Calcium supplementation of chocolate: effect on cocoa butter digestibility and blood lipids in humans[1–3]

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ABSTRACT

Background: The digestibility of cocoa butter was reported in animal but not human studies to be low (60–70% and 89–94%, respectively). These differences could be due to the much higher ratio of calcium to fat (by wt) in the diet of rats (0.04–0.18) than in that of humans (0.01).

Objective: We investigated whether supplementation of chocolate with 0.9% calcium (by wt), as an integral part of a Western diet, reduces absorption of cocoa butter and hence the digestible energy value of chocolate. We also assessed the effect of calcium supplementation on the blood lipid profile.

Design: Ten men were fed control diets containing 98–101 g chocolate/d with or without a 0.9%-Ca supplement (0.9 g Ca/d) for 2 periods of 2 wk each. The study was conducted with use of a randomized, double-blind crossover design under free-living conditions but with strict control of food intake.

Results: Calcium supplementation of chocolate increased fecal fat 2-fold (from 4.4 to 8.4 g/d; $P < 0.0001$) and reduced the absorption of cocoa butter by 13.0%. This was due mainly to an increase in the excretion of palmitic and stearic acids (3.4 g/d), which reduced the absorbable energy value of the chocolate by ≈9%. This supplementation also reduced plasma LDL cholesterol by 15% ($P < 0.02$); HDL cholesterol was unchanged.

Conclusions: Calcium supplementation can be used as a means of reducing the absorbable energy value of chocolate. Supplementation with 2.25% $CaCO_3$ had no effect on the taste of chocolate, was well tolerated by the subjects, and reduced LDL cholesterol in a short-term study.   *Am J Clin Nutr* 2001;73:246–52.

KEY WORDS   Calcium, chocolate, cocoa butter digestibility, energy content of chocolate, confectionery, fat digestibility, blood lipids, healthy men

INTRODUCTION

Cocoa butter is a vegetable fat that is rich in saturated fatty acids; palmitic acid (24–27% by wt) and stearic acid (32–36% by wt) occupy the terminal positions (*sn*-1 and *sn*-3) of triacylglycerol and oleic acid (33–37%) occupies the middle position (*sn*-2) of triacylglycerol (1). The digestibility of true lipids in the human diet is generally considered to be ≈95%. However, animal studies showed that 5–20% cocoa butter (by wt) in the diet has a lower digestibility (60–70%) (2–3) and may therefore be regarded as a low-energy fat. Cocoa butter was reported to be well absorbed (89–94%) in humans at high intakes (80–130 g/d), in nonrealistic diets (monotonous cookie or liquid diets) (4–6), or at moderate intakes of 31 g/d, in the form of chocolate, within a normal Western diet (7).

The discrepancy in cocoa butter absorption between rats and humans could be related to differences in the ratio of calcium to fat in their diets. This ratio may play an important role in absorption of cocoa butter because 1 mol Ca binds 2 mol saturated fatty acid (eg, stearic acid) to form poorly digested calcium stearate; the ratio of calcium to stearic acid (by wt) is 0.07. In the rat studies that showed poor digestibility of cocoa butter, the ratio of dietary calcium to fat was in the range of 0.04–0.18; the diets contained 0.9% calcium and 5–20% fat (3). In typical human diets, the daily consumption of calcium and fat is ≈1 and 100 g, respectively, with a ratio of calcium to fat of 0.01, which is 4–18 times lower than in the diet of rats. Consequently, the possibility that calcium binds with the saturated fatty acids of cocoa butter to form an insoluble soap is much higher with a rat diet than with a human diet. In fact, in rats the digestibility of stearic acid in the terminal positions of triacylglycerol [eg, stearic-oleic-stearic (S-O-S), 26% in cocoa butter] is lower with a diet that is rich in calcium (37% digestibility) than with a diet that is poor in calcium (70% digestibility) (8).

Furthermore, it was shown in humans that supplementation of the normal diet with large doses of calcium (2–4 g/d) resulted in increased fecal excretion of fat and saturated fatty acids (9, 10). In a recent study (11), a high intake of chocolate (184 g/d) enriched with calcium (1.87% Ca, by wt) as part of a very-low-fat diet resulted in higher fecal fat and saturated fatty acid excretion than did consumption of the same diet without calcium supplementation. However, the extent to which the absorption of cocoa fat and the energy value of chocolate was reduced under these unrealistic experimental conditions (high intake of chocolate, very high calcium supplementation, and very-low-fat control

diet) could not be quantified during the short (3 d) study with a parallel design and no adaptation to a test diet.

In the present study, the effect of a lower amount of calcium supplementation of chocolate (0.9% Ca, by wt) on digestibility of cocoa butter and energy value of chocolate was measured quantitatively when ≈100 g chocolate/d, with or without added calcium, was consumed as part of a typical Western diet after 7 d of adaptation. In addition, given several reports that diets supplemented with calcium reduce blood cholesterol in both humans (9, 12) and animals (13), we examined the effect of calcium supplementation (0.9 g/d) on the blood lipid profile.

SUBJECTS AND METHODS

Subjects

Ten male volunteers were selected for the study. The subjects fulfilled the following criteria: normal health as judged from a medical history, completion of a medical examination, no medication use, no history of gastrointestinal disorders, and normal results of liver function tests [aspartate aminotransferase activity (ASAT), alanine aminotransferase activity (ALAT), bilirubin, γ-glutamyl transpeptidase (GGT), alkaline phosphatase (PAL), and glucose] performed during the recruitment period. In addition, the defecation frequency of selected subjects, determined by a 7-d record during the recruitment period, was set to be ≥5 times/wk. The protocol was submitted and approved by the Nestlé Research Center ethical committee for human studies. Written consent was obtained from the volunteers after they had been informed about the exact nature of study. The mean (±SD) age and physical characteristics of the subjects were 34 ± 6 y, 73.9 ± 7.3 kg weight, 176 ± 6 cm height, and 24 ± 2.5 body mass index (in kg/m^2).

Experimental design and diet

The effect of calcium consumption on the digestibility of cocoa butter was assessed in a randomized, double-blind, 2-period crossover study conducted under free-living conditions but with strict control of food intake. A 7-d menu cycle (basal diet) composed of 14 varieties of normal Western menus was prepared in the kitchen of our research center. Each menu for the entire experimental period was prepared from the same ingredients, cooked in the same batch, portioned quantitatively, and deep frozen until consumption to ensure similar quality and quantity of all nutrients in all portions. The 10 volunteers were divided randomly into 2 groups. Each group consumed the basal diet, which was supplemented with either 98 g control dark chocolate/d (control diet) or 101 g calcium-supplemented dark chocolate/d that provided 0.9 g added Ca/d (calcium diet) for 2 wk in a crossover design study of 2 periods, with a 2-wk washout period between treatments. During the washout period the subjects consumed their habitual diets, which did not contain any calcium-supplemented foods. Group A consumed the control diet during the first 2 wk of the experiment (period 1) followed by a 2-wk washout period and then the calcium diet during the second 2-wk period of the experiment (period 2). Group B consumed the calcium diet first (period 1) and then the control diet (period 2).

The control chocolate (control Choc) and the calcium-supplemented chocolate (Ca-Choc) were prepared by Nestlé (Broc, Switzerland) from identical ingredients and had similar compositions except for the calcium supplementation. The Ca-Choc was prepared by incorporating 0.9% Ca from 2.25% fine CaCO$_3$

TABLE 1
Composition of the control and calcium-supplemented chocolates

	Control	Calcium-supplemented
Ingredients (% by wt)		
Sugar	54.90	52.65
Cocoa liquor (55.5% fat)	31.50	31.50
Cocoa butter	13.40	13.40
Lecithin	0.10	0.10
Vanillin	0.10	0.10
Vanilla	0.06	0.06
CaCO$_3$	0.00	2.25
Composition (% by wt)		
Protein	4.37	4.41
Carbohydrate	56.51	55.04
Fat (cocoa butter[1])	31.54	30.69
Fiber	3.80	3.80
Calcium	0.02	0.90
Magnesium	0.09	0.09
Phosphorus	0.13	0.13
Energy (kJ/100 g)	2170	
Fatty acids (% by wt of total)		
16:0	26	26
18:0	35	35
18:1	34	34

[1] Cocoa butter intake in all subjects was 31 g/d, calculated on the basis of a daily intake of 98 g control chocolate and 101 g calcium-supplemented chocolate with cocoa butter contents of 31.54% and 30.69%, respectively.

powder (with a particle size of 60% < 20 μm, 30% between 20 and 35 μm, and 10% > 35 μm; Merck, Dietikon, Switzerland) into dark chocolate in an equal exchange for sucrose on a weight-for-weight basis. The ratio of calcium to fat (by wt) of the control Choc (with 31.5% fat) and the Ca-Choc (with 30.7% fat) was 0.0006 and 0.03, respectively. The compositions of the chocolates, including their nutrient and fatty acid contents, are shown in **Table 1**. The chocolates were consumed as 2 separate portions between meals. One portion (49–50.5 g chocolate) was consumed 2.5 h after breakfast (between 1000 and 1030) and the other 2.5 h after lunch (between 1500 and 1530).

Individual differences in the energy intakes of different subjects were adjusted by increasing intakes of carbohydrate-rich foods (eg, bread, jam, fruit, and juice) and drinks during the first week of the experiment. Consumption of these extra carbohydrate-rich foods and drinks (alcoholic and nonalcoholic) was recorded by each subject during the first week of the study in a diary and was kept constant during all other experimental weeks. Under these conditions, the amount and composition of the diet was monitored thoroughly and was similar during both experimental periods, except for the calcium supplementation of the chocolate. The subjects consumed their breakfast, lunch, and chocolates under supervision at set times in our research center and were provided with preweighed packed food for the evening meal. During the weekends, the subjects received preweighed packed foods for their breakfast, lunch, and dinner and the test chocolates, which they consumed at home. The subjects continued their habitual activities during the entire study.

Sample collection and records

All feces excreted were collected in preweighed containers during the last 8 d of each experimental period. The fecal sample from the first day was discarded (training for fecal collection) and the samples from the last 7 d were weighed and kept

frozen ($-20\,°C$) until analyzed. Blood samples were taken after a 12-h overnight fast during the recruitment period and on the first and last day of each experimental period for assessment of liver function and the blood lipid profile.

The subjects kept a record of all the extra carbohydrate-rich foods and drinks that they consumed, the time at which the food and chocolate were consumed at home during supper and the weekend, the duration and type of exercise, the frequency of defecation (time and number of defecations/d), stool consistency (hard, smooth, soft, or liquid), any discomfort or health problems, and an evaluation of the diets and the chocolates during both experimental periods.

Fecal lipid analysis

For each subject, the fecal samples that were collected during the last 7 d of each experimental period were pooled, homogenized (after addition of adequate water), freeze-dried, and then extracted. Total fecal lipids, including the soap fraction, were measured in triplicate on dry fecal samples by the Van De Kamer method as described by Ellefson and Caraway (14). The samples were saponified with KOH in ethanol containing 0.4% isoamyl alcohol (by vol) ($80\,°C$ for 1 h). The samples were then acidified (pH < 2) and extracted 3 times with petroleum ether by mixing in a rotomixer for 1 h, followed by a 10-min incubation at $40\,°C$, then mixing again in a vortex mixer for 2 min and separating by centrifugation ($503 \times g$ for 10 min at $25\,°C$). The 3 extractions from each sample were pooled in a clean preweighed dry round-bottom flask, measured gravimetrically after evaporation of the solvent to dryness in a rotovapor (Büchi, Flawil, Switzerland), and dried overnight in a desiccator under vacuum. For each subject, all the samples were analyzed in the same run. A preliminary test showed that 3 extractions were sufficient because no fat was found in the fourth extraction. The recovery efficiency of cocoa butter was checked in each series of analyses by adding to a few fecal samples a known amount of cocoa butter and stearic acid (corresponding to the maximum amount of cocoa butter expected if none of the cocoa butter was absorbed). The recovery of cocoa butter and stearic acid was found to be 98.2% (CV: 8%) and 98.9% (CV: 3%), respectively.

Fecal fatty acid analysis

Extracted fecal fat was dissolved in chloroform:ethanol (2:1, by vol) and an aliquot was taken for analysis of fecal fatty acids. A known quantity of 19:0 was added to all samples as an internal standard, the solvent was evaporated under nitrogen, and the fatty acids were methylated with 2% H_2SO_4 in methanol in a water bath ($80\,°C$ for 1 h). The fatty acid methyl esters were then neutralized with K_2CO_3 (6% solution) and extracted into Baker resianalyzed hexane. A known quantity of 23:0 methyl ester (the same as that of 19:0) was added to all samples before separation of the fatty acid methyl esters by gas chromatography (model 5890; Hewlett-Packard, Palo Alto, CA). The gas chromatograph was equipped with an automatic on-column injector, a fused silica precolumn of 1 m $\times$ 0.53 mm inner diameter (J&W Scientific Fisons, Folsom, CA), a Stabilwax fused silica capillary column of 30 m $\times$ 0.32 mm inner diameter, 0.25-μm film thickness (Restek Corporation, Port Matilda, PA), and a flame ionization detector. Helium was used as the carrier gas. The oven temperature program was set at $40\,°C$, 2 min isothermal; $30\,°C$/min to $145\,°C$, 1 min isothermal; $3\,°C$/min to $227\,°C$, 5 min isothermal; $1.5\,°C$/min to $240\,°C$, 2 min isothermal. The detector was set at $320\,°C$. Chromatograms were recorded with a data system integrator (HP 3365 Chemstation; Hewlett-Packard). Identification of peaks was made by comparison of retention times with those of a known standard (standard 85; NuChek Prep, Elysian, MN) run under identical conditions. The quantity of fatty acids in each sample was calculated from the known quantity of 19:0 in each sample by comparing the area of each fatty acid methyl ester with that of 19:0 methyl ester after correction for response factors. The response factor for each fatty acid relative to 19:0 was calculated by comparing the areas of known quantities of standard fatty acid methyl esters (NuChek Prep) with the area of the same quantity of 19:0 methyl ester added to the standard solution and run under the same condition as were the samples. The recoveries of fatty acids, based on the corrected area of 19:0 to that of 23:0, were 89.6% (CV: 2.2%) and 92.8% (CV: 1.2%) during analysis of control Choc and Ca-Choc samples, respectively.

Digestibility of cocoa butter in calcium-supplemented chocolate

Given that, for a given subject, the food intake was the same during the 2 experimental periods (ie, with or without supplementation of the chocolate with calcium), any change in the fecal fat during these 2 periods was assumed to be due to the effect of calcium on the digestibility of cocoa butter. Consequently, the digestibility of cocoa butter in the Ca-Choc relative to that in the control Choc (ie, the relative digestibility of cocoa butter during calcium supplementation; RD), can be calculated with use of the following equation:

$$RD\ (\%) = [(\text{Cocoa butter intake}) - (\Delta\ \text{fecal fat during the 2 dietary periods})] \times 100/\text{Cocoa butter intake} \qquad (1)$$

where cocoa butter intake, in absolute terms, was the same for all subjects and during both dietary periods and amounted to 31 g/d (sum of 17.5 g in cocoa liquor and 13.4 g added; Table 1). On the basis of previously reported data (7) indicating that the digestibility of cocoa butter in chocolate is the same as that of corn oil ($\approx$95%), an apparent digestibility of cocoa butter in the Ca-Choc can be calculated as 0.95 RD.

Blood analysis

Blood samples were collected in tubes containing sodium fluoride for glucose analysis and in plain tubes for serum lipid analysis and liver function tests. Samples were analyzed in duplicate with a centrifugal analyzer (Cobas Fara; Roche Diagnostica, Basel, Switzerland). Lyophilized commercial quality controls (BioMérieux, Roche, Switzerland) and a human serum pool were analyzed with each run of samples to control day-to-day variations. Total cholesterol, HDL and LDL cholesterol, and triacylglycerol were measured by peroxidase antiperoxidase (PAP) enzymatic methods using appropriate kits and calibration solutions from BioMérieux and Roche Diagnostica.

Plasma glucose, ASAT, and ALAT in serum were measured by using ultraviolet kinetic methods. Serum bilirubin, GGT, and PAL were measured with use of colorimetric methods. All liver function tests were performed with kits from Roche Diagnostica adapted for Cobas Fara.

Statistical analysis

All data are presented as means ± SDs. Statistical differences were determined by using analysis of variance with diet, peri-

TABLE 2
Nutrient intakes during the study

	Control period	Calcium period
Protein		
(g/d)	107 ± 3[1]	107 ± 3
(% of energy)	14	14
Carbohydrate		
(g/d)	354 ± 37	363 ± 41
(% of energy)	43	44
Fat		
(g/d)	134 ± 0.7	134 ± 0.8
(% of energy)	39	39
Saturated fat		
(g/d)	57 ± 0.4	57 ± 0.4
(% of energy)	16.7	16.6
Monounsaturated fat		
(g/d)	45 ± 0	45 ± 0
(% of energy)	13.6	13.4
Polyunsaturated fat		
(g/d)	23 ± 0	23 ± 0
(% of energy)	6.7	6.7
Cholesterol (mg/d)	333 ± 0.4	333 ± 0.4
Calcium (mg/d)	950 ± 59	1855 ± 64
Alcohol		
(g/d)	15.2 ± 13	13.5 ±14
(% of energy)	3.5	3.0
Energy		
(kJ/d)	12880 ± 770	12990 ± 883
(kcal/d)	3070 ± 183	3090 ± 209

[1] $\bar{x}$ ± SD; n = 10.

ods, and sequence as fixed factors and subjects as a nested factor (15). In all cases, the period and sequence effects were not significant ($P > 0.05$). The statistical analyses were performed with use of NUMBER CRUNCHER (NCSS 2000; Number Cruncher Statistical Systems, Kaysville, UT).

RESULTS

Food intake

The nutrient intakes during the experiment, calculated from food-composition tables (16), is presented in **Table 2**. The control diet, designed to have a composition similar to that of habitual food intake in Western countries, had the following energy composition: 14% protein, 43% carbohydrate, 39% fat, and 3.5% alcohol, with a cholesterol intake of 330 mg/d and a calcium intake of 950 mg/d. The ratio of polyunsaturated to saturated fat in the diets was 0.4 because of the high intake of saturated fat from chocolate (≈31 g/d). The nutrient intake during the calcium period was similar to that during the control period, except for the intake of calcium, which was higher during the calcium period than during the control period (1850 and 950 mg/d, respectively).

Fecal moisture content, defecation frequency, and fecal weight

The fecal moisture content was <80% during both test periods in all but one subject. In this individual (subject 8), fecal moisture content, although normal (80%) during the control period, was found to be as high as 87% during the calcium period. This, together with the observation that one-third of his stools was classified as very soft (almost liquid) during the same period, raised the strong possibility that the subject had a gastrointestinal problem during the calcium period. Thus, in line with our exclusion criteria described previously in the protocol, the results from this subject were omitted in the calculation of mean values and in the statistical analyses. For all the other subjects, the fecal consistency reported by the subjects and observed during the analysis was similar during both periods. In addition, the defecation frequency was similar in 9 of 10 subjects during the control and calcium periods (8.4 ± 2.6 and 8.6 ± 2.1 defecations/wk, respectively), and no gastrointestinal discomfort was reported during the study. These results suggest that the supplementation of dark chocolate with 0.9% Ca (0.9 g/d) did not change the physical characteristics of fecal samples and did not lead to any adverse gastrointestinal effects.

The fecal wet weight was significantly higher during the calcium period than during the control period (161 ± 40 and 134 ± 31 g/d, respectively; $P < 0.02$). This difference was due partially to an increase in fecal solids (fecal dry weight) during the calcium period relative to the control period (39.7 ± 5.4 and 34.2 ± 4.0 g/d, respectively; $P < 0.0001$). Although the total fecal water content was also higher during the calcium period, this was in proportion with the increase in the solid content of the feces in most subjects. In fact, the percentage of fecal moisture content was similar during both periods (74.5 ± 3.7% and 73.4 ± 6.3% during the calcium and control periods, respectively).

Fecal fat

Fecal fat excretion during both experimental periods is shown in **Table 3**. Fecal fat excretion was higher by nearly 2-fold during the calcium period (8.4 ± 1.0 g/d) than during the control period (4.4 ± 0.4 g/d; $P < 0.0001$). Examination of individual data indicated that this increase in fecal fat excretion during Ca-Choc intake occurred in all of the subjects (range: 2.0–5.5 g increase/d). The proportion of fat in the dry fecal sample was 21% during the calcium period and 13% during the control period ($P < 0.0001$). Nonetheless, the fecal samples during the calcium period did not appear to be oily or different from those of the control samples, suggesting that most of the extra fecal fat excreted during the calcium period was in the form of fatty acids rather than triacylglycerol.

TABLE 3
Fecal fat and cocoa butter digestibility of the calcium-supplemented chocolate relative to that of the control chocolate (RD)[1]

	Fecal fat			
Control period	Calcium period	Difference	Cocoa butter intake[2]	RD
g/d			*g/d*	
4.36 ± 0.43	8.40 ± 1.01[3]	4.04 ± 1.11	31 ± 0	87 ± 3.6

[1] Calculated as RD = [(cocoa butter intake) − (difference in fecal fat between 2 dietary periods)] × 100/cocoa butter intake. $\bar{x}$ ± SD; n = 9 (data from subject 8 not included).

[2] Cocoa butter intake during both periods and for all subjects was 31 g/d (*see* Table 1).
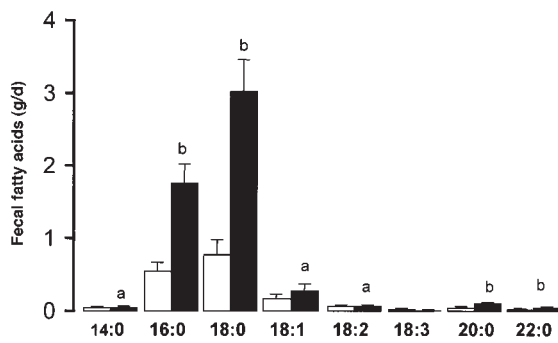
[3] Significant diet effect, $P < 0.0001$ (ANOVA).

FIGURE 1. Mean (±SD) fecal fatty acids during consumption of the calcium-supplemented chocolate (■) and the control chocolate (□). $n = 9$. [a,b]Significant difference between dietary periods (ANOVA): [a]$P < 0.05$, [b]$P < 0.001$.

Thus, in the calculation of cocoa butter absorption from Ca-Choc, no correction was made for the weight of glycerol.

Digestibility of cocoa butter and absorbable energy value in calcium-supplemented chocolate

The digestibility of cocoa butter in the Ca-Choc relative to that of the control Choc was calculated for each subject from the difference of fecal fat excreted during the 2 dietary periods expressed as a percentage of cocoa butter intake (31 g cocoa butter/d for all subjects and during both study periods). The mean relative digestibility was found to be 87%, with individual values ranging from 82% to 94% (Table 3). On the basis of these data, it was possible to estimate for each subject *1*) the apparent digestibility of cocoa butter in the Ca-Choc and *2*) the absorbable energy value of the Ca-Choc, as described below.

Apparent digestibility of cocoa butter in the calcium-supplemented chocolate

Because the digestibility of cocoa butter in chocolate was shown previously to be the same as that of corn oil ($\approx$95%; 7), the apparent digestibility of cocoa butter in the Ca-Choc, calculated as 0.95 RD, has a mean value of 83%. This translates to a 13% reduction in the apparent digestibility of cocoa butter in the Ca-Choc (from 95% to 83%).

Absorbable energy value of the calcium-supplemented chocolate

The presence of calcium in the chocolate reduced the apparent digestibility of cocoa butter by 13%. Consequently, the absorbable energy value of cocoa butter in the Ca-Choc would also have been reduced by 13%. Therefore, the absorbable

energy value of cocoa butter in the Ca-Choc would have been reduced from the generally accepted value of 37 kJ (9 kcal)/g to 32.2 kJ/g [37 kJ/g $\times$ 87% = 32.2 kJ (7.8 kcal)/g], ie, a difference of 4.8 kJ/g cocoa butter.

As indicated in Table 1, the chocolate used in this study had a cocoa butter content of 31% (by wt; 17.5% cocoa liquor and 13.4% cocoa butter) and an energy value of 2170 kJ/100 g chocolate. It follows, therefore, that during consumption of the Ca-Choc the absorbable energy value of cocoa butter was reduced by 149 kJ/100 g chocolate (4.8 kJ/g $\times$ 31 g). Furthermore, the exchange of 2.25% sugar (2.25 g $\times$ 1.05 = 2.36 g as monosaccharide equivalents) with 2.25% $CaCO_3$ (with zero energy value) would have reduced the energy value of the Ca-Choc by a further 38 kJ/100 g chocolate (2.36 g monosaccharide $\times$ 16 kJ/g). These changes (energy reduction of 149 kJ from cocoa butter and 38 kJ from sugar) would result in a total energy reduction of 187 kJ/100 g Ca-Choc. Thus, calcium supplementation would have reduced the absorbable energy value of the chocolate by 9% (from 2170 to 2021 kJ/100 g).

Fecal fatty acid profile

Fecal fatty acid excretion was much higher during the calcium period than during the control period (5.3 $\pm$ 0.7 and 1.7 $\pm$ 0.4 g/d, respectively; **Figure 1**). This difference was due mainly to the high rate of fecal excretion of saturated fatty acids, especially those of the main fatty acids of cocoa butter, namely palmitic acid (16:0) and stearic acid (18:0). During the calcium period, 16:0 excretion increased >3-fold (from 0.55 $\pm$ 0.12 to 1.75 $\pm$ 0.27 g/d) and that of 18:0 increased by $\approx$4-fold (from 0.78 $\pm$ 0.19 to 3.0 $\pm$ 0.42 g/d; $P < 0.0001$ in both cases). Apart from these fatty acids, the fecal output of other saturated fatty acids, such as 14:0, 20:0, and 22:0, and mono- and diunsaturated fatty acids, such as 18:1 and 18:2, also increased during the calcium period. These differences, although significant ($P < 0.05$), represent quantitatively only a small increase in total fatty acid excretion (0.2 g/d).

Body weight and blood analysis

Mean body weights and serum lipid profiles at the beginning of each period and their evolution during each period (day 14 minus day 0) are shown in **Table 4**. There was no significant period or sequence effect on any of these variables and no significant changes in body weight within or between the 2 experimental periods.

LDL cholesterol decreased on average by 0.43 mmol/L (15%) during the calcium period but by only 0.01 mmol/L during the control period ($P < 0.02$), suggesting that the changes in blood lipid values (reduction of LDL cholesterol) were more favorable during the calcium period than during the control period. Because

TABLE 4
Body weights and blood lipid concentrations of the subjects[1]

	Baseline (day 0)		Evolution (day 14 − day 0)	
	Control period	Calcium period	Control period	Calcium period
Body weight (kg)	73.40 ± 7.80	73.20 ± 7.50	0.2 ± 0.6	0.2 ± 0.6
Cholesterol (mmol/L)	4.81 ± 1.65	4.82 ± 0.72	−0.09 ± 0.35	−0.44 ± 0.62
LDL cholesterol (mmol/L)	2.73 ± 1.17	2.82 ± 0.84	−0.01 ± 0.25	−0.43 ± 0.39[2]
HDL cholesterol (mmol/L)	1.09 ± 0.42	1.08 ± 0.27	0.02 ± 0.12	−0.01 ± 0.12
Triacylglycerol (mmol/L)	1.07 ± 0.66	1.11 ± 0.60	−0.17 ± 0.38	−0.10 ± 0.41

[1]$\bar{x}$ ± SD; $n = 9$ (data from subject 8 not included).
[2]Significant diet effect, $P < 0.02$ (ANOVA).

HDL cholesterol and triacylglycerols did not change significantly during either of the experimental periods, the total cholesterol concentration decreased according to LDL cholesterol: a reduction of 0.44 mmol/L (9%) during the calcium diet but by only 0.09 mmol/L during the control diet. However, the effect of diet on total cholesterol was not significant ($P = 0.11$). Plasma glucose and serum bilirubin concentrations and PAL, GGT, ASAT, and ALAT were within the reference ranges during both periods, indicating that liver function was normal throughout the study.

DISCUSSION

In a previously reported study from our laboratory (7), it was shown that cocoa butter, when consumed in moderate amounts ($\approx$31 g/d) and as an integral part of a typical Western diet, has a digestibility as high as that of conventional oils and fats ($\approx$95%) and, hence, has an absorbable energy content of $\approx$37 kJ/g. In rats, by contrast, the digestibility of cocoa butter was reported to be poor (60–75%) (3). This apparent discrepancy between rats and humans could be related to the ratio of dietary calcium to fat, which is much higher in the diet of rats than in that of humans. Therefore, we investigated the extent to which human digestibility of cocoa butter may be reduced when dark chocolate is supplemented with 0.9% Ca and is consumed as an integral part of a typical Western diet ($\approx$100 g/d).

Our results show that supplementation of chocolate with 0.9% Ca (0.9 g/d) increases fecal fat excretion in humans and hence reduces the absorbed energy value of cocoa butter. In all the subjects, fecal lipid excretion was higher during the calcium period than during the control period, with increases ranging from 40% to 135% ($\overline{x}$: 93%) and the reduction in absorption of cocoa butter ranging between 6% and 18% ($\overline{x}$: 13%). Analysis of the fatty acid composition of the fecal lipids showed that the extra lipid losses during calcium supplementation were due mainly to higher losses of stearic and palmitic acids (ie, in saturated fatty acids, which are the main constituents of cocoa butter). These increases in the excretion of total fat (4 g/d) and stearic acid (2.2 g/d) are much larger (2-fold) than those found in 2 recent human studies (1.6–2.2 g fat and 1 g stearic acid/d) with much larger amounts of supplementary calcium (2–4 g/d) (9, 10) than that used in our study (0.9 g/d). It seems, therefore, that when calcium is added directly to chocolate, which is rich in saturated fatty acids, such that both are ingested together, calcium is more effective in reducing the absorption of lipids, particularly stearic acid.

On the basis of our data that showed that the absorption of cocoa butter in calcium-supplemented chocolate is reduced by 13%, it can be calculated that the absorbable energy value of cocoa butter in calcium-supplemented chocolate is 32 kJ (7.8 kcal)/g. This reduction in the absorbable energy value of cocoa butter in calcium-supplemented chocolate, together with zero energy value for 2.25% CaCO$_3$ (as an exchange for sugar, by wt), results in a reduction in the absorbable energy value of calcium-supplemented chocolate (31% cocoa butter; energy value of 2170 kJ/100 g) by $\approx$9%.

The mechanism of action by which calcium increases fat excretion is likely to be an interaction between calcium and saturated fatty acids, resulting in the formation of insoluble calcium–fatty acid soaps and hence reduced fat absorption. The hypothesis about the formation of calcium and magnesium soaps in the intestine was proposed by Givens (17) as early as 1917 and has since been confirmed in both animal studies (18) and human studies (19). In

fact, the absorption of fat from triacylglycerol involves lipase-mediated hydrolysis of fatty acids from the 1 and 3 position of the triacylglycerol, leaving a monoglyceride in position 2 and 2 fatty acids (20). However, the 2-monoglycerides are solubilized into biliary micelles (21); the free fatty acids have variable incorporation rates into biliary micelles (22). Saturated fatty acids are absorbed more slowly from the intestine than are unsaturated fatty acids (23), such that the interaction of saturated fatty acids with other gut contents is prolonged. Although a small amount of secreted intestinal calcium is necessary for the incorporation of unesterified fatty acids into biliary micelles (24), a large amount of calcium in the gut may actually reduce the absorption of saturated fatty acids by removing them from solution by precipitation of insoluble calcium–fatty acid soaps (19). In fact, it has been reported that the absorption of certain saturated fats rich in stearic acid, such as S-O-S (26% in cocoa butter), is lower in rats fed a high-calcium diet than in those fed a low-calcium diet (46–56% and 79–85% digestibility, respectively) (8, 25). In one of these studies (25), the low stearic acid digestibility of S-O-S fat in rats fed a high-calcium diet was found to reduce the apparent energy digestibility of the diet by $\approx$7% compared with that of a low-calcium diet (85% and 91.6% energy digestibility with high- and low-calcium diets, respectively).

Another important finding in the present study was that the reduced digestibility of cocoa butter with calcium was accompanied by a significant reduction in LDL cholesterol (with no changes in HDL cholesterol) in the short term. Although reduced absorption of saturated fatty acids may contribute to this LDL cholesterol–lowering effect of calcium, it is unlikely to be the entire explanation because the increased excretion of the saturated fatty acids was quantitatively small. At present, therefore, the mechanism by which calcium lowers blood cholesterol remains unclear. Although a cholesterol-lowering effect during calcium supplementation has often been reported (9), our result is of particular interest because *1*) cocoa butter is a saturated fat with a neutral effect on blood lipids (26) and *2*) a significant reduction in LDL cholesterol was achieved in our study with a normal mixed diet and with an amount of supplemented calcium (0.9 g/d) that was much lower than that in other studies (1.8 g/d) (9).

From an organoleptic standpoint, it should be noted that the addition of 0.9% Ca as 2.25% CaCO$_3$ in dark chocolate—and this was done in exchange for sugar—did not change the taste of the chocolate. In fact, the subjects participating in this study, as well as a few experts in the area of confectionery products, could not differentiate between the nonsupplemented and the calcium-supplemented chocolates.   ❧

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