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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 ( $57.6 \pm 4.8$ 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 ( $1355 \pm 750$  kJ) compared with both milk ( $1693 \pm 969$  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  $57.6 \pm 4.8$  years;  
189 body mass  $66.67 \pm 11.13$  kg; body mass index  $24.3 \pm 4.1$  kg·m<sup>2</sup>); 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  $7370 \pm 976$  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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**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). <sup>†</sup> 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 <sup>a</sup> white and dark, <sup>b</sup> white and milk, and <sup>c</sup> milk and dark chocolate ( $P \leq 0.05$ ; mean  $\pm$  SEM).