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Consumption of dark chocolate attenuates subsequent food intake compared with milk and white chocolate in postmenopausal women

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Short running head: Appetite responses to chocolate

1 Abstract

2 **Background:** Chocolate has a reputation for contributing to weight gain due to its high fat,
3 sugar and calorie content. However, the effect of varying concentrations of cocoa in
4 chocolate on energy intake and appetite is not clear. **Objective:** To compare the acute effect
5 of consuming an isocaloric dose of dark, milk and white chocolate on subsequent energy
6 intake, appetite and mood in postmenopausal women. **Methods:** Fourteen healthy
7 postmenopausal women (57.6 ± 4.8 yr) attended an introductory session followed by three
8 experimental trials performed in a counterbalanced order at a standardised time of day, each
9 separated by one week. *Ad libitum* energy intake, perceived appetite, mood and appetite-
10 related peptides were assessed in response to consumption of 80% cocoa [dark chocolate],
11 35% cocoa [milk chocolate] and cocoa butter [white chocolate] (2099 kJ), prepared from a
12 single-origin cacao bean. **Results:** *Ad libitum* energy intake was significantly lower following
13 dark (1355 ± 750 kJ) compared with both milk (1693 ± 969 kJ; $P = 0.008$) and white ($1842 \pm$
14 756 kJ; $P = 0.001$) chocolate consumption. Blood glucose and insulin concentrations were
15 transiently elevated in response to white and milk chocolate consumption compared with the
16 dark chocolate ($P < 0.05$), while pancreatic polypeptide was elevated in response to higher
17 cocoa content chocolate (dark and milk) compared with white chocolate ($P < 0.05$). No
18 differences in active ghrelin or leptin were observed between conditions, nor was mood
19 altered between conditions ($P > 0.05$). **Conclusions:** Dark chocolate attenuates subsequent
20 food intake in postmenopausal women, compared to the impact of milk and white chocolate
21 consumption.

22

23 **Key words:** appetite, *ad libitum* energy intake, cocoa, polyphenols, mood, ghrelin

24

25 **Abbreviations:** UWA: The University of Western Australia; POMS-A: Profile of Mood
26 States – Adolescents; VAS: Visual analogue scale; PP: Pancreatic polypeptide

27 INTRODUCTION

28 Chocolate is a highly palatable and indulgent confection, with American's consuming 5-6 kg
29 per capita in 2010 (1). Notwithstanding this high rate of consumption, chocolate is generally
30 considered 'unhealthy'; however, growing evidence suggests that some types of chocolate
31 may provide benefits to consumers ranging from protection against biomarkers of
32 cardiovascular disease risk (2-4), to enhanced cognition (5) and reduced overall mortality rate
33 (6). Such benefits have been attributed to the high polyphenol content (particularly flavanols)
34 contained within the component of cocoa liquor termed non-fat cocoa solids (7-8). Dark
35 chocolate contains a greater proportion of this cocoa liquor, and therefore non-fat cocoa
36 solids (~5-fold greater) compared with milk chocolate (9), with the remainder comprising
37 mainly sugar and a small amount of other constituents, as well as the addition of milk in milk
38 chocolate (10). In comparison, white chocolate is comprised of cocoa butter extracted from
39 cocoa liquor and is therefore devoid of the non-fat cocoa solids that contain flavanols, with
40 the remainder comprised of sugar and sweeteners (3). Accordingly, dark chocolate is
41 generally promoted over milk and white chocolate.

42

43 Despite these potential benefits of dark chocolate consumption, it is important to note that
44 most commercially available chocolate is high in fat, simple sugar and calories (11). This
45 may contribute to excess energy intake and subsequent weight gain in the long-term, which in
46 turn may increase the risk of cardiovascular disease and type 2 diabetes (12). However, there
47 is some preliminary evidence to suggest that dark chocolate may also have beneficial effects
48 on appetite. More specifically, Sørensen and Astrup (2011) found that consumption of 100 g
49 of dark chocolate (70% cocoa) promoted satiety, reduced hunger and *ad libitum* energy intake
50 at the next meal, compared with an equivalent volume of milk chocolate (30% cocoa) in
51 young healthy men (13). It is important to note that this study compared two commercially

52 available chocolate bars that were not matched for energy content (217 kJ difference between
53 conditions) and were unlikely to be from a cacao bean of similar origin, which would
54 influence the biochemical composition of the cocoa liquor and mixture of polyphenols
55 present. More recently, Akyol and colleagues (2014) demonstrated that substituting milk
56 chocolate for dark chocolate in a traditional Turkish recipe reduced subsequent *ad libitum*
57 energy intake at a lunch meal; however, the specific origin of the chocolate used in this study
58 was unclear (14). Furthermore, no previous studies have included a white chocolate
59 comparison in order to assess the dose-response to chocolate containing distinct
60 concentrations of cocoa, and the mechanisms for the proposed effect of dark chocolate on
61 appetite are yet to be studied. Accordingly, the present study aimed to assess the acute effect
62 of consuming an isocaloric dose of chocolate with varying cocoa concentrations (80% cocoa
63 dark chocolate, 35% cocoa milk chocolate and a cocoa butter white chocolate devoid of non-
64 fat cocoa solids) produced from the same batch of single-origin cacao beans (to ensure a
65 consistent biochemical profile of the cocoa liquor portion) on appetite, subsequent energy
66 intake and the circulating concentration of a number of appetite-related peptides and
67 metabolites (active ghrelin, insulin, leptin, pancreatic polypeptide, glucose). **These issues**
68 **were examined in postmenopausal women, as the hormonal changes accompanying**
69 **menopause are associated with an increased risk of weight gain (15-16).** It was hypothesised
70 that acute consumption of dark chocolate would reduce subsequent food intake to a greater
71 extent than both milk and white chocolate.

72

73 **MATERIALS AND METHODS**

74 **Participants**

75 Healthy, postmenopausal (defined as absence of menstruation for at least 12 months) women
76 aged 50-65 yr were recruited from The University of Western Australia (UWA) and the local

77 community via email announcements and flyers. Postmenopausal women were studied due to
78 their increased risk of weight gain resulting from the reduced production of endogenous
79 oestrogen during the menopausal transition (15-16). Exclusion criteria included taking any
80 prescribed medication, diabetes, a current eating disorder or weight loss diet, smoking, or not
81 enjoying regular consumption of all types of chocolate (white, milk and dark). Of those who
82 responded, fourteen women were eligible for inclusion in the study and consented to
83 participate. It was estimated that a sample size of 12 participants would provide 80% power
84 to detect a difference of approximately 300 kJ in *ad libitum* dietary intake from our
85 laboratory test meal with an alpha value of 0.05. This study was approved by the UWA
86 Human Research Ethics Committee (Perth, WA, Australia) and each woman provided written
87 informed consent.

88

89 **Experimental Design**

90 Using a within-subjects counterbalanced design, each participant was required to attend four
91 separate laboratory sessions at the School of Sport Science, Exercise and Health, UWA. The
92 first visit, an introductory session, was followed by three 2 h experimental trials administered
93 in a counterbalanced order involving the consumption of three energy-matched (2099 kJ)
94 chocolate conditions; (a) 84 g of a high concentration cocoa (80%) ‘dark’ chocolate, (b) 87 g
95 of a lower concentration cocoa (35%) ‘milk’ chocolate and (c) 85 g of a cocoa butter ‘white’
96 chocolate (0% cocoa solids). This amount was based on previous studies examining the effect
97 of an acute dose of chocolate on appetite and cardiovascular outcomes (90-100 g; 1735-2500
98 kJ; 5, 13-14, 17). All chocolate was specifically manufactured in a single batch using a
99 single-origin cacao bean from The Sambirano Valley, Madagascar, in the desired
100 concentrations of 35% and 80%, with the white chocolate condition containing the cocoa
101 butter extracted from the same bean (Gabriel Chocolate Factory, Yallingup, WA, Australia).

102 The nutritional composition of each chocolate was analysed by an independent agency
103 (Australian National Nutritional Measurement Institute, Melbourne, Australia; **Table 1**). Of
104 note, the precise macronutrient content of the chocolate could not be matched as it is the
105 proportion of cocoa liquor, cocoa butter and sugar that distinguishes dark, milk and white
106 chocolate.

107

108 **Introductory Session**

109 Participants were instructed to complete a food diary and abstain from caffeine, alcohol,
110 chocolate and vigorous physical activity in the 24 h prior to the introductory session and to
111 replicate this in the 24 h prior to each experimental session. The replication of energy intake
112 was confirmed verbally upon arrival at each session and **later via quantitative analysis of their**
113 **individual 24 h food diary** (Foodworks 7; Xyris Software, Queensland, Australia). The
114 abstinence from caffeine and chocolate was intended to amplify any potential effect of
115 chocolate administration in the experimental trials. Body mass and height were recorded
116 before participants were familiarised with the questionnaires to be used in the subsequent
117 experimental sessions, with explanation, demonstration and opportunity to complete each
118 questionnaire. In addition, the laboratory test meal to assess energy intake was explained.

119

120 **Experimental Trials**

121 The three experimental testing sessions were conducted approximately one week apart at a
122 standardised time in the morning, after an overnight fast. Upon arrival at the laboratory, each
123 participant underwent baseline measures of mood, perceived appetite and had a fasting blood
124 sample taken to determine the circulating concentrations of blood glucose and appetite-
125 related hormones (detailed below). The assigned chocolate treatment was then administered
126 in a counterbalanced order at the same time of the morning during each experimental testing

127 session, with a fixed time of 15 min allowed for consumption. The participant was
128 blindfolded to prevent visual recognition of the condition being administered in an attempt to
129 allow for the assessment of the physiological effects of the different types of chocolate on
130 appetite, rather than potential cognitive effects. Immediately following chocolate
131 consumption, perceived appetite was assessed, before 30 min of passive rest in a
132 standardised, temperature controlled laboratory environment **where they were allowed to read**
133 **the same reading material of their choice at each session.** Repeat measures of mood,
134 perceived appetite and the circulating concentrations of blood glucose and appetite-related
135 hormones were taken at 30 and 90 min after consumption. **Following** these measures at 90
136 min post-ingestion, *ad libitum* energy intake was assessed over a fixed time of 20 min using a
137 laboratory test meal.

138

139 **Outcome measures**

140 ***Perceived appetite and mood***

141 Perception of appetite was assessed using a modified 100 mm visual analogue scale (VAS)
142 that is well validated and used extensively in the appetite-literature (18). Briefly, this
143 involved answering four questions anchored with words representing opposing extreme states
144 of fullness, hunger, desire to eat and prospective food consumption respectively (i.e. “*how*
145 *hungry do you feel?*” anchored by “*not hungry at all*” and “*as hungry as I have ever felt*”).
146 Mood was assessed using the profile of mood states – adolescents (POMS-A) questionnaire
147 which has been validated for use with adult populations (19). With a response set of “*How do*
148 *you feel right now?*” participants rated the 24 mood states on a scale from “not at all” to
149 “extremely”.

150

151 ***Ad libitum energy intake***

152 The *ad libitum* laboratory test meal consisted of a standardised mixture of ~140 g of instant
153 oats (Oats Quick Sachet—Creamy Honey, Uncle Tobys, Nestle Australia, Sydney, NSW,
154 Australia) and ~300 ml milk (HiLo Milk, Pura, Melbourne, VIC, Australia), provided in
155 excess of expected consumption (~440 g) in a large bowl. Participants were instructed to eat
156 until “comfortably full” within a fixed time of 20 min. The amount of food provided was
157 standardised within participants and always presented in the same manner, including use of
158 the same large bowl to make it difficult for participants to consciously perceive how much
159 they had eaten if under normal conditions. This *ad libitum* test meal was weighed before and
160 after consumption to determine the amount ingested (g) and calculate energy intake (kJ). This
161 form of laboratory test meal has been previously reported to have a test–retest correlation of
162 0.91 for assessing *ad libitum* food intake (20).

163

164 ***Circulating appetite-related hormones***

165 Venous blood was sampled from an antecubital vein and collected in a lithium heparin tube
166 (2 mL) for immediate analysis of blood glucose (ABL™ 725, Radiometer, Copenhagen) or
167 collected with EDTA (3 mL) and immediately combined with 160 µL of serine protease
168 inhibitor (Pefabloc SC, Roche Diagnostics, NSW, Australia) before being centrifuged at
169 1000 g for 10 min at 4°C with the plasma stored at -80°C. Samples were later analysed in
170 duplicate for a range of appetite-related peptides including active ghrelin, insulin, leptin and
171 pancreatic polypeptide (PP) using a commercially available assay kit (Milliplex Human Gut
172 Hormone Panel, Millipore Corporation, Billerica, MA, USA) according to the manufacturer's
173 instructions on a Luminex 200 system (Luminex Corp., Austin, Texas, USA). Fluorescence
174 data were analysed using Luminex xPONENT software (Luminex Corp.).

175

176 **Statistical analysis**

177 Two extreme under-reporters of daily energy intake were identified using the Goldberg
178 method as per Black (21) and excluded from the assessment of typical daily energy
179 consumption. The effect of the chocolate conditions on *ad libitum* energy intake was assessed
180 using one-way (condition) repeated measures analysis of variance (ANOVA). **Mood,**
181 **perceived appetite, blood glucose and appetite-related hormones were compared using two-**
182 **way (condition x time) repeated measures ANOVA. Post-hoc comparisons with Bonferroni**
183 **adjustments were used, as appropriate, to determine where any differences lay.** Significance
184 was accepted at $P \leq 0.05$ (SPSS version 20.0 for Windows).

185

186 **Results**

187 *Participant Characteristics*

188 Fourteen women completed all three experimental trials (mean \pm SD age 57.6 ± 4.8 years;
189 body mass 66.67 ± 11.13 kg; body mass index 24.3 ± 4.1 kg·m²); however, one participant
190 declined to consume the test meal (n = 13 for this measure) as she did not feel comfortable
191 with the prospect of food wastage (leaving left-overs). Energy intake in the 24 h prior to each
192 trial was well-matched within participants ($P = 0.71$) with a mean reported daily energy
193 intake of 7370 ± 976 kJ.

194

195 *Ad Libitum Energy Intake*

196 There was a significant main effect of condition on energy intake at the *ad libitum* test meal
197 following chocolate consumption ($P = 0.003$). Post hoc analysis revealed lower energy intake
198 following dark chocolate consumption (1355 kJ), compared with both milk (1693 kJ; $P =$
199 0.024 ; 20% reduction) and white chocolate (1842 kJ; $P = 0.003$; 26% reduction; **Figure 1**).

200 There was no effect of the order of trial administration on *ad libitum* energy intake ($P =$
201 0.981) and no participant consumed the entire meal.

202

203 *Perceived Appetite and Mood*

204 There were no differences in perceived appetite between chocolate conditions at baseline
205 prior to chocolate consumption ($P > 0.05$). Following chocolate consumption, there was no
206 significant interaction of condition and time for perceived hunger ($P = 0.433$), perceived
207 fullness ($P = 0.129$), desire to eat ($P = 0.848$), or prospective food consumption ($P = 0.954$)
208 between conditions (**Figure 2**). However, there was a main effect for time, with feelings of
209 hunger, desire to eat and prospective food consumption decreasing, and feelings of fullness
210 increasing immediately following chocolate consumption ($P < 0.001$). With respect to mood,
211 there were no differences between conditions at baseline ($P > 0.05$; **Table 2**). In response to
212 chocolate consumption, there was no change in feelings of anger, confusion, depression or
213 tension; however, there was a main effect of time on feelings of fatigue ($P = 0.001$) and
214 vigour ($P = 0.015$) which decreased and increased respectively, although there was no
215 difference between conditions.

216

217 *Blood Glucose and Appetite-Related Hormones*

218 Baseline fasting concentrations of blood glucose and appetite-related hormones (ghrelin,
219 insulin, leptin and pancreatic polypeptide [PP]) were similar between conditions ($P > 0.05$;
220 **Figure 3**). In response to chocolate consumption, blood glucose concentrations were higher
221 30 min after ingestion of the white ($P = 0.004$) and milk ($P = 0.022$) chocolate compared with
222 the dark chocolate, with levels returning to baseline by 90 min post-consumption resulting in
223 no difference between conditions at this time. The higher blood glucose response to white
224 and milk chocolate ingestion corresponded with a higher insulin response compared with the

225 dark chocolate at 30 min post-consumption ($P = 0.001$ and $P = 0.003$, respectively). Plasma
226 insulin remained elevated in response to milk chocolate compared with white ($P = 0.002$) and
227 dark chocolate ($P = 0.002$) at 90 min post-consumption. There was no difference in the
228 response of ghrelin or leptin to chocolate consumption between conditions ($P > 0.05$). In
229 contrast, PP was elevated to a greater extent at 30 min post-consumption of both dark and
230 milk chocolate compared with white chocolate ($P = 0.035$ and $P = 0.005$ respectively). At 90
231 min post-consumption PP remained higher following dark compared with white chocolate (P
232 $= 0.018$).

233

234 **Discussion**

235 **This study shows that** the consumption of dark chocolate attenuates subsequent energy intake
236 compared with consumption of an equivalent amount of both milk and white chocolate, and
237 **is the first to** investigate the potential mechanisms underlying this observation. Importantly,
238 the chocolate used in this study was precisely matched for energy content, and was produced
239 from a single-origin cacao bean which fundamentally ensured a consistent biochemical
240 profile of constituents between chocolate conditions. **This was integral in allowing for**
241 **assessment of the dose-response to chocolate containing distinct concentrations of cocoa and**
242 **ensured that differences could be attributed to the proportion of each constituent, rather than**
243 **variation in the types of constituents present, as would be expected in chocolate from distinct**
244 **types of cacao beans grown in different geographic locations and exposed to varied methods**
245 **of post-harvest treatment (22).** While this study does not promote the consumption of
246 chocolate, these findings suggest that for postmenopausal women that do consume chocolate,
247 dark chocolate should be the preferred choice in relation to moderating overall energy intake.

248

249 The observation of reduced energy intake following consumption of dark chocolate is
250 consistent with that of the two previous studies that compared energy intake following dark
251 and milk chocolate ingestion (13-14). The first study reported a decrease in *ad libitum* energy
252 intake of a laboratory test meal (pizza) by 548 kJ (17%) following dark compared with milk
253 chocolate consumption in young healthy men (13). Meanwhile, Akyol and colleagues (2014)
254 demonstrated that substituting **dark chocolate in place of milk** chocolate in a traditional recipe
255 reduced subsequent *ad libitum* energy intake (by 20%; -719 kJ) of a test meal in young
256 healthy women (14). However, the current study is unique in including a white chocolate
257 comparison, precisely matching the energy content of the chocolate dose provided, and
258 ensuring consistency in constituents by sourcing all chocolate from a single-origin cacao
259 bean. Unfortunately, previous research has not addressed these issues, with Sørensen and
260 Astrup (2011) comparing commercially available milk and dark chocolate from Denmark and
261 France, respectively, which were likely derived from different cacao beans with differing
262 mixtures of polyphenols and other constituents, and providing a difference in caloric load of
263 217 kJ (13). The source of the chocolate used in the study of Akyol and co-workers (2014)
264 was not clear (14).

265

266 The reduced energy intake following consumption of dark chocolate was not associated with
267 significant alterations in perceived appetite, with similar ratings of perceived hunger, fullness
268 and prospective food consumption between trials. This may not be surprising given that
269 ratings of perceived appetite do not always correspond with actual energy intake (23);
270 **although it should be acknowledged that the study was powered to detect differences in the**
271 **primary outcome (*ad libitum* energy intake), and it is therefore possible that the study was**
272 **underpowered for this particular outcome.** Of note, Sørensen and Astrup (2011) reported

273 greater satiety, lower perceived hunger and lower ratings of prospective food consumption
274 after consumption of dark compared with milk chocolate (13).

275

276 The mechanisms contributing to the lower energy intake following consumption of dark
277 compared with an isocaloric dose of milk or white chocolate are not clear. One potential
278 contributing factor relates to the macronutrient composition of the chocolate (24-25). The
279 amount of total fat, carbohydrate and protein was reasonably consistent between conditions.

280 **Nonetheless, whether the small difference in protein (< 3 g) between conditions may have**
281 **affected satiety is not known. Furthermore,** the type of carbohydrate varied, with sugar
282 contributing the majority of the carbohydrate content in the white chocolate, while
283 accounting for a much lower proportion of carbohydrate in the dark chocolate condition. This
284 difference in sugar content could not be avoided and indeed reflects the difference in the
285 general composition of commercially available white, milk and dark chocolate and hence was
286 important for ecological validity. Regardless, there is some evidence to suggest that the type
287 of carbohydrate may influence satiety given the likely different rates of gastric emptying and
288 small intestinal transit and absorption (26). The sugar content of the chocolate likely also
289 contributed to the varied response of blood glucose following **consumption. However,** this is
290 unlikely to have affected energy intake in the current study given that blood glucose had
291 returned to similar levels between chocolate conditions by the time the *ad libitum* meal was
292 administered. Likewise, the **lower insulin response to dark chocolate compared with the milk**
293 **and white chocolate consumption** is unlikely to have contributed to the reduced energy intake
294 following dark chocolate consumption (27).

295

296 With respect to other appetite-related peptides (ghrelin, leptin and PP), this study is the first
297 to compare their responses to the ingestion of different types of chocolate. Our results suggest

298 that ghrelin and leptin did not mediate the reduction in *ad libitum* food intake following dark
299 chocolate consumption, since there was no difference in the circulating concentrations of
300 these peptides between conditions. In contrast, PP was elevated to a greater extent in response
301 to dark and also milk chocolate compared with white chocolate. These alterations in PP may
302 have influenced subsequent food intake, given the role of PP to reduce appetite and energy
303 intake (28). However, the reason for the varied response of PP to each chocolate condition is
304 unclear. Postprandial release of PP is generally considered to be proportional to caloric intake
305 (29); however, all chocolate conditions were isocaloric. An alternative possibility is that PP
306 was elevated in a dose-response manner to the polyphenol content of the chocolate. Indeed,
307 there is some evidence to suggest that polyphenols can influence the gastrointestinal
308 hormones released in response to food intake (30), although evidence specific to PP is
309 lacking.

310

311 The higher polyphenol content of the dark chocolate may have also influenced subsequent
312 energy intake by altering carbohydrate metabolism. Specifically, a variety of polyphenols
313 have been shown to inhibit the action of two key enzymes required for starch digestion,
314 alpha-glucosidase and alpha-amylase (31). In turn, this may attenuate the digestion of
315 carbohydrate in the fore-gut, delaying digestion further down the gastrointestinal tract,
316 thereby inducing satiety and reducing food intake at a later meal. Alternatively, there is some
317 limited supporting evidence to suggest that polyphenols may have a direct inhibitory effect
318 on appetite centres in the brain (30). Whether any of these potential mechanisms played a role
319 in the present study remains to be determined. It should also be acknowledged that while an
320 independent measure of overall polyphenol concentration was obtained for each kind of
321 chocolate used in the present study, it is unclear whether the observed effects were associated
322 with specific individual polyphenols, or the combined mixture. For instance, there is evidence

323 to suggest that epicatechin acutely reduces *ad libitum* energy intake in healthy, young
324 volunteers (32). Future research is needed to identify the role of specific polyphenols, as well
325 as their interactions when present in various combinations.

326

327 Other potential mechanisms for the reduced appetite following dark chocolate consumption
328 may relate to the sensory characteristics of the chocolate itself. Like previous studies
329 assessing energy intake in response to chocolate consumption, we did not attempt to match
330 for, or measure, perceived sweetness, palatability, enjoyment or preferences for each
331 chocolate (13-14). Only women who enjoyed regular consumption of all types of chocolate
332 (dark, milk and white) were included in the present study, and these women had varied
333 preferences in their favourite type of chocolate, however, their specific preferences within the
334 study were not assessed. Furthermore, despite the use of a blindfold to prevent visual
335 recognition of the chocolate, taste could not be completely blinded. Accordingly, further
336 research is needed to determine the potential contribution of consumer expectation to
337 subsequent energy compensation (33), as well as to assess the independent effects of
338 sweetness and palatability on subsequent appetite responses.

339

340 Regardless of the specific mechanism at play, the reduction in energy intake of ~400 kJ (20-
341 26%) following dark chocolate consumption is likely meaningful when one considers that an
342 additional energy intake of just 125 kJ per day has been found to cause a small, consistent
343 degree of positive energy balance that results in gradual weight gain (34). Of course, these
344 results do not intend to promote the consumption of chocolate for weight management, but
345 rather show that for women that *do* consume chocolate, it may be preferable to choose types
346 that are rich in cocoa liquor (i.e. darker). However, it must be acknowledged that participants
347 consumed a volume of chocolate (~80 g) that is larger than the average daily intake. It is also

348 important to highlight that energy intake was only assessed at the subsequent meal, so the
349 effect on energy intake later in the day remains to be determined. Furthermore, the present
350 results may be specific to postmenopausal women, and future research is needed to confirm
351 these findings in other populations, as well as investigate the longer-term effect of chronic
352 chocolate consumption on appetite. Nonetheless, the present study suggests that for
353 postmenopausal women who *do* consume chocolate, dark chocolate may be the chocolate of
354 preference.

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359 measurement of the appetite-related peptides.

360

361 **Statement of Authorship**

362 DG, LN and KG designed research; CM conducted research and analysed data; KG
363 conducted the blood analyses; all authors were involved in interpretation of data, drafting
364 manuscript for publication, read and approved final manuscript.

365

366 **Conflict of Interest**

367 The authors have no conflict of interest to declare.

368

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References

1. Caobisco (Association of the Chocolate Biscuit and Confection Industries of the European Union. Statistical bulletin review 2013: Ranking of consumption (chocolate confectionary) Statistical Bulletin Review. Brussels: 2013.
2. McFarlin BK, Venable AS, Henning AL, Prado EA, Sampson JNB, Vingren JL, Hill DW. Natural cocoa consumption: Potential to reduce atherogenic factors? *J Nutr Biochem* 2015;26(6):626-32.
3. Ellam S, Williamson G. Cocoa and human health. *Annu Rev Nutr* 2013;33:105-28.
4. Grassi D, Lippi C, Necozione S, Desideri G, Ferri C. Short-term administration of dark chocolate is followed by a significant increase in insulin sensitivity and a decrease in blood pressure in healthy persons. *Am J Clin Nutr* 2005;81(3):611-4.
5. Francis S, Head K, Morris P, Macdonald I. The effect of flavanol-rich cocoa on the fMRI response to a cognitive task in healthy young people. *J Cardiovasc Pharmacol* 2006;47:S215-S20.
6. Buijsse B, Feskens EJ, Kok FJ, Kromhout D. Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen Elderly Study. *Arch Inter Med* 2006;166(4):411-7.
7. Kay CD, Kris-Etherton PM, West SG. Effects of antioxidant-rich foods on vascular reactivity: review of the clinical evidence. *Curr Atheroscler Rep* 2006;8(6):510-22.
8. Lazarus SA, Hammerstone JF, Schmitz HH. Chocolate contains additional flavonoids not found in tea. *The Lancet* 1999;354(9192):1825.
9. Miller KB, Hurst WJ, Flannigan N, Ou B, Lee C, Smith N, Stuart DA. Survey of commercially available chocolate-and cocoa-containing products in the United States. 2. Comparison of flavan-3-ol content with nonfat cocoa solids, total polyphenols, and percent cacao. *J Agric Food Chem* 2009;57(19):9169-80.

10. Scholey, A., & Owen, L. (2013). Effects of chocolate on cognitive function and mood: a systematic review. *Nutrition Reviews*, 71(10), 665-681.
11. Greenberg JA, Manson JE, Buijsse B, Wang L, Allison MA, Neuhouser ML, Tinker L, Waring ME, Isasi CR, Martin LW. Chocolate-candy consumption and 3-year weight gain among postmenopausal US women. *Obesity*. 2015;23(3):677-83.
12. Salmeron J, Hu FB, Manson JE, Stampfer MJ, Colditz GA, Rimm EB, Willett WC. Dietary fat intake and risk of type 2 diabetes in women. *Am J Clin Nutr* 2001;73(6):1019-26.
13. Sørensen LB, Astrup A. Eating dark and milk chocolate: a randomized crossover study of effects on appetite and energy intake. *Nutr Diab* 2011;1(12):e21.
14. Akyol A, Dasgin H, Ayaz A, Buyuktuncer Z, Besler TH. β -Glucan and dark chocolate: A randomized crossover study on short-term satiety and energy intake. *Nutrients* 2014;6(9):3863-77.
15. Toth MJ, Tchernof A, Sites CK, Poehlman ET. Menopause-related changes in body fat distribution. *Ann N Y Acad Sci* 2000;904(1):502-6.
16. Rebuffe-Scrive M, Andersson B, Olbe L, Björntorp P. Metabolism of adipose tissue in intraabdominal depots of nonobese men and women. *Metab* 1989;38(5):453-8.
17. Grassi, D., Desideri, G., Necozione, S., Lippi, C., Casale, R., Properzi, G., Blumberg, J. B., & Ferri, C. (2008). Blood pressure is reduced and insulin sensitivity increased in glucose-intolerant, hypertensive subjects after 15 days of consuming high-polyphenol dark chocolate. *The Journal of Nutrition*, 138(9), 1671-1676.
18. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes Relat Metab Disord* 2000;24(1):38.
19. Terry PC, Lane AM, Fogarty GJ. Construct validity of the Profile of Mood States—Adolescents for use with adults. *Psychol Sport Exerc* 2003;4(2):125-39.

20. West JS, Ayton T, Wallman KE, Guelfi KJ. The effect of 6 days of sodium phosphate supplementation on appetite, energy intake, and aerobic capacity in trained men and women. *Int J Sport Nutr Exerc Metab* 2012;22(6):422.
21. Black AE. Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. *Int J Obes* 2000;24(9):1119-30.
22. Kongor JE, Hinneh M, Van de Walle D, Afoakwa EO, Boeckx P, Dewettinck K. Factors influencing quality variation in cocoa (*Theobroma cacao*) bean flavour – A review. *Food Res Int* 2016;82:44-52
23. Mattes R. Hunger ratings are not a valid proxy measure of reported food intake in humans. *Appetite* 1990;15(2):103-113.
24. Rolls BJ, Hetherington M, Burley VJ. The specificity of satiety: the influence of foods of different macronutrient content on the development of satiety. *Physiol Behav* 1988;43(2):145-53.
25. Poppitt SD, McCormack D, Buffenstein R. Short-term effects of macronutrient preloads on appetite and energy intake in lean women. *Physiol Behav* 1998;64(3):279-85.
26. Feinle C, O'Donovan D, Horowitz M. Carbohydrate and satiety. *Nutr Rev* 2002;60(6):155-69.
27. Chapman IM, Goble EA, Wittert GA, Morley JE, Horowitz M. Effect of intravenous glucose and euglycemic insulin infusions on short-term appetite and food intake. *Am J Physiol-Regul Integr Comp Physiol* 1998;274(3):R596-R603.
28. Batterham R, Le Roux C, Cohen M, Park A, Ellis S, Patterson M, et al. Pancreatic polypeptide reduces appetite and food intake in humans. *J Clin Endocrinol Metab* 2003;88(8):3989-92.

29. Adrian T, Bloom S, Bryant M, Polak J, Heitz P, Barnes A. Distribution and release of human pancreatic polypeptide. *Gut* 1976;17(12):940-4.
30. Panickar KS. Effects of dietary polyphenols on neuroregulatory factors and pathways that mediate food intake and energy regulation in obesity. *Mol Nutr Food Res* 2013;57(1):34-47.
31. Hanhineva K, Törrönen R, Bondia-Pons I, Pekkinen J, Kolehmainen M, Mykkänen H, Poutanen K. Impact of dietary polyphenols on carbohydrate metabolism. *Int J Mol Sci* 2010;11(4):1365-402.
32. Greenberg JA, O'Donnell R, Shurpin M, Kordunova D. Epicatechin, procyanidins, cocoa, and appetite: a randomized controlled trial. *Am J Clin Nutr* 2016;104(3):613-9.
33. Appleton KM, McKeown PP, Woodside JV. Energy compensation in the real world: Good compensation for small portions of chocolate and biscuits over short time periods in complicit consumers using commercially available foods. *Appetite* 2015;85:104-10
34. Hall KD, Sacks G, Chandramohan D, Chow CC, Wang YC, Gortmaker SL, Swinburn BA. Quantification of the effect of energy imbalance on bodyweight. *The Lancet*. 2011;378(9793):826-37.

TABLE 1.

Nutritional composition of white, milk and dark chocolate

Nutritional component	White chocolate	Milk chocolate (35% cocoa)	Dark chocolate (80% cocoa)
Energy (kJ/100 g)	2470	2420	2490
Amount consumed (g)	85	87	84
Energy consumed (kJ)	2099	2099	2099
Carbohydrate (g)	44.2	42.6	36.1
Sugar (g)	42.5	35.7	19.3
Fat (g)	34.1	34.0	36.3
Saturated fat (g)	21.3	21.1	22.1
Mono-unsaturated fat (g)	9.9	10.2	11.4
Poly-unsaturated fat (g)	1.1	1.0	1.0
Protein (g)	4.9	7.1	7.8
Total polyphenols (mg)	35	200	395

TABLE 2

Mood responses over time to white, milk and dark chocolate consumption [mean (SD); n = 14]

	White chocolate			Milk chocolate			Dark chocolate		
	Pre	30min	90min	Pre	30min	90min	Pre	30min	90min
Anger	0.3(0.6)	0(0)	0.1(0.3)	0.1(0)	0.1(0.3)	0(0)	0.1(0)	0(0)	0(0)
Confusion	0.7(2.5)	0.5(0.7)	0.3(0.6)	0.9(1.9)	0.9(0.8)	0.2(0.6)	0.6(1.4)	0.4(0.7)	0.1(0.6)
Depression	0.4(1.6)	0.1(0)	0.1(0.3)	0.4(1.0)	0.2(0.3)	0(0)	0.4(0.3)	0(0)	0(0)
Fatigue*	2.9(4.4)	2.1(2.3)	2.1(2.0)	2.8(2.8)	2.0(2.1)	0.9(1.6)	2.5(2.2)	1.2(1.7)	0.7(1.3)
Tension	1.1(2.1)	0.5(1.2)	0.4(0.7)	1.1(2.3)	0.9(1.7)	0.4(1.3)	0.9(2.2)	0.6(1.1)	0.4(1.1)
Vigour*	5.2(3.0)	5.9(2.9)	6.1(2.5)	5.1(3.2)	6.8(3.1)	7.3(3.4)	5.5(3)	6.6(2.2)	7.4(2.9)

* indicates significant main effect for time

Figure Legends

Figure 1. *Ad libitum* energy intake of a laboratory test meal following consumption of white, milk and dark chocolate (n = 13; mean \pm SEM). [†] indicates significantly lower energy intake following consumption of dark compared with both milk and white chocolate ($P < 0.05$).

Figure 2. Perceived hunger (A), fullness (B), desire to eat (C) and prospective food consumption (D) in response to white, milk and dark chocolate consumption. No significant interaction of time and condition ($P > 0.05$; mean \pm SEM).

Figure 3. Blood glucose (A), plasma insulin (B), plasma ghrelin (C), plasma leptin (D) and plasma pancreatic polypeptide (E) in response to white, milk and dark chocolate consumption. Significant differences are indicated between ^a white and dark, ^b white and milk, and ^c milk and dark chocolate ($P \leq 0.05$; mean \pm SEM).