

The influence of probiotic supplementation on selected athletic performance-related blood markers in men

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Abstract

It has been speculated that probiotics can improve athletic performance. Significant increases in haemoglobin concentration and oxygen consumption have also been shown to follow the ingestion of lactic acid bacteria in athletes. The aim of this study was to determine whether the administration of commercially prepared probiotics could influence haemoglobin concentration and other haematological parameters in moderately active males. Fifty healthy, moderately active male volunteers were divided into two groups (Test Group and Control Group). A randomized, double-blind, placebo-controlled, pre-test/post-test, group comparison study was done. The study showed no significant differences ($p \geq 0.05$) between the two groups on any of the dependent measurements. There were statistically significant changes ($p \leq 0.05$) between pre- and post-test results within the two groups. These changes were within physiological limits. Sodium increased significantly ($p = 0.001$) from 139.29 mmol/L to 140.96 mmol/L in the Control Group. In the Test Group sodium increased significantly ($p = 0.035$) from 139.60 mmol/L to 140.90 mmol/L, and potassium decreased ($p = 0.010$) from 4.67 mmol/L to 4.44 mmol/L. Study results thus indicated that the ingestion of a lactic acid bacteria preparation for 42 days did not increase the haemoglobin concentration in moderately active males.

Keywords: Lactic acid bacteria, athletic performance, ergogenic aids, VO_2 max.

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Introduction

Although numerous scientific studies have been conducted on probiotic supplementation, their use has not been largely applied in medicine (Joint FAO/WHO Expert Consultation, 2001). The ongoing scientific debate on the rationale for using probiotics medicinally has, however, promoted their therapeutic use over the last few years (Joint FAO/WHO Expert Consultation, 2001).

Probiotics are defined by Guarner and Schaafsma (1998) as live micro-organisms which are potentially beneficial to the health of the host when ingested in adequate quantities. The lactic acid bacteria (LAB), *lactobacillus* and *bifidobacteria*, are commonly used as probiotics (Joint FAO/WHO Expert Consultation, 2001). These live organisms have displayed the ability to be beneficial to the human host and do not exhibit any pathogenic properties (Aguirre & Collins, 1993). The natural occurrence of these products has added to their popularity. Reports from clinical and pharmacological studies have indicated that probiotics support anti-diarrhoeal action - both prophylactically and therapeutically (Arvola, Laiho, Torkkeli, Mykkänen, Salminen, Maunula & Isolauri, 1999). They have anti-carcinogenic effects (Hosada, Hashimoto, He, Morita & Hosono, 1996), improve mucosal immunity (Perdigon, Vintini, Alvarez, Medina & Medici, 1999), have a therapeutic effect on vascular ischaemia and lipid lowering properties (Bukowska, Pieczul-Mroz, Jastrzebska, Chelstowski & Naruszewicz, 1998), (Joint FAO/WHO Expert Consultation, 2001).

Although generally considered safe for human consumption (Naidu, Biblack & Clemens, 1999), the use of probiotics is based on the premise that they exist as living micro-organisms. The viability of these micro-organisms is often hindered by the preparation and production process that tends to diminish their survival during manufacture (Cortizo, 1999). Progress in this regard was made by scientists who managed to allow the manufactured probiotics to remain viable and functional for supplemental use (Ohhira & Nakae, 1987).

Until recently, probiotics were considered to offer an indirect effect on athletic performance, for example, when used to reduce gastro-intestinal disturbances and in the treatment of upper respiratory tract infections. The use of probiotics as direct ergogenic aids in athletes is currently under scrutiny (Ohhira, Kawasaki, Araki, Inokihara, Matsubara & Iwasaki, 1997). The Australian Institute of Sport has categorised probiotics as Group B supplements due to the lack of evidence supporting efficacy (Lazic, Dikic, Radivojevic, Mazic, Radovanovic, Mitrovic, Lazic, Zivanic & Suzic, 2009). This classification was adopted by the American College of Sports Medicine, the American Dietetic Association and the Dieticians of Canada. This concept may be relevant given the multifaceted nature of athletic training and performance. Ohhira *et al.* (1997) conducted a two-week study to emphasize the ergogenic effects of LAB and concluded that the most significant contribution of LAB to maximum oxygen consumption is attributable to haemoglobin synthesis. This supposition was supported by the 11, 6% increase in the haemoglobin value in subjects taking LAB daily over the two weeks (Ohhira *et al.*, 1997).

Should this lead to an improvement in athletic performance, even the most prudent athlete would consider the use of probiotics as a dietary supplement. With this in mind, the purpose of this study was to investigate the influence of LAB on selected

performance-related blood markers over a six-week period (de Roos, Schouten & Katan, 1998).

Methods and Material

A randomized, double-blind, placebo-controlled, pre-test/post-test, group comparison study was performed whereby the participants were equally but randomly divided into a test group (TG) and a control group (CG) (Joint FAO/WHO Expert Consultation, 2001). The sample consisted of fifty moderately active male student volunteers (aged between 18 and 30 years) from the University of Pretoria, South Africa. Being moderately active was defined by the American College of Sports Medicine (ACSM) and the Centre for Disease Control and Prevention (CDC) as performing a physical activity at 3.0 to 5.9 times the intensity of rest for 30 minutes on 5 days of the week (Haskell Lee, Pate, Powell, Blair, Franklin, Macera, Heath & Bauman, 2007).

Volunteers who did not comply with the activity criteria, as well as those indicating a history of cardiovascular, hepatic, respiratory, or renal impairment, were excluded from the study. Subjects using medication and other nutritional supplementation within the six weeks prior to the commencement of the study, as well as those reporting an illness within seven days prior to the trial, were also excluded.

The research protocol was approved by the Research Proposal and Ethics Committee of the Faculty of Health Sciences, University of Pretoria. An initial pre-study orientation and screening session was held to ensure compliance with criteria for participation in the study. This included a basic medical history and information regarding physical activity patterns and use of nutritional supplements. The exclusion criteria were enforced and the selected volunteers were thoroughly informed about the benefits and risks associated with the study. Informed written consent was obtained thereafter. The trial was conducted in compliance with the protocol, good clinical practice guidelines and the applicable regulatory requirement(s). The blood tests were done at the onset of the experiment (pre-test) and after completion (post-test).

The CG received three placebo capsules in the morning on an empty stomach with a glass of filtered water. The same procedure was followed for the TG, using the proposed supplement. The product used contained *bifidobacteria* and *lactobacillus* strains, as well as certain vitamins, minerals and amino acids (Kawakami, Ohhira, Araki, Inokihara, Matsubara & Iwasaki, 2003). At the start of the trial the subjects were provided with a logbook in which they had to record the time of treatment ingestion and the occurrence of any side-effects (e.g. nausea, headache, muscle cramps) and/or flu-like symptoms experienced. .

The participants were required to rest and refrain from strenuous activity at least 24 hours before the first day of the test, and to fast for at least 8 -12 hours prior to giving blood samples. Requisition forms were completed prior to the taking of samples which were drawn under sterile conditions using the median cubital vein in either the right or left cubital fossa. The necessary volumes were collected as per laboratory protocol (Ampath Laboratories, 2009). An appropriate technique for the disposal of sharps was employed. The haematological parameters measured were haemoglobin, leucocyte count, urea and electrolytes, uric acid/urate, lactate and lipid profile. The blood lactate samples were placed on ice, while the other blood samples were stored at room temperature upon delivery to the pathology laboratories for testing. Ampath laboratories in Pretoria, South Africa, carried out the biochemical assays. The same procedure was followed after 42 days.

Data analysis was done using the Statistical Package for the Social Sciences version 17.0. Only complete records were analysed, thus the base size differs for the different types of tests depending on the number of participants that completed the test. The mean, range and standard deviations were calculated. The Mann-Whitney-U Test was used to determine significant differences between the TG and CG on all variables measured. The Wilcoxon Signed Ranks Test was employed to determine whether statistically significant differences existed between the pre- and post-tests scores obtained for all variables measured within the same group ($p \leq 0.05$).

Results

No statistically significant differences were found between the TG and CG on any pre-test or post-test measurements (Table 1 and 2, Figure 3 and 4). However, statistically significant changes between pre- and post-test scores did occur within the two groups. The CG showed a statistically significant increase ($p=0,001$) in Na^+ -test scores from pre-test to post-test (Figure 1). The TG also showed a significant increase on Na^+ ($p=0,035$), as well as a significant decrease ($p=0,010$) in K^+ -test scores from pre-test to post-test (Figure 2).

Discussion

Athletes are constantly seeking ways to improve performance and gain the advantage over other competitors. Ergogenic aids are used to enhance performance (Morgan, 1972). The current strict anti-doping legislation has discouraged the use of inhibited substances amongst competitive elite athletes somewhat, while natural means to enhance performance has become more popular, even amongst non-competitive athletes.

Table 1: Descriptive statistics of blood test results: Control Group (CG)

Descriptive Statistics						
Variables	Units	n	Minimum	Maximum	Mean	Std. Deviation
Hb pre-test	g/dl	25	15.4	18.1	16.952	0.7627
Hb post-test	g/dl	23	14.7	19.2	16.961	1.0845
K pre-test	mmol/L	24	4.1	4.9	4.483	0.2220
K post-test	mmol/L	23	4.1	5.0	4.487	0.2160
Na Pre-test	mmol/L	24	137	143	139.29	1.681
Na post-test	mmol/L	23	138	143	140.96	1.745
Cl pre-test	mmol/L	24	97	105	101.63	2.143
Cl post-test	mmol/L	23	99	106	102.48	1.729
Urea pre-test	mmol/L	24	4	8	5.64	0.995
Urea post-test	mmol/L	23	2.7	8.0	5.757	1.1847
Creatinine pre-test	mmol/L	24	86	122	96.54	8.361
Creatinine post-test	mmol/L	23	77	120	93.09	9.922
Urate pre-test	mmol/L	25	0.26	0.49	0.3748	0.05394
Urate post-test	mmol/L	23	0.25	0.50	0.3652	0.05999
Cholesterol pre-test	mmol/L	25	2.6	5.9	4.172	0.7935
Cholesterol post-test	mmol/L	23	2.8	5.7	4.057	0.8923
Triglycer Pre-test	mmol/L	25	0.26	1.89	1.0368	0.49649
Triglycer Post-test	mmol/L	23	0.43	2.24	1.1291	0.50810
HDL pre-test	mmol/L	25	0.7	1.7	1.300	0.2533
HDL post-test	mmol/L	23	0.7	1.7	1.274	0.2179
LDL pre-test	mmol/L	25	1.5	4.1	2.612	0.6760
LDL post-test	mmol/L	23	1.5	3.7	2.543	0.7044
Lactic Acid pre-test	mmol/L	25	0.57	2.34	1.0112	0.38001
Lactic Acid post-test	mmol/L	22	0.61	3.00	1.1782	0.58136
WCC pre-test	$\times 10^9/L$	25	3.85	9.96	6.7372	1.36503
WCC post-test	$\times 10^9/L$	23	4.59	9.29	7.0039	1.37226
Valid n (list wise)		21				

As oxygen is transported via haemoglobin in the red blood cells, it would be reasonable to argue that an increase in the haemoglobin value could infer a greater capacity for oxygen delivery and subsequent utilisation. Since the oxygen content of blood is directly proportional to the haemoglobin concentration (Powers & Howley, 2009), a reciprocal improvement in aerobic performance may be expected. Ekblom (2000) confirmed that an acute increase in the athlete's haemoglobin concentration is more than likely to improve $VO_2\max$, resulting in an improvement in athletic performance. However, an increase in haemoglobin during exhaustive exercise may

also be due to haemoconcentration resulting from an extra-cellular fluid shift secondary to capillary hydrostatic pressure increase and the osmotic activity of metabolic by-products (McArdle, Katch & Katch, 2010). Any increase in blood haemoglobin concentration, haematocrit and red blood cell counts must be supported by a reciprocal increase in mean cell volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) to fully demonstrate extra-medullary erythropoiesis (Cordova, Sainz, Cuervas-Mons, Tur & Pons, 2010).

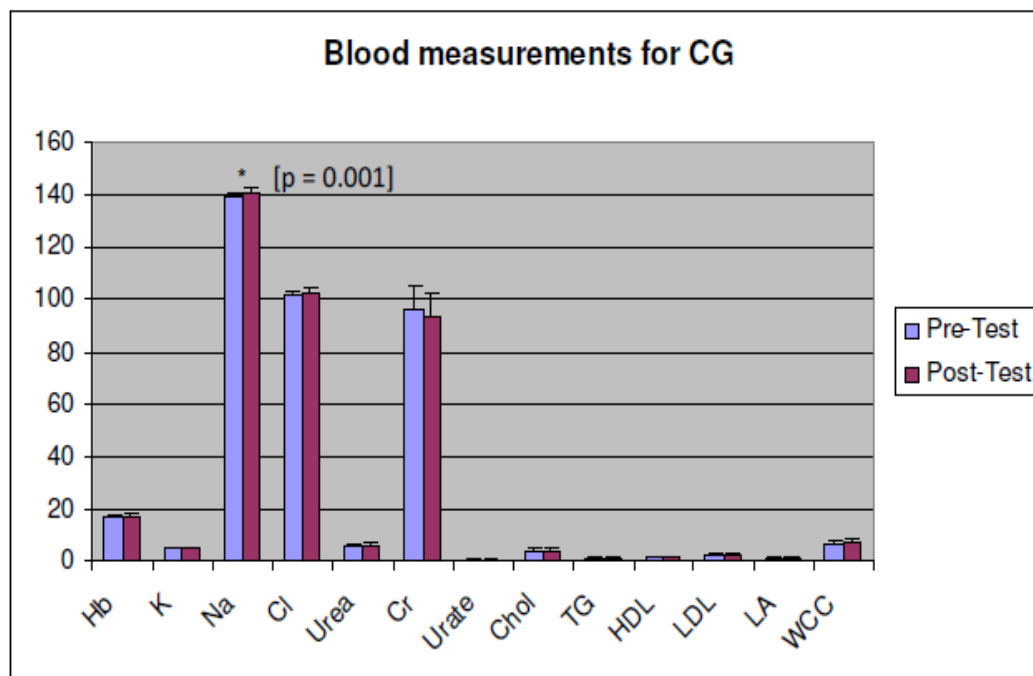


Figure 1: Pre- and Post-test scores of blood parameters measured in the Control Group (CG); sodium test scores were significantly different within the CG but were within physiological limits

The study by Ohhira *et al.* (1997) reported an 11, 6% increase in the haemoglobin value in selected athletes. Previous studies also reported a significant increase in the volume of haemoglobin in red blood cells following the ingestion of LAB (Kawakami *et al.*, 2003). This was postulated as a reason for the athletes' improved oxygen consumption values and athletic performance (Ohhira *et al.*, 1997). An increase in haemoglobin scores have also been reported in animal studies (Cetin, Guclu & Cetin, 2005). However, the present study provided no significant differences in haemoglobin scores between the two groups.

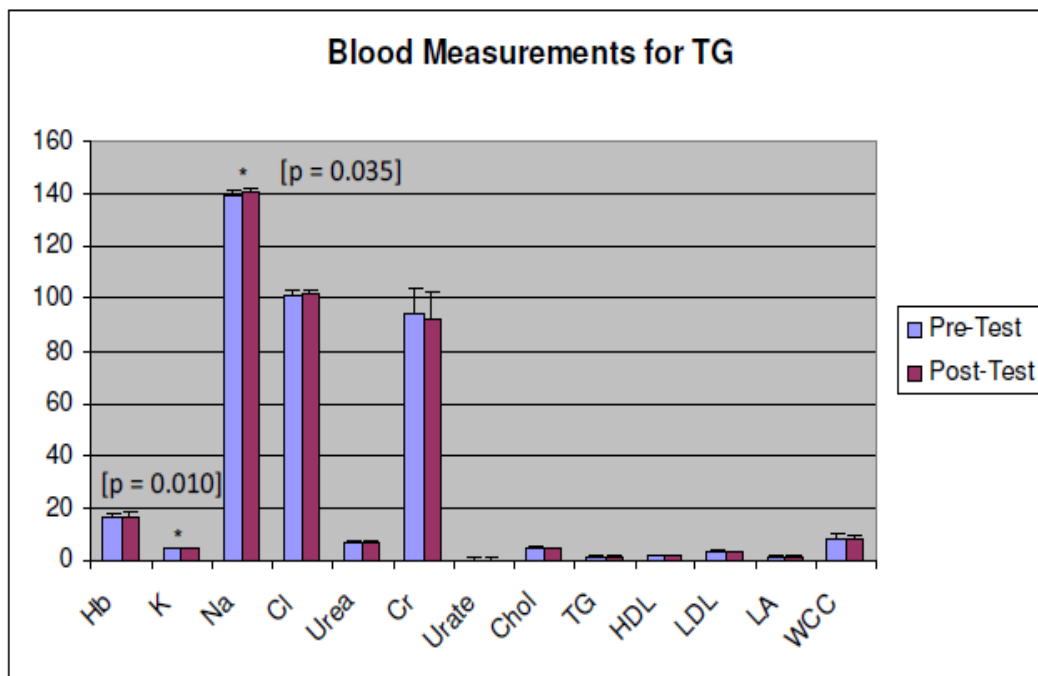


Figure 2: Pre-and post-test scores of blood parameters measured in the Test Group (TG); significant differences were found within the group. This was within physiological limits for that entity

Red blood cell production (erythropoiesis) occurs primarily in the red bone marrow of adults due to the action of the erythropoietin hormone secondary to hypoxia. Erythropoietin is present mainly in the kidneys; however, a small portion of this hormone is also found in the liver. Although extramedullary erythropoiesis occurs in the liver and spleen during foetal development, such production in adults is usually limited to disease states involving bone (Barret, Barman, Boitano & Brooks, 2010). The ability of these matured red blood cells to carry oxygen is made possible by the presence of haemoglobin. This protein molecule is made up of 2 important parts – the globin portion and the iron-containing porphyrin part. Whilst there are factors that govern the production of red blood cells, haemoglobin production requires other factors. These include the presence of amino acids necessary for globin formation, minerals such as iron, copper, cobalt and nickel, as well as vitamins, including vitamin C, riboflavin, nicotinic acid and pyridoxine (Sembulingam & Sembulingam, 2006)

One cannot consider the presence of haemoglobin as an isolated entity: it is an important part of the red blood cell and the absence of either renders both ineffective. The red blood cell is the vehicle that allows the haemoglobin molecule to perform its function. It is also prudent to surmise that haemoglobin production must precede red blood cell synthesis and that the normal process of erythropoiesis remains.

Table 2: Descriptive statistics of blood test results: Test Group (TG)

Variable	Units	n	Minimum	Maximum	Mean	Std. Deviation
Hb pre-test	g/dl	25	15.4	18.8	17.004	0.7689
Hb post-test	g/dl	21	15.1	19.8	17.057	1.1360
K pre-test	mmol/L	25	4.2	5.6	4.672	0.3506
K post-test	mmol/L	23	4.0	4.9	4.435	0.2228
Na Pre-test	mmol/L	25	135	143	139.60	2.160
Na post-test	mmol/L	23	138	144	140.91	1.593
Cl pre-test	mmol/L	25	97	105	101.28	2.227
Cl post-test	mmol/L	23	99	104	101.83	1.267
Urea pre-test	mmol/L	25	5	9	6.18	1.328
Urea post-test	mmol/L	23	4.6	8.9	6.170	1.1929
Creatinine pre-test	mmol/L	25	82	123	94.28	9.889
Creatinine post-test	mmol/L	23	73	114	92.00	10.737
Urate pre-test	mmol/L	25	0.26	0.45	0.3776	0.05182
Urate post-test	mmol/L	23	0.25	0.49	0.3870	0.05217
Cholesterol pre-test	mmol/L	25	3.2	6.2	4.564	0.8276
Cholesterol post-test	mmol/L	23	3.2	6.0	4.478	0.6424
Triglycer Pre-test	mmol/L	25	0.44	2.18	1.0952	0.39464
Triglycer Post-test	mmol/L	23	0.49	3.03	1.1657	0.61157
HDL pre-test	mmol/L	25	0.8	1.9	1.268	0.2462
HDL post-test	mmol/L	23	0.9	1.9	1.313	0.2455
LDL pre-test	mmol/L	25	2.1	4.5	3.048	0.7001
LDL post-test	mmol/L	23	2.0	4.1	2.939	0.5639
Lactic Acid pre-test	mmol/L	24	0.10	2.32	0.9104	0.56774
Lactic Acid post-test	mmol/L	21	0.10	3.15	0.9990	0.57081
WCC pre-test	$\times 10^9/L$	25	4.68	13.10	7.7500	2.20909
WCC post-test	$\times 10^9/L$	21	4.21	13.83	7.6562	2.14057
Valid n (list wise)		17				

Ohhira *et al.* (1997) attributes the increase of haemoglobin levels seen in their study to the production of new red blood cells. The authors maintain that this early erythrocyte production was potentially due to extra-medullary synthesis promoted by the presence of amino acids, vitamins and minerals available in the probiotic formulation prescribed. These important ingredients are made available to the liver by the ability of LAB to aid enteric absorption. Theoretically, this is plausible, given that the ingredients required for both red blood cell and haemoglobin synthesis was available. The stimulus for the production of erythropoietin would be the possible hypoxia during exhaustive exercise (Ohhira *et al.*, 1997). With this in mind, one would expect the presence of reticulocytes and other products of red blood cell production to at least postulate that early erythropoiesis did indeed take place, secondary to the administration of LAB.

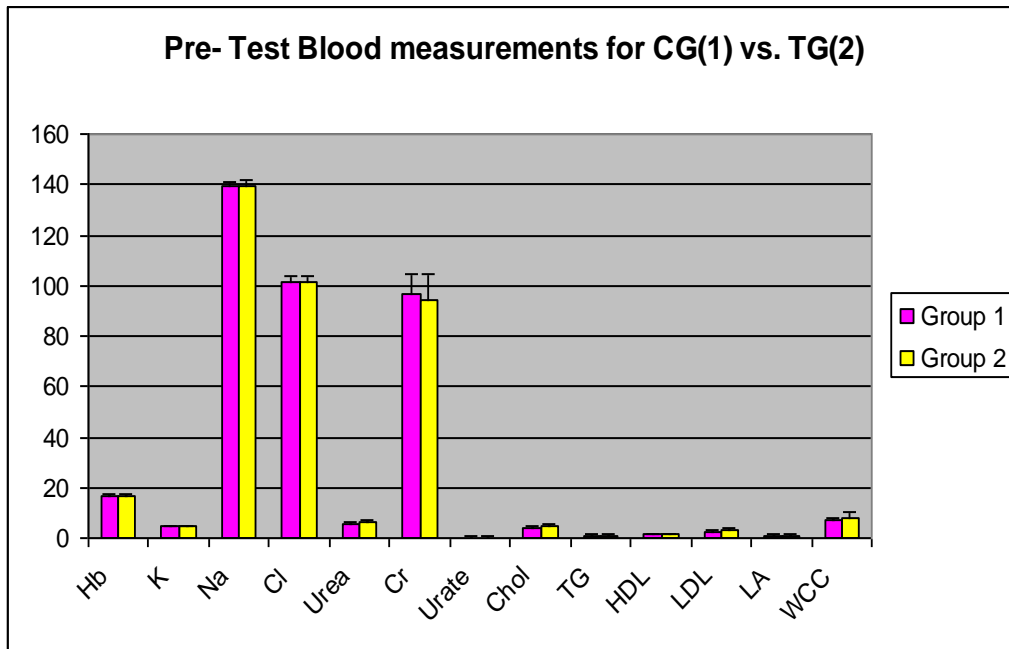


Figure 3: Pre-Test Blood measurements for CG versus the TG; no statistically significant differences were evident ($p \geq 0.05$)

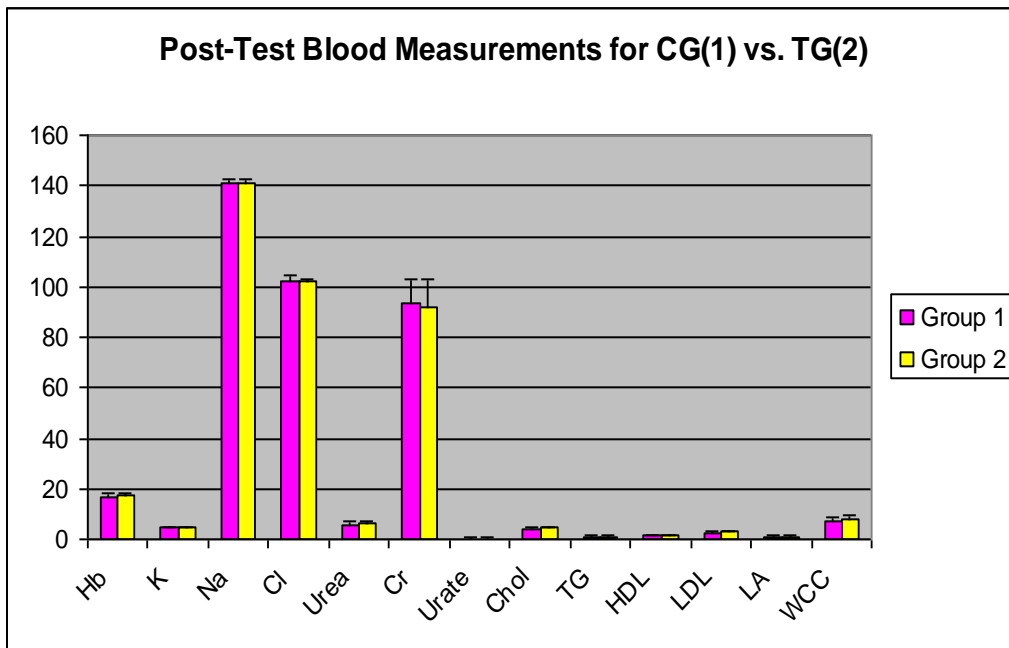


Figure 4: Post-test blood measurements for CG versus TG; no significant post-test differences were found between the CG and TG ($p \geq 0.05$)

The absence of detail regarding a change in MCV, MCHC and MCH makes it difficult to postulate that extra-medullary erythropoiesis is the probable cause of the haemoglobin increase: the effect of haemoconcentration cannot be excluded in this regard (Cordova *et al.*, 2010).

Conclusion

The lactic acid bacteria administered over a six-week period did not affect the haematological parameters investigated in moderately active males. The performance-enhancing variables alluded to, notably haemoglobin concentration, were not altered in this study. Previous studies (Ohhira *et al.*, 1997; Kawakami *et al.*, 2003) reported an increase in haemoglobin levels following the administration of lactic acid bacteria. The absence of information regarding the results of the athlete controls, as well as other important investigations such as a full blood count highlighting the mean cell volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration, makes it difficult to exclude another reasonable rationale for the haemoglobin increase. The effects of haemoconcentration cannot be excluded. The need for a placebo-controlled, double-blind study measuring the above blood parameters in endurance athletes undergoing maximum exercise is strongly advised.

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