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Abstract

The aim of this study was to investigate the effects of guarana supplementation on cognitive performance before and after a bout of maximal intensity cycling, and to compare this to an equivalent caffeine dose. Twenty-five participants completed the randomised double-blind crossover trial by performing cognitive tests with 1 of 3 supplements, on 3 different days: guarana (125 mg/kg), caffeine (5 mg/kg) or placebo (65 mg/kg protein powder). After 30-minutes of rest, participants performed simple (SRT) and choice reaction time (CRT) tests, an immediate word recall test and Bond-Lader mood scale. This was followed by a cycling $\dot{V}O_{2\text{max}}$ test, cognitive tests were then immediately repeated. Guarana supplementation decreased CRT before exercise (407 ± 45ms) in comparison to placebo (421 ± 46ms, P=.030) but not caffeine (417 ± 42ms). SRT after exercise decreased following guarana supplementation (306 ± 28ms) in comparison to placebo (323 ± 32ms, P=.003) but not caffeine (315 ± 32ms). Intraindividual variability on CRT significantly improved from before (111.4 ± 60.5ms) to after exercise (81.85 ± 43.1ms) following guarana supplementation, no differences were observed for caffeine and placebo (P>.05). Alertness scores significantly improved following guarana supplementation (63.3 ± 13.8) in comparison to placebo (57.4 ± 13.4, P=.014) but not caffeine (61.2 ± 12.8). There were no changes to $\dot{V}O_{2\text{max}}$, immediate word recall or any other Bond-Lader mood scales. Guarana supplementation appears to impact several parameters of cognition. These results support the use of guarana supplementation to possibly maintain speed of attention immediately following a maximal intensity exercise test ($\dot{V}O_{2\text{max}}$).

Key words: Paullinia Cupana, Reaction time, Alertness, Cognitive Performance, Nutrition
Guarana (Paullinia Cupana) is a native fruit of the Amazonian basin which has been used as a traditional medicine for centuries by indigenous populations (1). The purported biological effects of supplementation are extensive. Reports demonstrate guarana to possess psychoactive and diuretic properties, as well as exhibiting antioxidant and anti-fungal effects (2). Positive stimulating and cognitive effects of guarana have also been reported, with the high caffeine content of its seed (up to 6% dry weight) being the predominant suggested mechanism of action (1). Indeed, caffeine supplementation possesses a strong efficacy for improvements in a variety of cognitive (3) and physical performances (4). The ubiquitous psychostimulant is predominantly metabolised in the liver by the cytochrome P450 enzyme system (5). Cytochrome P450 isoform 1A2 (CRP1A2) mediates the main caffeine demethylation reaction of N-3 demethylation to paraxanthine, which equates up to 90% of caffeine demethylation (5,6). The antagonism of adenosine receptors also modulates the rapid movement across of the blood-brain barrier which consequently augments dopamine concentrations in the brain (6), resulting in heightened alertness, vigilance, attention, and reaction time (3). However, despite the possible ergogenic effects derived from the caffeine content in guarana, reports suggest that the caffeine content is small in typically consumed doses, and in some cases lower than the pharmacological threshold required for humans (7). As such, some studies indicate that additional components may also be the contributing mechanism (1,7,8). Consequently, guarana has become increasingly popular in commercial markets worldwide, particularly in the soft drink and supplementation industry (1,2).

Initial studies conducted by Galduróz and colleagues (9,10) evaluated the acute (1g/day for 3-days) and chronic (1g/day for 150-days) cognitive effects of guarana supplementation in young and elderly individuals, but found no changes to cognition, sleep, or anxiety in either group. No further studies have assessed the long-term effects, but several studies have since reported significant acute cognitive changes following guarana supplementation (7,11-13). Kennedy et al. (2004) found both speed of attention and secondary memory were improved by a 75 mg dose of guarana. The increase in speed of attention is reflected in a decrease of reaction time observed in several other studies (8,11,14,15). Haskell et al. (2007) corroborated that secondary memory was improved with both 75 mg and 37.5 mg of guarana. Notably, the lower dose contained less than 9 mg of caffeine, which is considered less than the threshold for pharmacological activity in humans (7). It is therefore speculated that other pharmacokinetic components of guarana (tannins, saponins, and flavonoids such as catechins and epicatechins; methylxanthines such as theobromine and theophylline) may be working synergistically with caffeine to enhance the psychoactive effects of the supplement (1,7-9,11). Comparing the effects of guarana to caffeine alone may therefore help elucidate the contribution of the other components within the supplement.

Successful performance in many modern sports necessitates an athlete’s capacity to simultaneously control a variety of physiological and cognitive loads. Such sports include Modern Pentathlon whereby optimal cognitive functioning is essential during combined events (laser-run) (16) or team sports where many relevant external attentional cues demand quick and accurate decisions. Though it is recognised that moderate exercise improves cognitive performance, higher/maximal intensities are said to worsen cognitive performance (17). Yet to the best of our knowledge, only four studies so far have considered how physical activity may
alter the cognitive effects of guarana supplementation, all of which have used moderate
intensity exercise (60% $\dot{V}O_{2}\text{max}$/peak power and/or RPE 13/20) for 30-40 minutes (6,8,13,16).
An interaction between physical activity and guarana supplementation was reported in only
one of the studies, where mean reaction time decreased following guarana supplementation in
pentathletes after running at an RPE of 13 on the Borg scale (16). Moreover, only one study
reported any significant changes to cognition following guarana supplementation alone, both a
decreased reaction time and stable autonomic nervous system (heart rate variability) were
reported (8). It is therefore difficult to determine whether the absence of significant changes
were the result of combining physical activity and guarana, or due to other extenuating factors.

It is apparent that guarana supplementation affects several areas of cognition which could be
highly appealing to athletes who compete in sports that rely heavily on accurate and efficient
decision-making processes for successful performance. However, thus far, evidence on how
guarana supplementation affects cognitive performance during physical activity is equivocal
and limited, particularly as all previous studies have only considered moderate intensities.
Increasing the intensity of physical activity is likely to alter the associated fatigue and may
therefore interact differently with supplementation compared to moderate intensities of
exercise. Therefore, the aim of this study was to evaluate the effects of guarana
supplementation on cognitive performance, before and after a bout of maximal intensity
exercise. It was hypothesised that guarana supplementation would improve cognitive
performance in comparison to caffeine and placebo following a bout of maximal intensity
exercise.

Methods

Subjects

Twenty-seven physically active participants were recruited from the local university and
cycling clubs, two dropped out due to injury or personal extenuating circumstances leaving
twenty-five (age: 21 ± 1 years, height: 172.4 ± 15.3 cm, mass: 68.5 ± 12.6 kg). The participants
comprised 18 males and 7 females. Participants were considered physically active if they met
the UK recommended physical activity guidelines (at least 30-minutes of moderate intensity
activity 5-times per week). The mean daily caffeine intake of participants was 122 ± 114 mg.
All participants completed both informed consent and physical activity readiness questionnaire
(PAR-Q) forms prior to participation. This study was approved by the UCL Research Ethics
Committee in line with the Declaration of Helsinki.

Supplementation

Employing a double-blind randomised crossover design, participants attended the exercise
laboratory on 3-separate days, each 1-week apart. Participants were asked not to eat in the 2-
hours before testing, and not to consume caffeine or alcohol in the 24-hours prior to testing. During the intervention participants were also asked to maintain their regular diet and not to consume any supplements. On each visit participants drank a different supplement in a randomised order: caffeine (5 mg/kg) (Caffeine, Loughborough, Fisher Scientific), guarana (125 mg/kg) (Guarana Powder, Brighton, The Guarana Company Ltd.) or placebo (65 mg/kg) (protein powder containing undenatured whey protein isolate and instatising agent). The caffeine dose in both caffeine and guarana supplement is in line with the suggested protocol of use by the International Olympic Committee (4). Supplements were diluted in 250 ml of room temperature water and placed into opaque water bottles by an independent member of staff. To minimise olfactory and taste input, participants were instructed to hold their nose whilst drinking. To enhance the blinding and reduce bias further, participants were initially informed of the broad scope of the study (‘investigating the influences of different supplements on exercise and cognitive function’) but were not informed about the specific supplements and their purported effects. High performance liquid chromatography (HPLC) was performed to determine the caffeine content of the guarana supplement. This was found to be 3.99%. The guarana dose was then calculated to match the caffeine content of the caffeine supplement given. After drinking the supplement, participants relaxed quietly for 30-minutes. Following this, participants completed the first set of cognitive tests (see below for protocol).

A $\dot{V}O_{2\text{max}}$ test was then completed on a cycle ergometer (CareFusion, Vyaire Medical, Basingstoke, United Kingdom) using a ramp protocol of 25-40W per minute based on the participants reported level of physical activity. First, a 3-minute warm up with no resistance (0W) was completed. Thereafter, participants started at 0W and continued until volitional fatigue. The criteria for achieving $\dot{V}O_{2\text{max}}$ were: (1) to attain an RER above 1.1, (2) to achieve a plateau in $\dot{V}O_2$, and (3) heart rate reading ± 10 beats/min of the age-predicted max (18). The $\dot{V}O_{2\text{max}}$ was determined by the highest $\dot{V}O_2$ value that was recorded from the 15-second averages, at this point the peak power output was also recorded. After a 5-minute cool down (consisting of unloaded cycling), cognitive tests were repeated.

Cognitive Tests

All cognitive tests were completed on the Computerised Mental Performance Assessment System (COMPASS) (Northumbria University, Newcastle Upon Tyne, UK) computer software. This included simple reaction time (SRT), choice reaction time (CRT), secondary memory and mood. For SRT and CRT, 50-stimuli were presented on the screen at random intervals of 1-3 ms. Participants were seated 1 meter away from the screen, with their hands placed on the response keys. They were asked to respond as quickly as possible by selecting the appropriate response key (spacebar for SRT, ‘M’ and ‘Z’ keys for CRT), reaction time was recorded in milliseconds (ms). For SRT participants had to hit the spacebar as soon as they saw the stimuli whereas the CRT required a different response depending on the stimulus presented. For example, the screen would show an arrow pointing either left or right. Participants would have to press the ‘Z’ key if the arrow was pointing left, or the ‘M’ key of the arrow was pointing right. The accuracy of responses were recorded as percentage (%). Secondary memory was
tested using an immediate word recall test. Fifteen words were presented, each for one second, after which participants recalled as many words as possible within 60-seconds. The number of words and accuracy of recall were recorded. Mood was assessed using the Bond-Lader mood scales (19). At the beginning of each visit, prior to the first set of cognitive tests and any supplementation, researchers explained and familiarised every participant the above cognitive testing battery with a short familiarisation test designed on the COMPASS software. Thus, dissipating the influence of learning effects (11).

Intraindividual Variability - Inconsistency (IIV-I)

Interindividual variability (IV) refers to short term fluctuations in behaviour which, when measured across trials of a same session, can be used as an accurate measure of inconsistency (IIV-I), defined as trial-to-trial variability from a single person (20). In line with Costa et al. (2009) IIV-I was calculated for SRT and CRT using the standard deviation of item-by-item response times; therefore, each individual’s standard deviation was calculated from the 50-stimuli during the CRT and during the SRT. It has been conceived that inconsistency is determined by higher-order cognitive processes (attentional and executive control) rather than being random noise or result of error variance, (20-22) and is therefore worth comparing between supplements to identify their effect on short term changes in attention.

Statistical Analysis

All variables are expressed as mean ± standard deviation. Statistical analysis was performed using SPSS 26 (IBM, New York, USA). All data sets were analysed for normality using a Shapiro Wilk test. Each outcome measure was analysed using a repeated measures ANOVA (alpha ≤ .05). Where statistically significant differences were identified, a Bonferonni post-hoc test was conducted. If parametric assumptions were not met, Friedmans was employed. Analysis of the effect of daily caffeine intake on performance was analysed using Pearson Correlation Coefficients.

Results

Simple Reaction Time

A significant main effect was detected for SRT between supplements (P = .049, ES = .11, Observed Power = .58). There was no Time effect or Supplement X Time interaction (P > .05). Before exercise, Post Hoc tests revealed no differences for SRT between supplements (P > .05). Whereas, after exercise, Post Hoc tests revealed SRT to be significantly lower following guarana supplementation (306 ± 28ms) in comparison to placebo (323 ± 32ms) (P = .003). No
differences were reported between caffeine (315 ± 32 ms) vs. placebo or caffeine vs. guarana (P > .05) (Figure 1).

Therefore, guarana supplementation significantly reduced SRT after exercise compared to placebo, but not compared to caffeine.

Choice Reaction Time

A significant main effect was detected for CRT between supplements (P = .05, ES = .11, Observed Power = .57). There was no Time effect or Supplement X Time interaction (P > .05).

Post Hoc tests revealed CRT to be significantly lower before exercise following guarana supplementation (407 ± 45 ms) in comparison to placebo (421 ± 46 ms) (P = .030). No differences were reported between caffeine (417 ± 42 ms) vs. placebo or caffeine vs. guarana before exercise (P > .05) (Figure 2). Guarana had the same effect vs. placebo when only the correct responses were selected (P = .018).

After exercise (including when only the correct responses were selected), Post Hoc tests revealed no differences between supplements (P > .05).

There were no differences in response accuracy percentage between placebo, guarana, and caffeine (P > .05).

Therefore, guarana supplementation significantly reduced CRT response time compared to placebo following 30-minutes of rest, but there were no differences between supplements after exercise.

Intraindividual Variability - Inconsistency (IIV-I)

For SRT and CRT IIV-I there was no significant main effect between supplements or Supplement X Time interaction (P>.05).

A significant Time effect was detected in SRT (P = 0.05, ES = .15, Observed Power = .50) where post hoc analysis demonstrated a significant increase in IIV-I from pre to post exercise for placebo (P = 0.007), whereas for guarana and caffeine there were no differences (P>.05).

A significant Time effect was also detected in CRT (P = 0.007, ES = .26, Observed Power = 0.89) where post hoc analysis demonstrated guarana supplementation to significantly decrease IIV-I from pre to post exercise (P = 0.038). No differences were detected in placebo and caffeine, see Table 1.

Therefore, SRT IIV-I increased significantly after exercise with placebo but not under supplementation with caffeine or guarana, and CRT IIV-I decreased significantly after exercise with guarana only.

Memory recall
There was no significant main effect, time effect or Supplement X Time interaction (P > .05) for memory recall between supplements.

**Bond-Lader Mood Scales**

Parametric assumptions were not met for alert scores therefore Friedman’s was employed. Friedman’s reported a significant increase in alert scores following guarana supplementation (63.3 ± 13.8) when compared to placebo (57.4 ± 13.4) following exercise only (P = .014). Kendall’s W reported .097, indicating a small effect size. No differences were reported between caffeine (61.2 ± 12.8) vs. placebo and caffeine vs. guarana (P > .05). There was no significant main effect, time effect or Supplement X Time interaction (P > .05) for all other Bond-Lader Mood Scale variables (content & calm) between supplements.

**Physical performance**

No differences in \( \dot{V}O_{2\text{max}} \) were detected between placebo (52.2 ± 10.7 ml/min/kg), guarana (51.4 ± 10.0 ml/min/kg), and caffeine (51.7 ± 9.0 ml/min/kg). Equally, no differences in peak power output were detected between placebo (300 ± 80 Watts), guarana (299 ± 81 Watts), and caffeine (305 ± 81 Watts).

**Daily Caffeine Intake**

There was no correlation found between daily caffeine intake and performance on any task or supplement before or after physical activity, with the Pearson Correlation Coefficient less than 0.08 for all outcome measures.

**Correlations**

Spearman’s Rho was employed to investigate possible correlations between changes in alert scores and changes in SRT post-exercise (a) and changes in alert scores and changes in CRT IIV-I post-exercise (b). Despite a small trend for (a), the threshold was not met (P = 0.058). Similarly, there was no correlation identified for (b) (P > .05).

**Discussion**

The key novel findings of this study demonstrate that guarana supplementation decreased both SRT (after exercise) and CRT (before exercise) in comparison to placebo, supporting previous literature that guarana increases speed of attention (6-8,11,12,16). The supplementation of guarana also improved CRT IIV-I and alert scores, both after exercise. The protective effect
against cognitive fatigue was further demonstrated by guarana whereby SRT IIV-I was maintained following exercise whilst with placebo it was significantly higher. The improvement in SRT following guarana supplementation is consistent with previous research and further supports the notion that guarana may possess cognitive stimulant-like effects which therefore enhances information processing speed (7,8,11,12). These findings are also in line with previous investigations that sought to examine the possibility of guarana influencing cognition during/after physical activity whereby significant decreases in mean reaction time and improvements in temporal performance were reported (6,16). IIV-I in the placebo group was significantly higher for SRT following exercise, whereas guarana and caffeine exhibited a plateau with no difference. These results allude to the fact that guarana supplementation can not only improve reaction time but may do so without hindering cognitive consistency, as measured by trial-to-trial variability which could be caused by physical fatigue. In addition to this, although CRT did not improve after exercise, the IIV-I for CRT following guarana supplementation was significantly reduced (see Table 1), perhaps demonstrating that while participants did not improve in their choice reaction times, they became more consistent with their response times after exercise (20). A mean improvement in consistency after exercise was observed in all conditions but was only significant with guarana supplementation, further suggesting that guarana supplementation in combination with exercise may improve cognitive performance. It is therefore possible that exercise may promote an increase in focus and alertness, which is further augmented by supplementing with a stimulant.

It’s important to note that true comparisons to previous literature are difficult as key differences exist in exercise intensity and duration, as well as when cognitive tests commenced. For example, during the Pomportes et al. (2007) study, participants were exercising submaximally at 60% peak power output for 40-minutes and completed cognitive tasks on the cycle ergometer during this time frame. Guarana supplementation led to an improvement in temporal performance (production) but did not positively influence cognitive control. It is likely that participants would have been in a heightened state of arousal throughout the testing, beyond what is optimal for cognitive performance (23). Whereas in this study and previous research, testing was completed after 5-minutes of recovery or immediately after physical activity (16). Considering participants cooled down for 5-minutes before being taken to a quiet room for cognitive testing in the current study, this may have allowed for a sufficient amount of recovery before testing. It is also plausible that contrasting results in previous studies are due to differing administration methods. Some studies have combined the consumption of guarana with multivitamin complexes (6) as well as just mouth rinsing (16), whilst others have employed contrasting caffeine content in comparison to this study (7,8,12,13). In those studies, it is possible that other constituents contained in the multivitamin complexes may have interacted with the effects of guarana.

In the current study, guarana elicited a greater magnitude of effects than the equivalent caffeine dose of 5 mg/kg, suggesting that the combination of caffeine and guarana provide superior stimulant-like effects. Despite this, the pharmacokinetics and central nervous system stimulant contributions of caffeine in the current study cannot be ignored. It is well known that caffeine supplementation results in heightened alertness, vigilance, attention and reaction time (3). The rapid absorption of caffeine is mediated by the antagonism of adenosine receptors and the inhibition of phosphodiesterase (1). By competing with and blocking A1 and A2 adenosine
receptors, indirectly, the release of norepinephrine, dopamine, acetylcholine, and serotonin could occur (Kennedy, 2019). The release of such neurotransmitters results in excitatory effects which are believed to alter cognitive performance in both habitual and non-habitual consumers (24). This is in keeping with an earlier study by Haskell et al. which demonstrated that both habitual consumers and non-consumers had a positive cognitive response from caffeine supplementation at both 75 and 150 mg doses (25). However, daily caffeine intake did not appear to affect performance for any outcome measure in the current study. Guarana’s stimulant-like properties have historically been accredited to the caffeine content of guarana’s seed, but the results from this study add to growing evidence that caffeine cannot be acting exclusively (1,7). Haskell et al. (2007) observed guarana to be pharmacologically active at caffeine doses as low as 4.5 mg, below the threshold for activity in humans.

The interaction caffeine has with the other components within guarana are not yet clear. Evidence suggests that synergistic relationships when co-consumed with other bioactive compounds may result in greater stimulant-like effects. For example, guarana contains traces of several other purine alkaloids (methylxanthines), such as theobromine and theophylline (24). Theobromine, a caffeine derivative, has a higher affinity for A1 receptors in comparison to caffeine yet has a longer half-life (26). Studies into these compounds do not suggest a consistent cognitive effect when administered alone but have shown that theobromine appears to act synergistically with caffeine (27). As evidenced by Smit and colleagues (2004) whereby caffeine (19mg) and theobromine (250mg) combined capsules improved simple reaction time tests, consistent with our current findings. There are also high levels of polyphenols such as tannins (total tannin concentrations estimated between 5-14%) and saponins (2,24). Saponins derived from lychee seeds (28) and ginseng tea leaves (29) have previously demonstrated to possess neuroprotective activities in-vitro and in-vivo. The interaction and target of HRAS (HRas Proto-Oncogene, GTPase), MAPK1(Mitogen-Activated Protein Kinase 1), and MAPK8 (Mitogen-Activated Protein Kinase 8) in the blood-brain-barrier via the AGE-RAGE (Advanced Glycation Endproducts - Receptor For Advanced Glycation Endproducts) and PI3K-Akt (Phosphatidylinositol 3-Kinase - Protein Kinase B) signalling pathway are the purported mechanisms of action (29). When co-consumed, saponins and tannins may also interact with caffeine and increase the bioavailability of phenols (24). Indeed, there is some promising data to suggest that phenols may improve some cognitive brain functions (30), though the data is not particularly consistent. Although the ingestion of guarana may exert similar stimulant-like effects to caffeine, the presence of tannins and saponins found in guarana may prolong this effect (31). It’s therefore plausible to assume that the additional bioactive components of guarana may be acting concomitantly, investigating the psychoactive contribution of each requires more research in order to understand guarana’s full mechanism of action.

The study acknowledges several key limitations. Firstly, the 5-minute period between exercise and cognitive tests may not necessarily be translatable and applicable in the field, where athletes or professionals (police/military) are required to make crucial decisions while under physical exertion. Further research should therefore try to eliminate or reduce the time between exertion and testing and attempt also apply relevant tests to real-world sporting or professional situations, such tests could place further emphasis on problem solving and higher executive functioning rather than only SRT and CRT. Secondly, the sample size in the current study was small, therefore caution must be taken when interpreting the results, particularly when
considering the wider applicability of the outcomes. The authors also acknowledge that the study population was specifically young active individuals, some of which came from a cycling club, this therefore impacts the generalizability of the results. It is also plausible to suggest that variations in the ergogenicity of caffeine supplementation, and by extension guarana supplementation, may be as a result of inter-individual subtle differences in CYP1A2 and ADORA2A genes (32), measurement of which was beyond the scope of the current study. Though recently there is increasing conflicting evidence that indicates caffeine provides a positive response irrespective of the aforementioned genes (33,34). Finally, although supplements were blinded, exit questionnaires were not employed to distinguish whether the participants knew or could guess which supplement they were on. Consequently, this may have influenced the result. Guarana has a distinct taste, but it is unlikely participants would have taken any on its own before and therefore be able to identify it. To combat this, future research could therefore investigate guarana using capsule form.

**Practical and wider applications**

This study provides promising evidence that guarana supplementation may be useful by sustaining, or possibly improving, decision making following maximal exertion. This may be particularly pertinent to activities that require bursts of high intensity exercise accompanied by rapid decision making, including team sports, law enforcement, and military applications. Previous studies in law enforcement have in fact reported negative effects of stress and exertion on cognitive performance after physically demanding operational tasks (35). Further research could explore the applicability of supplementation strategies in these more specific settings. The magnitude of effect observed with guarana appears to be higher than an equivalent dose of pure caffeine only. Future research should consider the effect of high intensity activity and cognitive performance simultaneously, in conjunction with guarana supplementation, and should consider these effects on higher executive functions that may be applicable to broader fields.

**Conclusion**

The findings of this study indicate that acute guarana supplementation leads to significant improvements in both CRT and SRT before and after a maximal bout of exercise, respectively, which is consistent with a heightened feeling of alertness. This improvement, with respect to placebo, is evident in guarana alone, despite a matched dose of caffeine content in the caffeine supplement. These effects appear promising for the use of guarana in sports which require fast response times to be maintained through high intensity physical activity.

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Authorship contributions

T.G contributed to the statistical and data analysis, writing and drafting of manuscript, formation of figures. N.B and D.I collected data and helped with the drafting of the manuscript. F.R supervised the study, contributed to the conception, design, drafting of manuscript and data analysis. All authors approved the final version of the manuscript.

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Table 1: Intraindividual variability inconsistency for both choice and simple reaction time cognitive tests

Figure 1: Mean and individual simple reaction time before and after exercise. * Signifies a significant difference (P≤.05) between supplements and simple reaction time.

Figure 2: Mean and individual choice reaction time before and after exercise * Signifies a significant difference (P≤.05) between supplements and choice reaction time.