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Caffeine release and absorption from caffeinated gums

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COMMUNICATION

Caffeine release and absorption from caffeinated gums

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The objectives of this study were to estimate the impact of chewing time on caffeine release from gum and to understand caffeine pharmacokinetics. Caffeine release increased with chewing time (2 min < 5 min < 10 min). Furthermore, two plasma caffeine concentration peaks were observed suggesting that caffeine absorption occurs both through the oral mucosa and gastrointestinal tract. This is of practical relevance to maximise caffeine doses and to synchronise effort with peak caffeine concentration.

There is convincing evidence that caffeine improves both cognitive and physical performance [1, 2]. Caffeine has commonly been administered in capsules or tablets, but recently, caffeine has become available in energy drinks, gels, bars and gums. Caffeinated gum has generated much interest from athletes and researchers, because it is viewed as a convenient fast acting vehicle to administer caffeine [3]. A number of recent studies have investigated the extent to which caffeinated chewing-gum improves exercise performance [4-11]. Whilst most studies have reported ergogenic effects some have reported null findings [9, 12]. Interestingly, those studies failing to report a benefit of caffeine gum have combined relatively low doses of caffeine (200 mg) with a chewing time of 5 minutes [9, 12], whereas those reporting clear benefits have commonly administered higher doses [6, 8, 10] or asked their participants to chew the gum for 10 minutes [5, 13]. A chewing time of 5 minutes probably results in a lower release of caffeine from gum than a chewing time of 10 min, and when combined with a low initial dose may result in caffeine failing to reach a sufficient concentration in the circulation to exert an ergogenic effect.

Despite the increasingly widespread use of caffeine gum by researchers and athletes, surprisingly, only fragmented information is available on the effect of different chewing times on the release of caffeine from gum [14, 15]. Sport and exercise scientists often cite information provided by a gum manufacturer (Amural confectioners, Yorkville, IL) indicating that approximately 85% of the caffeine dose is released during the initial 5 min of chewing [16]. However, the experimental details and results of the study (including information on inter-individual variation) are not publicly available. A study reporting the release of caffeine from a 3 layered chewing gum with chewing durations of 10, 20, 30 and 40 min showed that approximately 90% of the caffeine was released after 10 min of chewing, rising to approximately 98% after 40 min [14]. More recently, the dose of caffeine delivered through caffeine gum (chewing duration 10 minutes) and the dose delivered through a caffeinated beverage (instant coffee) were compared [15]. A considerable inter-individual variation in the amount of caffeine remaining in the gum was observed, ranging from 3 to 32%, with a mean of 18%. This variability was attributed to differences in saliva production and chewing rates. The variable release of caffeine from gum following a 10 min chewing protocol and lack of publicly available data for the most common chewing duration adopted by researchers (5 min) is of concern because the validity of studies assessing the effects of caffeine relies on administering a known amount of caffeine.

Caffeine delivery timing:

Researchers often attempt to synchronise peak caffeine concentration with the start of the event for which enhanced performance is required [6, 9, 17] and have so far based their estimation on findings comparing plasma caffeine concentrations upon administration of 0, 50, 100 and 200 mg of caffeine via chewing-gums (5 min chewing) or capsules [18]. They concluded that caffeine absorption was significantly faster with gums than capsules. The explanation may be that, as with other drugs [19, 20], it is likely that caffeine can be absorbed through the oral mucosa during chewing as well as through the gastrointestinal tract following swallowing. Two caffeine peaks following chewing of caffeinated gums have been reported in passing [18, 21], this is consistent with

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absorption taking place at both sites. In order to synchronise caffeine delivery for specific events (for example during half-time in team sports), it is important, from a scholarly and practitioner's point of view, to fully understand when plasma caffeine concentrations peak following chewing of caffeinated gums for 5 min. Furthermore, confirmation of substantial absorption from the gastrointestinal tract is of practical importance because gum has been proposed as a suitable vehicle to avoid the gastrointestinal irritation that can accompany caffeine ingestion [8].

The aim of this project was to address the critical gaps in publicly available data relating to caffeine extraction from gums upon chewing and resulting absorption into the blood stream with time and act as a decisive document to inform further research and caffeinated gum use in general. The objectives were:

- To understand the impact of chewing time on caffeine extraction rates; gaining in the process an insight into inter-subject variation. To this purpose 15 participants were instructed to chew a caffeinated gum for 2, 5 and 10 min. The remaining caffeine in the discarded chewing-gums was then quantified.
- To determine whether caffeine delivered through gums is absorbed through the buccal mucosa, the gastrointestinal tract or both and confirm the time of peak plasma caffeine concentration. To this purpose, plasma caffeine concentrations of 14 participants were followed at numerous time points over 24 hours following chewing for 5 min of a caffeinated gum (100 mg).

Materials and methods

Study design:
Participants were asked to refrain from ingesting any caffeine containing food or drink for 2 days prior to starting the study and during the 2 days of the study. On the morning of the first day, participants were asked about their oral health as mastication function can be impaired by compromised dentition [22], their age and their typical caffeine intake. Caffeine intake was estimated from their intake of coffee, tea, caffeinated soft drinks (including energy drinks) and chocolate using a method adapted from [23].

A finger prick baseline blood sample was then taken after which participants were asked to chew one piece of caffeinated gum for exactly 5 min. In order to obtain data which relates to studies in the field and which is relevant to recreational users, no specific chewing instructions with respect to chewing patterns were issued but participants were asked to refrain from speaking during those 5 min. The chewed gum was kept to quantify the remaining caffeine. Further blood samples were collected at 10, 20, 30, 45, 75, 120 and 420 min after gum expectoration. On the second day, participants were asked to come back to take a final blood sample (24 hours after gum expectoration), they were then asked to chew a caffeinated gum for 2 min and, after a short break, another gum for 10 min. Both expectorated gums were kept for caffeine quantification.

Participants:
Fifteen participants were recruited; all 15 participants completed the chewing duration trial (mean age 34.2 years [range 21-49]; 10 females; 3 participants with 1 molar missing, mean daily caffeine intake: 113 mg [range 1 to 270 mg]) however, 1 participant dropped out of the blood collection aspect of the study leaving 14 participants completing the plasma caffeine concentration part of the study (mean age 35.0 years [range 21-49]; 9 females; 3 participants with 1 molar missing, mean daily caffeine intake: 121 mg [range 3 to 270 mg]).

The inclusion criteria were to be 18 years of age or more, not to be pregnant or breastfeeding, to not suffer from food allergies or have a history of anxiety, caffeine hypersensitivity, Type I or Type II diabetes, heart disease, kidney disease, gastrointestinal problems or high blood pressure. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and approved by the Faculty Ethics Committee of Sheffield Business School, Sheffield Hallam University (Ref: SBS-260). Written informed consent was obtained from all participants.

Materials:
Caffeinated chewing-gums (100 mg / stick) were obtained from MilitaryEnergyGum®. HPLC grade methanol and hexane were purchased from Fisher Chemical (Loughborough, UK). The water used in the project was obtained using an Elga Purelab system. The caffeine (>99%) was purchased Sigma-Aldrich (Gillingham, UK).

Caffeine extraction from gums:
Caffeine is a polar molecule and is ionised at pHs lower than 10; it is therefore soluble in water and other polar solvents, but poorly soluble in non-polar solvents such as hexane [24]. Based on this the extraction of caffeine from chewing gum was developed using liquid-liquid extraction (LLE) with water (adjusted to pH 6.5) and hexane. Un-chewed caffeinated gums were used to optimise the caffeine extraction method. In brief, the caffeine from the chewed gums was extracted by mixing the sample using a pestle and mortar in 70 mL hexane, this was transferred into a beaker and the mortar was rinsed with 70 mL water also transferred to the beaker. The mixture was homogenised for 10 min at 5000 rpm using a Silverson L4RT homogenizer and transferred into a separating funnel (10 min manual agitation) to recover the aqueous phase. The organic phase was washed three more times with 70 mL water. The pooled aqueous phase recovered was then completed to 500 mL and filtered (0.22 µm syringe from Merck Millipore; Tullagreen, Ireland) before injection.

Human plasma collection and preparation (adapted from [25]):
Finger prick blood samples (approximately 250 µL) were collected in BD Microtainer K2E tubes which were then

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centrifuged (Hermle Z 233 MK-2) for 15 min at 5000 g and 4 °C. The supernatant (plasma) was then collected for further analysis. 100 µL of methanol was added to 30 µL of plasma and centrifuged for 5 min at 17500 g and 4 °C. The resulting supernatant was injected directly into the HPLC.

Caffeine quantification - adapted from [26]:
Caffeine from the samples was quantified using an HPLC-UV (Shimadzu Prominence-I LC 2030 Plus) and a 3 µm Shim-pack GIST C18 column (100 x 4.6 mm) in isocratic mode with a mobile phase water:methanol (80:20) at a flow rate of 1 mL/min. The wavelength for detection was set at 275 nm. Caffeine standards at 0.05, 0.1, 1, 2, 5, 7.5, 10 and 25 mg/L were used for the calibration curve. Injection volume for all samples was 10 µL.

Data analysis:
The percentage of caffeine released by different chewing time was estimated by subtracting the amount of caffeine remaining in the gum following chewing from the nominal amount of caffeine present in the gum (100 mg). The elimination rate was calculated from the slope of the natural logarithm of the plasma caffeine concentration vs time and used to estimate caffeine half-life. A repeated measures ANOVA was used to analyse the caffeine release and plasma caffeine concentration results, post-hoc, a Bonferroni test was applied. All were performed using SPSS (v24, IBM Corp, Armonk, NY).

Results and discussion

Figure 1 presents the percentage of caffeine released for different chewing time.

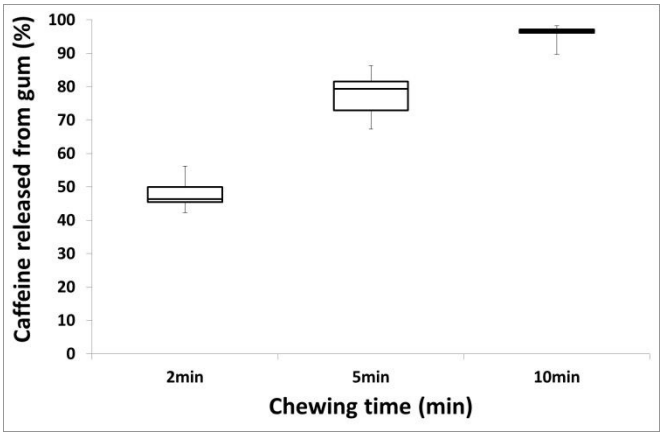

Fig. 1 Caffeine release from gums chewed for 2, 5 and 10 minutes (n = 15).

A clear increase in caffeine release was observed with chewing time ($p<0.001$) with each duration resulting in significantly different ($p<0.001$) caffeine release from the others. Whilst 2 min only resulted in a mean release of 47.9% and is therefore entirely inadequate for the purpose of dosing participants for trials, chewing for 10 min resulted, on average, in an extraction of 96.2% of the caffeine from the gum which is broadly consistent with the results obtained elsewhere [14, 15] (approximately 90% and 82%). The differences observed may stem from different gum composition or caffeine extraction methods although the caffeine extraction method was not always reported [15]. Our results for a 5 minute chewing duration (77.6% caffeine release) are in broad agreement with others' [16] (approximately 85%) although the experimental details of their study are not available and in particular, there are no data on the inter-subject variation in caffeine release. We have found a rather large inter-subject variation in the amount of caffeine released following all chewing durations although it was largest following the 5 minute chewing condition ranging from 67.3% to 86.2%.

Figure 2 presents the average plasma caffeine concentration with time following chewing of a gum (100 mg caffeine) for 5 min.

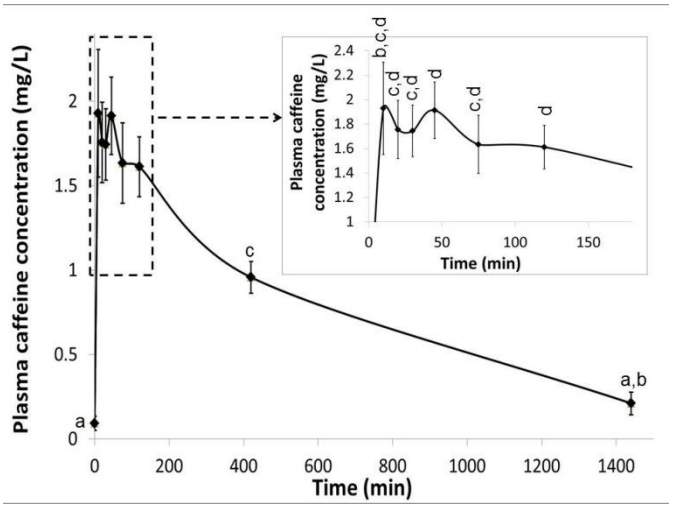

Fig. 2 Plasma caffeine concentration following chewing for 5 min of a caffeinated gum (100 mg, n = 14). Error bars represent +/- 1 SE; letters indicate statistically different plasma caffeine concentrations.

Significant differences in plasma caffeine concentration were observed with time ($p<0.001$). Two peaks for plasma caffeine concentration were observed at approximately 10 and 45 min for 9 of the participants. Thanks to its sufficiently lipophilic nature, caffeine can pass though the buccal mucosa leading to rapid absorption [27-29]. As such, the peak timings are consistent with part of the caffeine being absorbed immediately through the buccal mucosa but also through the gastrointestinal tract following dissolution into the saliva and swallowing. These results confirm that, as with other drugs [19, 20], both absorption routes co-exist for caffeine. This is the first time that the actual peak times are explicitly reported although, It is worth mentioning that factors such as gastric emptying and the presence of other components from the diet are likely to have an impact on the gastrointestinal tract absorption and resulting plasma caffeine concentration peak time [30]. This partially supports the view that gastrointestinal distress can be

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minimized by delivering caffeine using chewing-gums [8, 31] as some of the caffeine is absorbed through the buccal mucosa. However, there is now evidence that some of the caffeine is also swallowed and absorbed through gastrointestinal tract which may still induce some gastrointestinal discomfort. A large variation in the relative importance of the two absorption routes was observed between participants; this can be explained by large inter-subject differences in saliva production as well as chewing and swallowing patterns [32]. Indeed, "saliva wash out" whereby saliva flow leads to premature swallowing of the drug [33] is one of the key challenges in delivering drugs through the buccal mucosa to avoid harsh conditions in the gastrointestinal tract and bypass hepatic metabolism. In addition to chewing intensity, swallowing patterns and volume of saliva produced, inter-subject physiological differences such as mouth pH are likely to result in different propensities to absorb caffeine through the buccal mucosa [29].

The average half-life and elimination rate were respectively 7.55 h and 0.092 h^{-1}. Caffeine half-life is generally accepted to range from 1.5 to 9.5 h in adults [27] but more recent reports place it between 4.70 and 7.58 h [15, 34, 35]. The elimination rate was estimated at 0.11 h^{-1} [30] and 0.150 h^{-1} [18]. The large variation in reports is likely due to different modes of delivery and although our results are in broad agreements with previous reports; the co-existence of two absorption routes is likely to impact on the overall half-life and elimination rate as the gastrointestinal absorption route effectively releases more caffeine to the blood stream later.

Study limitations and future work: although consistent with practices elsewhere, the number of participants remained low and it is impossible to generalise the results to the wider population, especially in terms of inter-subject variation. In particular, there appears to be a wide variation in how participants absorb caffeine which can be explained by inter-subject differences such as mouth pH as well as chewing and swallowing patterns; however, those may be explored more systematically by comparing a "chew and spit" protocol to a "chew and swallow" protocol (as reported for loratadine [20]) in order to estimate the variation in relative contribution of each absorption site.

Conclusions

Our findings are in broad agreement with previous reports, but present a more complete and detailed picture of how and when caffeine is released from gums and the impact of chewing duration. This should support researchers and practitioners to make decisions about how to administer caffeine for their specific purposes. Based on this study, we confirm that almost 80% of caffeine is released by a 5 minute chewing protocol although adopting a 10 minute chewing protocol is likely to maximise caffeine release and minimize the variation in doses delivered to the participants. Moreover, it may be advisable to take into account the two caffeine absorption sites (buccal vs. gastrointestinal tract) to synchronise effort with peak caffeine concentration.

Conflicts of interest

There are no conflicts to declare.

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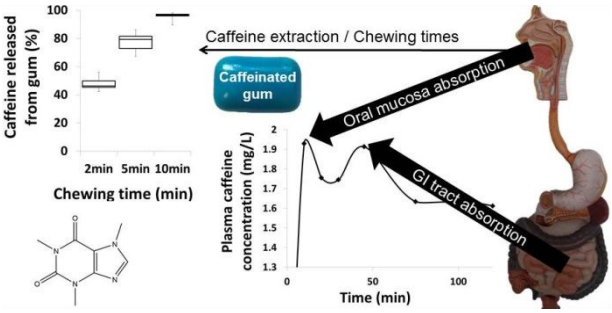

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