Glucose control upon waking is unaffected by hourly sleep fragmentation during the night, but is impaired by morning caffeinated coffee.

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Sleep fragmentation and caffeinated coffee

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Abstract

Morning coffee is a common remedy following disrupted sleep yet each factor can independently impair glucose tolerance and insulin sensitivity in healthy adults. Remarkably, the combined effects of sleep fragmentation and coffee on glucose control upon waking *per se* have never been investigated.

In a randomised cross-over design, 29 adults (Mean ± SD; age: 21 ± 1 years, BMI: 24.4 ± 3.3 $kg \cdot m^{-2}$) underwent three oral glucose tolerance tests (OGTT). One following a habitual night of sleep (*Control*; in bed, lights-off trying to sleep ~2300-0700 h) the others following a night of sleep fragmentation (as *Control* but waking hourly for 5 min), once with and once without morning coffee ~1 h after waking (~300 mg caffeine as black coffee 30 min prior to OGTT).

Peak plasma glucose and insulin concentrations were unaffected by sleep quality but were higher following coffee consumption (Mean [normalised confidence interval] for Control, Fragmented, and Fragmented+Coffee, respectively; Glucose: 8.20 [7.93-8.47] $mmol \cdot L^{-1}$ *versus* 8.23 [7.96-8.50] $mmol \cdot L^{-1}$ *versus* 8.96 [8.70-9.22] $mmol.L^{-1}$; Insulin: 265 [247-283] $pmol \cdot L^{-1}$; and 235 [218-253] $pmol \cdot L^{-1}$; and 310 [284-337] $pmol \cdot L^{-1}$). Likewise, iAUC for plasma glucose was higher in the Fragmented+Coffee trial compared to Fragmented.

Whilst sleep fragmentation did not alter glycaemic or insulinaemic responses to morning glucose ingestion, if a strong caffeinated coffee is consumed then a reduction in glucose tolerance can be expected.

Introduction

Sleep curtailment is a risk factor for obesity-associated metabolic diseases, possibly due to the important role of sleep in maintaining glucose homeostasis [1]. For example, impaired glucose clearance and whole-body insulin sensitivity can occur following a single night of either sleep deprivation (e.g. limiting the habitual duration), broken sleep (i.e. 2 periods of sleep separated by an extended waking interval - e.g. 2300-0100 h & 0500-0730 h), or nonspecific sleep-fragmentation (e.g. random arousal stimuli throughout the night) [2-5]. Studies to date have employed differing protocols to curtail sleep however, to our knowledge, only one previous study has examined the influence of hourly sleep-fragmentation on postprandial metabolism [6]. In this study, postprandial insulin was lower following breakfast in the fragmentation condition relative to a normal night of sleep. However, whilst the use of metabolic units used in this study allowed for a highly controlled situation in which subjects are in a stable environment, it is important to consider the first night effect of an unfamiliar environment on sleep quality [7], and therefore to see whether these findings translate into more ecologically valid contexts (i.e. participants sleep in their own beds).

From an ecological standpoint, coffee is a commonly used means of combatting feelings of lethargy and fatigue [8], therefore acting as a 'therapeutic tool' following disrupted sleep. However, whilst moderate habitual caffeinated coffee consumption is associated with reduced risk of cardiovascular mortality and cancer incidences [9], studies by Moisey *et al* Moisey, Robinson [10] (~62 mg caffeine per 100 mL coffee) and Robertson *et al* Robertson, Clifford [11] (100-400 mg caffeine) demonstrate the potential for a single serving of caffeinated coffee to acutely impair postprandial glucose metabolism in both normal-weight and overweight individuals. This raises the possibility that that coffee consumption could potentiate any negative effects of sleep disruption on glucose metabolism. Furthermore, this effect of caffeine upon postprandial glycaemia seems to be modulated by a single nucleotide polymorphism in the CYP1A2 gene, which codes for an enzyme responsible for caffeine metabolism in the liver [12]. Caffeine is primarily metabolised (>95%) through CYP1A2 activity [13]. However, this modulating role has yet to be investigated under a variety of scenarios [14, 15].

It is remarkable therefore that no study to date has investigated their combined effects upon waking, although one study has investigated the combined influence of caffeinated coffee (65 mg caffeine) *prior to* sleep deprivation on next-day glucose control. Higher fasting serum insulin and increased levels of glucose and insulin were observed after an oral glucose tolerance test (OGTT) following sleep deprivation (4 h in bed) with consumption of caffeinated coffee relative to decaffeinated coffee [16]. However, coffee was consumed prior to sleep, which is not an ecologically valid model of when coffee is usually consumed, especially as a remedy following disrupted sleep. In addition, caffeine is

a fast-acting pharmacological agent, with metabolic effects that occur rapidly and may subside within hours (especially amongst habitual caffeine consumers; [17]), so there is also a clear physiological rationale to examine the acute effects of caffeine intake upon waking immediately prior to the first intake of nutrients following the overnight fast.

The aim of this study, therefore, was to determine the effects of one night of sleep fragmentation with and without morning caffeinated coffee on glycaemic control relative to an undisturbed night of sleep in healthy young adults. An exploratory aim of the study was to examine whether individual responses were mediated by the CYP1A2 genotype of participants. It was hypothesised that sleep fragmentation *per se* would impair insulin sensitivity and that morning coffee would exacerbate this response, with the latter effect modulated by the relevant polymorphism of CYP1A2.

Methods and Materials

Participants

Twenty-nine healthy men and women (Age: 21 ± 1 years, BMI: 24.4 ± 3.3 $kg \cdot m^{-2}$) participated in the study. Exclusion criteria included, body mass index outside of the range of 18.5-29.9 $kg \cdot m^{-2}$, any diagnosed metabolic disease (e.g. type 1 or type 2 diabetes), reported use of substances which may pose undue personal risk to the participants or introduce bias into the experiment, and non-standard sleep-wake cycle (e.g. shift worker). All were informed of any potential risks and discomfort involved in the study prior to providing written and oral informed consent. The study was given a favourable ethical opinion by the Research Ethics Approval Committee for Health (REACH) at the University of Bath (SES/HES: 18R1-019). The measurements of *CY1PA2* gene polymorphism were completed as part of a wider screening project for which ethical approval was granted by the National Health Service Research Ethics Committee (18/NW/0573). All procedures were performed in accordance with the Declaration of Helsinki.

Experimental Design

Participants underwent three trials in a randomised cross-over design, with a washout interval of 7-14 days. For 48 h prior to each trial, participants standardised diet and physical activity and refrained from consuming caffeine and alcohol. Main trials involved an oral glucose tolerance test (OGTT) following a habitual night of sleep (Control; in bed, lights-off trying to sleep 8 consecutive hours, waking <1 hour prior to arrival at the lab) and a night of sleep fragmentation (Fragmented*;* as control but waking hourly for 5 min – prompted and verified by repeated text messaging), with and without morning coffee (Fragmented+Coffee*;* 300 mg caffeine as black coffee 30 min prior to OGTT).

Experimental Protocol

Participants arrived in the laboratory at between 0800 h and 1000 h (within 1-hour of waking) in an overnighted fasted state (~10 h). Height, body mass, waist:hip circumference were assessed before participants completed baseline subjective assessments of sleep quality, mood, and appetite on a 0-100 mm scale. Waist and hip circumferences were measured using a tape measure around the mid-point between bottom rib and top of the iliac crest and at the largest circumference between the waist and thighs respectively [18]. An intravenous cannula was placed into an antecubital vein and a baseline sample of 5 mL venous blood collected (BD Venflon Pro; BD, Eysins, Switzerland). Cannulae were kept patent throughout all trials by flushing with 0.9% NaCl infusion (B. Braun; Sheffield, UK). Participants would then either consume a cup of caffeinated coffee (8.8 g Nescafé Original, Nestlé, SUI with 300 mL water; ~300 mg caffeine, ~163 mg total caffeoylquinic acids) or a matched volume of hot water over a 10-min period, 30-minutes prior to undergoing an OGTT. At the end of this 30-minute period a 5 mL blood sample was obtained before ingesting a 75 g oral load of glucose (113 mL Polycal; Nutricia, UK with 87 mL water). Further blood samples were then taken at 15- and 30-minute intervals for the first and second hour of the protocol respectively, alongside hourly assessments of subjective mood and appetite.

Sleep Fragmentation

Participants were asked to achieve 8-h time in bed, trying to sleep (~2300-0700 h –modifiable according to individual preference and time of testing the next day). Audible alarms were set on the hour every hour throughout the night. Upon waking, participants would receive a series of 10 text messages from a member of the research team, at a rate of 1 every 30 seconds, which required simple responses prior to being able to fall asleep again (e.g. simple arithmetic). This would be repeated until wake time. Participants rated subjective sleep fragmentation using visual analogue scales upon entering the laboratory.

Blood analysis

All blood samples were immediately transferred into tubes treated with ethylenediaminetetraacetic acid (EDTA) prior to centrifugation (10 min, 4000 x g, 4°C) before the plasma supernatant was aliquoted and stored at -80°C for subsequent metabolite analysis. In the control trial the buffy coat layer of the centrifuged bloods was removed and stored at -80°C for later genetic profiling. All plasma samples were later analysed for plasma glucose, using a spectrophotometric analyser (RX, Daytona, Randox Laboratories Ltd., Crumlin, UK; Inter-assay CV: <2 %) and insulin via enzyme-linked

191 immunosorbent assay (ELISA; Crystal Chem, IL, USA; Inter-assay CV: 13 ± 3%, Intra-assay CV: 6
192 ± 2%).

193 ***DNA extraction and analysis***

194 DNA was extracted from the buffy coat layer using QIAamp DNA Blood Mini Kit following
195 manufacturer's instructions (Qiagen, Germany) and frozen at -80°C until analysis. Extracted DNA
196 was then analysed for the rs762551 SNP using a 5'-nuclease allelic discrimination assay (Taqman
197 drug metabolism genotyping assay SNP ID rs762551, C___8881221_40 [C/A], gene CYP1A2;
198 ThermoFisher Scientific, US).

199 ***Statistical Analysis***

200 Sample size estimations were performed using G*Power software v3.1.9.4. Based on differences in
201 plasma insulin following sleep deprivation (D=1.58), and considering the multi-level (i.e. 3 condition)
202 design of the study a sample size of 30 was deemed adequate to provide a 95% chance of detecting
203 such an effect at α=0.05.

204 All in text values are reported as means with normalised confidence intervals, unless otherwise stated.
205 Normality of data was assessed using the Shapiro-Wilk test, with a paired t test or Wilcoxon's test
206 employed to analyse parametric data and non-parametric data respectively. A mixed model ANOVA
207 (condition, time, and condition x time) was used to examine differences in blood glucose and insulin
208 data, with *post-hoc* Bonferroni corrections applied in GraphPad Prism (GraphPad Software Inc.,
209 California, USA). Statistical significance was accepted at $p < 0.05$. Error bars shown on figures are
210 also normalised confidence intervals (CI) corrected for between-participant variation, such that the
211 magnitude of these CI's therefore directly infers the contrast between paired means at each time-point
212 rather than variance of individual values around the mean [19]. Using this approach, error bars not
213 overlapping their respective comparison would typically be deemed significantly different according
214 to conventional null hypothesis testing (i.e. p<0.05). Incremental area under the curve (iAUC –
215 Trapezoid method [20]) and Matsuda Insulin sensitivity index (Matsuda index; [21]) were calculated
216 from plasma glucose and insulin data using Microsoft excel (Version 16.04848.1000, Microsoft,
217 Redmond, WA, USA). Updated homeostatic model of insulin resistance (HOMA2-IR; [22]) was
218 calculated using publicly available online software (https://www.dtu.ox.ac.uk/homacalculator/).
219 Distribution of the rs762551 SNP was tested for fit against the global expected distribution using a
220 Pearson's χ^2 test with 1 degree of freedom. All values for genotype data were taken from the
221 Fragmented+Coffee condition, with ΔiAUC calculated as the difference between Fragmented+Coffee
222 and Fragmented. As data for genotype was not paired, differences between means was compared

using standard 95% confidence intervals. Effects of trial order were assessed using two-way ANOVA testing for effects of Condition, Sequence, or Sequence x Condition interactions [23, 24].

Results

Glycaemia, Insulinaemia, and Insulin Sensitivity

Mixed model ANOVA revealed main effects for glucose for condition ($p < 0.01$), time ($p < 0.01$), and condition x time ($p < 0.01$). Plasma glucose concentrations did not differ between conditions at baseline and remained similar prior to ingestion of the oral glucose load (i.e. following caffeine or hot water ingestion; **Figure 1A**). After ingestion of the oral glucose load, plasma glucose concentrations rose to a greater extent in the Fragmented+Coffee *versus* the Fragmented and Control conditions (8.61 [CI: 8.25-8.96] mmol·L^{-1} *versus* 7.92 [CI: 7.57-8.28] mmol·L^{-1}, and 7.57 [CI: 7.21-7.92] mmol·L^{-1} respectively; $p < 0.05$). Plasma glucose concentration remained higher in the Fragmented+Coffee condition relative to Control and Fragmented conditions at 120-mins ($p < 0.05$). As such, plasma glucose incremental area under the curve (iAUC) was higher in the Fragmented+Coffee condition relative to the Fragmented ($p = 0.02$) but not Control ($p > 0.05$) Conditions (196.6 [175.4-217.7] *versus* 130.3 [114.3-146.4] *versus* 153.1 [137.0-169.1] mmol·L^{-1}·120min, respectively).

Plasma insulin displayed effects of time ($p < 0.01$), but not condition ($p = 0.06$) or condition x time ($p = 0.053$). Baseline plasma insulin concentrations were similar between conditions pre-OGTT (**Figure 1B**). Following ingestion of the glucose load, insulin rose to a greater extent in the Fragmented+Coffee condition, relative to both Control and Fragmented conditions (272 [251-293] pmol·L^{-1} *vs* 227 [206-248] pmol·L^{-1} and 223 [202-244] pmol·L^{-1}) at 30-minutes ($p < 0.05$) (**Figure 1B**). Plasma glucose incremental area under the curve (iAUC) was not different in the Fragmented+Coffee condition relative to the Fragmented ($p = 0.06$) and Control ($p = 0.08$) Conditions (10035 [8892-11178] *versus* 7837 [7260-8980] *versus* 8425 [7848-9001] pmol·L^{-1}·120min, respectively. Time to peak insulin, HOMA2-IR and Matsuda insulin sensitivity index were not different between conditions (**Table 2**).

Order effect

Analysis of Sequence x Condition interactions revealed no effect of first trial on the observed effect of Fragmented+Coffee ($p = 0.101$). However, an order effect in plasma glucose iAUC was observed whereby values in participants first trial were higher than both the second and third trial (197.0 [173.4-220.6] *versus* 147.5 [125.7-169.3] *versus* 132.2 [106.5-158.0] mmol·L^{-1}·120 min).

Subjective Sleep Quality

Time to sleep (Median [Inter Quartile Range]; 23:30 [23:00-00:00] h *vs* 23:30 [23:00-00:01] h*, vs* 23:45 [23:05-24:10] h) and wake time (07:00 [06:52-07:17] h *vs* 07:00 [07:00-07:16] h *vs* 07:00 [06:55-07:20] h) did not differ between Control, Fragmented and Fragmented+Coffee conditions, respectively. Subjective ratings of sleep fragmentation (i.e. "How fragmented was your night's sleep?") were greater in the Fragmented, and Fragmented+Coffee conditions, relative to the Control condition (83 [78-87] *versus* 81 [77-85] *versus* 8 [3-11] mm/100, respectively).

Genotyping

Of the n=26 genotyped, 15 participants were homozygous for the A allele, with the remaining 11 carrying the C allele (n=2 CC; n=9 AC). The distribution of genotypes was therefore as expected within the given population and therefore did not deviate from the Hardy – Weinberg equilibrium (Pearson's $\chi 2$ test with 1 df, P >0·05).

Secondary Analysis: Glycaemia and insulinaemia by genotype.

The glucose incremental area under the curve (iAUC - AA: 208.5 [95%CI: 126.2-290.8] *versus* AC/CC*:* 188.7 [95%CI: 103.9-273.4] mmol·L^{-1}·120min; p = 0.54) and peak plasma glucose concentrations (AA: 8.86 [95%CI: 7.94-9.78] *versus* AC/CC*:* 9.13 [95%CI: 8.33-9.93] mmol·L^{-1}; p = 0.71) did not differ between "fast metaboliser" (AA) and "slow metaboliser" (AC/CC) genotype. Furthermore, ΔiAUC for glucose between the sleep fragmentation and coffee trials was similar between "fast" and "slow" genotypes respectively (81.8 [18.5-145.1] *versus* 55.7 [-8.1-119.4] mmol·L^{-1}·120 min; p = 0.26).

Similarly, peak insulin concentration (AA: 309.9 [220.7-399.1] *versus* AC/CC: 345.5 [283.7-407.4] pmol·L^{-1}; p = 0.84), insulin iAUC (AA: 10234.5 [6720.2-13748.8] *versus* AC/CC: 10842.9 [6558.2-15127.7] pmol·L^{-1}·120min; p = 0.80), and ΔiAUC for insulin did not differ between "fast metaboliser" or "slow metaboliser" genotype respectively (2680.3 [423.0-4937.6] *versus* 2047.8 [-3447.06-7542.6] pmol·L^{-1}·120min; p = 0.75).

Discussion

The current study demonstrates that one night of hourly sleep fragmentation had no effect on next-day insulin sensitivity or glucose tolerance, relative to a habitual night of sleep, in young, healthy men and women. However, consumption of caffeinated coffee after sleep fragmentation increased glucose iAUC by ~50%.

A recent survey found that ~40% of people in the UK drink caffeinated coffee upon waking, and therefore the coffee condition in the current study provided an important ecological comparison to the sleep-fragmentation alone condition [8]. Previous studies have demonstrated acute reductions in glucose tolerance when caffeinated coffee is ingested prior to assessment of glucose metabolism [10]. In the present study, glucose iAUC was ~50% greater following caffeinated coffee relative to sleep fragmentation alone; this agrees with the one other study to have investigated the combination of caffeinated coffee and sleep disruption [16], albeit in the reverse order (i.e. caffeine before *versus* after sleep) and in relation to overall sleep deprivation (i.e. lower total duration) rather than the fragmentation reported here. Indeed, in that previous study there was no habitual sleep control condition and the caffeinated coffee was ingested during the sleep deprivation period; the present study therefore extends those findings by isolating the independent effects of sleep deprivation and caffeinated coffee, with the latter consumed following, rather than prior to sleep disruption.

Whilst not investigated in the current study, there are several potential mechanisms following caffeinated coffee ingestion that may explain the reduction in glucose tolerance. Primarily, within a circulating range of 25-40 $mmol \cdot L^{-1}$ caffeine acts as an antagonist for adenosine receptors, particularly in skeletal muscle where caffeine may inhibit glucose uptake via A1 adenosine receptor antagonism [25-27]. Additionally, caffeine ingestion is a stimulant for the release of adrenaline, which suppresses the action of insulin through β-adrenergic receptor activation [28-30]. It is also worth considering the lipolytic effects of caffeine ingestion on glucose uptake into skeletal muscle. The dose of caffeine provided in the current study is likely sufficient to induce lipolysis *prior* to the OGTT, subsequently impairing glucose uptake into the muscle [31, 32]. Finally, the role of cortisol in disruption of glucose metabolism must also be considered. Specifically, cortisol is elevated following both caffeine ingestion [33], and sleep disruption [3], which elevates postprandial glucose responses [34].

The rate of caffeine metabolism is affected by the rs762551 single-nucleotide polymorphism in the CYP1A2 gene, with individuals classed as either fast (AA) or slow (AC/CC) metabolisers [35]. Recent evidence suggests that postprandial glycaemic and insulinaemic responses to both chronic and acute caffeine ingestion are modulated by this polymorphism [14]. Despite observing the expected distribution of the rs762551 SNP, no difference in the insulinaemic or glycaemic response to the glucose load was found between genotype following caffeinated-coffee ingestion. While this may suggest that the modulating effect of the rs762551 SNP was not present, this analysis was included on an exploratory basis and thus the study was not directly powered to detect differences between

316 "fast" and "slow" metabolisers. Future work should therefore investigate the acute effect of this SNP
317 on the response to coffee both after a night of disrupted *versus* habitual sleep in a larger sample.

318 The current study observed no difference in insulin sensitivity or glucose tolerance following sleep
319 fragmentation relative to a habitual night of sleep. This is interesting considering that previous work
320 has shown even a single night of sleep restriction is sufficient to induce reductions in both peripheral
321 and hepatic insulin sensitivity relative to a habitual night of sleep [2]. In similar fashion to Gonnissen
322 *et al* [6], the current study observed one time-point at which postprandial plasma insulin concentration
323 was lower following Fragmented *versus* Control, which in the former was speculated to be due to a
324 difference in night-time glycogen use from waking. However, this cannot be directly concluded from
325 one time point alone. Speculatively, lack of postprandial differences in the current and previous study
326 [6] may be explained by the total magnitude of sleep disruption achieved through hourly sleep
327 fragmentation. In the current study, wake time was ~70-80 minutes total. Comparatively, Donga *et*
328 *al* [2] employed a broken sleep protocol (i.e. sleep time 2300-0100 h and 0500-0730 h) in healthy
329 lean subjects, observing a ~22% increase in endogenous glucose production alongside a ~20%
330 decrease in the rate of glucose disposal, indicative of reduced hepatic and peripheral insulin
331 sensitivity, respectively. Similarly, *two* nights of sleep reduction (~50% of habitual sleep duration:
332 442 ± 78 min *versus* 235 ± 34 min) also reduced Matsuda insulin sensitivity index by ~19% relative
333 to habitual sleep [4]. The total duration of sleep loss accumulated over one or more nights may
334 therefore be proportionate to the effect on postprandial glycaemia. Speculatively, this could be
335 explained by lesser disruption of slow-wave sleep (SWS - *i.e.* stage III of non-rapid eye movement
336 sleep) with the current protocol compared to previous studies. As SWS is thought to be the most
337 important mediator of metabolic, hormonal, and neurophysiological changes during sleep, studies
338 observing greater reductions in insulin sensitivity are likely to have done so through employment of
339 sleep disruption protocols that provide a greater degree of disruption to SWS [2, 4, 36]. Conversely
340 in the current study, participants were aroused from sleep every hour, which based on the average
341 length of each stage of sleep (~5-15 mins) and one sleep cycle (~90-110 mins) would potentially not
342 provide as much SWS disruption as previous literature [37].

343 Whilst the dose of caffeine ingested in the current is above that typically consumed in one cup of
344 coffee on a daily basis (~100-200 mg) [38, 39], studies employing lower doses (~60 mg) have
345 observed disrupted glucose control [10]. Despite this, a strength of the current study is the perhaps
346 more ecologically valid model of consumption following one night of sleep curtailment relative to
347 previous research [16]. Furthermore, the effects shown in this study are present in a relatively large
348 sample size for an acute study of this nature. One potential limiting factor in the interpretation of the
349 present results is the apparent order effect whereby mean plasma iAUC was higher in participants

first trial compared to both the second and third trials (197 [173-221] *versus* 148 [126-169] *versus* 132 [106-158] mmol·L^{-1}, respectively). However, repeated measures ANOVA revealed no interaction effect between which trial participants performed first and the effect of Coffee on postprandial glycaemia relative to Fragmented and habitual sleep. Simply put, the higher responses in the Fragmented+Coffee condition were not due to the participants who performed the Fragmented+Coffee trial first. Furthermore, the addition of a 4th condition (i.e. habitual sleep+coffee) to the study would have provided an interesting comparison to fully assess the independent effects of caffeinated coffee relative to sleep disruption. The lack of assessment of hormones potentially linked to the mechanism of disruption (e.g. cortisol) also limit further discussion surrounding the effects reported. Finally, the current study also did not strictly control for the menstrual phase in female participants and this may have influenced our primary outcomes [40].

In summary, no effect of hourly fragmented sleep (totalling <80 mins) was found on postprandial glucose and insulin responses to breakfast the next morning. However, the common approach of consuming a strong caffeinated coffee following disrupted sleep resulted in a reduction in ~~insulin sensitivity and~~ glucose tolerance. Following a night of disrupted sleep, individuals should balance the potential stimulating benefits of caffeinated coffee consumption with the potential to increase postprandial glucose excursions.

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Conflict of interest

None of the authors have any conflicts of interest to declare.

Author contributions

HAS –Design, data collection, analysis, write up
AH – Data collection, analysis, write up
JT – Data collection, write up
JPW – Analysis, write up
PH – Data collection, write up
OP – Data collection, write up
YCC – Data collection, write up
JGT – Design, data collection, write up
JAB – Design, data collection, analysis, write up

References

1. Briancon-Marjollet, A., et al., *The impact of sleep disorders on glucose metabolism: endocrine and molecular mechanisms.* Diabetol Metab Syndr, 2015. **7**: p. 25.
2. Donga, E., et al., *A single night of partial sleep deprivation induces insulin resistance in multiple metabolic pathways in healthy subjects.* J Clin Endocrinol Metab, 2010. **95**(6): p. 2963-8.
3. Stamatakis, K.A. and N.M. Punjabi, *Effects of sleep fragmentation on glucose metabolism in normal subjects.* Chest, 2010. **137**(1): p. 95-101.
4. Sweeney, E.L., et al., *Skeletal muscle insulin signaling and whole-body glucose metabolism following acute sleep restriction in healthy males.* Physiol Rep, 2017. **5**(23).
5. Wang, X., et al., *Short-Term Moderate Sleep Restriction Decreases Insulin Sensitivity in Young Healthy Adults.* Sleep Health, 2016. **2**(1): p. 63-68.
6. Gonnissen, H.K., et al., *Effects of sleep fragmentation on appetite and related hormone concentrations over 24 h in healthy men.* Br J Nutr, 2013. **109**(4): p. 748-56.
7. Tamaki, M., et al., *Night Watch in One Brain Hemisphere during Sleep Associated with the First-Night Effect in Humans.* Curr Biol, 2016. **26**(9): p. 1190-4.
8. Statista. *Why do you usually drink coffee?* 2017 February 14, 2019.]; Available from: https://www.statista.com/statistics/681692/coffee-consumption-drivers-united-kingdom-uk/.
9. Poole, R., et al., *Coffee consumption and health: umbrella review of meta-analyses of multiple health outcomes.* Bmj, 2017. **359**: p. j5024.
10. Moisey, L.L., L.E. Robinson, and T.E. Graham, *Consumption of caffeinated coffee and a high carbohydrate meal affects postprandial metabolism of a subsequent oral glucose tolerance test in young, healthy males.* Br J Nutr, 2010. **103**(6): p. 833-41.
11. Robertson, T.M., et al., *A single serving of caffeinated coffee impairs postprandial glucose metabolism in overweight men.* Br J Nutr, 2015. **114**(8): p. 1218-25.
12. Sachse, C., et al., *Functional significance of a C-->A polymorphism in intron 1 of the cytochrome P450 CYP1A2 gene tested with caffeine.* Br J Clin Pharmacol, 1999. **47**(4): p. 445-9.
13. Thorn, C.F., et al., *PharmGKB summary: caffeine pathway.* Pharmacogenet Genomics, 2012. **22**(5): p. 389-95.
14. Robertson, T.M., et al., *Postprandial glycaemic and lipaemic responses to chronic coffee consumption may be modulated by CYP1A2 polymorphisms.* Br J Nutr, 2018. **119**(7): p. 792-800.
15. Banks, N.F., et al., *Genetic Polymorphisms in ADORA2A and CYP1A2 Influence Caffeine's Effect on Postprandial Glycaemia.* Sci Rep, 2019. **9**(1): p. 10532.
16. Rasaei, B., et al., *Simultaneous coffee caffeine intake and sleep deprivation alter glucose homeostasis in Iranian men: a randomized crossover trial.* Asia Pac J Clin Nutr, 2016. **25**(4): p. 729-739.
17. Bell, D.G. and T.M. McLellan, *Exercise endurance 1, 3, and 6 h after caffeine ingestion in caffeine users and nonusers.* J Appl Physiol, 1985. **93**(4): p. 1227-34.
18. Rimm, E.B., et al., *Validity of self-reported waist and hip circumferences in men and women.* Epidemiology, 1990. **1**(6): p. 466-73.
19. Loftus, G.R. and M.E. Masson, *Using confidence intervals in within-subject designs.* Psychon Bull Rev, 1994. **1**(4): p. 476-90.
20. Wolever, T.M.S., *Effect of blood sampling schedule and method of calculating the area under the curve on validity and precision of glycaemic index values.* British Journal of Nutrition, 2004. **91**(2): p. 295-300.
21. Matsuda, M. and R.A. DeFronzo, *Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp.* Diabetes Care, 1999. **22**(9): p. 1462-70.

22. Levy, J.C., D.R. Matthews, and M.P. Hermans, *Correct homeostasis model assessment (HOMA) evaluation uses the computer program.* Diabetes Care, 1998. **21**(12): p. 2191-2.
23. Wellek, S. and M. Blettner, *On the proper use of the crossover design in clinical trials: part 18 of a series on evaluation of scientific publications.* Dtsch Arztebl Int, 2012. **109**(15): p. 276-81.
24. Betts, J.A., et al., *PRESENT 2020: Text Expanding on the Checklist for Proper Reporting of Evidence in Sport and Exercise Nutrition Trials.* Int J Sport Nutr Exerc Metab, 2020. **15**: p. 1-12.
25. Thong, F.S. and T.E. Graham, *Caffeine-induced impairment of glucose tolerance is abolished by beta-adrenergic receptor blockade in humans.* J Appl Physiol, 1985. **92**(6): p. 2347-52.
26. Fredholm, B.B., *Astra Award Lecture. Adenosine, adenosine receptors and the actions of caffeine.* Pharmacol Toxicol, 1995. **76**(2): p. 93-101.
27. Vergauwen, L., P. Hespel, and E.A. Richter, *Adenosine receptors mediate synergistic stimulation of glucose uptake and transport by insulin and by contractions in rat skeletal muscle.* J Clin Invest, 1994. **93**(3): p. 974-81.
28. Avogaro, A., et al., *Epinephrine exerts opposite effects on peripheral glucose disposal and glucose-stimulated insulin secretion. A stable label intravenous glucose tolerance test minimal model study.* Diabetes, 1996. **45**(10): p. 1373-8.
29. Baron, A.D., P. Wallace, and J.M. Olefsky, *In vivo regulation of non-insulin-mediated and insulin-mediated glucose uptake by epinephrine.* J Clin Endocrinol Metab, 1987. **64**(5): p. 889-95.
30. Deibert, D.C. and R.A. DeFronzo, *Epinephrine-induced insulin resistance in man.* J Clin Invest, 1980. **65**(3): p. 717-21.
31. Kovacs, E.M., J. Stegen, and F. Brouns, *Effect of caffeinated drinks on substrate metabolism, caffeine excretion, and performance.* J Appl Physiol (1985), 1998. **85**(2): p. 709-15.
32. Gonzalez, J.T., *Paradoxical second-meal phenomenon in the acute postexercise period.* Nutrition, 2014. **30**(9): p. 961-7.
33. Lovallo, W.R., et al., *Caffeine stimulation of cortisol secretion across the waking hours in relation to caffeine intake levels.* Psychosom Med, 2005. **67**(5): p. 734-9.
34. De Feo, P., et al., *Contribution of cortisol to glucose counterregulation in humans.* Am J Physiol, 1989. **257**(1 Pt 1).
35. Yang, A., A.A. Palmer, and H. de Wit, *Genetics of caffeine consumption and responses to caffeine.* Psychopharmacology, 2010. **211**(3): p. 245-57.
36. Tasali, E., et al., *Slow-wave sleep and the risk of type 2 diabetes in humans.* Proc Natl Acad Sci U S A, 2008. **105**(3): p. 1044-9.
37. Roebuck, A., et al., *A review of signals used in sleep analysis.* Physiol Meas, 2014. **35**(1): p. R1-57.
38. Desbrow, B., et al., *An examination of consumer exposure to caffeine from retail coffee outlets.* Food Chem Toxicol, 2007. **45**(9): p. 1588-92.
39. Ludwig, I.A., et al., *Coffee: biochemistry and potential impact on health.* Food Funct, 2014. **5**(8): p. 1695-717.
40. Valdes, C.T. and K.E. Elkind-Hirsch, *Intravenous glucose tolerance test-derived insulin sensitivity changes during the menstrual cycle.* J Clin Endocrinol Metab, 1991. **72**(3): p. 642-6.

Table 1. Summary of Participant Characteristics.

	Male (*n*=16)	**Female (*n*=13)**	**Combined (*n*=29)**
Age (y)	22 ± 1	21 ± 1	21 ± 1
Height (m)	1.81 ± 0.07	1.69 ± 0.07	1.78 ± 0.09
Body mass (kg)	82.2 ± 10.5	66.3 ± 11.5	75.3 ± 13.47
Body Mass Index ($kg \cdot m^{-2}$)	25.0 ± 2.6	23.2 ± 3.8	24.2 ± 3.3
Waist circumference (cm)	84.0 ± 6.4	73.3 ± 8.2	80.4 ± 8.7
Hip circumference (cm)	100.7 ± 6.8	97.1 ± 12.9	99.1 ± 10.0
Waist:Height (cm)	0.46 ± 0.03	0.43 ± 0.05	0.45 ± 0.04

Values are Mean ± Standard Deviation.

Table 2. Peak and time to peak glucose and insulin values in each condition alongside Matsuda insulin sensitivity index. Values are mean [normalised confidence interval].

	Control	Fragmented	Fragmented+Coffee
Peak Glucose (mmol·L^{-1})	8.20 [7.93-8.47]	8.23 [7.96-8.50]	8.96 [8.70-9.22]*
Peak Insulin (pmol·L^{-1})	265.1 [247.3-283.0]	235.4 [217.6-253.3]	310.5 [283.5-337.4]*
Time to Peak Glucose (min)	33 [29-36]	28 [25-32]	30 [27-33]
Time to Peak Insulin (min)	36 [32-40]	35 [31-38]	38 [35-42]
ISI Matsuda (au)	15.4 [14.3-16.3]	15.3 [14.2-16.3]	13.9 [12.9-14.9]
HOMA2-IR (au)	0.40 [0.26-0.54]	0.55 [0.41-0.68]	0.43 [0.28-0.58]

* Indicates difference from Fragmented based on nCIs not overlapping the mean of the comparator.

Figure 1. A) Time course plasma glucose and B) Time course plasma insulin response to an oral glucose load following a habitual night of sleep (Control), a night of fragmented sleep (Fragmented), or sleep fragmentation + caffeinated coffee (Fragmented + Coffee). *$p < 0.05$