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Digestibility of cocoa butter from chocolate in humans: a comparison with corn-oil

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Objective: To compare, in humans, the digestibility of moderate amounts of cocoa butter (30.7 g/d) consumed in the form of chocolate as part of a normal western diet with that of a well-absorbed fat (corn oil); and hence determine whether, by virtue of its apparent low absorption, cocoa butter can be considered to be a low calorie fat.
Design: Randomised, two-period crossover metabolic study, conducted under free-living conditions, but with strict control over food intake.
Setting: Metabolic Unit, Nestlé Research Center Lausanne.
Subjects: Twelve healthy men were selected from volunteers at the Nestlé Research Center and all subjects completed the study.
Intervention: Two treatment periods of two weeks each: cocoa butter and control periods, with strict dietary control separated by a two week wash out period.
Results: No differences ($P > 0.05$) were observed in faecal weight (wet or dry), faecal fat nor in defecation frequency between treatments (cocoa butter and corn oil). Cocoa butter at a dose of 30.7 g/d in the form of black chocolate, consumed between two meals, was found to have a similar digestibility to that of corn oil (99% of corn oil digestibility).
Conclusion: Cocoa butter, consumed as black chocolate within a normal mixed diet, has a high digestibility, similar to that of corn oil, and a digestible energy value of 37 kJ/g in man. Thus, cocoa butter cannot be considered to be a low-calorie fat.
Sponsorship: Nestec Ltd, Switzerland.
Descriptors: cocoa butter digestibility; faecal fat; defecation frequency; chocolate; corn oil; human study
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Introduction

Cocoa butter is primarily consumed in cocoa-containing products such as chocolate, confectionery products and certain beverages (for example hot chocolate). Although of vegetable origin, it is rich in saturated fatty acids, namely palmitic acid (24–27% by wt) and stearic acid (32–36% by wt) which are primarily in the terminal positions of triacylglycerols (sn-1 and sn-3), as well as oleic acid (33–37% by wt) which is mainly in the middle position (sn-2) of triacylglycerols (Bracco, 1994) . Despite the high degree of saturation, cocoa butter differs from other saturated fats (for example butter, animal fats) in its neutral effect on blood cholesterol. This effect is well documented in both animal (Chen *et al*, 1989) and human studies (Denke & Grundy, 1991; Kris-Etherton *et al*, 1993), and could be due to both reduced absorption and differential postabsorptive metabolism of its fatty acids (Pearson, 1994).

The absorption of saturated fatty acids has been shown to be stereotype-dependent, and to be reduced when they occupy — as in cocoa butter — the terminal position of triglycerols (sn-1 and sn-3) (Bracco, 1994, Decker, 1996). Indeed, the absorption of stearic acid, which makes up about a third of the fatty acids in cocoa butter, is often reported to be low both in animal and human studies. Low absorption of stearic acid in the terminal positions of glycerol (SOS) has been demonstrated in rats fed with either Ca-deficient diets (70%) or Ca-sufficient diets (37%) (Mattson *et al*, 1979). The chylomicron recovery rate of stearic acid in the sn-3 position has also been shown to be lower than that in the sn-2 position in rats (Redgrave, 1988). Furthermore, using stable isotope techniques, Jones *et al* (1985) reported lower absorption of free steric acid in human (78%) than that of oleic acid (98%).

In line with these findings about the low absorption of stearic acid, and particularly of the stereotype found in cocoa butter, the digestibility of cocoa butter in the rat has also been shown to be much lower than that of corn oil, namely with digestibility values of 60–70% and 93–97%, respectively (Chen *et al*, 1989, Apgar *et al*, 1987). In contrast, human studies have reported cocoa butter to be relatively well absorbed (89–94% digestibility) (Denke & Grundy, 1991; Mitchell *et al*, 1989; Mitchell *et al*, 1990). These human studies, however, do not reflect a normal consumption of cocoa butter since excessive quantities of cocoa butter (80–130 g/d) were consumed as virtually the sole source of dietary fat. In addition the cocoa butter was provided in unusual diets such as a liquid-formulated diet containing cocoa butter (Denke & Grundy, 1991), a monotonous diet of cookies made with cocoa butter (Mitchel *et al*, 1989) or diets very low in meat and dairy products (Mitchell *et al*, 1990).

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Given the uncertainties of extrapolating values for cocoa butter digestibility obtained with such unusual diets to normal consumption of cocoa butter and the current interest in the development of confectioneries with a lower energy content, we have re-evaluated, in humans, the digestibility of cocoa butter under more normal dietary conditions. To this end, the digestibility of moderate amounts of cocoa butter (30.7 g/d) consumed in the form of dark chocolate within a normal mixed diet was compared with that of a well-absorbed fat (corn oil) in a cross-over design experiment, with strict control over food intake. At the same time, the effect of the experimental diets on the faecal lipid profile was also studied.

Methods and design

Subjects

Twelve male volunteers were selected for the study. The subjects fulfilled the selection criteria which were as follows: normal health as judged from their medical history, a medical examination, free from medication, no history of gastrointestinal disorders and normal liver function tests (aspartate amino-transferase (ASAT), alamine amino-transferase (ALAT), bilirubin, gamma-glutamyl-transferase (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 at least 5 times per week. 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 values (and standard deviation) for age and physical characteristics of the subjects were age (y) = 37 ± 7.8; weight (kg) = 77 ± 7.0; height (cm) = 177 ± 4.1; body mass index (BMI, kg/m^2) = 25 ± 2.4.

Experimental design and diet

Cocoa butter digestibility was assessed in a randomised, double-period, crossover metabolic study, conducted under free-living conditions, but with strict control over food intake. A 7-d menu cycle (basal diet) composed of 14 varied normal western menus was prepared in the Nestlé Research Center kitchen. Each menu for the whole experimental period was prepared from the same ingredients, cooked in the same batch and portioned quantitatively, in order to ensure similar quality and quantity of all nutrients in all portions. The twelve volunteers were randomly divided into two groups (A and B). Each group consumed the basal diet, which was supplemented either with black chocolate (chocolate diet) or with a dessert containing corn oil (control diet) in a crossover design for a period of two weeks, with a 2-week washout period between treatments. The control and chocolate diets were consumed in periods I and II respectively by group A, while group B consumed the chocolate diet during period I and the control diet during period II. During the chocolate period, the basal diet was supplemented with 75 g of black chocolate daily (Nestlé Noir), which provided 30.7 g of cocoa butter/d. During the control period, the chocolate bar was replaced with an isocaloric dessert (control mousse) which had a similar composition to the chocolate bar except that cocoa butter was replaced quantitatively with corn oil and hence had a 'mousse like' soft texture. The composition of the chocolate and the control mousse as well as their nutrient and fatty acid content is shown in Table 1. The chocolate or control mousse was consumed as two separate portions daily (37.5 g/portion) between meals. One portion was consumed 2.5 h after breakfast (between 10.0 h and 10.30 h) and the other 2.5 h after lunch (between 15.0 h and 15.30h). Individual differences in the energy intake of subjects were adjusted with ad libitum intake of carbohydrate-rich foods (for example bread, jam, fruit juices, etc.) and drinks during the first week of the experiment. These extra intakes of carbohydrate-rich foods and all drinks (alcoholic and non-alcoholic) were recorded by each subject during the first week of the study and were kept constant during all experimental weeks. Under these conditions, the amount and composition of the diet was almost identical during both experimental periods (chocolate and control periods), except for the substitution of cocoa butter with corn oil. The subjects consumed their breakfast, lunch and chocolate or control dessert, under supervision, at set times in the dinning room of the metabolic unit and were provided with packed food for supper during the week. During the weekends the subjects were not supervised, however, they did receive preweighed packed foods for their breakfast, lunch and dinner and test desserts for home consumption. They continued their habitual activities during the entire study.

Table 1 Composition of black chocolate and control mousse

	Black chocolate	*Control mousse*
Ingredients	*% dry weight*	*% dry weight*
Sugar	35.30	35.30
Nestlé cocoa liqor (55% Fat)	52.50	0.00
Defatted cocoa powder (1.5% Fat)	0.00	23.60
Cocoa butter	12.10	0.00
Corn oil	0.00	41.00
Lecithin	0.09	0.09
Vanillin	0.05	0.05
Composition		
Protein	6.0	6.0
Carbohydrate	43.5	43.5
Fiber	6.3	6.3
Fat	41.0	41.0
Fatty acids	*% total fatty acids*	*% total fatty acids*
C16 : 0	25.4	12.2
C18 : 0	36.4	2.2
C18 : 1	33.6	27.5
C18 : 2	2.9	57.0

Sample collection and records

Total faecal excretion was collected during the last 8 d of each 2-week experimental period, and the samples from the last 7 d were analysed for fat content and fatty acid composition. The subjects kept a record of all of the extra foods (carbohydrate-rich food) and drinks which they consumed, duration and type of exercise, defecation frequency (time and number of defecation per day), consistency of faeces (hard, smooth, diarrhoea), any discomfort or health problem during both experimental periods.

Faecal lipid analysis

For each subject, the 7-d faecal samples that were collected during each experimental period were pooled, homogenised after addition of adequate water, freeze-dried and then extracted. Total faecal lipids, including the saponifiable (soap) fraction, were measured in triplicate on dry faecal samples as described by Ellefson & Caraway (1976). The samples were saponified with KOH in ethanol containing

0.4% isoamyl (v/v), cooled, acidified (pH < 2) and then extracted three times with petroleum-ether after mixing in a rotomixer for 1 h, incubation at 40 °C for 10 min followed by mixing with a vortex for 2 min and centrifugation. The faecal lipids (mainly as free fatty acids & sterols) from three extractions were pooled, evaporated and measured gravimetrically. For each subject, all the samples were analysed in the same run. A preliminary test showed that three extractions were sufficient since no fat was found in the fourth extraction. The recovery efficiency of cocoa butter was checked in each series of analyses by adding a known amount of cocoa butter (corresponding to the maximum amount of cocoa butter expected, if none of the cocoa butter was absorbed) to the faecal samples. The recovery of cocoa butter was found to be 100 % (coefficient of variation of ± 3.3 %).

Faecal fatty acids analysis
Faecal lipids were dissolved in chloroform-ethanol (2 : 1, v/v) and an aliquot was taken for analysis. The free fatty acids were methylated with acetyl chloride (at 100 °C for 1 h) as described by Lepage & Roy (1986), cooled to room temperature and neutralised with K_2CO_3 (6 % solution). The fatty acids methyl esters were extracted into hexane (Baker resianalyzed) and separated by gas chromatography (Carlo Erba model 8180, Milan, Italy). The gas chromatograph was equipped with an automatic on-colum injector, a flame ionization detector and a fused silica capillary column (25 m × 0.32 mm ID) coated with carbowax 20 M (0.225 μm). Hydrogen was used as the carrier gas (0.27 kg/cm^2). The oven temperature program was set at 50 °C, 4 min isothermal; 15 °C/min to 145 °C, 1 min isothermal; 3 °C/min to 195 °C, 1 min isothermal; 5 °C/min to 220 °C, 4 min isothermal. The detector was set at 320 °C. Chromatograms were recorded with a Waters 740 Data System Millipore integrator. Identification of peaks was made by comparison of retention times with those of a standard (Nucheck Prep, Elysian, MN) run under the same conditions.

Digestibility of cocoa butter (as % of corn oil digestibility)
The food intake of the subjects during both experimental periods (chocolate and control diet) was similar except for the exchange of cocoa butter (30.7 g/d) for the same amount of corn oil. Thus, any change in the faecal fat of each subject, during chocolate and control period was assumed to be due to differences between the digestibility of cocoa butter and the cornoil of the control dessert. The percentage digestibility of cocoa butter relative to that of corn oil was calculated by subtracting the difference in faecal fat of each subject during the two dietary treatments, and expressing the results as a percentage of cocoa butter intake per day, that is

$$\mathrm{RD} = \frac{[(\text{cocoa butter intake}) - (\text{differences of faecal fatty acids during chocolate and control diets* } 1.046\dagger)]*100}{\text{Cocoa butter intake}}$$

where CBI = cocoa butter intake and FL = faecal lipids during chocolate and control diets. §: The factor of 1.046 was used to correct for the weight of glycerol.

Statistical analysis
All data are presented as mean ± s.d. Statistical differences were determined using paired *t*-test. Differences were considered statistically significant at $P < 0.05$.

Results

Food intake
The nutrient intake during the experiment, calculated from food composition tables (Holland *et al*, 1992), is presented in Table 2. The control diet, designed to have a composition similar to that of habitual food intakes in western countries, had the following energy composition: protein (14 %), carbohydrates (43 %), fat (38 %), and alcohol (4 %), with a cholesterol intake of 273 mg/d, and Ca intake of 950 mg/d. The nutrient intake during the chocolate period was similar to that during the control period, except for differences in the quality of fat due to the different fatty acid composition of corn oil and cocoa butter. The fatty acids of cocoa butter are main saturated, while those of corn oil are mainly unsaturated (Table 1). Thus the main difference in nutrient intake between the two test periods was a higher intake of saturated fatty acids and a lower intake of polyunsaturated fatty acids during the chocolate period (52 and 20 g/d, respectively), compared with the control (corn oil) period (36 and 37 g/d, respectively).

Faecal weight and defecation frequency
Table 3 shows the data for faecal weights (wet and dry) and defecation frequency during the last 7 d in both experimental periods. They were similar during both experimental periods in all subjects, except one (subject 5) who had much higher faecal wet and dry weights during the chocolate period (155 and 38 g/d, respectively) than during the control period (106 and 27 g/d, respectively). Nonetheless the mean values of wet and dry faecal weights were similar during the control and chocolate periods (140 ± 29 *vs* 145 ± 28 and 36 ± 5 g/d, respectively). In addition defecation frequency was also found to be similar during the control and chocolate periods (7.5 ± 1.8 and 7.8 ± 2.3 defecation/week, respectively) and no gastrointestinal discomfort was reported during the experiment.

Faecal fat and cocoa butter digestibility
The results for total faecal fat excretion during both dietary periods of chocolate and control are presented in Table 4. The mean values of faecal fat were similar during chocolate and control periods (4.7 ± 1.0 *vs* 4.4 ± 0.7 g/d, respectively). Examination of individual data revealed that 11 out of the 12 subjects had similar faecal fat excretion during the chocolate and control periods. Only one subject (subject 5) showed a higher faecal fat excretion during the cocoa butter period (6.9 g/d) relative to control period (3.7 g/d). Cocoa butter digestibility in this subject was found to be 89 % of that of corn oil. The mean value of cocoa butter relative digestibility was found to be higher and similar to digestibility of corn oil (99 ± 4 % relative to corn oil digestibility).

Faecal fatty acid (FA) profile
The results presented in Figure 1 indicate that the proportion of palmitic acid in faecal samples (as % of total fatty acids) was similar during chocolate and control corn oil periods (26.6 ± 2.9 % *vs* 26.0 ± 3.6 %, respectively). However, the proportion of stearic acid was much higher in the

Table 2 Nutrient intake during study (mean ± s.d., $n = 6$)

	Group A		*Group B*	
	Control (Period I)	*Chocolate (Period II)*	*Control (Period II)*	*Chocolate (Period I)*
Protein				
g/d	100 ± 2	101 ± 2	100 ± 5	99 ± 2
% Energy	14.2	14.3	14.5	14.3
Carbohydrate				
g/d	321 ± 20	323 ± 22	319 ± 29	318 ± 20
% Energy	42.7	42.9	43.3	43.0
Fat				
g/d	120 ± 0.5	120 ± 0.7	120 ± 1.5	120 ± 0.7
% Energy	38.0	38.2	39.0	39.0
Saturated Fat				
g/d	364 ± 0.07	520 ± 0.15	365 ± 0.2	519 ± 0.14
% Energy	11.6	16.6	11.9	16.9
Monounsaturated Fat g/d	368 ± 0.07	398 ± 0.15	370 ± 0.27	396 ± 0.15
% Energy	11.8	12.7	12.0	12.9
Polyunsaturated Fat g/d	371 ± 0.1	203 ± 0.2	372 ± 0.2	201 ± 0.2
% Energy	11.9	6.5	12.1	6.5
Cholesterol				
mg/d	273 ± 0	273 ± 0	274 ± 2.5	273 ± 0
Calcium				
mg/d	963 ± 29	959 ± 27	945 ± 39	941 ± 27
Alcohol				
g/d	18 ± 10	17 ± 10	12 ± 7	14 ± 10
% Energy	4.5	4.2	3.0	3.5
Energy				
KJ/d	11798 ± 399	11818 ± 531	11592 ± 534	11617 ± 389
(kcal/d)	(2816 ± 96)	(2826 ± 127)	(2763 ± 127)	(2770 ± 93)

Table 3 Defecation frequency and faecal weights

			Defecation	*Faecal Weight (g/d)*	
Subject	*Period*	*Diet*	*# Sample/week*	*Wet Wt*	*Dry Wt*
1	I	Control	7.0	150.6	38.3
	II	Chocolate	8.0	158.3	41.1
2	I	Control	8.0	135.4	32.3
	II	Chocolate	7.0	129.9	28.9
3	I	Control	10.0	208.7	39.2
	II	Chocolate	7.0	212.4	38.7
4	I	Control	10.0	160.1	39.9
	II	Chocolate	10.0	154.4	33.8
5	I	Control	9.0	106.4	27.5
	II	Chocolate	10.0	155.1	38.4
6	I	Control	6.0	113.8	31.8
	II	Chocolate	4.0	119.9	33.3
7	II	Control	5.0	120.0	35.2
	I	Chocolate	5.0	110.2	30.9
8	II	Control	8.0	118.6	35.1
	I	Chocolate	9.0	129.7	39.0
9	II	Control	5.0	110.8	30.3
	I	Chocolate	7.0	117.4	30.8
10	II	Control	6.0	149.7	41.4
	I	Chocolate	6.0	158.9	41.0
11	II	Control	9.0	159.3	42.8
	I	Chocolate	12.0	160.0	42.4
12	II	Control	7.0	149.7	39.9
	I	Chocolate	8.0	135.6	36.2
Mean ± s.d. ($n = 12$)		Control	7.5 ± 1.8	140 ± 29	36.1 ± 4.8
		Chocolate	7.8 ± 2.3	145 ± 28	36.2 ± 4.6

*No significant differences between dietary treatments by paired *t*-test.

faeces during the chocolate period than during the control period (41 ± 2.7 % *vs* 27 ± 4.4 %, $P < 0.0001$, respectively), while that of other faecal fatty acids (for example: oleic and linoleic acids) was decreased during this chocolate period ($P < 0.01$).

Table 4 Faecal fat and relative digestibility of cocoa butter (RD)

	Faecal lipids § (g/d)		*RD*
Subject	*Control*	*Chocolate*	*(as % of corn oil digestibility)*
1	4.38	4.82	99
2	3.69	3.11	102
3	3.56	3.62	100
4	4.93	4.11	103
5	3.65	6.86	89
6	4.01	4.34	99
7	4.69	4.19	102
8	4.49	5.24	97
9	3.79	4.20	99
10	5.16	5.17	100
11	5.62	5.58	100
12	4.60	5.38	97
Mean (n = 12)	4.38	4.72	99
s.d.	0.66	1.00	4

$$RD = \frac{[(\text{cocoa butter intake}) - (\text{differences of faecal fatty acids during chocolate and control diets}^{*}\ 1.046^{\dagger})]^{*}100}{\text{Cocoa butter intake}}$$

†The factor of 1.046 is used to correct for the weight of glycerol.
§No significant differences between dietary treatments by paired *t*-test ($P > 0.05$).

Discussion

This study examined, in a cross-over design in humans, the digestibility of cocoa butter relative to a well-absorbed fat (corn oil) under normal dietary conditions, that is in moderate amounts (30.7 g/d) given in the form of chocolate consumed between two meals, and as an integral part of a normal mixed diet. Under these conditions, the digestibility of cocoa butter was found to be as high as that of conventional fats, such as corn oil.

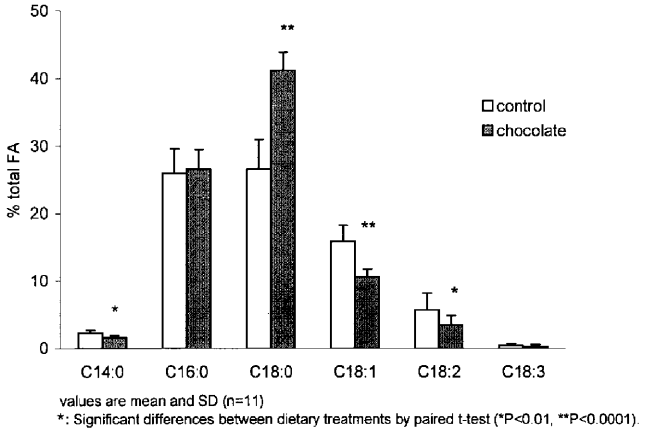

Figure 1 Faecal fatty acids (FA) composition.

Indeed, no differences were observed in faecal weight (wet or dry), in faecal fat nor in defecation frequency, suggesting that cocoa butter at a dose of 30.7 g/d did not change the physical characteristics of faecal samples and no gastrointestinal side-effects were reported by the subjects. Examination of the fatty acid profile of the faeces indicated that the proportion of stearic acid was much higher in the faeces during the chocolate period than during the control period (41% and 27%, $P < 0.001$, respectively) and hence indicates that high intakes of stearic acid leads to an increase in the proportion of stearic acid in faecal fat. This increase in percentage of faecal stearic acid during the chocolate period was compensated for by a decrease in the proportion of other faecal fatty acids (for example oleic and linoleic acids) during this period. However, the proportion of palmitic acid did not change during the two diet periods. Thus, in spite of changes in fatty acid composition of faecal fat, the total faecal fat output and thus the digestible energy value of cocoa butter and cornoil were not different.

The digestibility of cocoa butter was found to be 99 ± 4% of the digestibility of corn oil. Thus unlike in the rat, the digestibility of cocoa butter in humans is similar to that of a well-absorbed fat (corn oil), and its metabolisable energy value is hence similar to that of other fats (37 kJ/g). The results of this study, and those of previous studies with much larger amounts of cocoa butter (80–130 g/d) (Denke & Grundy, 1991; Mitchell *et al*, 1989; Mitchell *et al*, 1990), therefore suggest that cocoa butter both at normal or high doses, in the form of confectioneries or as the main source of dietary fat, has a high digestibility in man, and cannot be considered to be a low calorie fat.

The discrepancies observed in cocoa butter digestibility in rats and humans may be due to differences in the calcium to fat (Ca : fat) ratio in their diets. It is known that long chain saturated fatty acids form calcium soaps that are 10–20 times less soluble than the calcium salts of unsaturated fatty acids. Denke *et al* (1993) reported reduced absorption of saturated fat in humans when the diet was fortified with large amounts of calcium (2200 mg/d). The rat diet used for cocoa butter digestibility studies contained 0.9% Ca and 5–20% fat (Apgar *et al*, 1987), so that the ratio of Ca/fat is 0.04–0.18. The reduction in cocoa butter digestibility was greater with a Ca/fat ratio of 0.18 than with a ratio of 0.04 (60% and 70% digestibility, respectively) (Apgar *et al*, 1987). This Ca/fat ratio is, however, much lower in human diets since the daily consumption of Ca and fat is about 1 g and 100 g, respectively, such that the Ca : Fat ratio of the human diet (0.01) is 4 to 18 times lower than that in the rat (0.04–0.18). Consequently, the possibility that Ca binds with saturated fatty acids of cocoa butter to form insoluble soap, is much higher with a rat diet than with a human diet. This contention is supported by a recent report that cocoa butter digestibility is reduced in humans when large amounts of Ca are added to chocolate (Murata *et al*, 1998). However, in this study large amounts of chocolate were consumed (184 g/d) and the level of calcium supplementation in chocolate was high (1.87% wt/wt). Thus, it remains to be investigated whether lower levels of Ca supplementation may have a significant impact on cocoa butter digestibility.

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