Introduction

The primary biological role of nucleotides is to form the backbone of nucleic acids, such as DNA and RNA (Ostojic, Idrizovic & Stojanovic. 2013). As such, nucleotides also aid in regulation of energy metabolism, cellular signaling and protein homeostasis (Carver & Walker. 1995; Grimble & Westwood. 2001; Hess & Greenberg. 2012). Nucleotides are important for growth and development of cells with a rapid turnover rate, such as those in the immune or gastrointestinal system (Dancey, Attree & Brown. 2006; Grimble & Westwood 2001). However, under certain circumstances (I.e. insufficient dietary intake, presence of disease, high levels of growth and repair are necessary) nucleotides synthesized endogenously may be insufficient to meet the demands of cells with a high turnover rate (Gil. 2002). As such, the use of exogenous nucleotides may be warranted to maintain immune function, cell growth and repair (Carver & Walker. 1995; Gil. 2002; Hess & Greenberg. 2012). High intensity exercise temporarily attenuates immune cell function and results in very high recovery demands. Additionally, exercise-induced increases in specific metabolic demands and responses, as well as muscle damage may attenuate subsequent exercise performance. Therefore, exogenous nucleotide supplementation and its effect on recovery is of growing interest.

Recent research has demonstrated greater levels of serum and salivary immunoglobin after acute exercise when supplementing with exogenous nucleotides (McNaughton et al. 2006; McNaughton et al. 2007; Ostojic & Obrenovic. 2012; Ostojic & Obrenovic. 2013). Therefore, suggesting an attenuation of transient immunosuppression after exercise. Furthermore, several researchers have shown a similar decrease in cortisol response to both endurance and resistance exercise, which may explain some of the immunosuppression (McNaughton et al. 2006; McNaughton et al. 2007; Sterczlala et al. 2016). As cortisol can increase protein degradation and inhibit protein synthesis (Kraemer & Ratamess. 2005), it is possible that elevated cortisol may attenuate recovery efforts post high-intensity exercise.

Muscle damage is a common characteristic of unaccustomed high-intensity exercise and can attenuate subsequent athletic performance (Aoi et al. 2004; Hoffman et al. 2004) It has been suggested that nucleotide supplementation improves recovery indirectly through attenuating exercise-induced muscle damage (Sterczala et al. 2016). For instance, leakage of muscle proteins or creatine kinase (CK) into the blood stream is widely used as an indirect marker of muscle damage (Damas et al. 2017). Sterczlala et al. (2016) demonstrated that creatine kinase (CK) values were significantly lower in the nucleotide-supplemented groups 24 hours post a muscle-damaging resistance training protocol and remained lower than the controls for 72 hours. In contrast, McNaughton et al. (2007). Reported no effect on nucleotide supplementation and subsequent post-exercise CK response. However, the latter only assessed CK levels immediately post exercise bout. Thus, differences in findings could be due to the time points at which CK was assessed.

To the authors' knowledge, there is no study to date that has assessed the effects of exogenous nucleotide supplementation on perceptual and hematological recovery parameters. In addition, we seek to determine whether exogenous nucleotide supplementation can reduce decrements in power output during bouts of repeated sprinting.

Methods

Subjects

A total of 10 subjects were recruited by word of mouth, email contact, and website advertisements. The study was completed by 9 subjects as one subject was lost from the sample due to voluntarily withdrawing from the study without adverse effects to supplementation or exercise protocols.

The subject inclusion criteria for study were: males 18-20 years old with a least 3 years of consistent resistance training (i.e. 3 days • week^{-1}), free of musculoskeletal and metabolic disorders, free of cardiovascular disease, no musculoskeletal injuries with the last six months, no history of smoking or drug use, no history of excessive alcohol consumption (e.g. more than 2 drinks per day for men or 1 drink per day for women), not taking prescription medication; have not used a thermogenic-, protein-, amino acid-, or creatine supplement within the last month, have not habitually used caffeine (e.g. more than 2 cups of coffee per day). Table 1 contains subjects characteristics.

Table 1. Subject Characteristics.

Age (years)	26.50 ± 3.59
Height (cm)	177.17 ± 7.41
Total Mass (kg)	90.55 ± 7.50
BMC (kg)	3.079 ± .543
Fat Mass (kg)	16.20 ± 2.89
Lean Mass (kg)	71.27 ± 7.12
Lean + BMC (kg)	74.35 ± 7.45
Fat (%)	17.9 ± 3.4

Data are mean ± SD. BMC = bone mineral content.

Wingate Cycle Ergometer

Absolute anaerobic peak power (PP), relative anaerobic peak power (RP), and power fatigue (the difference between peak and minimum power as a percentage [%%FT]) was assessed via Monark Wingate cycle ergometry (Monark™, Model 894E, Vansbro, Sweden). During the cycling test, the volunteer was instructed to cycle against a predetermined resistance (7.5% of body mass) as fast as possible for 30 seconds. The saddle height was adjusted for each subject in order to produce a 5–10° knee flexion angle while the foot was in the low position of the central void. A standardized verbal stimulus was provided to the participant. Power measures were recorded in real time by a computer directly connected to the Monark standard cycle ergometer during each bout. Power measures were calculated using Monark Anaerobic test software (Monark Anaerobic Wingate Software, Version 1.0, Monark, Vansbro, Sweden). All subjects were familiarized with the operating mechanics and testing on the ergometer prior to the experimental period.

Perceptual Measures

The perceptual measures collected during the study in perceived recovery status scale (PRS) and rating of perceived exertion (RPE). PRS was collected in a manner similar to Laurent et al

(2011). The PRS scale consisted of a scalar representation numbering from 0-10. Visual descriptors of "very poorly recovered", "adequately recovered" and "very well recovered" for perceived recovery were presented at numbers 0, 5, and 10, respectively. RPE was collected using a traditional Borg Scale (index of 6-20). The Borg category scale (4) is designed to describe perceptions of physical exertion during a wide range of exercise modes. The scale consists of numbered categories, 6–20, and verbal cues, from "very, very light" to "very, very hard" (Borg, 1982).

Blood Measures

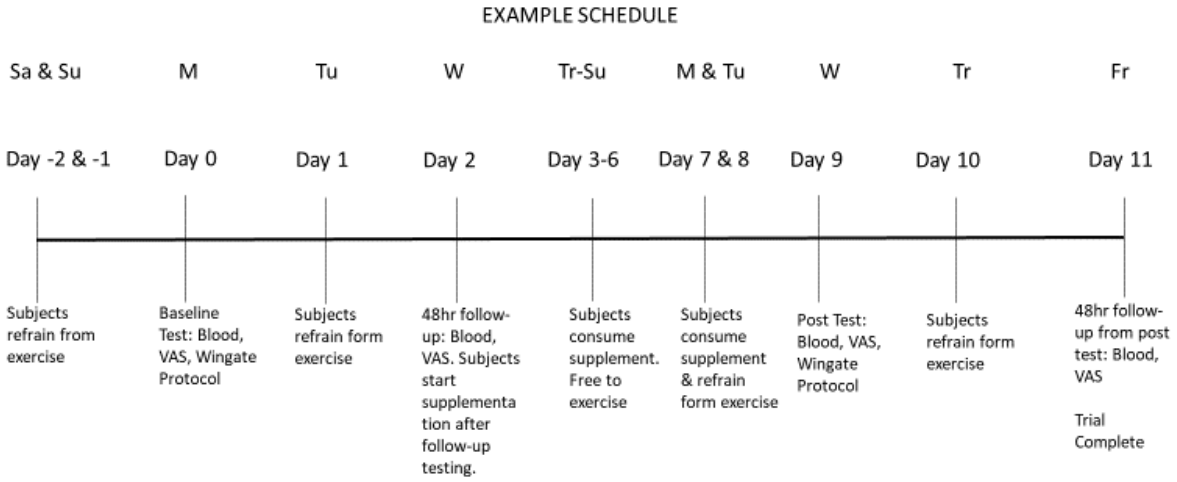
Blood draws were obtained via standard venipuncture procedures by a trained phlebotomist following a 10-hour fast. All subjects submitted a blood sample for analysis in the morning to control for diurnal variations. Whole blood was collected, transferred into appropriate tubes for obtaining serum and plasma, and subsequently centrifuged at 1,500 g for 15 min at 4°C. Resulting serum and plasma were then aliquoted and stored at -80°C until analysis. All hematological variables were measured in the same assay on the same day to avoid compounded inter-assay variance. Variables measured include immunoglobulin A (IgA), immunoglobulin M (IgM), immunoglobulin G (IgG), cortisol (COR), creatine kinase (CK), and blood urea nitrogen (BUN). Blood data are reported as mean and standard error of the mean.

Testing Protocol

Upon arriving to the laboratory following a 10-hour overnight fast, subjects checked in with a research technician for pre-testing. Perceptual measures (described above) were collect immediately following check-in and subjects then donated approximately 10mL of whole blood. Thereafter, subjects were properly fitted with a BioModule for physiological monitoring according to manufacturer instructions. Once subjects remained seated for approximately 5 minutes to allow for BioModule data initialization, they were instructed to proceed to a standardized general warm-up where they pedaled against a 1kg resistance on the cycle ergometer for 3 minutes. Following the warm-up, a one-minute rest period was allotted and RPE was collected prior to the initiation of the exercise protocol which consisted of four, 30 Wingate sprints separated by a 2-minute rest period. Following the second sprint, RPE was collected. Upon completing the exercise protocol perceptual measures were collected and power data was recorded. Forty-eight hours after completion of the exercise protocol, subjects returned to the laboratory to donate a blood sample, assess PRS, and receive the supplement.

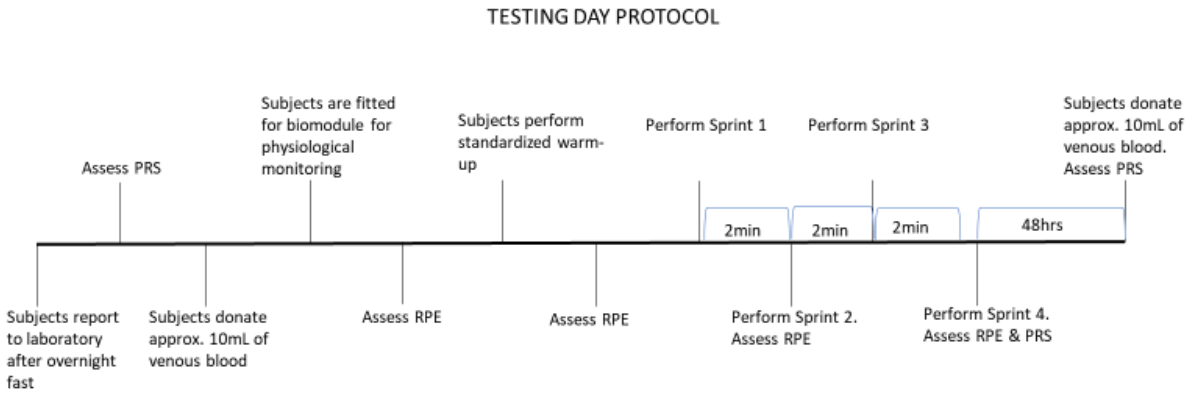
Subjects were instructed to consume two capsules totaling 500mg of nucleotide supplementation with their first meal of the day for the next seven days. Following the supplementation period (i.e. 7 days), subjects return to the laboratory following a 10 hour overnight fast to repeat the testing protocol (post-testing). Following the completion of post-testing, subjects returned to the laboratory 48 hours later to donate a blood sample and assess PRS.

Figure 1. Study Timeline of Events.



VAS = Visual Analogue Scales (RPE and PRS in this study)

Figure 2. Order of Procedures for Testing Day Protocol.



Statistical Analysis

Before carrying out the parametric statistical analysis, dependent variables were examined for a normal distribution and outliers through investigation of boxplots and a normality test (i.e. Shapiro Wilk). No outliers were detected, and data pasted normality testing ($p>0.05$). One-way repeated measures analysis of variance (ANOVA) was used to scrutinize the effects of supplementation on dependent variables (GraphPad Prism 7®, La Jolla, CA). Whenever a significant F-value was obtained, a Tukey post-hoc test was performed for multiple comparisons purposes. In addition, absolute delta change were calculated on select variables and were analyzed with paired t-tests. The significance was previously set at $p\leq0.05$. Results are expressed as mean ± standard deviation unless otherwise stated.

Results

Wingate Cycle Ergometer

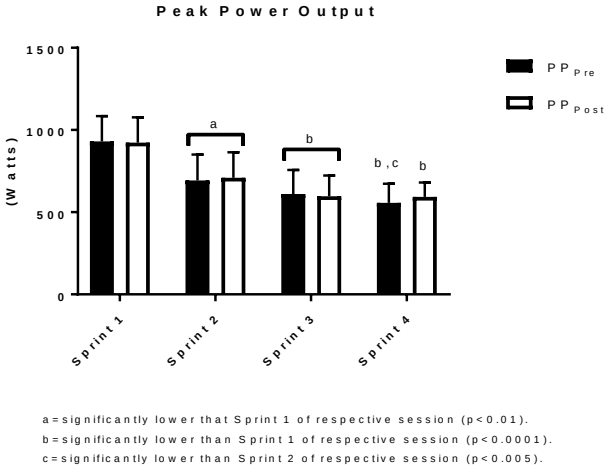
There were no between-session (pre to post) differences for PP, RP, or %FT ($p>0.05$). In regards to within-session differences for power measures, PP (Figure 3) and RP (Figure 5) was significantly lower on Sprint 2, Sprint 3, and Sprint 4 relatively to Sprint 1 in each respective testing session (Sprint 1 vs. Sprint 2: $p<0.01$; Sprint 1 vs. Sprint 3: $p<0.0001$; Sprint 1 vs. Sprint 4: $p<0.0001$). However, PP and RP on Sprint 4_{pre} was significantly lower than Sprint 2_{pre} ($p<0.005$), whereas PP and RP on Sprint 4_{post} was not significantly less than Sprint 2_{post} or Sprint 3_{post} ($p>0.05$). When considering the absolute mean difference between sessions (pre to post), PP and RP was significantly lower from Sprint 3_{pre} to Sprint 4_{pre} when compared to Sprint 3_{post} to Sprint 4_{post} ($p<0.02$; Figure 4). Significant within-session differences of %FT (Figure 6) were demonstrated at pre-testing whereby Sprint 3_{pre} and Sprint 4_{pre} were greater than Sprint 1_{pre} and Sprint 2_{pre} ($p<0.05$). There were no significant within-session differences at post-testing for %FT ($p>0.05$). Raw data for power measures are presented in Table 2.

Table 2. Wingate Power Measures.

	PP (W)	RP (W • kg^{-1})	%FT
Sprint 1_{Pre}	930.99 ± 153.88	10.41 ± 1.22	63.58 ± 4.86
Sprint 2_{Pre}	699.91 ± 140.82 [a]	7.81 ± 1.21[a]	63.92 ± 8.61
Sprint 3_{Pre}	610.4 ± 146.81[b]	6.81 ± 1.39[b]	70.51 ± 8.64[d]
Sprint 4_{Pre}	557.28 ± 116.27[b,c]	6.23 ± 1.07[b,c]	69.91 ± 7.95[d]
Sprint 1_{Post}	923.14 ± 153.67	10.32 ± 1.07	66.12 ± 4.96
Sprint 2_{Post}	708.93 ± 155.46 [a]	7.9 ± 1.27[a]	63.09 ± 5.89
Sprint 3_{Post}	597.44 ± 125.67[b]	6.69 ± 1.18[b]	67.69 ± 8.10
Sprint 4_{Post}	593.22 ± 86.92[b]	6.68 ± 0.96[b]	68.33 ± 8.66

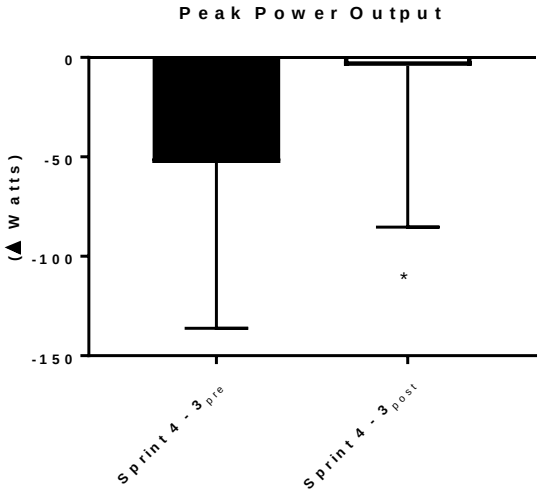
Data are mean ± SD. a=significantly lower that Sprint 1 of respective session ($p<0.01$). b=significantly lower than Sprint 1 of respective session ($p<0.0001$). c=significantly lower than Sprint 2 of respective session ($p<0.005$). d=significantly greater than Sprint 1 and Sprint 2 of respective session ($p<0.05$).

Figure 3. Peak Power Output (W) for Pre- and Post-Testing Exercise Protocol.



a = significantly lower that Sprint 1 of respective session ($p < 0.01$).
b = significantly lower than Sprint 1 of respective session ($p < 0.0001$).
c = significantly lower than Sprint 2 of respective session ($p < 0.005$).

Figure 4. Delta Change in PP from Sprint 3 to Sprint 4 at Pre- & Post-Testing.



* = significantly lower from pre value ($p < 0.02$)

Figure 5. Relative Peak Power Output (W • kg^{-1}) for Pre- and Post-Testing Exercise Protocol.

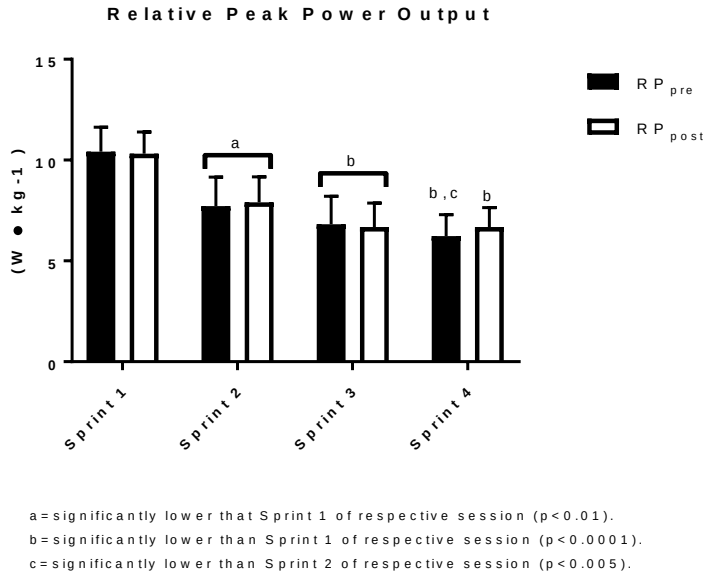
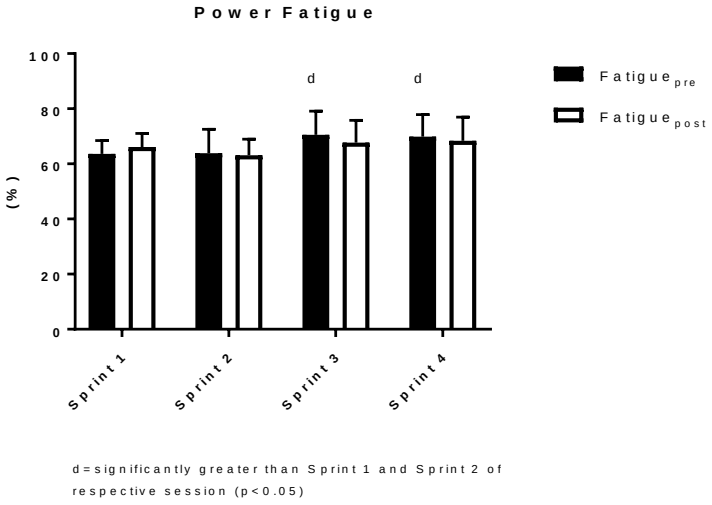


Figure 6. Power Fatigue (%) for Pre- and Post-Testing Exercise Protocol.



Perceptual Measures

There were no significant between-session (pre to post) differences for PRS ($p>0.05$). However, within-session differences did occur for PRS (Figure 7) whereby both pre- and post-testing sessions demonstrated significant decreases from Baseline to Post-Sprint 4 ($p<0.0001$) and significant increases from immediately Post-Sprint 4 to 48 hours Post-Sprint 4 ($p<0.0001$). However, PRS at 48 hours Post-Sprint 4 was significant lower than baseline during the pre-testing (non supplemented) session ($p<0.05$). This was not indicated for the post-testing supplemented session ($p>0.05$). Raw data for PRS is represented in Table 3.

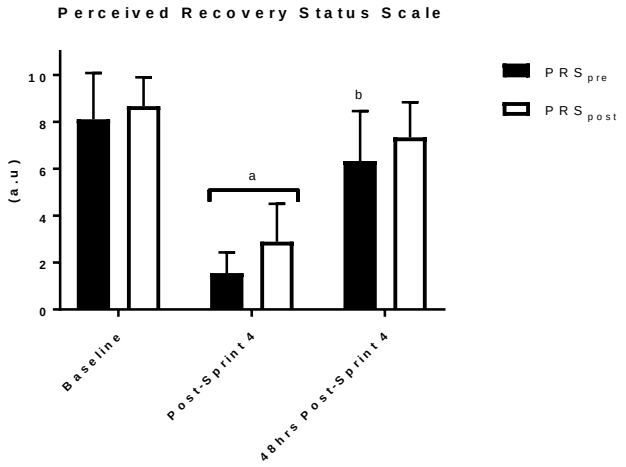
In regards to RPE, there were no significant between-session differences ($p>0.05$). Within-session differences indicated the RPE (Figure 8) increased from Baseline to Post-Warm up ($p<0.05$) to Post-Sprint 2 ($p<0.0001$) to Post-Sprint 4 ($p<0.001$) during pre- and post-testing. Raw data for RPE is represented in Table 4.

Table 3. Perceived Recovery Status Scale (PRS) Data.

	PRS_{Pre}	PRS_{Post}
Baseline	8.11 ± 1.96	8.67 ± 1.22
Post-Sprint 4	1.56 ± 0.88[a]	2.89 ± 1.62[a]
48 hrs Post- Sprint 4	6.33 ± 2.12[b]	7.33 ± 1.50

Data are mean ± SD. a=significantly lower than Baseline and 48 hrs Post-Sprint 4 at respective session ($p<0.0001$). b=significantly lower than Baseline at respective condition ($p<0.05$).

Figure 7. Perceived Recovery Status Scale (a.u.).



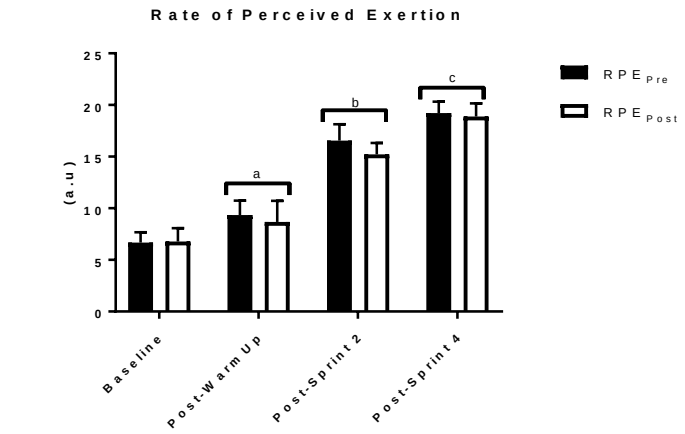
a=significantly lower than Baseline and 48 hrs Post-Sprint 4 at respective session ($p<0.0001$).
b=significantly lower than Baseline at respective condition ($p<0.05$).

Table 4. Rate of Perceived Exertion (RPE) Data.

	RPE_{Pre}	RPE_{Post}
Baseline	6.67 ± 1.00	6.78 ± 1.30
Post-Warm up	9.33 ± 1.41[a]	8.67 ± 2.06[a]
Post-Sprint 2	16.56 ± 1.59[b]	15.22 ± 1.09[b]
Post-Sprint 4	19.22 ± 1.09[c]	18.89 ± 1.27[c]

Data are mean ± SD. a = significantly higher than Baseline of respective session ($p<0.05$). b= significantly higher than Baseline and Post-WarmUp of respective session ($p<0.0001$). c= significantly higher than Baseline, Post-WarmUp, Post-Sprint 2 of respective session ($p<0.001$).

Figure 8. Rate of Perceived Exertion (a.u.)

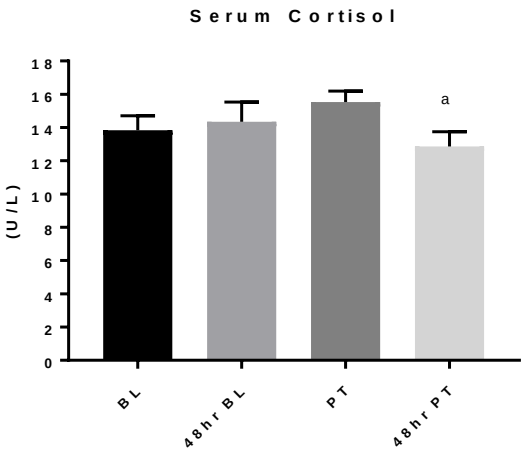


a = significantly higher than Baseline of respective session ($p<0.05$).
b= significantly higher than Baseline and Post-Warm Up of respective session ($p<0.0001$).
c= significantly higher than Baseline, Post-Warm Up, Post-Sprint 2 of respective session ($p<0.001$)

Blood Measures

There were no significant between-session (pre to post) or within-session differences for IgA, IgM, IgG, CK, or BUN detect over the course of the study ($p>0.05$). For Cortisol, a significant within-session difference was noted whereby the 48hr post-test supplemented group (48hr PT) was significantly lower than the baseline (PT) levels ($p<0.005$; Figure 9). It is likely that the anticipation of the exercise pre-test demonstrated high cortisol levels in the baseline non-supplemented group, while the post-test levels were the same due to the stress of the exercise. However, the lower response of cortisol 48hrs post exercise in the supplement condition indicates that the stress of exercise was no longer demonstrated in hormone levels as cortisol had decreased. These values support the perceived recovery values.

Raw data for all blood variables are displayed in Table 5.



a=significantly lower that PT ($p<0.005$).

Table 5. Raw Data for Blood Measures.

	BL	48hr BL	PT	48hr PT
IgA (mg/dL)	192.44 ± 22.00	195.11 ± 21.27	191.44 ± 22.39	194.56 ± 24.16
IgM (mg/dL)	114.67 ± 11.69	116.67 ± 11.88	115.78 ± 12.31	116.56 ± 11.54
IgG (mg/dL)	953.78 ± 59.76	962.56 ± 61.27	979.56 ± 70.86	995.00 ± 65.52
Cortisol (ug/dL)	13.84 ± 0.87	14.34 ± 1.19	15.53 ± 0.66	12.86 ± 0.90[a]
Creatine Kinase (U/L)	709.56 ± 183.40	668.44 ± 224.56	534.67 ± 135.90	457.11 ± 111.32
Blood Urea Nitrogen (mg/dL)	18.44 ± 1.60	18.33 ± 1.92	18.89 ± 1.82	18.89 ± 2.06

Values are reported as mean ± standard error of the mean. BL = baseline; 48hr PL = 48hr after BL testing; PT = post-testing following supplementation period; 48hr PT = 48hr after post-testing; a = significantly lower than PT ($p<0.005$).

Summary

- Supplementing with nucleotides reduced declines in performance at the most difficult parts of interval training (the second half of sprinting).
- Supplementing with nucleotides prevented an increase in % fatigue compare to non- supplemented.
- Supplementing with nucleotides allowed subjects to recover faster than not supplementing.
- Supplementing with nucleotides appeared to reduce the cortisol response to exercise.

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