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

Abstract

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

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

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

INTRODUCTION

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

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

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

Experimental Design

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

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

Introductory Session

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

Experimental Trials

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

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

Outcome measures

Perceived appetite and mood

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

Ad libitum energy intake

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

Circulating appetite-related hormones

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

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 ± SD age 57.6 ± 4.8 years;
189 body mass 66.67 ± 11.13 kg; body mass index 24.3 ± 4.1 kg·m^2); 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**).

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

Perceived Appetite and Mood

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

Blood Glucose and Appetite-Related Hormones

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

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

Discussion

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

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

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

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

The mechanisms contributing to the lower energy intake following consumption of dark compared with an isocaloric dose of milk or white chocolate are not clear. One potential contributing factor relates to the macronutrient composition of the chocolate (24-25). The amount of total fat, carbohydrate and protein was reasonably consistent between conditions. Nonetheless, whether the small difference in protein (< 3 g) between conditions may have affected satiety is not known. Furthermore, the type of carbohydrate varied, with sugar contributing the majority of the carbohydrate content in the white chocolate, while accounting for a much lower proportion of carbohydrate in the dark chocolate condition. This difference in sugar content could not be avoided and indeed reflects the difference in the general composition of commercially available white, milk and dark chocolate and hence was important for ecological validity. Regardless, there is some evidence to suggest that the type of carbohydrate may influence satiety given the likely different rates of gastric emptying and small intestinal transit and absorption (26). The sugar content of the chocolate likely also contributed to the varied response of blood glucose following consumption. However, this is unlikely to have affected energy intake in the current study given that blood glucose had returned to similar levels between chocolate conditions by the time the *ad libitum* meal was administered. Likewise, the lower insulin response to dark chocolate compared with the milk and white chocolate consumption is unlikely to have contributed to the reduced energy intake following dark chocolate consumption (27).

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

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

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

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

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

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

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

Acknowledgements

The authors acknowledge the facilities, and the scientific and technical assistance at the Centre for Microscopy, Characterisation & Analysis, The University of Western Australia, a facility funded by the University, State and Commonwealth Governments for the measurement of the appetite-related peptides.

Statement of Authorship

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

Conflict of Interest

The authors have no conflict of interest to declare.

Financial Support

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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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 ± 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 ± 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 ± SEM).