

# Human Nutrition and Metabolism Research Communication

## Cocoa Powder Increases Postprandial Insulinemia in Lean Young Adults<sup>1</sup>

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**ABSTRACT** We hypothesized that chocolate products elicit higher insulin responses than matched products with alternate flavoring. To test this, we used a within-subject, repeated-measures comparison of six pairs of foods, one flavored with chocolate (cocoa powder) and the other not. Healthy subjects ( $n = 10$ , 4 men, 6 women) tested each pair of foods. Postprandial glucose and insulin levels were determined at intervals over 2 h using standardized glycemic index (GI) methodology. The product categories were chocolate bars, cakes, breakfast cereals, ice creams, flavored milks and puddings. Although the GI did not differ within each pair, the insulin index (II) of the chocolate product was always higher, by a mean of 28%, than the alternate flavored product ( $P < 0.001$ ). The greatest difference occurred within the flavored milk category in which the chocolate version elicited 45% greater insulinemia than the strawberry flavored milk ( $P = 0.021$ ). Macronutrient composition (fat, protein, sugar, fiber or energy density) accounted for nearly all of the variation in GI among the foods, but did not explain differences in insulinemia. The presence of cocoa powder in foods leads to greater postprandial insulin secretion than alternate flavorings. Specific insulinogenic amino acids or greater cephalic phase insulin release may explain the findings. *J. Nutr.* 133: 3149–3152, 2003.

**KEY WORDS:** • chocolate • glycemic index  
• postprandial hyperinsulinemia

Studies on the glycemic index (GI)<sup>3</sup> of foods indicate that chocolate and chocolate-containing confectionery elicit rela-

tively low levels of postprandial glycemia compared with equicarbohydrate amounts of starchy staples such as bread, rice and potatoes (1). Block chocolate, for example, has a GI of 50 compared with many varieties of bread, rice and potato that have GI values  $> 70$ . The low glycemic response can be attributed at least in part to the sugar content of chocolate confectionery. Sucrose itself has a GI of  $\sim 60$  because it contains only half the glucose-equivalents of an equal amount of glucose or starch.

Interestingly, however, we have noted that insulin responses to chocolate confectionery have often been disproportionately higher than expected for the glycemic response (2). In some cases (e.g., chocolate-coated peanuts and chocolate-coated caramel bar), the insulin response was twice that expected for the level of glycemia. Foods with a similarly high fat content such as potato chips and croissants do not induce as much insulin secretion as some chocolate-containing products (3,4). This raises the hypothesis that specific insulinogenic compounds in cocoa powder might directly stimulate  $\beta$ -cell insulin secretion and thereby reduce the accompanying glycemia. It is also possible that the high sensory quality of chocolate might promote early cephalic phase insulin secretion (5).

To test the hypothesis that cocoa powder has unique insulin-stimulating properties, separate from those induced by the presence of large amount of fat and sugar, we studied six pairs of commercially available foods. Within each pair, one was flavored with cocoa powder and the other with an alternate flavor, but both were of substantially equivalent macronutrient composition. Postprandial glucose and insulin levels were assessed at frequent intervals over a 2-h period using standardized glycemic index (GI) methodology (6).

### SUBJECTS AND METHODS

**Subjects.** Healthy subjects ( $n = 11$ ) were recruited from the staff and student population of the University of Sydney (4 men, 7 women). Exclusion criteria were as follows:  $> 40$  y of age, BMI  $> 25$  kg/m<sup>2</sup>, prescription medication other than oral contraception, food intolerance and family history of diabetes. The subjects were  $24.7 \pm 3.3$  y old (range, 21–33 y) and their BMI was  $21.8 \pm 1.6$  kg/m<sup>2</sup> (range, 20–24 kg/m<sup>2</sup>). The study was approved by the institutional ethics committee and all subjects gave written informed consent.

**Test foods.** Six pairs of foods were studied. Within each pair, the appearance and macronutrient content were similar, but one was flavored with chocolate (cocoa powder) and the other with an alternate flavor. They represented a wide range of food types: 1) *breakfast cereals*: chocolate-coated puffed rice (Coco Pops, Kellogg's, Page-wood, NSW, Australia) and plain puffed rice (Rice Bubbles, Kellogg's, Australia); 2) *cakes made from mixes*: chocolate cake topped with ready-made chocolate frosting (Betty Crocker chocolate fudge super moist cake mix and Betty Crocker creamy deluxe dark Dutch fudge frosting, General Mills, Minneapolis, MN) and vanilla cake topped with ready-made vanilla frosting (Betty Crocker French vanilla super moist cake mix and Betty Crocker creamy deluxe vanilla frosting, General Mills); 3) *block chocolate*: plain chocolate (classic full cream milk chocolate, Nestlé, Sydney, NSW, Australia) and "white" chocolate (Milky Bar, Nestlé); 4) *flavored reduced-fat milk*:

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<sup>3</sup> Abbreviations used: AUC, area under the curve; CPIR, cephalic phase insulin response; GI, glycemic index; II, insulin index.

TABLE 1

The macronutrient composition for the 50-g available carbohydrate portions of the reference food and six pairs of test foods containing chocolate or an alternate flavor

Food	Portion weight	Energy	Protein	Fat	Total carbohydrate <sup>1</sup>	Sugar	Fiber
	<i>g</i>	<i>kJ</i>	<i>g</i>				
Reference food	Glucose, 50 Water, 250	800	0.0	0.0	50.0	50.0	0.0
Plain rice cereal	58.3	934	3.9	0.2	50.6	5.3	0.6
Chocolate rice cereal	57.1	921	3.1	0.2	50.7	20.9	0.7
Vanilla cake	Cake, 68.3 Frosting, 28.9	1400	2.8	13.5	50.9	36.2	0.9
Chocolate cake	Cake, 75.4 Frosting, 31.8	1519	4.1	16.0	51.8	35.9	1.8
White chocolate	86.2	1983	6.2	27.8	50.0	50.0	0.0
Milk chocolate	84.0	1865	6.0	24.7	50.0	48.7	0.0
Vanilla ice cream	277.6	3026	14.6	52.6	50.0	32.1	0.0
Chocolate ice cream	264.1	2800	12.2	47.5	50.0	31.9	0.0
Vanilla pudding	310.6	1398	9.0	10.9	50.0	40.4	0.0
Chocolate pudding	314.5	1415	9.4	11.0	50.0	38.7	0.0
Strawberry milk	Powder, 26 Milk, 412	1362	17.0	6.0	50.0	50.0	0.0
Chocolate milk	Powder, 28 Milk, 439	1441	19.0	7.0	50.0	48.0	0.0

<sup>1</sup> The total carbohydrate values include fiber.

reduced-fat (1.5%) cow's milk (Lite White, NSW Dairy Farmers, Sydney, Australia) flavored with chocolate drinking powder (chocolate Nesquik, Nestlé) or strawberry drinking powder (strawberry Nesquik); 5) *ice cream*: premium vanilla ice cream (French vanilla classic ice cream, Sara Lee, Gosford, NSW, Australia) and premium chocolate ice cream (Ultra chocolate classic ice cream, Sara Lee); and 6) *puddings*: chocolate and vanilla flavored versions of the same instant pudding made from a packet mix with full-cream milk (White Wings Foods, Smithfield, NSW, Australia).

Test foods and the reference food, anhydrous glucose, were fed as portions containing 50 g of available carbohydrate with 250 mL water. The macronutrient composition was calculated using the manufacturer's data (Table 1).

**Study design.** Each pair of foods was tested by 10 subjects using a within-subject, repeated-measures design. The reference food was consumed twice (at the first and last session) by each subject, and the test foods were given in a random, counterbalanced order with at least 1 d between tests. After a 10–12 h overnight fast, a baseline capillary blood sample (~1 mL) was collected by fingerprick using an automatic lancet device (Autoclix; Boehringer Mannheim, Frenchs Forest, NSW, Australia). Subjects consumed the test food and water within 12 min. Additional finger-prick blood samples were taken at 15, 30, 45, 60, 90 and 120 min after eating commenced.

Plasma glucose concentrations were analyzed in duplicate using a glucose-hexokinase enzymatic assay (Roche Diagnostics, Frenchs Forest, NSW, Australia) and a Cobas Fara automatic spectrophotometric analyser (Roche Diagnostica, Basel, Switzerland). Mean within- and between-assay CV were 0.7 and 1.1%, respectively. Plasma insulin concentration was analyzed using a solid-phase, antibody-coated tube RIA kit (Coat-A-Count insulin, Diagnostic Products Corporation, Los Angeles, CA) with internal controls. The mean within- and between-assay CV were 2.5 and 3.3%, respectively.

Subjects rated how much they liked the food on a 15-cm 7-point category rating scale anchored from the left-hand end with the category "dislike extremely" (−3) with a midpoint descriptor of "neither like nor dislike" (0) and anchored at the right-hand end with the descriptor "like extremely" (+3).

**Data analysis.** The incremental area under each 120-min plasma glucose and insulin response curve (AUC) was calculated using the trapezoidal rule with fasting values as the baseline (7). Any negative area (below the fasting baseline level) was ignored. The GI was calculated for each individual according to the equation:

$$\text{GI} = (120\text{-min glucose AUC value for the test food} \times 100) / \text{AUC value for the reference food}$$

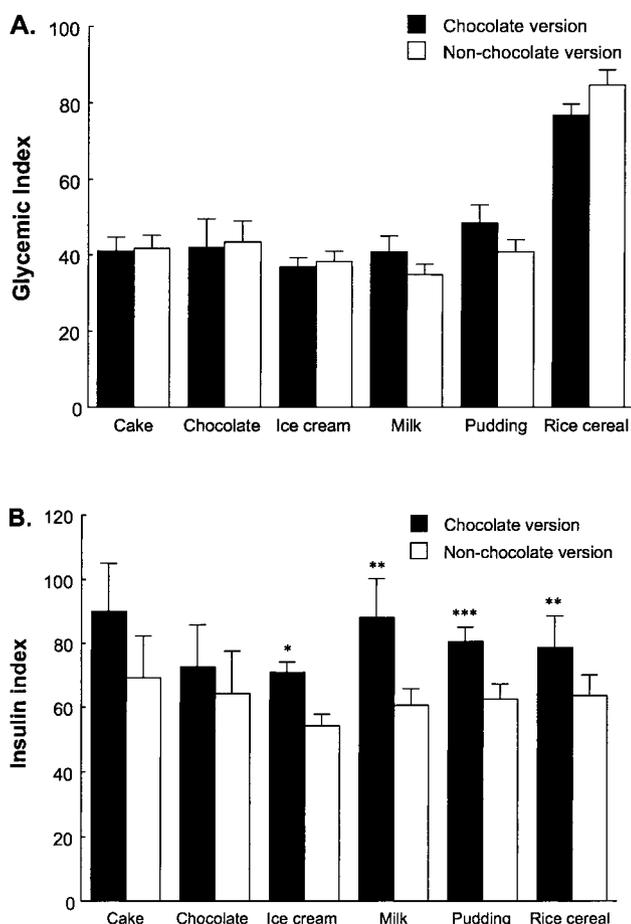
Insulin index (II) values were calculated similarly using the insulin AUC. GI and II values (means  $\pm$  SEM) for each food were calculated using all 10 subjects.

ANOVA using general linear models with food group and presence of chocolate as fixed factors and subjects as a random factor was used to investigate the effects of chocolate on GI and II (SPSS for Windows 10.0, Chicago, IL; StatView software, version 4.02, Abacus Concepts, Berkeley, CA). Interactions between the factors were investigated, but found not to be significant. With 10 subjects, the study had 80% power to detect a difference of 1.5 SD at the 0.05 level of significance. Multiple linear regression analysis was used to examine the extent to which different nutrient variables (protein, fat, sugar, fiber per test portion) accounted for the variability in GI and II values for the 12 test foods.

## RESULTS

The within-pair GI values did not differ for any of the six product pairs (Fig. 1A, Table 2). For all of the chocolate products, the mean GI value ( $47 \pm 2$ ) did not differ from that of all six nonchocolate products ( $48 \pm 2$ ). In contrast, as hypothesized, the insulin index for the chocolate version of each product was invariably higher than that of the alternate flavor (Fig. 1B, Table 2). The greatest difference occurred within the flavored milk category in which the chocolate version elicited 45% greater insulinemia than the strawberry flavored milk ( $P = 0.021$ ). The smallest difference was within the block chocolate category in which the dark chocolate II was only 13% higher than the white chocolate II ( $P = 0.61$ ). The mean II of the six chocolate products ( $79 \pm 3$ ) was 28% higher than the mean of the six nonchocolate products ( $62 \pm 3$ ) ( $P < 0.001$ ).

Surprisingly, the median GI and II of all 12 products were not correlated ( $r = 0.03$ ,  $P = 0.93$ ). The foods with the most marked difference between the II and the corresponding GI



**FIGURE 1** The glycemic index (A) and insulin index (B) of six pairs of foods, one containing chocolate and the other an alternative flavor determined in lean young adults. Values are means  $\pm$  SEM,  $n = 10$ . Asterisks indicate different from the nonchocolate version, \* $P < 0.001$ , \*\* $P < 0.05$ , \*\*\* $P < 0.01$ .

were chocolate cake (difference +47), chocolate milk (+45), chocolate ice cream (+34) and chocolate pudding (+33).

The mean insulin AUC/glucose AUC ratio (a measure of

insulinemia relative to glycemia) was 30% greater for the chocolate flavored version of each pair of foods ( $P = 0.001$ ). However, none of the individual food pairs differed significantly.

Multiple regression analysis using the median GI for each food as the dependent variable and protein, fat, sugar and fiber per serving as independent variables showed that sugar was the strongest determinant of the GI ( $P < 0.001$ ), followed by fat ( $P = 0.009$ ), fiber ( $P = 0.049$ ) and protein ( $P = 0.081$ ). Together, these variables accounted for 93% of the variance in GI. In contrast, when median II was the dependent variable, there were no significant associations with any one nutrient (sugar,  $P = 0.96$ ; fat,  $P = 0.56$ ; fiber,  $P = 0.77$  and protein,  $P = 0.65$ ), and this regression model explained only 8% of the variance in the II.

Within food pairs, the palatability rating for the chocolate product was always greater than that of the alternate flavor, with the greatest difference within the breakfast cereals ( $P < 0.001$ ) and the puddings ( $P < 0.01$ ).

**DISCUSSION**

To our knowledge, this is the first study to demonstrate that chocolate (cocoa powder), has a specific insulinotropic effect, irrespective of food source or the overall macronutrient composition of the food. We found that the chocolate-flavored product within six product categories produced greater insulinemia, whether expressed as the AUC over a 2-h period or as an insulin index relative to a reference food. In general, the chocolate product produced 28% greater insulinemia than the alternate flavor, ranging from 45% greater in the chocolate milk vs. the strawberry milk, to 13% greater in dark vs. white block chocolate. Within-pair differences in glycemia were minimal and did not explain the marked difference in insulinemia. Macronutrient composition accounted for nearly all of the variation in GI among the foods, but did not explain differences in insulinemia.

Our findings are consistent with those of other studies in healthy and diabetic individuals (2,4,8,9). Although those studies were not designed specifically to demonstrate an insulinogenic effect of chocolate, comparisons with other foods tested simultaneously allow a similar conclusion. For example,

**TABLE 2**

*The glucose, insulin and sensory responses of lean adults to the six pairs of foods containing chocolate or an alternate flavor<sup>1,2</sup>*

Food	Glucose AUC	Insulin AUC	Insulin AUC / glucose AUC	GI	II	Palatability
	mmol/(L · min)	pmol/(L · min · 10 <sup>3</sup> )				
Plain rice cereal	173 $\pm$ 17	10.2 $\pm$ 1.1	62 $\pm$ 6	84 $\pm$ 4	64 $\pm$ 6	0.4 $\pm$ 0.4
Chocolate rice cereal	177 $\pm$ 23	12.2 $\pm$ 1.0	76 $\pm$ 9	87 $\pm$ 11	79 $\pm$ 10**	1.9 $\pm$ 0.2
Vanilla cake	80 $\pm$ 10	7.3 $\pm$ 1.4	99 $\pm$ 17	41 $\pm$ 4	67 $\pm$ 12	1.8 $\pm$ 0.5
Chocolate cake	76 $\pm$ 6	9.2 $\pm$ 1.0	127 $\pm$ 17	41 $\pm$ 4	88 $\pm$ 14*	2.1 $\pm$ 0.5
White chocolate	84 $\pm$ 14	6.6 $\pm$ 1.1	92 $\pm$ 14	43 $\pm$ 6	63 $\pm$ 13	2.1 $\pm$ 0.4
Milk chocolate	78 $\pm$ 13	9.0 $\pm$ 2.4	125 $\pm$ 25	42 $\pm$ 7	71 $\pm$ 13	2.6 $\pm$ 0.1
Vanilla ice cream	80 $\pm$ 10	9.3 $\pm$ 1.3	123 $\pm$ 16	38 $\pm$ 3	54 $\pm$ 4	2.7 $\pm$ 0.1
Chocolate ice cream	78 $\pm$ 11	11.2 $\pm$ 1.2	169 $\pm$ 23	37 $\pm$ 3	71 $\pm$ 3*	2.8 $\pm$ 0.1
Vanilla pudding	87 $\pm$ 11	10.2 $\pm$ 1.1	125 $\pm$ 17	43 $\pm$ 5	62 $\pm$ 5	-0.2 $\pm$ 0.5
Chocolate pudding	95 $\pm$ 11	13.3 $\pm$ 1.6	159 $\pm$ 27	47 $\pm$ 4	80 $\pm$ 5***	1.6 $\pm$ 0.3
Strawberry milk	64 $\pm$ 4	6.9 $\pm$ 1.0	112 $\pm$ 17	35 $\pm$ 3	59 $\pm$ 5	1.1 $\pm$ 0.5
Chocolate milk	74 $\pm$ 6	9.4 $\pm$ 1.2	137 $\pm$ 24	41 $\pm$ 4	86 $\pm$ 11**	2.1 $\pm$ 0.3

<sup>1</sup> Values are the means  $\pm$  SEM,  $n = 10$ . Asterisks indicate different from the nonchocolate product: \* $P < 0.001$ , \*\* $P < 0.05$ , \*\*\* $P < 0.01$ .

<sup>2</sup> Abbreviations: AUC, area under the curve; GI, glycemic index; II, insulin index; RS, rating scale.

Holt et al. (4) tested postprandial blood glucose and insulin responses to isoenergetic portions of 40 common foods. Foods such as potato crisps, cheese and croissants, which are also rich in fat and energy dense, had insulin scores (insulin AUC relative to that of a reference food) that were comparable to their glucose scores. On the other hand, the chocolate confectionery (Mars Bar) had an insulin score that was 50% higher than its glucose score. In another study (8), a chocolate-flavored breakfast cereal (Coco Pops) had an insulin index 60% higher than its GI.

Cocoa powder is a complex substance containing several biologically active compounds, including caffeine, theobromine, serotonin, phenylethylamine and cannabinoid-like fatty acids (10,11). These intrinsic factors might affect glucose homeostasis not only by directly promoting insulin secretion, but also by producing insulin resistance. Some amino acids, particularly arginine, and amino acid mixtures have been found to be strongly insulinotropic when consumed simultaneously with carbohydrate (12). Van Loon et al. (13) showed that a mixture of free leucine, phenylalanine and arginine produced twice the insulin response compared with carbohydrate alone. The presence of protein and amino acids in the cocoa powder but not the alternative flavor might therefore explain our findings.

Cocoa butter, the fat component of the cocoa bean, is also one of the richest food sources of triglycerides containing stearic acid, 18:0. Stearate has been found to be a powerful stimulant of insulin secretion in perfused rat pancreas compared with four other fatty acids (oleate, linoleate, oleate and palmitate) (14). However, this in itself does not explain our findings because two of the product pairs in the present study (rice cereal and low fat milk) were devoid of cocoa butter. Further research is therefore required to identify the source of the high insulin response to cocoa powder.

Chocolate may not be unique in its insulinogenic capacity. We and others have noted that dairy products produce hyperinsulinemia despite a low GI (15–17). In the present study, the milk-based categories (liquid low fat milk, pudding and ice cream) displayed the highest insulin AUC/glucose AUC ratios (5).

Together with its chemical components, the sensory characteristics of chocolate may also potentiate insulin secretion. Chocolate is an extremely palatable food, the mere thought of which can trigger a “Pavlovian” response, and therefore enhance cephalic phase insulin release (CPIR), particularly in people with a high preference for chocolate (5). Because our subjects rated the chocolate version of each product as more palatable, it is conceivable that greater CPIR contributed to our findings.

The physiologic importance of postprandial hyperinsulinemia is unknown, particularly if the corresponding level of glycemia is low, as in this case. Hyperinsulinemia may be pathogenic when associated with dyslipidemia, hypertension,

impaired fibrinolysis and other features of the metabolic syndrome (18). However, other components in chocolate may play a protective role in the disease process (19).

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