYR	1.15 ± 0.26	1.10 ± 0.34	0.03 for group \times time
CHL/HDL	PL	3.80 ± 1.30	3.40 ± 0.70	0.16 for group
				0.38 for time
	PYR	4.20 ± 1.20	4.40 ± 1.30	0.04 for group \times time
LDL (mM/L)	PL.	2.78 ± 0.60	2.84 ± 0.61	0.69 for group
				0.54 for time
	PYR	2.80 ± 0.77	2.60 ± 0.84	0.31 for group \times time
VLDL (mM/L)	PL	0.72 ± 0.17	0.64 ± 0.23	0.31 for group
				0.75 for time
	PYR	0.76 ± 0.36	0.87 ± 0.48	0.18 for group \times time
Triacylglycerols (mM/L)	PL	1.56 ± 0.38	1.37 ± 0.51	0.29 for group
				0.87 for time
	PYR	1.64 ± 0.77	1.88 ± 1.04	0.14 for group \times time

Table 3. Pre- and postsupplementation fasting lipid profiles for the PYR and PL groups*

CHL: total cholesterol; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; PL: placebo; PYR: calcium pyruvate; VLDL: very low-density lipoprotein cholesterc

*Data are presented as mean ± standard deviation

 $= 0.08$ for group \times time). However, there was some evidence that mean RER values tended to increase after supplementation in the PYR group (day 0: PL 0.855 ± 0.04 , PYR 0.841 ± 0.04 ; day 30: PL 0.837 ± 0.03, PYR 0.855 ± 0.03, *P* = 0.10). Interestingly, the trend toward higher RER values after PYR supplementation (PL −0.018 ± 0.04, PYR 0.05 ± 0.05 , $P = 0.10$ for group \times time) occurred despite a slight decrease in mean oxygen uptake values to perform the walk (PL −0.24 ± 0.27, PYR −0.07 ± 0.14, *P* = 0.08 for group × time). These findings suggest that the subjects may have developed a slightly higher rate of carbohydrate oxidation during exercise after PYR supplementation. No significant differences were observed in concentrations of glycerol, free fatty acid, and β-hydroxybutyrate between groups.

DISCUSSION

The purpose of this study was to determine whether calcium pyruvate supplementation significantly affects body composition, metabolic

Respiratory Exchange Ratio

Figure 2. Changes in oxygen uptake and respiratory exchange ratio responses from baseline values observed during the walk test for the placebo group (squares) and pyruvate group (diamonds). Data are presented as mean ± standard deviation.

responses to exercise, or substrate utilization during aerobic exercise in mildly overweight women who engage in a basic exercise program. The rationale for this study was based on previous findings that indicated that pyruvate supplementation taken in dosages of up to 44 g/d enhances weight and fat loss among morbidly obese subjects (1), (2) and (13), shifts the resting substrate utilization in favor of an increased proportion of energy derived from fats (1), and elicits a carbohydratesparing effect in athletes, thus enabling them to improve exercise performance (3) and (4).

Although some statistical trends were observed, the present results indicated that PYR supplementation (10 g/d for 30 d) does not sig-

nificantly affect overall changes in body composition during training. The trends observed were relatively minor and appear to have been influenced to a greater degree to an unexplained increase in body mass and fat mass observed in the PL group rather than to changes observed in the PYR group. In addition, changes in body composition values were within the typical test-to-test reliability of hydrostatic weighing (i.e., 5% to 8%) and therefore may have simply been a result of standard assessment error. The present findings are in contrast to previous studies that reported that obese subjects who received large doses of pyruvate (e.g., 16 to 30 g/d) lost 0.8 to 1.3 kg more fat than did the PL group, with no significant decreases in lean body mass (1), (2), (14) and (15). Moreover, because of the relatively high cost of calcium pyruvate, one must question whether the minor effects observed on fat loss (i.e., -0.4 ± 0.5 kg of fat mass) would be worth the costs associated with taking 10 g/d of calcium pyruvate.

Although the mechanisms by which pyruvate might promote weight and fat loss are unknown, researchers have postulated that it may alter the manner and efficiency in which ingested foods are used, resulting in enhanced lipolysis and an increased proportion of energy derived from fats (1). Stanko et al. proposed that pyruvate supplementation activates a futile cycle, such as the pyruvate-phosphoenolpyruvate cycle, resulting in excess energy and lipid expenditure (1). A futile cycle is produced when a non-equilibrium reaction in the forward direction of a pathway is opposed by another unequal reaction in the reverse direction. Because a substrate is continuously converted and reconverted back to its original form, chemical energy is created and converted into heat, which is lost in the environment (8). This theory suggests that exogenously provided pyruvate may exceed the amount in which the cells could convert into acetyl coenzyme A. As a result, supplemental pyruvate may be transformed into oxaloacetate within the Krebs cycle and then converted back to phosphoenolpyruvate, the precursor of pyruvate. Through such a futile cycle, excess energy could theoretically be expended, thereby promoting fat loss.

In the present study, there was no evidence that the fat loss was attributed to an increase in fat metabolism. Serum concentrations of free fatty acids and glycerol, which are indicative of mobilization and breakdown of triacylglycerols, did not differ significantly between the PYR and PL groups. Fewer non-esterified fatty acids were mobilized in response to PYR supplementation, suggesting less fat mobilization and/or metabolism. Further, concentrations of βhydroxybutyrate, which is a ketone indicative of fat metabolism, did not statistically differ between trials or groups. Although the etiology of these observations remains to be determined, the exogenous pyruvate could simply have served as a carbohydrate fuel source during exercise, thereby decreasing the need to mobilize fat as a fuel. In support of this contention, previous studies have indicated that pyruvate enabled subjects to prolong their exercise times by enhancing fractional glucose extraction during rest and exercise that resulted in the provision of additional energy substrates (3) , (4) and (16) . Although this measurement was not assessed in the present investigation, RER values tended to be higher during the walk test in the PYR group $(P = 0.10)$ despite a slightly lower oxygen uptake, suggesting greater carbohydrate oxidation. These findings may suggest that the pyruvate can serve as an exogenous source of carbohydrate during exercise, which may increase carbohydrate oxidation and energy expenditure. However additional research is necessary to evaluate the effects of PYR supplementation on substrate utilization during exercise.

Interestingly, subjects who used PYR developed an increase in fasting serum levels of triacylglycerol and very low-density lipoprotein, whereas levels of high-density lipoprotein cholesterol were significantly decreased. In this regard, fasting serum triacylglycerol levels were 14% higher after PYR supplementation, in contrast to a 14% lower value after training in the PL group. This pattern was also observed after the subjects performed the walk test after supplementation. These results contradict previous findings suggesting that pyruvate supplementation (36 to 53 g) decreases total cholesterol and low-density lipoprotein concentrations by 4% and 5%, respectively, in obese hyperlipidemic patients (17). Further, they are in agreement with findings of Ivy et al. (18) who reported that pyruvyl-glycine supplementation increased plasma triacylglycerols in obese Zucker rats. Ivy et al. (18) hypothesized that the increase in triacylglycerols was be due to a greater mobilization or decreased clearance of blood lipids in response to consuming the pyruvyl-glycine diet. Although pre-exercise feeding of calcium pyruvate may have only temporarily increased blood lipids, additional research should evaluate this potentially negative side effect of PYR supplementation.

The present results indicated that PYR supplementation does not significantly affect maximal exercise capacity, ventilatory anaerobic threshold, or time to exhaustion. These findings indicated that PYR supplementation during training does not appear to be an effective ergogenic aid in women who initiate training.

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