

Pyruvate ingestion for 7 days does not improve aerobic performance in well-trained individuals

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Morrison, Michael A., Lawrence L. Spriet, and David J. Dyck. Pyruvate ingestion for 7 days does not improve aerobic performance in well-trained individuals. *J Appl Physiol* 89: 549–556, 2000.—The purposes of the present studies were to test the hypotheses that lower dosages of oral pyruvate ingestion would increase blood pyruvate concentration and that the ingestion of a commonly recommended dosage of pyruvate (7 g) for 7 days would enhance performance during intense aerobic exercise in well-trained individuals. Nine recreationally active subjects (8 women, 1 man) consumed 7, 15, and 25 g of pyruvate and were monitored for a 4-h period to determine whether blood metabolites were altered. Pyruvate consumption failed to significantly elevate blood pyruvate, and it had no effect on indexes of carbohydrate (blood glucose, lactate) or lipid metabolism (blood glycerol, plasma free fatty acids). As a follow-up, we administered 7 g/day of either placebo or pyruvate, for a 1-wk period to seven, well-trained male cyclists (maximal oxygen consumption, $62.3 \pm 3.0 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in a randomized, double-blind, crossover trial. Subjects cycled at 74–80% of their maximal oxygen consumption until exhaustion. There was no difference in performance times between the two trials (placebo, $91 \pm 9 \text{ min}$; pyruvate, $88 \pm 8 \text{ min}$). Measured blood parameters (insulin, peptide C, glucose, lactate, glycerol, free fatty acids) were also unaffected. Our results indicate that oral pyruvate supplementation does not increase blood pyruvate content and does not enhance performance during intense exercise in well-trained cyclists.

ergogenic aid; cycling; blood metabolites

PYRUVATE AND DIHYDROXYACETONE are three-carbon carbohydrate (CHO) metabolites that are reported to enhance weight loss (4, 11, 13, 14) and improve endurance during aerobic exercise (9, 12). On the basis of these reports, pyruvate is currently being marketed as a weight-loss and ergogenic supplement. Several studies have documented accelerated weight loss or prevention of weight regain in both animals (4, 11, 13, 14) and humans (9, 12). However, few studies have examined the effects of pyruvate or of a pyruvate and dihydroxyacetone combination (DHAP) on aerobic endurance. One study examined the effects of an acute intravenous injection of pyruvate on running performance in rats, and reported a decrease in run time to exhaustion,

which coincided with greater muscle and liver glycogen utilization (1).

Only two studies have examined the effects of chronic supplementation of DHAP on aerobic endurance in humans (15, 16). No studies have examined the isolated ergogenic effect of pyruvate in humans. In an initial study, Stanko et al. (16) tested the hypothesis that pyruvate supplementation, which was previously shown to increase carcass glycogen in rats (11), would increase muscle glycogen content and improve endurance. After 7 days of supplementation with 100 g DHAP/day in untrained individuals, triceps muscle glycogen increased 48% and time to exhaustion during arm cranking improved by 23%. Muscle glycogen concentrations at exhaustion were similar in both trials, indicating greater glycogen utilization in the DHAP trial (95 vs. 60 mmol/kg dry wt). Arm glucose extraction was also increased twofold during exercise after DHAP supplementation.

In a second study, Stanko et al. (15) assessed endurance during cycling at 70% peak oxygen consumption ($\dot{V}O_{2 \text{ peak}}$) in active, but untrained, individuals receiving placebo or DHAP (100 g) supplementation for 7 days. Dietary CHO content was increased to 70% of total caloric content in both trials, eliminating the disparity in resting glycogen content observed in their initial study. Similar to their previous finding (16), DHAP improved endurance by 20% and increased muscle glucose extraction. Contrary to their previous study, total muscle glycogen use was similar in both trials, indicating that the rate of glycogen utilization was decreased during the DHAP trial. It was hypothesized that the performance-enhancing effect of DHAP was due to enhanced muscle glucose uptake and oxidation. Increased availability of exogenous glucose late in prolonged aerobic exercise between 65 and 85% maximal oxygen consumption ($\dot{V}O_{2 \text{ max}}$), when muscle glycogen stores are nearly depleted, could extend endurance.

Despite these positive findings indicating a possible ergogenic effect of pyruvate, it should be noted that both studies utilized high combined dosages of pyruvate (25 g) and dihydroxyacetone (75 g). Such dosages

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are impractical in that they are cost prohibitive and may be distressful to the stomach. Furthermore, well-trained individuals were not used in these studies and there was little mechanistic information, although enhanced glucose oxidation was proposed as a possible means for the improved performance.

Commercially available pyruvate supplements do not contain dihydroxyacetone, are recommended at considerably lower dosages (3–6 g) than those used in scientific studies, and yet are still advertised as a “scientifically proven” ergogenic aid. Therefore, it is important to establish whether pyruvate, as available in commercial preparations, can elicit a significant ergogenic effect in trained individuals. There have been no human studies documenting the efficacy of oral pyruvate supplementation; consequently, there is no scientific basis on which to recommend an optimal supplementation dosage. Indeed, there have been no attempts to date to determine the efficacy with which oral pyruvate supplementation increases its content in the blood. Any mechanism by which pyruvate alters muscle metabolism during exercise (e.g., enhanced glucose uptake) would require an elevation in blood pyruvate. Clearly, further research is required to establish the value of pyruvate as an ergogenic aid. The reported metabolic and ergogenic effects of pyruvate have been summarized in two recent reviews (7, 17); Sukala (17), in particular, points out the limitations of previous research and the need to substantiate the putative ergogenic effect of pyruvate with further research.

Therefore, the present study had two purposes. The initial purpose was to determine whether modest dosages of pyruvate significantly elevate blood pyruvate concentrations. There are no current data to indicate the most effective supplementation regime or even whether pyruvate supplementation increases its content in the blood or muscle. Therefore, blood and urine pyruvate concentrations were monitored for 4 h after the acute ingestion of 7, 15, and 25 g of calcium pyruvate. In addition, we also measured various parameters of lipid [glycerol, free fatty acids (FFAs)], and CHO (glucose, lactate, insulin, peptide C) metabolism. Our second purpose was to test the hypothesis that chronic pyruvate supplementation, as available in commercial preparations, enhances aerobic performance in well-trained individuals. Therefore, we administered 7 g/day of pyruvate for 1 wk to well-trained cyclists to determine whether this improved performance during aerobically intense cycling at 75–80% $\dot{V}O_{2\max}$.

METHODS

Study 1

Subjects. Nine recreationally active individuals (8 women, 1 man) volunteered to participate in this study [age, 22.8 ± 0.5 (SE) yr; body mass, 64.9 ± 3.1 kg]. All subjects were screened by questionnaire for health risks, as well as to determine whether they were currently taking any medication or oral supplements that might interfere with the results of the study. All procedures were approved by the Human Subjects Committee at the University of Guelph, and written

consent was obtained from each subject before the trials were initiated.

Procedures. Subjects reported to the laboratory on three separate occasions at the same time of day, separated by 7 days. Subjects were instructed to avoid alcohol, caffeine, and strenuous exercise 48 h before each trial. In addition, subjects followed a prescribed high-CHO diet (~65% of total kcal) for 24 h before each trial. In addition, subjects were asked to consume a high-CHO meal 2–3 h before commencing each trial. This high-CHO meal was recorded (366 ± 47 kcal as CHO, $76 \pm 3\%$ of total kcal) and was followed before each trial. On entering the laboratory, subjects voided their bladder and were fitted with a catheter (20 gauge, 1 in.; Intima, Becton Dickinson) in their antecubital vein. Catheter patency was maintained with a saline drip. A 7-ml aliquot of blood was sampled and collected in a heparinized vacutainer for subsequent analysis of pyruvate, glucose, lactate, glycerol, FFAs, insulin, and peptide C (time = 0 min). Subjects then received one of three pyruvate dosages (7, 15, or 25 g). Because we were aware of reports of possible gastrointestinal distress with larger dosages of pyruvate, we chose to administer the dosages in order of increasing amounts. In fact, two of our subjects were barely able to consume the entire 15-g dosage, and they did not return for the third trial (25-g dosage). Because the purpose of this study was to determine whether acute pyruvate consumption elevated its content in the blood relative to preingestion levels, we believed it unnecessary to blind subjects to the treatment or to include a control trial in which subjects consumed nothing. Pyruvate capsules (1 g) were administered orally in the form of calcium pyruvate (Natrol, Chatsworth, CA). Subjects were instructed to consume all capsules within a 20-min period and with as much water as necessary. Thereafter, blood samples were collected every 30 min for a 4-h period. Urine was collected throughout the duration of the 4-h period for analysis of the pyruvate content. Urine samples were deproteinized with 0.6 N perchloric acid and neutralized with 0.5 N KOH before they were analyzed fluorometrically.

Analyses. A 200- μ l aliquot of blood was immediately deproteinized in 1 ml of 0.6 N perchloric acid for subsequent analysis of pyruvate, glucose, lactate, and glycerol. These were determined in duplicate fluorometrically by using procedures previously reported (2). Of the remaining blood, a small aliquot was used for determination of hematocrit, and the remainder was centrifuged at 10 g for 2 min for plasma collection. An 800- μ l aliquot of plasma was added to 200 μ l of 5 N NaCl and heated for 30 min at 56°C to destroy any lipoprotein lipase, which would falsely elevate plasma FFA levels (5). Plasma FFAs were analyzed in duplicate from this sample by using a colorimetric kit (NEFA C kit, Wako Chemicals, Richmond, VA). The remainder of the plasma was retained for determination of pyruvate, insulin, and peptide C by using appropriate RIA kits (Linco Research, St. Charles, MO; Diagnostic Products, Los Angeles, CA).

Statistics. Repeated-measures ANOVAs (dosage \times time) were used to detect significant differences in all measured parameters. A Tukey's post hoc test was used to test for significance revealed by the ANOVAs.

Study 2

Subjects. Seven well-trained male cyclists, (mean age 31 ± 2 yr and weight 80.7 ± 4.3 kg) volunteered for the experiment. Maximal aerobic power of the subjects was 62.3 ± 3.0 ml \cdot kg \cdot min, as determined by a progressive maximal aerobic power ($\dot{V}O_{2\max}$) test. Subjects were informed of the experimental procedure and potential risks both verbally and in

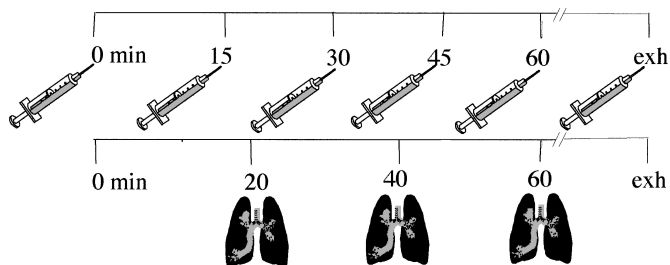


Fig. 1. Time course of dependent measurements in performance study at 75–80% maximal oxygen consumption. Syringes represent venous blood sampling; lungs represent respiratory measurements (oxygen consumption and respiratory exchange ratio). exh, Exhaustion.

writing, and they gave their consent. The experiment was approved by the University Ethics Committee.

Procedures. All subjects initially reported to the laboratory to undergo a $\dot{V}O_{2\max}$ test and to determine a power output corresponding to 75–80% of their $\dot{V}O_{2\max}$ during cycling. During the $\dot{V}O_{2\max}$ test, power output was increased incrementally by 50 W, approximately every 3–4 min, or until oxygen consumption ($\dot{V}O_2$) measurements plateaued at each power output. Respiratory exchange ratios (RERs) were >1.1 at $\dot{V}O_{2\max}$. Subjects also performed a practice ride at 75–80% $\dot{V}O_{2\max}$ to exhaustion to confirm the proper power output and to become familiar with the protocol. The mean power output for the trials was 251 ± 12 W.

The order of the treatments (placebo, pyruvate) was randomized and double blind, and the trials were separated by 14 days. The placebo capsules were filled with a mixture of equal parts gelatin and glucose and were visually indistinguishable from the calcium pyruvate capsules. Each day's supply of capsules was divided into four aliquots, and subjects consumed the capsules with each meal (2 g, 3 times a day), as well as with a small snack before going to bed (1 g). Subjects were asked to maintain their normal activity patterns and to refrain from intense exercise as well as from caffeine and alcohol consumption 48 h before each trial. In addition, subjects followed a high-CHO diet for 3 days before each trial ($65 \pm 3\%$ of total kcal; $2,982 \pm 313$ total kcal/day). Subjects maintained dietary records over this period so that the same diets could be followed on the subsequent trial.

Subjects reported to the laboratory on the eighth day of treatment, 10–14 h after their last aliquot of placebo or pyruvate capsules and 2–3 h after consuming a small CHO-rich meal ($65 \pm 3\%$ kcal, 745 ± 62 kcal). Subjects were encouraged to bring their own bike seats and pedals, which were then fitted on the cycle ergometer. Subjects were weighed, a catheter (as in *study 1*) was placed in the antecubital vein, and a 7-ml blood sample was drawn. Subjects were then moved to an electronically braked cycle ergometer (Excalibur, Quinton Instrument, Seattle, WA) and were permitted a 3 min warm-up at a power output corresponding to 50% of their $\dot{V}O_{2\max}$. The power output was then increased to 75–80% of their $\dot{V}O_{2\max}$ (time = 0 min). Subjects pedaled at a cadence (90–100 rpm) selected during their practice trial, and this was maintained throughout the trial. Blood samples were drawn at 15-min intervals until exhaustion, and respiratory measurements were obtained every 20 min by using a metabolic cart (Sensor Medics, Yorba Linda, CA), as indicated in Fig. 1. Fatigue was defined as the point at which subjects could no longer maintain a cadence equivalent to 85% of their preselected value. As subjects approached fatigue, they were verbally encouraged to cycle for as long as

possible. Subjects were permitted to drink as much water as desired during the experiments. The volume of water consumed was recorded in each trial and was similar in both trials for all subjects (*trial 1*, $1,502 \pm 203$ ml; *trial 2*, $1,683 \pm 203$ ml).

After 7 days, subjects began supplementation (placebo or pyruvate) for the second trial, and returned to the laboratory after 1 wk to repeat the experiment. A posttrial questionnaire was completed by each subject to confirm whether the treatment or placebo trials could be distinguished. Monetary prizes were awarded to the three subjects with the best total performance times (sum of both trials) to encourage subjects' maximal effort in both trials.

Analyses. Blood samples were analyzed for pyruvate, glucose, lactate, FFAs, glycerol, insulin, and peptide C as described for *study 1*. Blood hematocrit was measured in duplicate at each time point.

Statistics. Blood metabolites were analyzed by using a repeated-measures ANOVA [treatment (placebo, pyruvate) \times time]. Performance time (placebo vs. pyruvate) was assessed using a paired *t*-test. Statistical significance was accepted at $P < 0.05$. All results are reported as means \pm SE. A Tukey's post hoc test was used to test for significance revealed by the ANOVAs.

RESULTS

Study 1

Total fluid consumption throughout the trial increased with each pyruvate dosage (960 ± 64 , $1,383 \pm 157$, and $1,458 \pm 277$ ml). Increased fluid consumption corresponded to greater total urine production (811 ± 110 , 842 ± 132 , and $1,200 \pm 146$ ml, respectively) but did not affect hematocrit, which remained unchanged over time and between trials (overall range 39.1–1.9).

Whole blood pyruvate concentration (Fig. 2) was unaffected by acute oral supplementation ($P = 0.090$) and did not increase over time ($P = 0.921$). Pyruvate concentration was also determined in plasma (Fig. 2) and was unaffected by oral supplementation. Whole blood pyruvate concentrations were ~ 2.5 -fold lower

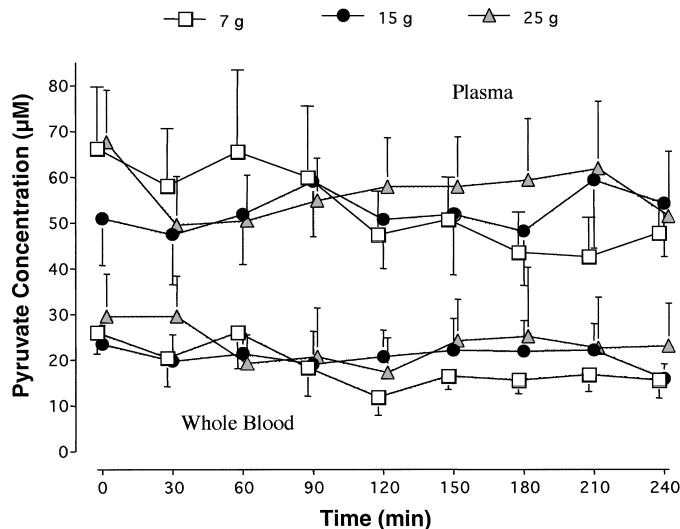


Fig. 2. Whole blood and plasma pyruvate concentrations after acute, oral ingestion of 7, 15, or 25 g of calcium pyruvate.

Table 1. Whole blood glucose and lactate after acute supplementation with 7, 15, or 25 g of calcium pyruvate

Parameter	Time, min								
	0	30	60	90	120	150	180	210	240
Glucose, mM									
7 g Pyruvate	3.8 ± 0.1	4.3 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.3 ± 0.1	4.4 ± 0.1	4.4 ± 0.1	4.3 ± 0.2	4.4 ± 0.2
15 g Pyruvate	4.2 ± 0.2	4.4 ± 0.2	4.3 ± 0.1	4.5 ± 0.2	4.7 ± 0.3	4.6 ± 0.2	4.4 ± 0.3	4.6 ± 0.2	4.3 ± 0.3
25 g Pyruvate	4.1 ± 0.2	4.3 ± 0.2	4.4 ± 0.4	4.4 ± 0.2	4.5 ± 0.1	4.6 ± 0.2	4.8 ± 0.2	4.2 ± 0.2	4.5 ± 0.2
Lactate, mM									
7 g Pyruvate	1.4 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	1.0 ± 0.1
15 g Pyruvate	1.1 ± 0.2	1.2 ± 0.2	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.2
25 g Pyruvate	1.3 ± 0.2	1.2 ± 0.2	1.1 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.3 ± 0.1	1.2 ± 0.2	1.3 ± 0.2	1.3 ± 0.3

Values are mean ± SE. Note that 0 min refers to blood sample before pyruvate ingestion and that each corresponding time point refers to after pyruvate ingestion.

than plasma concentrations, indicating that the majority of the pyruvate was confined to the plasma compartment, with minimal amounts carried in the erythrocytes. Whole blood glucose and lactate were unaffected by oral pyruvate supplementation (Table 1). Plasma FFA and whole blood glycerol increased over time (Table 2), and insulin and peptide C (Table 3) significantly decreased over time, but all were unaffected by pyruvate supplementation. Pyruvate content in the urine was negligible in all trials (i.e., <0.1 mmol; data not shown), corresponding to <0.1% of the total pyruvate consumed.

Study 2

The major finding was that time to exhaustion was unaffected by pyruvate consumption (Fig. 3).

$\dot{V}O_2$ (Table 4), lactate (Fig. 4A), glycerol, and FFAs (Fig. 5) became significantly elevated during exercise, but they were unaffected by pyruvate supplementation. RER was significantly lower at 40 and 60 min in the placebo trial; however, RER was unaffected in both trials by pyruvate supplementation (Table 4). Blood glucose (Fig. 4B) remained unchanged during exercise. Plasma insulin and peptide C decreased significantly during the initial 15 min of exercise (Table 5) but were also unaffected by pyruvate supplementation.

DISCUSSION

Pyruvate, a naturally occurring CHO metabolite, has been reported to improve endurance during aerobic

exercise. However, no studies have examined the isolated ergogenic effect of pyruvate in humans. Two studies have demonstrated improved aerobic endurance after chronic supplementation of DHAP in humans (15, 16), but they were performed on untrained individuals using high combined dosages of pyruvate and dihydroxyacetone. Only one plausible theory has been put forth to explain the putative ergogenic effect of pyruvate. If, as suggested, pyruvate stimulates glucose uptake by the muscle (15, 16), then certainly pyruvate content must first be elevated in the blood. Possibilities for chronic pyruvate administration to alter muscle metabolism could include 1) increased activity or translocation of GLUT-4 to enhance glucose uptake, 2) increased monocarboxylate transporter activity to increase pyruvate uptake into the cell, and, subsequently, 3) an increase in muscle pyruvate content might potentially stimulate the activity of pyruvate dehydrogenase, thereby increasing glucose oxidation. All of these possibilities would require either increased exposure of the muscle to pyruvate or an increase in actual muscle pyruvate content. Therefore, for pyruvate to have any direct effect on the muscle, its delivery to the muscle would have to be increased, which would necessitate at least transient increases in blood pyruvate. However, no studies have determined the efficacy of elevating blood or muscle pyruvate content after chronic supplementation. The results of the present studies indicate that oral dosages of isolated pyruvate, similar to those that are widely reported to

Table 2. Plasma FFA and whole blood glycerol after acute supplementation with 7, 15, or 25 g of calcium pyruvate

Parameter	Time, min								
	0	30	60	90	120	150	180	210	240
Glycerol, mM									
7 g Pyruvate	47.6 ± 10.8	50.4 ± 7.7	55.4 ± 3.8	59.3 ± 4.8	70.7 ± 6.5†	66.7 ± 5.1	70.6 ± 5.7†	73.8 ± 5.0†‡	81.0 ± 5.7†‡§
15 g Pyruvate	47.4 ± 6.1	53.0 ± 7.1	63.2 ± 7.6	57.0 ± 5.4	69.7 ± 10.7	68.5 ± 12.5	68.2 ± 7.3	73.0 ± 12.6†	65.3 ± 6.8
25 g Pyruvate	52.8 ± 6.4	54.5 ± 3.7	59.9 ± 6.8	76.5 ± 10.2	67.2 ± 11.2	66.4 ± 4.3	62.1 ± 7.3	61.6 ± 8.2	81.4 ± 11.4†‡
FFA, mM									
7 g Pyruvate	0.36 ± 0.05	0.39 ± 0.06	0.49 ± 0.08	0.55 ± 0.08	0.71 ± 0.08†	0.76 ± 0.07†‡	0.83 ± 0.08†‡§	0.79 ± 0.07†‡§	0.87 ± 0.11†‡§
15 g Pyruvate	0.28 ± 0.03	0.38 ± 0.07	0.44 ± 0.08	0.58 ± 0.09†	0.75 ± 0.16†‡§	0.59 ± 0.09	0.70 ± 0.12†‡	0.70 ± 0.12†‡	0.75 ± 0.17†‡§
25 g Pyruvate	0.25 ± 0.03	0.31 ± 0.03	0.33 ± 0.04	0.44 ± 0.07	0.48 ± 0.08	0.55 ± 0.08	0.52 ± 0.07†	0.57 ± 0.09†	0.59 ± 0.03*†

Values are mean ± SE. Note that 0 min refers to blood sample before pyruvate ingestion and that each corresponding time point refers to after pyruvate ingestion. FFA, free fatty acids. *Significantly different from 7-g dosage, $P < 0.05$. †Significantly different from 0 min; ‡Significantly different from 30 min, $P < 0.05$. §Significantly different from 60 min, $P < 0.05$.

Table 3. Plasma insulin and peptide C after acute supplementation with 7, 15, or 25 g of calcium pyruvate

Parameter	Time, min								
	0	30	60	90	120	150	180	210	240
Insulin, $\mu\text{U/ml}$									
7 g Pyruvate	14.4 \pm 4.0	11.8 \pm 2.4	8.7 \pm 0.8	7.3 \pm 0.6*	6.8 \pm 0.3*	6.5 \pm 0.3*	6.0 \pm 0.3*†	6.2 \pm 0.6*	6.0 \pm 0.7*
15 g Pyruvate	ND	ND	ND	ND	ND	ND	ND	ND	ND
25 g Pyruvate	12.1 \pm 2.2	8.3 \pm 1.3	6.1 \pm 1.0*	5.7 \pm 0.6*	6.6 \pm 0.6*	6.6 \pm 0.5*	6.1 \pm 0.9*	5.7 \pm 1.1*	6.4 \pm 1.2*
Peptide C, ng/ml									
7 g Pyruvate	2.7 \pm 0.4	2.5 \pm 0.4	1.9 \pm 0.2	1.7 \pm 0.1*†	1.5 \pm 0.1*†	1.4 \pm 0.1*†	1.3 \pm 0.1*†	1.2 \pm 0.1*†‡	1.2 \pm 0.1*†‡
15 g Pyruvate	ND	ND	ND	ND	ND	ND	ND	ND	ND
25 g Pyruvate	2.5 \pm 0.2	1.9 \pm 0.1	1.5 \pm 0.1*	1.4 \pm 0.1*	1.3 \pm 0.1*	1.3 \pm 0.1*	1.2 \pm 0.1*	1.1 \pm 0.1*	1.1 \pm 0.1*

Values are means \pm SE. Note that 0 min refers to blood sample before pyruvate ingestion, and that each corresponding time point refers to after pyruvate ingestion. ND, not determined. *Significantly different from 0 min, $P < 0.05$. †Significantly different from 30 min, $P < 0.05$. ‡Significantly different from 60 min, $P < 0.05$.

be effective, do not elevate its content in the blood and that 7 days of consumption of 7 g/day of pyruvate do not improve aerobic endurance in well-trained cyclists.

Effect of Acute Oral Pyruvate Supplementation on Blood Pyruvate

To date, there has been no direct measurement of how effectively pyruvate is absorbed and what dosage is optimal to elevate its content in blood or muscle. For pyruvate to have a specific metabolic effect on muscle, such as stimulating glucose uptake, it is necessary for blood levels to increase. Only two published reports (15, 16) have demonstrated an ergogenic effect of pyruvate, and these studies used a total of 100 g of CHO, of which 25 g were pyruvate and 75 g were dihydroxyacetone. However, no blood samples were taken during the period of oral supplementation. Nevertheless, commercial marketing of pyruvate supplements typically advocate 3–6 g daily as an effective dosage. Therefore, the objective of our initial study was to determine whether a wide range of oral pyruvate dosages (7–25 g) would elevate blood pyruvate concentration. Contrary to our expectations, we were unable to demonstrate a significant increase in blood pyruvate over this range of oral dosages.

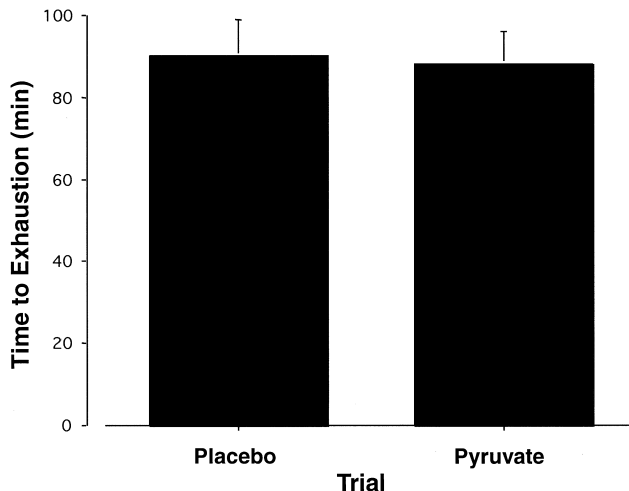


Fig. 3. Performance during intense aerobic cycling after 7 days of consumption of placebo or calcium pyruvate.

The inability to detect a significant elevation in blood pyruvate after acute supplementation might be attributable to 1) decarboxylation in the stomach and intestine; 2) elimination through the urine or feces; and 3) rapid clearance by other tissues, such the liver or muscle. Pyruvate content in urine represented $<0.1\%$ of the total pyruvate consumed in each of the trials, eliminating this as a significant means of loss in the present study. Fecal pyruvate content was not measured, but it has previously been shown to be negligible after DHAP supplementation in both rats and humans (11, 16). In the present study, all subjects complained of increased borborygmous and flatulence after the 15- and 25-g trials, indicating pyruvate decarboxylation and the production of gas. Alternatively, it is possible that some pyruvate was absorbed into the circulation, and rapidly cleared by either the muscle or liver. Because pyruvate is an excellent gluconeogenic precursor, it would be expected that the majority of pyruvate cleared by the liver would be either stored as glycogen or released as glucose. Although it was unfeasible to measure liver glycogen in the present study, we attempted to maximize liver glycogen stores by instructing subjects to consume a high-CHO diet and avoid strenuous exercise for 48 h before each trial, as well as to consume a CHO-rich meal 2–3 h before each trial. If the entire dosage of 25 g of pyruvate were absorbed and cleared by the liver, this would correspond to 12.5 g of glucose (2 pyruvate = 1 glucose) that could ultimately

Table 4. Respiratory exchange ratio and $\% \dot{V}O_{2max}$ during intense aerobic cycling after 7 days of placebo or calcium pyruvate consumption

Trial	Time, min		
	20	40	60
RER			
Placebo	0.90 \pm 0.01	0.88 \pm 0.01*	0.88 \pm 0.01*
Pyruvate	0.89 \pm 0.02	0.88 \pm 0.02	0.88 \pm 0.02
$\% \dot{V}O_{2max}$			
Placebo	74.2 \pm 1.3	76.6 \pm 2.1	79.7 \pm 1.7*
Pyruvate	74.0 \pm 0.9	77.8 \pm 1.5*	78.5 \pm 1.4*

Values are mean \pm SE. RER, respiratory exchange ratio; $\dot{V}O_{2max}$, maximal oxygen consumption. *Significantly different from 20 min, $P < 0.05$.

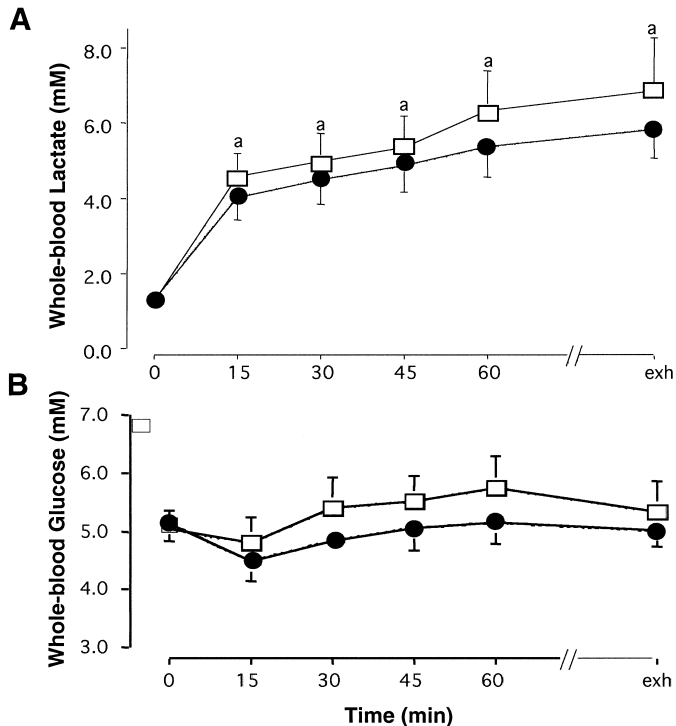


Fig. 4. Whole blood lactate (A) and glucose (B) concentrations during aerobic cycling after 7 days of consumption of placebo or calcium pyruvate. □, Placebo trial; ●, pyruvate trial. ^aSignificantly different from 0 min, $P < 0.05$.

be released to the muscle. Assuming a liver mass of ~2 kg, this would correspond to 6.25 g/kg, or ~35 mmol/kg. This represents only 7% of the total storage capacity of the liver for glycogen (>500 mmol/kg). Therefore, despite our efforts to maximize liver glycogen stores before exercise, it is quite conceivable that the liver was able to clear, on first pass, the majority of any pyruvate that had been absorbed. Despite this possibility, there were no significant differences in blood glucose or lactate. In a recent study by Constantin-Teodosiu et al. (3), intravenous pyruvate infusion elevated blood pyruvate approximately fivefold but failed to increase muscle pyruvate content. However, it may be possible to elevate muscle pyruvate by infusing large amounts of pyruvate directly into the arterial system (M. Gibala, unpublished observations, personal communication). Nevertheless, it appears that very high concentrations of systemically infused pyruvate are necessary to increase muscle content and that low oral dosages are likely ineffective.

One limitation to the design of the present study is the lack of a control group to monitor metabolic responses during the 4-h period in the absence of pyruvate supplementation. The only significant findings of this study were 1) a decrease in plasma insulin and peptide C and 2) a parallel increase in lipolysis, as indicated by elevated FFAs and glycerol. This response was expected, because subjects had consumed a small, CHO-rich meal 2–3 h before each trial, resulting in elevated plasma insulin and peptide-C levels. Thus, during the trial, which represents a range of 3–7 h

after their meal, a decline in insulin and peptide C and an increase in lipolysis would be expected. Although it could be argued that pyruvate may be partly responsible for the increased rate of lipolysis during the 4 h, the fact that there is no difference between the low (7-g) and high (25-g) dosages strongly suggests that its effect is negligible. Furthermore, the absence of an elevation in blood pyruvate would likely preclude an effect on hormonal release from the pancreas.

It seems clear that at least a portion of the ingested pyruvate is degraded in the stomach and/or intestine. Each of our subjects complained of increasing borborygmic, flatulence, and nausea as the dosage of pyruvate was increased, as has been previously reported (15, 16). Two of our subjects did not participate in the final trial with the highest pyruvate dosage (25 g) due to adverse effects. Although we cannot eliminate the possibility of clearance by the liver, the inability to elevate blood pyruvate content would seem to preclude the possibility of orally ingested pyruvate having an effect on target tissues, such as the pancreas or skeletal muscle, and cause altered insulin and/or peptide C release or an increase in muscle glucose uptake.

Effect of Pyruvate on Aerobic Endurance and Metabolism During Exercise

The findings of the present study do not support the hypothesis that low dosages of pyruvate have an ergogenic effect in well-trained individuals. Furthermore, changes in the blood metabolites glucose, lactate, FFAs, and glycerol and in the hormones peptide C and insulin during exercise were generally unaffected by pyruvate supplementation.

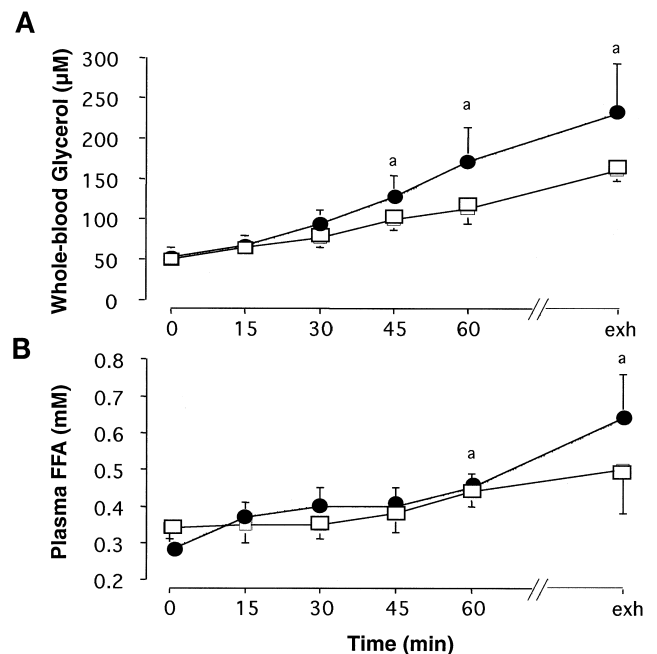


Fig. 5. Whole blood glycerol (A) and plasma free fatty acids (FFA; B) concentrations during aerobic cycling after 7 days of consumption of placebo or calcium pyruvate. □, Placebo trial; ●, pyruvate trial. ^aSignificantly different from 0 min, $P < 0.05$.

Table 5. Plasma insulin and peptide C during intense aerobic cycling after 7 days of placebo or calcium pyruvate consumption

Trial	Time, min					
	0	15	30	45	60	Exhaustion
Insulin, $\mu\text{U/ml}$						
Placebo	8.9 ± 2.2	$2.6 \pm 0.4^*$	$2.5 \pm 0.4^*$	$2.8 \pm 0.4^*$	$2.3 \pm 0.4^*$	$2.1 \pm 0.6^*$
Pyruvate	6.6 ± 2.2	$2.9 \pm 0.9^*$	$2.3 \pm 0.3^*$	$2.1 \pm 0.4^*$	$1.4 \pm 0.1^*$	$1.3 \pm 0.1^*$
Peptide C, ng/ml						
Placebo	1.7 ± 0.4	$1.2 \pm 0.3^*$	$0.9 \pm 0.2^*$	$0.8 \pm 0.2^*$	$0.7 \pm 0.2^*$	$0.4 \pm 0.1^*$
Pyruvate	1.4 ± 0.3	$0.9 \pm 0.2^*$	$0.7 \pm 0.2^*$	$0.5 \pm 0.2^*$	$0.3 \pm 0.1^* \dagger$	$0.2 \pm 0.1^* \dagger \ddagger$

Values are means \pm SE. *Significantly different from 0 min, $P < 0.05$. \dagger Significantly different from 15 min; $P < 0.05$. \ddagger Significantly different from 30 min, $P < 0.05$.

Previous research regarding the effects of pyruvate on substrate utilization at rest is scant and contradictory. In rodents, it has been demonstrated that high doses of DHAP may shift substrate utilization toward lipid (4) because of reduced plasma insulin (11). However, in contradiction, insulin sensitivity has been shown to improve after supplementation in rats (4, 8, 11). Fractional glucose extraction (arteriovenous difference) is enhanced at rest after DHAP supplementation in humans but is not accompanied by a significant increase in RER (15, 16). There is little information regarding substrate utilization in response to pyruvate supplementation during exercise. Bagby et al. (1) demonstrated greater depletion of muscle and liver glycogen in exercised rats after lactate and pyruvate infusions. In humans, Stanko et al. (15, 16) demonstrated greater fractional glucose extraction (on the basis of arteriovenous differences) during aerobic exercise after chronic DHAP supplementation. However, this was not supported by significantly elevated RER values in these studies. Furthermore, RER values determined in the initial arm cranking study (16) were in excess of 1.0 throughout exercise, which precludes their use in calculating fuel utilization. In the present study, no significant differences in RER or in blood glucose, lactate, FFAs, and glycerol existed between trials.

Differences Between the Present and Previous Studies

There are several differences between the present study and the studies by Stanko et al. (15, 16), which demonstrated an improvement in arm cranking and leg cycling after pyruvate supplementation.

Aerobic fitness of the subjects. The subjects in the present study were well trained ($\dot{V}O_{2\text{max}}$, 62.3 ± 3.3 ml \cdot kg $^{-1}$ \cdot min $^{-1}$) compared with those used in a previous study (Ref. 15; $\dot{V}O_{2\text{peak}}$, 47.3 ± 0.4 ml \cdot kg $^{-1}$ \cdot min $^{-1}$). The earlier study by Stanko et al. (16) only assessed $\dot{V}O_{2\text{peak}}$ during arm cranking; no information pertaining to whole body aerobic capacity was given. The greater aerobic fitness of the subjects used in the present study is also indicated by the duration of cycling to exhaustion of 88 ± 8 min in the placebo trial, compared with only 66 ± 4 min in the study by Stanko et al. (15), despite the subjects exercising at a greater power output and higher percentage of $\dot{V}O_{2\text{max}}$ in the present study. We have no explanation for why

pyruvate would have an ergogenic effect in untrained, but not in well-trained, individuals. However, it is well known that time to exhaustion is a more reliable measure of performance and is more reproducible in trained individuals than in untrained individuals who are unaccustomed to exercising to exhaustion (6, 10). In the study by Stanko et al. (15), there was no indication of a practice trial to exhaustion for the untrained subjects to become accustomed to exhaustive exercise, and no forceful motivation was used. This raises the question as to whether subjects were truly motivated to exercise to exhaustion in each trial. On the basis of information presented in their study (15), it is possible to separate the subjects into two groups of apparently different aerobic fitness. Subjects 1, 4, 5, and 8 had a $\dot{V}O_{2\text{max}}$ of 4.5 ± 0.3 l/min, whereas subjects 2, 3, 6 and 7 had a $\dot{V}O_{2\text{max}}$ that was 50% lower (3.0 ± 0.1 l/min). Interestingly, the group with the greater aerobic capacity only showed a 9.9% improvement in performance after pyruvate supplementation, whereas the group with the lower aerobic capacity demonstrated a 32.1% improvement.

Awareness of supplementation regime. In the present study, subjects were questioned after the completion of both trials as to whether they could distinguish between the pyruvate and placebo supplements. The results of the questionnaire indicated that five of the seven subjects did not know which supplement they were taking, whereas two of the subjects correctly identified the pyruvate and placebo trials. Interestingly, only three of our subjects had longer times to exhaustion during the pyruvate supplementation trial, and two of these individuals had correctly identified when they were taking the pyruvate supplements. Only one of the five subjects who were unable to distinguish between placebo and pyruvate supplementation demonstrated improved performance in the pyruvate trial. None of our subjects complained of gastrointestinal discomfort, presumably because of the relatively low dosage used in the present study (7 g/day divided over 4 meals). However, Stanko et al. (15, 16) reported in each of the studies that subjects developed borborygmous and flatus in response to consuming 100 g of DHAP. These side effects were also evident in our initial study when 25 g of calcium pyruvate were ingested. Although the studies by Stanko et al. were

well-controlled, double-blind, crossover designs, there is a high probability that the unavoidable side effects permitted subjects to correctly identify whether they were receiving placebo or treatment.

Supplementation dosage. One of the major differences between the present study and those by Stanko et al. (15, 16) is the dosage of pyruvate consumed. In all studies, including the present, pyruvate supplements were consumed four times daily with each meal for 7 days. In the studies by Stanko et al., 100 g of DHAP were consumed daily, compared with the present study in which 7 g of pyruvate were consumed. Commercially available pyruvate supplements are typically sold as 0.5- to 1-g capsules, and they do not contain dihydroxyacetone. Daily consumption of 25 g or greater of pyruvate is clearly prohibitive both in terms of cost and potential side effects. Manufacturer recommendations are typically 3–6 g daily. Therefore, there is little practical relevance in studying the ergogenic effect of high combined dosages of trioses (pyruvate, dihydroxyacetone). It is also highly unlikely that well-trained athletes would consider using dosages that cause the aforementioned side effects as an adjunct to training or in preparation for a competition. In their initial study, Stanko et al. (16) reported an increase in triceps muscle glycogen of ~40 mmol/kg after 7 days of DHAP supplementation. Muscle glycogen was not measured in the present study. However, subjects consumed a diet high in CHO (65%) for 3 days before each trial, as well as a CHO-rich meal before cycling. Increasing dietary CHO to ~70% (16) sufficiently increased resting muscle glycogen concentrations to eliminate any further effect of DHAP supplementation. Furthermore, assuming that the two triose molecules result in the synthesis of one glucose moiety in muscle glycogen, a total consumption of ~50 g of triose compounds over 7 days could theoretically increase total muscle glycogen by 25 g (139 mmol), or ~5 mmol/kg muscle in a 70-kg man. Therefore, it is highly unlikely in the present study, with lower dosages of pyruvate supplementation and a high-CHO diet, that muscle glycogen content would have differed between trials. It is possible that dosages of pyruvate of 25 g or greater may elicit an ergogenic effect that was not apparent in the present study. Furthermore, the ergogenic effect of high dosages of dihydroxyacetone have not been investigated.

Conclusion

A wide range of “practical dosages” of calcium pyruvate were ineffective in elevating blood pyruvate content over a 4-h period. Furthermore, 7 days of supplementation with 7 g/days of pyruvate failed to enhance performance during intense aerobic cycling in well-trained individuals.

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