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Caffeine metabolites are associated with different forms of caffeine supplementation and with perceived exertion during endurance exercise

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ABSTRACT: This investigation compared the urine caffeine metabolites produced from different forms of caffeine supplementation given to runners 15 minutes before a series of 5-km running trials. Fourteen amateur competitive runners completed a series of self-paced outdoor time trials following ingestion of placebo or one of three alternate forms of caffeine supplement. Trials were randomized in a crossover design with equivalent doses of caffeine (4.0 $mg.kg^{-1}$) administered 15 minutes before each trial via chewing gum, a novel dissolvable mouth strip or tablet. Runners produced a urine sample following each caffeinated trial that was tested for caffeine and its metabolites by high-performance liquid chromatography. The tablet form of caffeine produced a lower ($p = 0.04$) urinary ratio of the metabolite paraxanthine to caffeine compared with either gum or strip. Independently of caffeine delivery mode, subjects who metabolized a higher proportion of caffeine to paraxanthine recorded a lower ($p = 0.01$) perceived exertion. We demonstrate that oral swallowed caffeine administered 15 minutes before 5-km running is less metabolized compared with caffeinated products designed to be chewed or dissolved in the mouth. We suggest the metabolism of caffeine to paraxanthine has an inverse relationship with perceived exertion independently of caffeine delivery mode.

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INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is frequently used by athletes and is one of the most common endurance exercise performance-enhancing ergogenic aids [1–3]. Caffeine exerts its ergogenic effects via several suggested mechanisms including increases in (1) catecholamine's, (2) central nervous system drive, and (3) muscle recruitment and contractility [3, 4]. Caffeine also appears to reduce perceived exertion (RPE) during exercise, and this may partly explain its observed ergogenic effect [5]. Caffeine may be absorbed by oral ingestion (coffee, energy drinks, shots, tablets, and gels) or via oral mucosa (sublingual or buccal) methods (chewing gum, dissolvable mouth strips, aerosols and mouth rinses). A recent meta-analysis [1] which included 31 studies administering oral caffeine before endurance activity (energy drinks and gums were excluded) found that the majority of studies (84%), provided the supplement 60 minutes before endurance activity. Across all 31 studies, caffeine supplements were provided with a time range of 30–90 min before endurance activity. There is currently a lack of research examining the effects of caffeine on athletes, when provided less than 30 minutes before endurance activity.

While caffeine is clearly ergogenic overall, the level of performance enhancement varies widely between individual studies [1–3]. This is demonstrated in a recent meta-analysis on the effect of caffeine on time trial ($n = 56$) performance [3] reporting a wide range (−3.0% to 15.9%) between the caffeine and placebo groups. In four of these 56 time trials (7%), the placebo group performed better than caffeine group [3]. Large variance in performance changes with caffeine are also seen within studies at an individual level [6]. Occasionally, ergolytic effects on individual athletes are reported [6–8]. Individual athlete responses to caffeine include factors such as caffeine dose, dose-timing, genotype, gender, medication and dietary interactions, habitual caffeine intake, mode of delivery, event duration and training status [6, 9, 10]. When comparing oral ingestion with oral mucosa products, a similar dose of caffeine may produce different caffeine serum concentration levels [11] and different ergogenic effects [12]. Recent work with a caffeine gum [13] identified two separate plasma caffeine concentration peaks. This suggests that with caffeinated gum, plasma caffeine concentration peaks once via oral mucosa absorption, falls then peaks a second time via absorption in the lower gastrointestinal tract. The individual response to caffeine is often overlooked in caffeine performance studies.

One factor clearly involved with individual responses to caffeine is large individual variation in caffeine metabolism [14–16]. Caffeine

metabolism is largely dependent on the activity of four major enzymes, each displaying genetic polymorphism [16]. The most studied gene for caffeine metabolism is *CYP1A2* which encodes the CYP1A2 enzyme, and variation in this gene has been shown to modify the ergogenic effects of caffeine in athletes [17]. The caffeine metabolic ratio, which is the concentration of caffeine and its metabolites (primarily paraxanthine) excreted in urine, has been determined using a variety of high-performance liquid chromatography (HPLC) methods in several populations [16]. This ratio is dependent on the activity of the combined individual enzymes [14–16]. It has recently been concluded that the caffeine metabolic ratio offers enzyme metabolic phenotyping to a level that may be useful for personalized medicine [16]. We speculate that urine caffeine and urine caffeine metabolites may be useful analytes to explain some of the indivdual variance observed with caffeine in athletes.

To our knowledge, no previous study examining different modes of caffeine delivery in athletes has measured urine caffeine and metabolites. We previously published a randomised control trial with caffeine (~3–4.5 $mg.kg^{-1}$) administered 15 minutes before 5 km running via chewing gum, dissolvable mouth strips or tablet [18]. All caffeine supplements led to small (1.4% ± 0.9%) enhancements in running performance. While the caffeine supplements were not different from each other, only the tablet form of caffeine produced significantly faster run times ($p = 0.02$) compared with placebo. This current study aims to investigate the urinary metabolic differences from these different caffeine delivery modes from this performance study. We hypothesize that the orally absorbed caffeinated tablet will produce lower urinary concentration ratios of caffeine metabolites to caffeine when compared to mucosa absorbed products. Our secondary hypothesis is that the ratios of caffeine metabolites to caffeine will offer a enzyme metabolic phenotyping that will explain any individual caffeine effects on 5k run time independently of caffeine delivery mode.

MATERIALS AND METHODS

Subjects and trials

Fourteen subjects completed this study (mean ± SD age 40 ± 8 y; weight 69 ± 11 kg; height 177 ± 11 cm). Inclusion criteria of subjects (verified with a training questionnaire) were experienced (> 5 years) runners who regularly trained (3–10 hours per week) and were not currently injured. A caffeine use questionnaire identified ten subjects as habitual caffeine users (100–400 mg/day), and four as non-habitual caffeine users (< 100 mg/day). Sixteen subjects were initially recruited but two were excluded from the final analysis one due to injury (unrelated to the study) and one for non-compliance with scheduled testing. The cohort consisted of males (n = 10, 40 ± 9 y, 73 ± 10 kg, 181 ± 10 cm), and females (n = 4, 41 ± 9 y, 167 ± 7 cm, 59 ± 2 kg).

The trial protocol was a randomized crossover design [18]. Briefly, subjects completed five x 5-km running time trials spaced (4–9 days apart) over 4–9 weeks. Subjects were instructed to abstain from caffeine for 48 hours and replicate their 24-hour dietary intakes for each trial. All trials took place on an IAAF Tier 1 standard 400-meter running track under similar environmental conditions. All trials were performed at a self-paced maximal effort. Trial timing was conducted using Webscorer software (Webscorer Inc., Woodinville, WA, USA) on a portable tablet device. Heart rate was recorded at 1 Hz (Garmin 920 XT Garmin International, Olathe, KS, USA). Post-trial, subjects were asked to estimate their average rate of perceived exertion (RPE), using the Borg 6–20 scale [19].

Supplement administration

Supplement treatments were randomized using a 4 x 4 Latin Square model [20]. Caffeine was administered 15 minutes before each trial based on pre-study body mass: Subjects > 65 kg received 200 mg caffeine (female's n = 4, males n = 2), subjects > 65 kg received 300 mg caffeine (male's n = 8). This ensured all subjects received *3–4.5* $mg.kg^{-1}$. Caffeine was administered as either caffeine chewing gum 100 mg of caffeine per piece (Military Energy Gum, Marketright Inc. USA), caffeine dissolvable mouth strips 40 mg of caffeine per piece (Revvies Energy Strips, Caringbah, NSW, Australia) and caffeine tablets 100 mg of caffeine per tablet (NoDoz, Cedar Rapids, IA, USA). A placebo was also blindly administered (300 mg glucose powder in an opaque gelatin capsule).

Urine sampling

All participants provided a urine sample within 30 minutes of completing each trial. Samples were collected in a polypropylene airtight container (LabServ 70 mL ThermoFisher Scientific, Auckland, New Zealand). Samples were stored in a fridge at 4°C (± 2°C) and prepared within 72 hours of collection using the protocol outlined by Furge and Fletke [15]. Briefly, urine specific gravity was measured with a refractometer, adjusted with distilled water to account for concentration differences using specific gravity, then a 2.5 mL aliquot was adjusted to pH 3.1–3.3, by addition of acetic acid (HiPerSolv Chromoanorm VMR Chemicals, Radnor, PA, USA, Batch 12D030505). Samples were then was filtered with a cellulose acetate 0.45 μm syringe filter (Membrane Solutions, Kent, WA, USA), and 1.5 mL was transferred into an Interlab V923 clear screw cap glass vial (Interlab, Wellington, New Zealand). Samples were then stored at -80°C before analysis in one batch.

Prepared samples were analyzed using Ultra-High-Performance Liquid Chromatography (Shimadzu Nexera X2 LC-30AD) with a reverse-phase column (GraceSmart RP18 250 mm x 4.6 mm 5 μm, Fisher Scientific, Loughborough, England,). The mobile phase was 90:10 ammonium acetate/acetonitrile, a flow rate of 1.0 $mL.min^{-1}$, column oven was 40°C, and injections were 20 μL External standards of caffeine (ReagentPlus C0750, Sigma-Aldrich, Auckland, New Zealand, Lot BCBS9512V), and paraxanthine (D5385 Sigma 1,7-dimethylxanthine Sigma-Aldrich, Auckland, New Zealand, Lot 06740) were analyzed to determine retention times and method linearity. A stock standard solution of each metabolite was made, diluted 1:10

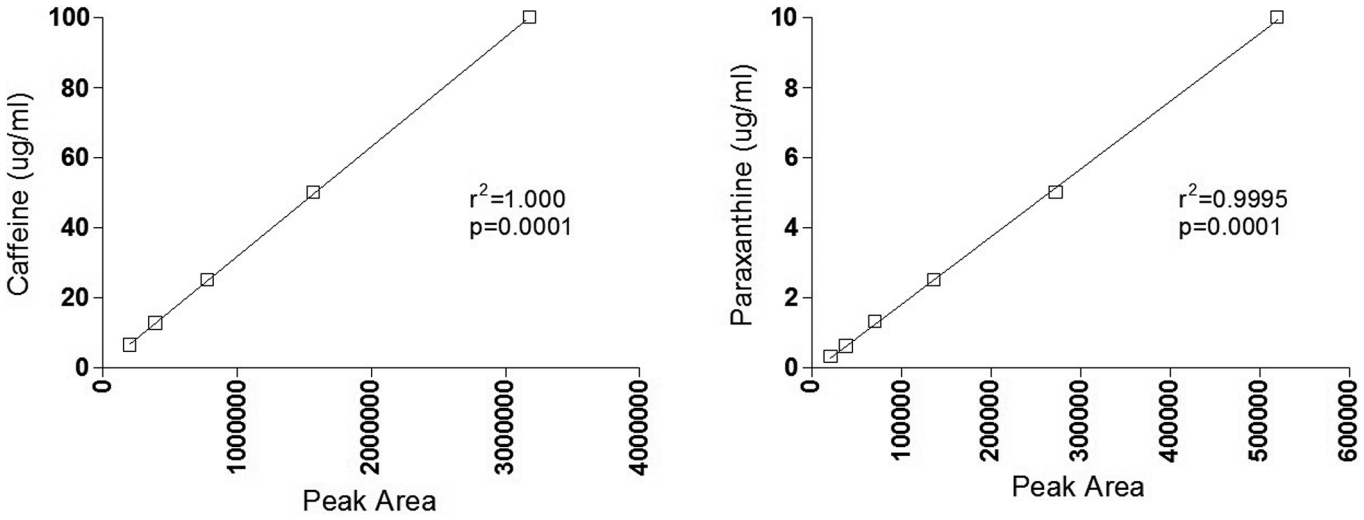

FIG. 1. Urine caffeine and paraxanthine method linearity.

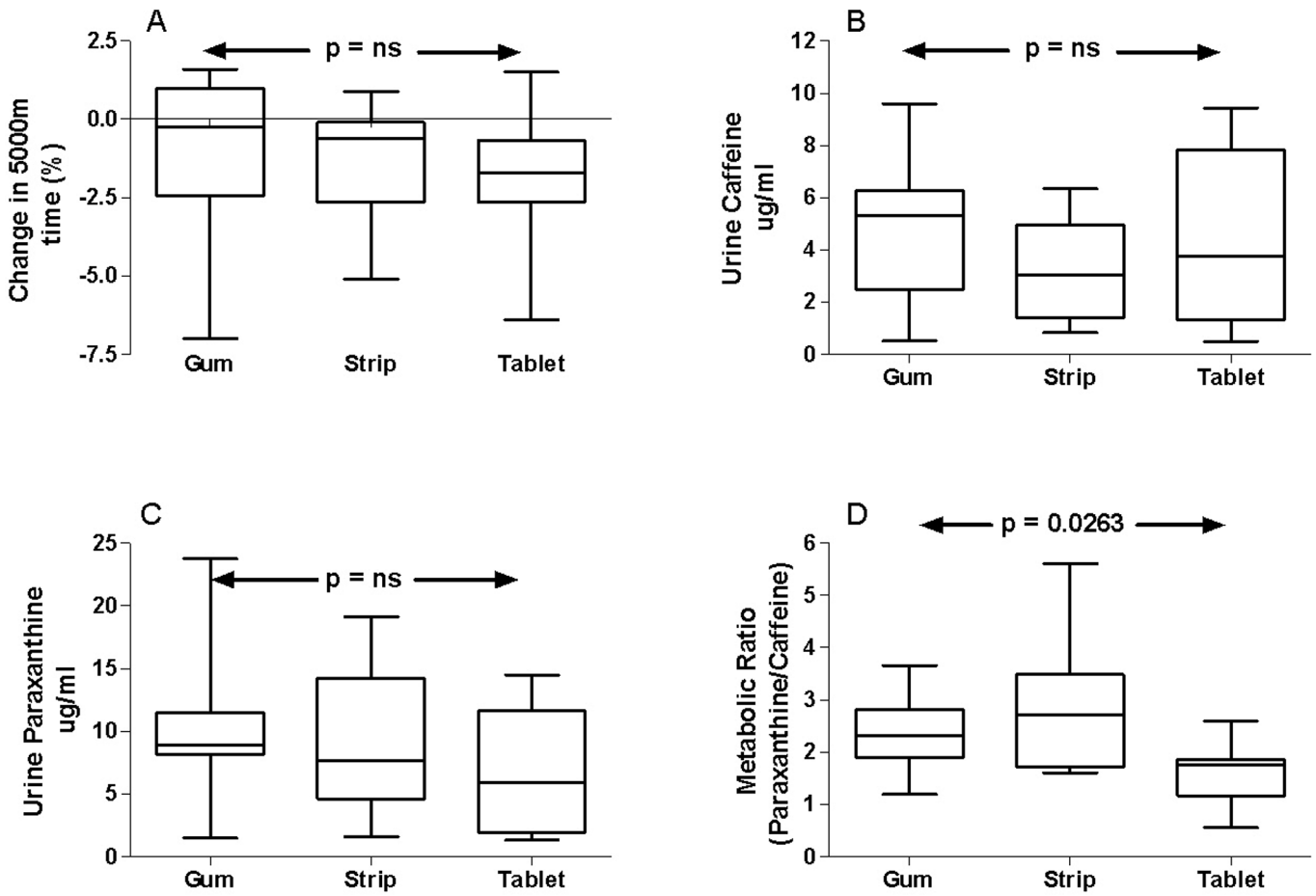

FIG. 2. Figure 2a. Mean change in 5-km performance time as percent (%) for caffeine modes.
Figures 2b, c, and d. Urine caffeine, paraxanthine and metabolic ratio (MR) between the three different forms of caffeine.

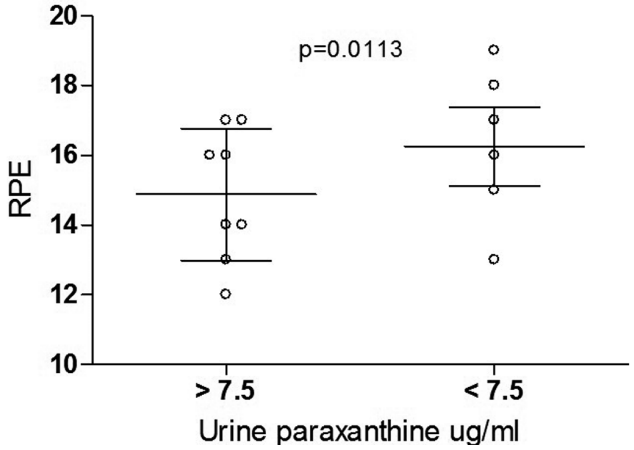

FIG. 3. RPE of subjects with a high (> 7.5 ug/ml) vs low urinary paraxanthine

with the mobile phase and then serially diluted to create 5 standards as described elsewhere [15].

Statistical analysis

Group statistics were calculated as means ± between-subject standard deviations (SD). Linearity for both caffeine and paraxanthine across the range of concentrations tested was established with linear regression. Differences in urinary caffeine metabolites between the three modes of caffeine were examined with one-way repeated measure analysis of variance (ANOVA), followed by Tukey's post-hoc analysis. Difference between oral and buccal modes of caffeine delivery were compared with student t-test. All statistical analysis was performed with GraphPad V4, San Deigo, CA, USA.

Ethics

All subjects gave their written informed consent to participate in the study, which was approved by the participating institutes' human research ethics committee in accordance with the declaration of Helsinki.

RESULTS

Figure 1 shows the urinary caffeine method proved highly linear for both caffeine and paraxanthine across the range of concentrations tested.

The performance data for these trials have been previously reported [18]. All caffeine supplements led to enhancements in running performance, though only runners taking the tablet were faster ($p = 0.02$) than placebo (figure 2a). The different forms of caffeine produced no differences in post-run urinary caffeine (figure 2b) or paraxanthine (figure 2b) concentrations. However, the metabolic ratio of caffeine to paraxanthine was higher ($p = 0.03$) with the tablet form compared with either gum or strip (figure 2d).

Neither urinary caffeine, paraxanthine nor the metabolic ratio were associated with changes in 5k run time. RPE was not different between trials, though for all forms of caffeine, urine paraxanthine negatively correlated with RPE ($r = -0.33$, $p = 0.04$). Subjects with a higher than median (> 7.5 ug/ml) urinary paraxanthine recorded a lower ($p = 0.01$) RPE (mean ± SD 14.9 ± 1.9 vs 16.2 ± , 1.1) compared with subjects below this threshold (figure 3).

The metabolic ratio of urinary caffeine to paraxanthine was lower ($p < 0.01$) in the tablet compared with other forms of caffeine (gum and strip). (Figure 4b), Unknown urinary compounds (Figure 4c and 4d) had different concentrations for the tablet compared with other forms of caffeine (gum and strip).

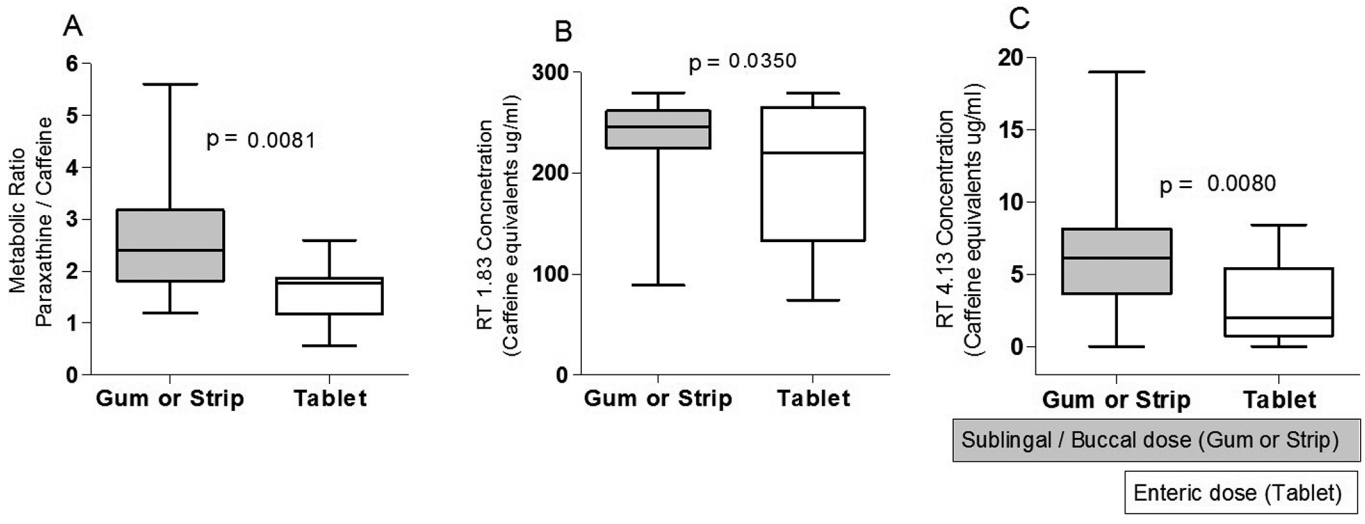

FIG. 4. Caffeine absorption and metabolic differences between the modes of caffeine delivery.

DISCUSSION

The purpose of this study was to investigate the urinary metabolic differences produced from caffeine (~3–4.5 mg.kg^{-1}) administered 15 minutes before 5 km running via chewing gum, dissolvable mouth strips or table. This administration time is a briefer time period than any previous study examining an oral caffeine tablet to our knowledge. Our hypothesis, that an orally absorbed caffeinated tablet will produce lower urinary concentration ratios of caffeine metabolites to caffeine when compared to mucosa absorbed products (gum and strip) was confirmed. However, our secondary hypothesis that the ratios of caffeine metabolites to caffeine will offer a enzyme metabolic phenotyping that will explain any individual caffeine effects on 5k run time independently of caffeine delivery mode was not substantiated. Our evidence suggests that caffeinated products designed to be chewed or dissolved in the mouth before exercise are metabolized more rapidly compared with a caffeine tablet. We also found that greater metabolism of caffeine to paraxanthine has an inverse relationship with RPE independently of caffeine delivery mode.

The urinary caffeine and paraxanthine method proved highly linear which provides a measure of reliability to our results. Post-trial we found a lower ($p < 0.01$) urinary ratio of the metabolite paraxanthine to caffeine for the tablet compared with gum and strip. As caffeine is metabolized predominately (84%) to paraxanthine [21], our findings demonstrate that a caffeinated tablet will be metabolized at a slower rate compared with gums and strips. The novel caffeinated strip we employed in this study was consistent with the caffeine absorption and metabolism rate we observed in the gum. Previous research [11] comparing caffeine delivered as gum against oral administration found an earlier onset pharmacological effect with gum. Our urinary metabolic results confirm this finding with both gum and strip caffeine.

Prior to this study, we speculated the the ratios of caffeine metabolites to caffeine may account for the large number of multifactorial factors (e.g. dose, dose-timing, genotype, gender, dietary interactions, modes, training status) involved in the individual caffeine response. [6, 9, 10]. However our secondary hypothesis, that the ratios of caffeine metabolites to caffeine will offer a enzyme metabolic phenotyping that will explain any individual caffeine effects on 5k run time independently of caffeine delivery mode was not substantiated. Nevertheless, the lower urinary ratio of metabolite to caffeine for the tablet compared with gum and strip may be a highly relevant finding. Previous studies (reviewed in [1]) used a range of 30 to 90 minutes for oral caffeine administration before exercise. Thus, the lower ratio with the tablet is somewhat expected given our study used a novel brief 15 minute time period between supplement administration and exercise. As serum caffeine levels peak between 90 and 240 min after administration [22], our subjects cannot have achieved peak serum caffeine levels from the tablet. Our urinary ratio of metabolite to caffeine data confirms that the caffeine in the tablet was not metabolized. However, in spite of this the tablet still clearly conferred an ergogenic effect. Indeed, while there was no difference with performance between the caffeine modes, the performance gain tended (figure 2a) to be greater with the tablet, and that mode was the only mode to result in faster running compared with placebo ($p = 0.02$). We conclude that neither time for caffeine metabolism to occur, nor time to achieve peak serum levels are requirements for athletes seeking benefits from caffeine. Further, we speculate that higher urinary caffeine to paraxanthine ratios may be desirable to achieve the greatest enhancement in performance.

Research has not yet established an optimal supplement administration timing for caffeine products delivered via the oral mucosa such as gum and strip formulations. In regards to oral swallowed caffeine, such as a tablet, approximately 80% of studies utilize a supplement administration timing of 60 minutes before endurance activity [1]. However, the higher relative paraxanthine to caffeine we observed for gum and strip compared to tablet suggests administration time frames may need to be brief for products delivered via oral mucosa. Indeed, our protocol of supplement administration 15 minutes before exercise may be too long a timeframe for gum and strip forms of caffeine. Our evidence clearly demonstrates these forms were more rapidly metabolized than a tablet which conferred a potentially greater ergogenic effect. We suggest further research employing gums or strip mediated caffeine delivery examine the effect of dosing on the start line or during actual performance. We speculate that with gums or strip caffeine, dose and timing interactions that result in a higher caffeine to paraxanthine ratio at the time of the event may be a method of optimising the use of these products for individual athletes. This needs to be tested in further research.

Caffeine's performance-enhancing effects are likely to have more than one mechanism of action [4]. One commonly reported finding thought to partly explain the ergogenic effect is that caffeine reduces RPE during exercise [5]. However, our data suggest that caffeine's ergogenic effect and RPE may not be related. RPE was not different between trials. The tablet had an ergogenic effect though subjects also exhibited a lower relative paraxanthine concentration. In contrast, a lower RPE ($p = 0.02$) was only observed in subjects with higher urinary paraxanthine. Thus, we suggest that caffeine's ergogenic effect is not mediated by a reduced RPE. Rather, a lower RPE may be a separate physiological mechanism associated with caffeine metabolites such as paraxanthine.

There are several imitations in our study. Firstly, we employed no genotyping of individuals for caffeine metabolism, which is possible factor for endurance performances with caffeine [17, 23]. Thus we cannot account for individual genetic differences. In addition, while 100 mg of caffeine has been shown [24] to exhibit linear pharmacokinetics, higher doses (250 to 500 mg) as used here (and indeed in most studies) are associated with reduced clearance, prolonged half-life and non-linear kinetics [25]: It cannot be assumed that an equivalent caffeine dose will control for these variances. This heterogeneity may be particularly pronounced for oral mucosa caffeine absorption. Research [26] investigating drug release from caffeinated

gum during mechanical chewing found that even after 30 min of chewing, 10% of caffeine remained unreleased. Additionally, measurement of caffeine remaining in discarded caffeinated gum cud after chewing found the mean dose of caffeine available for absorption, was ~18% lower than the intended 50-mg dose [27]. Thus, although our delivery modes were intended to contain equal caffeine doses, the full dose may not have been available for the gum and strip forms. Indeed, considering that the rate of chewing is a factor, no study utilising caffeinated gum can eliminate dose differences between individuals as a factor. However, this limitation is shared in all studies appraising similar products, although our use of the concentration ratio of metabolite to caffeine somewhat limits the impact of potential dose differences.

CONCLUSIONS

In conclusion, our evidence supports that oral swallowed caffeine administered 15 minutes before 5-km running is less metabolized compared with caffeinated products designed to be chewed or dissolved in the mouth. We suggest that caffeine's ergogenic effect may not be primarily mediated by a reduced RPE, rather a lower RPE is associated with the caffeine metabolite paraxanthine. Further research is warranted to identify the optimal dose timing with gum and strip caffeinated products and should include briefer time frames than have been traditionally used. We recommend that future investigations may consider increasing the administration doses in caffeinated gums to allow for the proportions of caffeine not released from that delivery form. We suggest that a time period to allow for caffeine metabolism are not requirements for athletes seeking benefits from caffeine. We suggest examining dose and timing interactions that result in a higher caffeine to paraxanthine ratio for an individual at the time of the event may be a method of optimising the individual use of these products. This needs to be tested in further research.

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Conflict of interest declaration

No potential conflict of interest was reported by the authors.

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