Influence of Acqua Lete[®] (Bicarbonate Calcic Natural Mineral Water) Hydration on Blood Lactate After Exercise

P. Brancaccio^{*,1}, F.M. Limongelli¹, I. Paolillo², C. Grasso¹, V. Donnarumma³ and L. Rastrelli²

¹Seconda Università di Napoli, Servizio di Medicina dello Sport, Via Costantinopoli 16 80138, Napoli, Italy

²Dipartimento di Scienze Farmaceutiche e Biomediche, Università di Salerno, Via Ponte Don Melillo, 84084 Fisciano, Salerno, Italy

³Laboratorio di Diagnostica e Ricerca Clinica Roecker, Marano di Napoli, Italy

Abstract: *Purpose*: This investigation examined the effects of Acqua Lete® bicarbonate calcic mineral water ingestion on blood lactate, glucose, and serum lactate dehydrogenase.

Methods: 88 amateur male athletes underwent two experimental trials with modified Wingate test: the first was carried out without hydration (Test C), the second (Test H) with hydration following this scheme: 44 subjects (Group A) hydrated by a very low mineral content water and 44 subjects (Group B) hydrated by Acqua Lete[®]. Measures of body temperature, [La], glucose, total LDH and its isoenzyme pattern, were taken before (T0), during (T1-T5), and after exercise (T6-T7).

Results: Blood lactate significantly increased after the session of exercises in both groups: after hydration and 30' of resting (T7) Group B returned a level of lactate lower than the Group A ($2.2\pm0.2 vs 2.9\pm0.3 mmol/L$; p<0.001). In Test H, LDH activities after exercise did not change but serum LDH5 isoenzyme activity decreased by 0.9% in athletes in Group B compared to Group A (p<0.05).

Conclusions: All the athletes (Group B) hydrated pre-exercise with Acqua Lete® showed a significant decrease in blood lactate levels post-exercise and changes in LDH isoenzymatic pattern compared with athletes hydrated pre-exercise with a low mineral content water.

Keywords: Acqua Lete[®] mineral water, Hydration, Blood glucose, Blood lactate, LDH isoenzymes, Urinary specific gravity.

INTRODUCTION

Lactic acid (La) at physiological pH is dissociated more than 99% into La⁻ anions and protons (H⁺). During exercise and muscle contractions, muscle and blood [La⁻] and [H⁺] can rise to very high levels. Most researchers have argued that any detrimental effects of La on muscle and exercise performance are due to H⁺ rather than La⁻ [1]. However, some studies have recently identified strategies that may help to lower blood lactate levels during exercise and have a better recovery [2, 3]. High activity of cytosolic LDH is considered to guarantee La formation in the cytosol under virtually all conditions but especially during exercise; total serum LDH and specific isoenzymes activities change with training status of the athlete. Variation in LDH isoenzymes profile might have a role in studying muscle response to training and particularly LDH5 is the isoenzyme involved in lactate production [4]. Lactate production is compensated by the displacement of bicarbonate into carbon dioxide, which is lost through the lungs during exercise more rapidly than it is produced by cell respiration [5]. Alkalinizing agents including sodium bicarbonate (NaHCO₃), mineral-based alkaline bottled water, nutritional drinks and mineral waters

containing more than 600 mg/L of bicarbonate, have been proposed for their potential effects on providing enhanced extracellular buffer capacity, leading to the elevated proton efflux from the contracting musculature [6-8] and elevated plasma HCO_3^- can improve exercise endurance in humans [9]. According to current EEC directives mineral waters, are of underground origin, protected from contamination, and microbiologically wholesome; present a peculiar and constant chemical composition, and have favorable effects on health; they must be bottled at source into safe and checked containers. Acqua Lete® mineral water shows calcium concentrations of 314 mg/L, magnesium levels of 15 mg/L and bicarbonate levels of 981 mg/L, implying very high calcium and bicarbonate mineral water. The Acqua Lete® exhibits other peculiarities, notably high levels of carbon dioxide, and low contents of sodium and potassium. Bicarbonate waters may neutralize acid secretion, accelerate gastric empting, and provoke the release of gastric peptides (like gastrin and endorphins). They are indicated in hydrochloric-peptic hypersecretion and gastro-esophageal reflux disease [10]. During physical activity they restore liquids and salts, facilitate nitrogen waste clearance and counterbalance metabolic acidosis, which is typical of the effort syndrome of the sportsman [11].

The aim of this study was to investigate the effectiveness of a hydration strategy prior to exercise involving the ingestion of Acqua Lete® mineral water on blood lactate

^{*}Address correspondence to this author at the Seconda Università di Napoli, Servizio di Medicina dello Sport, Via Costantinopoli 16 80138, Napoli, Italy; Tel: 0039 815665908; Fax: 0039 815665864; E-mail: pabranca@libero.it

concentration and serum levels of LDH in 88 amateur athletes.

METHODS

Participants

All testing procedures were approved by the institution's Human Research Ethics committee. Eighty-eight male amateur athletes volunteered to participate in the study. All potential participants attended a familiarization session where details of the test protocol and their time commitment were described. All participants were advised that they were free to withdraw from testing at any time without any adverse consequences. Upon completion of the consent form participants were randomly selected into one of two groups of 44 subjects:

Group A : aged 34.7 y \pm 7.4 (mean \pm S.D.); height 178.5 cm \pm 5.6; weight 79.6 kg \pm 6.9, and Body Mass Index (BMI) 24.6 \pm 1.2.

Group B : aged 33.7 y \pm 8.6 (mean \pm S.D.); height 174.6 cm \pm 5.4; weight 79.6 kg \pm 9.6, and Body mass Index (BMI) 25.7 \pm 3.4.

Study Design

Both groups underwent two experimental trials, performed on an electrically braked ergometer (Bycicle SECA Hamburg, Germany) with a modified repeated Wingate protocol: five bouts of cycling of 60" with a mean speed of 80 RPM and 60" of recovery between the sessions. The workload was 85% of their maximal workload calculated in a preliminary session a week before the first Test, with an incremental cycle test until exhaustion. Before the test all the athletes complete a 2 minutes of warm up on treadmill, with a speed of 4 Km/hr without grade.

The two Tests were:

- Test C of control, in basal conditions and without hydration the day of trial, for both groups.
- Test H, after one week of controlled hydration with 1.5 L/die of a very low mineral content water (dry residues 14.3 mg/l) in Group A and 1.5 L/die of a bicarbonate calcic water (Acqua Lete®) with a medium mineral content (dry residues >840 mg/l), in Group B. Moreover, athletes received 750 ml of water using freshly opened bottles one hour before the exercise and 250 ml of water in the following 30 minutes after effort, as recommended by National Athletic Trainer Association [8].

Before testing, all participants received a physical examination including medical history. In each session of work (Test C and Test H), was detected:

- At rest before the exercise (T0): blood concentration of lactate, glucose, total serum LDH and its isoenzymes, urinary specific gravity;
- During exercise, after each bouts of cycling (T1, after 1 minute cycling; T2, after 2 minutes cycling; T3, after 3 minutes cycling; T4, after 4 minutes cycling): blood glucose;
- *immediately after the last session of exercise (T5, after 5 minutes cycling):* blood glucose and blood lactate;

- 5 minute after exercise (T6): blood glucose and blood lactate;
- *30 minutes after exercise (T7):* blood concentration of lactate, glucose, total serum LDH and its isoenzymes, urinary specific gravity;

Testing Procedures

Blood Lactate Concentration

We have taken a drop of blood by pricking the fingertip after cleaning the sweat with cotton wool and wipe off the first drop of blood. Samples have been collected by inserting a La⁻ strip into a calculator-sized instrument and then touching the strip with a drop of blood: the sample is drawn into the strip by capillary action. Lactate concentration (mmol/L) have been measured on whole blood before and after exercise (T0, T5, T6, T7) using an amperometric method with an enzymatic electrode (Lactate Pro, Arkray, Kyoto, Japan).

LDH and Isoenzymes Analysis

Blood samples were taken from a forearm vein by a trained nurse. The post-exercise blood samples were taken immediately after the cycling. The blood samples were put in ice bath and sent to the laboratory for analysis. The relative value of each lactic dehydrogenase isoenzyme (iso LDH) was measured by electrophoretic separation on a cool serum within 12 hours after blood sample was taken. LDH enzymes were analyzed with a spectrometric monotest method, (Pharmacia LKB-vitrospec) at 25°C of temperature, taking into consideration the following values as normal: LDH= 113 - 189 U/L. Isoenzymatic evaluation was performed by agarose-gel electrophoresis and determined by Beckman Appraise Densitometer System method and expressed as a percentage of the total LDH activity.

Blood Glucose

Blood was collected by fingertip and blood glucose concentration (mmol/L) was measured using a One Touch Ultra 2 glucometer per the manufacturer's instructions Johnson & Johnson instrument.

Urinary Specific Gravity

The urine was collected in polyethylene containers and mixed with 5 ml/L of a 5% solution of thymol in isopropanol to preserve the urine. During the collection period, the containers and their contents were maintained at 5°C. Urine samples were tested for the presence of blood and infection. Nitrite-positive and hematuria samples were discarded. Urinary specific gravity was recorded by Bayern Ketostix

Water Analysis

The bicarbonate-rich mineral water Acqua Lete (Acqua Lete®; Società Generale delle Acque Minerali, Pratella, CE, Italy), was consumed by the experimental Group B and shipped directly to the testing lab from its bottling facility. The very low mineral content water used for Group A is commonly available throughout Italy; contains no significant minerals or electrolytes whatsoever. Very low mineral content and Acqua Lete waters were also analyzed for 15 chemical parameters in our laboratory. Most of the elements were analyzed by ion chromatography (IC) using a Dionex

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instrument, while a non-acidified aliquot was used to determine pH, electrical conductivity (EC), to titrate alkalinity. The 15 chemical and chemical-physical variables measured on each sample are listed in Table 1. Analytical methods are not further discussed here since they represent standard methods fixed by Italian regulations (IRSA - CNR methods 1994).

Parameter	Measurement Unit	Acqua Lete	Very Low Mineral Content
Conductivity	mS/cm	1321.40 ± 46.10	17.57 ± 0.91
pH	pН	6.14±0.11	5.00 ± 0.09
Fixed residue	mg/l	878.41 ± 25.21	14.31 ± 0.68
CO ₂	mg/L	1890.12 ± 72.51	15.22 ± 0.77
(HCO ₃ ⁻)	mg/l	981.11±33.82	3.51 ± 0.15
Cľ	mg/l	8.24±2.22	0.41 ± 0.02
SO4 ²⁻	mg/l	6.60 ± 0.91	1.40 ± 0.08
NO ₃ ⁻	mg/l	4.14 ± 0.20	1.91 ± 0.08
Na ⁺	mg/l	4.91 ± 0.33	1.21 ± 0.05
K^+	mg/l	2.10 ± 0.08	0.32 ± 0.01
Ca ⁺⁺	mg/l	313.70±9.81	1.11 ± 0.05
Mg ⁺⁺	mg/l	15.12 ± 3.92	0.42 ± 0.03
Fe	mg/l	0.02 ± 0.01	< 0.01
Sr ⁺⁺	mg/l	0.15 ± 0.01	< 0.1
Li ⁺	mg/l	< 0.01	< 0.01

 Table 1.
 Chemical Characteristics of Mineral Waters Used in the Study*

*Each result represents the mean \pm SD of three analysis for each water.

Statistical Analysis

Statistical analysis was performed by SPSS statistical package for Windows, release 17.0 (Chicago, IL, USA). We compared the data collected in each group at every step of work:

- in test C (without hydration) before and after exercises;
- in the test H (with hydration) before and after exercises;
- the two groups to each other.

Statistical significance between Group A and Group B was evaluated by Student's T Test for independent samples: descriptive statistics were calculated, and values reported are mean \pm standard deviation. Statistical significance within Group A and Group B, comparing Test C and Test H, was evaluated by Student's T Test for paired samples: descriptive statistics were calculated, and values are reported as mean \pm standard deviation. Differences were considered statistically significant when p<0.05.

RESULTS

All the subjects underwent the protocol previously described. Tests were performed at an environmental

temperature of 19.50 ± 0.53 °C with a wetness of 58.38 ± 0.52 %.

Blood Lactate

Table 2 identifies the lactate levels produced by participants under each test condition. In detail, without hydration (Test C), both groups started with the same mean values of [La] reached similar levels at peak of exercise and showed after 30 minutes resting the same blood lactate concentration, with a rate of decrease of 44.77 % in Group A vs 46.26 % in Group B. Some studies evaluate blood lactate after Wingate test, reporting a peak of blood lactate concentration between the 3_{rd} and the 8_{th} minute after effort [12, 13], with an almost complete recovery within the 10 minutes after the test [14]. In fact the shortness of exercise provides a delayed onset of lactate: in our study the modified Wingate test is longer than usual and therefore the lactate accumulation begins during exercise, reaching a peak at the end of it. Comparing Test C and Test H, we saw that after hydration, peak lactate values at T5 were increased by 10.4 % in Group A and of 44.2% in Group B probably for the higher rest levels detected in this Group in the Test H (Table 2). Moreover, comparing the response of two groups after hydration (Fig. 1), we found that Group B, despite reaching higher peak levels of lactate at the end of exercise (T5), when compared to Group A, showed a better blood lactate removal, with a decrease over the 30 minutes recovery period (T7) of 77.5 % vs 60.8 % (Table 2).

LDH and Glucose

Comparing the groups in Test C before and after exercise (Table 3), we found almost the same isoenzymatic pattern. In Test H, (Table 4) LDH5 decreased significantly after exercise $(4.0\pm0.7\% \ vs \ 6.2\pm0.9\%, \ p<0.05)$. Blood glucose showed a progressive decrease of its levels during the exercise in both groups during Test C and Test H (Table 5).

Urinary Specific Gravity

When the groups were tested without hydration, we found in both group a slight but significant increase of urine gravity after exercise (Group A: 1020 ± 4.7 g/L at rest vs 1022 ± 4.4 g/L after exercise; p=<0.001; Group B: 1018 ± 6.5 g/L at rest vs 1019 ± 5.5 g/L after exercise; p= ns): conversely we expected the decreasing of urinary specific gravity after acute hydration, but we found that group B reached after exercise a significantly lower level than group A (1008.1 ± 6.3 g/L vs 1014.6 ± 5.1 g/L; p=<0.001), reflecting a better hydrated condition (Fig. 2).

DISCUSSION

Many studies have used Wingate Test [15, 16] and modified Wingate Test [17], to evaluate physiological responses to anaerobic exercise. In our study we evaluate the response to anaerobic exercise before and after hydration with a bicarbonate-calcic mineral water, named Acqua Lete, compared to very low mineral content water (dry residues 14.3 mg/L).

The importance of [La] as a carbohydrate fuel source is now underscored [18]: in short term exhaustion exercise, muscle produces La quickly, while its clearance is slow. Lactate enters the plasma from interstitial fluid of active

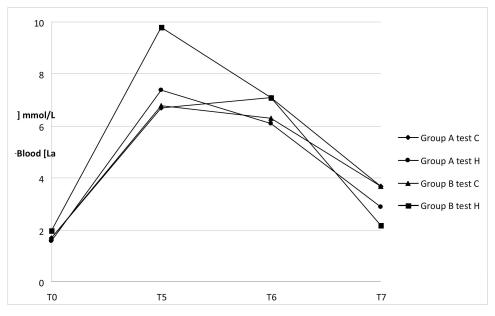


Fig. (1). Differences between not hydrated (Test C) and hydrated athletes (Test H) in terms of the [La-] response to exercise and recovery. Group A (n=44) hydrated by a very low mineral content, Group B (n=44) hydrated by Acqua Lete mineral water.

Table 2. I	Blood Lactate Levels [®]	During and After	· Session Exercise ^{**}
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Test C	Rest (T0)	Т5	Δ	Т6	Τ7	Δ^{*}
Group A	1.7±0.4	6.7±0.6 ^a	394%	7.1±0.9	3.7±0.7 ^b	44.77% ^e
Group B	1.7±0.3	6.8±0.8ª	400%	6.3±0.8	3.7±0.6 ^b	46.26% ^d
Test H	Rest (T0)	Τ5	Δ	Т6	Τ7	Δ^{*}
Test H Group A	Rest (T0)	T5 7.4±0.8ª,	Δ 462%	T6 6.1±0.7	T7	Δ* 60.80% ^{ce}

Group A: Control water.

Group B: Lete Water. Values are expressed in mmol/L.

**Mean±SE, n=88.

 Δ rate of increase measured in percentage (%) respect T0.

 Δ^* rate of decrease measured in percentage (%) respect T5.

^aSignificantly different from resting values, P < 0.05.

^bSignificantly different from resting values, P < 0.001.

 $^{\rm e} {\rm P} < 0.05$

Test C: test performed without hydration.

Test H: test performed with hydration.

Group A: subjects hydrated with control water.

Group B: subject hydrated with Lete water. T5: immediately after exercise.

T6: 5 minutes after exercise

T7: 30 minutes after exercise.

muscle and from the plasma into the red blood cells (RBC), which are in equilibrium with plasma, so that RBC/plasma ratio is almost constant. The tool we used for blood lactate evaluation was Lactate Pro, which measures lactate in whole blood lysing RBC, and has been found to be a reliable instrument for lactate detection [19]. In Test C, without hydration (Fig. 1), we found in both groups an increase of lactate levels immediately after exercise (T5) remaining elevated until the 5_{th} minute and returning at lower values 30 minutes after exercise (Table 2). In the second test with hydration (Test H, Table 2) the groups showed different responses: the Group B, despite reaching a higher increase of lactate at the end of exercise (490% vs 462%, p<0.05), had

significantly lower values after 30 minutes, than the Group A (2.2±0.2 mmol/L vs 2.9±0.3 mmol/L, respectively; p<0.001). Hydration status has been widely studied, detecting its incidence on lactate threshold, showing that, low levels of hydration; change the trigger of anaerobic metabolism [20, 21]. In fact, according with literature, the better hydration status improved the recovery after exercise in both groups of athletes, with a rate of decrease of lactate higher in test H respect the test C.

Besides in our study the mineral ion composition of water seems to have had an effect on blood lactate: the water administered during the second trial were very different (Table 1), the very low mineral content water had low levels

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of calcium and bicarbonate and a dry residues of 27 mg/l (Table 1), the Acqua Lete mineral water with significant contents of bicarbonate (981.1 mg/L), calcium (313.7 mg/L) and magnesium (15.12 mg/L), belongs to the group of the bicarbonate-calcics and exhibits other interesting peculiarities, notably high levels of carbon dioxide (1890.12 mg/L), interesting amount of Sr^{++} (0.15 mg/L) and low contents of sodium (4.91 mg/L) and potassium (2.10 mg/L).

Table 3. Enzyme activities (± s.d.) at each testing stage (n=88) in Test C

Enzyme	Rest (T0)	Τ7
Group A		
Serum total LDH*	287.6±59.6	279.7±64.1
LDH1**	25.4±1.6	24.6±1.78
LDH2**	37.3±0.8	36.4±1.31
LDH3**	24.0±0.7	25.9±1.01
LDH4**	7.8±1.3	8.0±1.26
LDH5**	5.5±1.1 ^b	5.1±1.31 ^b
Group B		
Serum total LDH*	347.5±42.0	334.7±53.1
LDH1**	26.3±1.3	25.3±1.8
LDH2**	36.2±0.6	35.8±1.2
LDH3**	24.3±1.8	25.9±0.5
LDH4**	7.9±1.3	8.4±1.4
LDH5**	5.3±1.3ª	4.6±0.8ª

*Values are expressed in U/L.

**% total LDH.

^aSignificantly different from resting value, P < 0.001.

^bSignificantly different from resting values, P < 0.05.

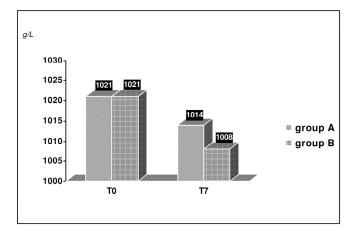


Fig. (2). Urinary specific gravity detected in Test H before and after exercise. T0=: before exercise; T7= 30 minutes after exercise. Data are expressed as mean \pm SD; n=88; Standard deviations were below 5%.

In athletes hydrated with Acqua Lete we found a significant decrease of specific urinary gravity after effort, in fact subjects who drank Acqua Lete mineral water (Group B) showed a significant lower mean values of specific urinary gravity when compared with athletes belonging to Group A (Fig. **2**).

Research has shown that the free intake of mineral rich alkalizing bottled water, could improve hydration status in young adults [22]. However, studies about the effect of bicarbonate ingestion on metabolic response are often conflicting. A study conduct on horses by Schuback et al. in 2002 reported no effect of sodium bicarbonate ingestion on metabolic response and duration of exercise [23]; contrariwise other study reported in athletes, show an improved performance in a way dose-dependent [24-26] and probably by increasing buffering capacity [27, 28]. Alkalinizing agents, including sodium bicarbonate, have been proposed as ergogenic aids for their potential effect on providing enhanced extracellular buffer capacity. In fact increased blood lactate, commonly observed with metabolic alkalosis, results from a complex series of events which modulate the activities of the key regulatory enzymes, resulting in a mismatch between the rates of pyruvate production and oxidation [29]; metabolic alkalosis leads to an increased lactate production and intramuscular accumulation resulting from the absence of downregulation of glycogenolysis and glycolysis that typically occurs as pH declines.

Table 4.Enzyme Activities (± s.d.) at Each Testing Stage
(n=88) in Test H

Cuann A					
Group A					
304.4±52.4	301.2±51.9				
25.2±1.0	23.1±1.9				
36.6±1.9	38.3±1.3				
23.5±1.5	24.8±0.4				
8.2±1.3	7.6±1.3				
6.5±1.3 ^b	6.2±0.9 ^{bc}				
Group B					
340.5±70.4	334.7±53.1				
26.8±1.2	27.8±1.7				
35.6±1.9	36.3±0.8				
24.7±1.9	25.0±1.4				
7.5±1.2	6.9±0.9				
5.4±1.2ª	4.0±0.7 ^{ac}				
	25.2±1.0 36.6±1.9 23.5±1.5 8.2±1.3 6.5±1.3 ^b Group B 340.5±70.4 26.8±1.2 35.6±1.9 24.7±1.9 7.5±1.2				

**% total LDH

^aSignificantly different from resting value, P < 0.001.

^bSignificantly different from resting values, P < 0.05.

 $^{\circ} P < 0.05$

In our study the specificity of the Acqua Lete water, with the combination of high calcium content and a buffering agent, may have affected the increase of lactate at peak of exercise and the restore after exercise, leading to minimal, but significantly lower levels of [La⁻] after effort. The intracellular lactate shuttle (ILS) hypothesis holds that lactate produced as the result of glycolysis and

Test C	то	T1	T2	Т3	T4	Т5	Т6
Group A	4.6±0.2	4.4±0.3	4.6±0.3	4.5±0.3	4.6±0.3	4.1±0.3 ^a	4.3±0.1
Group B	5.0±0.6	4.8±0.6	4.9±0.5	5.0±0.5	4.7±0.5	4.6±0.5 ^a	4.5±0.4
Test H	TO	T1	Т2	Т3	T4	Т5	Т6
Test H Group A	T0 4.8±0.5	T1 4.9±0.7	T2 4.4±0.3	T3 4.6±0.3	T4 4.3±0.3	T5 4.3±0.4 ^a	T6 4.3±0.4

Table 5. Blood Glucose Levels* During and After Session Exercise**

*Values are expressed in mmol/L.

**Mean±SE.

^aSignificantly different from resting values, P < 0.05.

glycogenolysis in the cytosol, is balanced by oxidative removal in mitochondria of the same cell. The presence of intracellular lactate shuttles gives rise to the notion that glycolytic and oxidative pathways can be viewed as linked processes, because lactate, the product of one pathway, is the substrate for the other [30]. After its continuous production in citosol by LDH5 (M4), lactate diffusing in mitochondrial matrix, where LDH1 (H4) would catalyse the conversion of lactate back to pyruvate, with concomitant interconversion of NADH and NAD. The evaluation of total LDH and its isoenzymes allows obtaining much information about the muscle metabolism [4]. In fact strenuous exercise induces a significant increase of LDH [31] and the degree of the rise depends on the intensity and duration of the effort [32, 33]. We did not found an increase of total enzyme in Group A, neither in Group B, in both tests (C and H), probably for the type and intensity of effort: In our study the time of examination (30 minutes after exercise) and the type of exercise (submaximal) did not allow to detect a significant increase of total enzyme (Tables 3 and 4). Besides we found after hydration a variation of isoenzymatic pattern after exercise, which showed in Group B, significantly lower values of LDH5 compared to Test C (4.0±0.7% vs 4.6±0.8%, p < 0.05) and compared to Group A in the same test (H) (4.0±0.7% vs 6.2±0.9%, p<0.05), and increasing, although non significant, levels of LDH1 and LDH2 (Table 4).

Blood glucose response to exercise has been widely studied because it reflects the lactate responses to effort [34]. At rest and during moderate intensity exercise adrenaline stimulates glycogenolysis and lactate production [35]. In our study during the Test C, the increase of lactate coincides with a decrease of blood glucose. In fact after exercise (T5) both groups had a blood glucose level significantly lower than before the exercise (T0) (Group A: 4.1 ± 0.3 vs $4.6 \pm$ 0.2 mmol/L, p<0.05; Group B: 4.6±0.5 vs 5.0±0.6 mmol/L, p<0.05). Hydration status can modify the hormonal and metabolic response to exercise, influencing carbohydrate metabolism [36]. In fact during moderate intensity exercise, lactate competes with blood-glucose as a metabolic substrate and may represent a mechanism of protection against premature hypoglycemia during prolonged exercise. The increase in the percentage oxidation from lactate coincided with the decrease in the percentage oxidation of blood glucose, resulting in a decreased glucose production to maintain blood glucose homeostasis [37].

CONCLUSION

The oral intake of Acqua Lete[®], a bicarbonate calcic natural mineral water with peculiar and exclusive mineral ion composition, before and after the Wingate test was associated with a better oxidation of lactate, LDH isoenzymatic variation, and an improved maintenance of physiological homeostasis in athletes. In particular, Acqua Lete water shown to improve the restore due to its buffering capacity. These results indicate that the habitual consumption of Acqua Lete water may be a valuable nutritional vector for influencing the restore and hydration status in athletes. Additional studies are warranted to fully explore the effects of Acqua Lete in specific sport skills such as football and tennis with the measurement of blood lactate levels and LDH isoenzymatic pattern.

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