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Human bioavailability of flavanols and phenolic acids from cocoa-nut creams enriched with free or microencapsulated cocoa polyphenols

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Abstract

Human bioavailability of cocoa flavanols and phenolic acids from a cocoa-nut cream (CC) and from CC enriched with a 1·5 % (w/w) cocoa polyphenol extract in free form (FPC) or encapsulated with a gastric-resistant high-amylose maize starch (EPC), was studied. In a randomised cross-over protocol, with 1-week wash-out in between, twelve healthy volunteers had three portions/d of each cream, providing approximately 190 μmol/d of total flavanols and 12 μmol/d of total phenolic acids with CC and 385 and 28 μmol/d with both FPC and EPC, respectively. Blood, urine and faecal samples were analysed by HPLC/MS/MS. Serum (epi)catechin was absent at baseline and after CC consumption, while 22·1 (SEM 2·62) and 1·59 (SEM 0·22) nmol ($P < 0·05$) were found after FPC and EPC, respectively. The EPC increased faecal excretion of total flavanols compared to FPC (151·0 (SEM 54·6) *v.* 28·0 (SEM 14·0) nmol; $P < 0·05$). Within 6 h after consumption, serum phenolic acid content was 50-fold higher than (epi)catechin; no difference between CC and FPC was observed, but a significant reduction after EPC (1954 (SEM 236·3) and 1459 (SEM 137·6) *v.* 726·8 (SEM 73·4) nmol, $P < 0·05$) was recorded. Short-term phenolic acid urinary excretions were significantly higher after FPC than CC and EPC, the values being 11·4 (SEM 5·1) *v.* 3·1 (SEM 1·7) and 0·9 (SEM 0·5) μmol, respectively. Faecal phenolic acids were approximately 60-fold reduced after FPC (8·1 (SEM 0·13) nmol) and EPC (14·7 (SEM 2·7) nmol) consumption compared to CC (641·4 (SEM 99·1) nmol) consumption. The data demonstrated that: (i) (epi)catechin was absorbed from CC; (ii) cocoa polyphenols' consumption increased circulating phenolic acids; and (iii) encapsulated ingredient increased flavanol delivering into the gut. Further studies should evaluate whether encapsulated cocoa polyphenols may be a functional prebiotic ingredient.

Key words: Bioavailability: Cocoa polyphenols: Encapsulation: Functional food

Epidemiological studies associate cocoa and chocolate consumption to a reduced risk of CVD, and attribute this effect to their polyphenol moiety[1,2]. Cocoa polyphenols include a sub-class of flavonoids, namely flavan-3-ols, occurring as monomers, mainly epicatechin and catechin, oligomers (procyanidins B_1, B_2 and C_1) and polymers (up to ten units), known as procyanidins[3–5]. Monomers account for 5–10 % of total cocoa flavanols, while oligomers and polymers constitute ≥90 %[6]. They are the major cocoa polyphenols, being estimated to be more than 3 g/100 g cocoa powder, while cocoa phenolic acids and flavonols are 100 times less abundant, estimated at 62 mg and 30 mg/100 g, respectively[7]. Small amounts of gallocatechin and epigallocatechin have also been quantified in cocoa[5]. The abundance of flavanols compared to the other polyphenols has justified the major scientific interest shown up till now in the bioavailability of these compounds. All in all, bioavailability studies demonstrated that, whereas monomers are readily absorbed in the stomach and small intestine, the absorption of dimeric procyanidins in human subjects is very limited[8,9]. Indeed, polymeric procyanidins mainly reach the colon, where they are largely metabolised by the local micro-organisms to produce several phenolic acids[10–12]. Once formed, they are absorbed, further metabolised in the liver and excreted in urine[8,13,14]. Thus, the general consensus is that cocoa flavanol bioavailability is dependent on the ingested dose, the glucuronidated, sulphated and methylated metabolites being the most abundant compounds within 6 h after consumption, while phenolic acids (in the free forms or further metabolised by the liver) predominate later on. These *in vivo* studies were mainly

Abbreviations: CC, control cream; EPC, cream enriched with 1·5 % (w/w) of the encapsulated cocoa polyphenol extract; FPC, cream enriched with 1·5 % (w/w) of the free cocoa polyphenol extract; HACS, high-amylose maize starch.

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performed using chocolate or cocoa, consumed alone and/or with milk, and often highlighted some differences in bioaccessibility and biotransformation of cocoa polyphenols, depending on the composition of the dietary matrices such as the presence of proteins that generally retard polyphenol absorption as well as the fat content that may modulate relative absorption of individual compounds and/or metabolites[8,15–21].

In some cases, the effects of cocoa or chocolate consumption were reported, a few hours after intake, on some markers of cardiovascular function (such as NO synthesis, flow-mediated vasodilation and peripheral arterial tonometry responses) and associated with the increased amount of catechin phase I/II metabolites in the blood[22]. However, the biological effects of flavanol-3-ol-conjugated metabolites have been mostly studied *in vitro* using concentrations that not always have physiological relevance; thus, the *in vivo* bioactivity of these compounds is still a highly debated matter[23]. On the other hand, the potential role of other classes of cocoa polyphenols, such as phenolic acids, in exerting short-term bioactive effects has never been explored, despite a growing interest in phenolic acids formed from the catabolism of flavanols by colon microflora emerging in the scientific literature[23].

The knowledge of the relationship between the physiological response to foods and their composition is fundamental to formulate new ingredients and foods having a nutritional advantage, compared to the existing ones[24]. In general, due to the extensive metabolism and rapid excretion of cocoa flavanols, increasing their intake without increasing overall energy intakes may be nutritionally advantageous. Enrichment of cocoa-containing foods (e.g. chocolate bars, creams or drinks) with flavanol-rich extracts obtained from different cocoa bean fractions may be a reliable appropriate solution[25]. Unfortunately, catechin and procyanidin enrichment over a certain level may impart an undesired bitter and astringent taste to the final product. Encapsulation of cocoa polyphenol extract may overcome this limitation. In fact, during the last 10 years, this technology has rapidly enlarged its application in the food industry, thus allowing food addition with several bioactive compounds, guaranteeing their protection during food processing, tailoring their release over time and/or at particular sites and masking unwanted tastes and flavours of core materials[26–28].

In this framework, the objectives of the present study were to evaluate human bioavailability of cocoa flavanols and phenolic acids upon consumption of nut creams containing 20 % of cocoa (CC) and to evaluate whether, and to what extent, cocoa-nut cream enrichment with a cocoa polyphenol extract in free (FPC) or in encapsulated (EPC) form influenced this issue. For this purpose, nut creams were developed and produced. A total of twelve healthy volunteers participated in the study, consumed the creams in a randomised manner and blood, urine and faeces were collected at specific time points over 24 h. Biological samples were analysed by HPLC/MS/MS to monitor free native flavanol-3-ols (monomers and dimers) and several phenolic acids (both present in cocoa-nut creams as well as known to originate from cocoa flavanols and flavonols).

Materials and methods

Chemicals and reagents

All chemicals and reagents were of analytical grade. Methanol, water, acetonitrile and hexane were from Merck; only for cream chemical characterisation, water was obtained from a MilliQ water purification system (Millipore Corporation). Ethyl acetate, glacial acetic acid and HCl were from Clean Consult International; formic acid (98 % purity), butylated hydroxytoluene and salts used for PBS preparation were obtained from Sigma. All analytical standards, 5-caffeoylquinic acid (95 %, chlorogenic acid), *trans*-4-hydroxy-3-methoxycinnamic acid (99 %, ferulic acid), 3,4-dihydroxybenzoic acid (≥97 %, protocatechuic acid), 4-hydroxybenzoic acid (99 %, *p*-hydroxybenzoic acid), 3,4-dihydroxycinnamic acid (≥98 %, caffeic acid), 4-hydroxyphenylacetic acid (98 %), 3-(4-hydroxyphenyl)propionic acid (98 %), 4-hydroxy-3-methoxybenzoic acid (97 %, vanillic acid), naringenin (98 %) and quercetin (≥98 %) were purchased from Sigma. (+)-Catechin (≥99 %), (−)-epicatechin (≥97 %), procyanidin B_1 and B_2 (≥90 %), apigenin (≥99 %), luteolin (≥97 %) and kaempferol (≥99 %) were obtained from Fluka.

Preparation of free and encapsulated cocoa polyphenol extract

Polyphenol extract from cocoa nibs was produced by La Morella Nut, according to the procedure described by Ortega *et al.*[25]. It was partly microencapsulated by KARMAT using a technological process based on the formation of nanocomplexes with a high-amylose maize starch (HACS) as coating agent of cocoa polyphenols and on their aggregation in microcomplexes. In particular, the following steps were performed: (1) solubilisation of the coating material in an alkaline solution (pH 12) kept at high temperature (80°C) and stirred continuously; (2) chilling this material up to 30°C; (3) addition, under stirring, of the cocoa extract up to 10 % of HACS (w/w); (4) pressurisation into an homogenator together with an acid solution until a pH of approximately 5 is reached and, finally, spray-drying using 200°C as inlet temperature, approximately 100°C outlet temperature and a flow rate of 10 l/h to obtain fine particles.

The final ingredient contained cocoa polyphenol extract–HACS in a 1:9 (w/w) ratio.

Preparation of cocoa-nut creams

Once the polyphenol-rich ingredients were obtained, a nut cream containing 20 % (w/w) cocoa (control cream, CC) and ten prototypes of polyphenol-enriched cocoa-nut creams containing free or encapsulated polyphenols ranging from 0·5 to 2·5 % (w/w) were prepared by a pilot-scale apparatus located at La Morella Nuts. The CC was prepared by the sequential addition of individual ingredients through continued mixing, in order to obtain a well-mixed and refined cream (particle sizes about 30 μm). To this basic cream, the free or encapsulated polyphenol extract was slowly added and mixed gently to homogenously disperse the ingredients.

Once the homogeneous products were obtained, creams were immediately packaged in 33-g portions and labelled with alpha-numeric codes.

Sensory analysis of creams

To establish what was the maximum enrichment of the creams achievable with the polyphenol-rich ingredients, a sensory analysis of the ten cream prototypes was performed. A total of thirty untrained subjects, selected among students and staff of the Department of Food Science at the University of Naples based on medical status, absence of allergies and habitual consumption of nut/chocolate creams and spreads, were enrolled to participate in the study. The selected subjects were healthy, of both sexes (fifteen male and fifteen female), between 25 and 35 years of age and were of normal weight (BMI 22 (SEM 2) kg/m^2). A total of ten prototype formulations, prepared as described earlier, were compared to CC: they contained 0·5, 1, 1·5, 2 and 2·5 % of the cocoa polyphenol extract, in free or encapsulated form. At different days for each test, and always at least 2 h after having breakfast, three types of creams were tested in blinded and randomised manner. Approximately 10 g of each cream, placed in transparent small cups, were provided to subjects together with a glass of room temperature water and a slice of white bread for palate cleansing between sample testing. The panelists were asked to taste and to assign a score to their hedonic of the following sensory attributes: sweetness, bitterness, creaminess, fatness, granularity and overall palatability. Ratings were based on a nine-point hedonic scale (0 = extremely dislike, 9 = extremely like).

Composition of the cocoa-nut creams

The composition of CC, FPC and EPC is summarised in Table 1, reporting for each parameter the mean of three measures obtained by triplicate analysis and SEM. Water, lipids, proteins and carbohydrates were determined by official methods of analysis (AOAC 9321.04, AOAC 963.15, AOAC 939.02, AOAC 980.13, respectively), while flavanols (monomers, oligomers and polymers) as well as phenolic acids and flavonols were measured according to the method described by Ortega *et al.*[25] with slight modifications. Briefly, approximately 3 g of sample were weighted and fat was removed by using 3 ml of hexane. Polyphenols were extracted from the defatted pellet using a total volume of 9 ml (3 × 3 ml) acetone–water (1:1) solution. After addition of extraction solvent to the pellet, the whole mixture was sonicated for 10 min at 4°C to improve the polyphenols' extraction efficiency and obtain a recovery of 98 % of polyphenols from the encapsulated ingredient (as found in preliminary experiments whose data are not shown). All acetone/water phases were collected and acetone removal by rotary evaporator was followed by freeze-drying of resulting aqueous solutions. Finally, approximately 10 mg of dried extracts were suspended in 2 ml of a methanol–water (1:1) solution and 20 µl of this suspension were injected in to a HPLC system (Shimadzu LC-10A Series) equipped with two pumps (LC-10AD), a controller (SCL-10A) and a diode-array detector (SPD-M10A). Chromatography separation was carried out with a Prodigy 5 µm ODS-3 100 Å column, size 250 × 4·60 mm C18 column, purchased from Phenomenex, with a mobile phase flow rate of 0·8 ml/min, consisting of acidified water with 0·2 % of formic acid (phase A) and an acetonitrile–methanol solution in the ratio 60:40 (v/v, phase B). The elution gradient was set as follows: time 0 = 20 % B; 0–6 min 30 % B; 6–16 min 40 % B; 16–24 min 50 % B; 24–32 min 98 % B; 32–35 min 98 % B; 35–40 min 20 % B; and 40–45 min 20 % B. Catechin, epicatechin, procyanidin B_2, *p*-hydroxybenzoic acid, vanillic acid, apigenin, naringenin, quercetin arabinoside, luteolin glucoside, quercetin glucoside and kampferol-rutinoside were detected at 280 nm; protocatechuic acid at 254 nm; and chlorogenic acid,

Table 1. Composition of experimental creams (per 100 g)
(Mean values with their standard errors (*n* 3))

	CC		FPC		EPC	
	Mean	SEM	Mean	SEM	Mean	SEM
Water (g)	1·5	0·1	1·5	0·1	1·3	0·2
Protein (g)	7·9	0·3	7·8	0·6	6·7	0·5
Carbohydrates (g)	49·7	2·1	50·0	1·8	57·3*	2·8
Dietary fibre	40·0	3·0	40·1	2·6	47·2*	2·0
Lipids (g)	40·9	2·9	40·8	3·1	34·8	1·9
Total flavanols (µmol)	190·0	2·2	385·1†	5·3	385·3†	4·9
Monomers‡	176·0	2·1	336·8†	5·0	337·0†	4·4
Dimers§	14·0	0·1	48·3†	0·3	48·3†	0·5
Total phenolic acids (µmol)‖	12·0	0·9	28·1†	1·0	27·9†	0·2
Total flavonols (µmol)¶	9·2	0·0	12·9†	0·1	13·0†	0·4

CC, control cream; FPC, cream enriched with 1·5 % (w/w) of the free cocoa polyphenol extract; EPC, cream enriched with 1·5 % (w/w) of the encapsulated cocoa polyphenol extract.
* Mean values were significantly different from that of FPC ($P<0·05$; Bonferroni test).
† Mean values were significantly different from that of CC ($P<0·05$; Bonferroni test).
‡ Catechin and epicatechin.
§ Procyanidin B_1 and B_2.
‖ *p*-Hydroxybenzoic acid, vanillic acid, protocatechuic acid, chlorogenic acid and caffeic acid.
¶ Apigenin, naringenin, quercetin arabinoside, luteolin glucoside, quercetin glucoside, kampferol-rutinoside and rutin.

caffeic acid and rutin at 330 nm. The peak area was integrated by means of Class-VP software (version 7.3) obtained from Shimadzu. Each compound was quantified using specific calibration curves obtained with the reference standard compounds, as reported previously; for glycosides, the calibration curves of the respective aglycones were used.

Bioavailability study

Study design. The present study was a single-blind study, and had a randomised, cross-over design with three arms.

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving subjects were approved by the Ethics Committee of 'Federico II' University of Naples (Ethic Approval Number 37/10). Written informed consent was obtained from all subjects before entering into the study.

A total of twelve healthy volunteers (four males/eight females, mean age 24 (SEM 3) years, BMI 23·1 (SEM 1·5) kg/m^2) were selected among the students of the Agricultural Faculty, University of Naples. Subjects reporting symptoms of gastrointestinal disorders (including frequent diarrhoea episodes or constipation), having metabolic diseases (diabetes, metabolic syndrome, etc.), taking non-steroidal anti-inflammatory drugs or having undergone controlled dietary regimens over the last 6 months were considered ineligible for the present study. The selected volunteers signed a written informed consent before starting the study. The experimental design is schematised in Fig. 2. For the 3 d before and over each experiment day, subjects were asked to follow a polyphenol-free diet. It consisted, in exclusion of the habitual diet, of all polyphenol-rich foods and beverages such as fruits, vegetables, chocolate, tea, coffee, wine, beer, supplements, herbal extracts and wholegrain-based foods. Consumption of non-steroidal anti-inflammatory drugs and antibiotics was also avoided during 1 week and 1 month before treatments, respectively. On the experiment day, after fasting for 12 h, the subjects reached the laboratory at 08.00 hours and were randomised to receive one of the three experimental creams, which was consumed within 15 min together with three slices of toasted bread and a glass of room temperature water. They left the research centre 6 h later and were allowed to have lunch (within 14.45 hours) and dinner (within 22.00 hours), including, in both occasions, another cream portion. Each subject was invited to consume lunch and dinner during the three experimental sessions, always constituting the same foods, so that no influence of dietary pattern on bioavailability of compounds in the three treatments might occur. In particular, they were invited to choose among the following foods: rice or pasta with butter and cheese or with tuna, meat or fish or bread with ham and/or cheese. After 24 h, subjects returned to consume their own habitual diet for a 1-week wash-out period. After this week, the experimental design was repeated. In particular, subjects again followed a 3-d polyphenol-free diet and were randomised for another treatment.

Thus, all subjects participated in the three experimental sessions (one for each type of cream) with a 1-week interval between each other, and on each test day, they consumed three portions (99 g) of the test cream they were randomised for.

Sample collection. At fasting conditions and at 30 min, 1, 2, 4 and 6 h after breakfast consumption, blood samples were drawn. Then, 24 h urine was collected over 0–2, 2–4, 4–6, 6–8, 8–10, 10–24 h time intervals, after ingestion of the first cream portion, and the volume was measured. The 10 ml samples of urine collected before breakfast and at each time interval were stored for analysis. The day after the experiment, participants returned to the laboratory after fasting for 12 h and their blood samples were taken (24 h from the first cream consumption), while the faecal sample was collected on the experiment day. No subject was constipated or had diarrhoea episodes over the study period; thus, faecal samples were always collected from each volunteer.

Biological sample treatment, storage and analysis. Blood samples were collected in a Vacutainer tube for gel separation, and immediately centrifuged at 4000 rpm for 10 min at 4°C. Urine samples were immediately treated with 0·005 % of butylated hydroxytoluene. Faeces were diluted in the ratio 1:10 (w/v) in PBS (10 mM) containing 0·005 % of butylated hydroxytoluene, vortexed and centrifuged at 4000 rpm for 15 min at 4°C. Serum, urine and faecal supernatants were stored at −40°C until the analysis. Procyanidins, metabolites and phenolic acids were extracted by 1·5 ml of ethyl acetate from 500 μl of serum and 1·5 ml of urine and faecal suspensions (twice and for three times, respectively). The collected supernatants were dried under nitrogen flow and the dry extracts were dissolved in 50 μl methanol–water (70:30); 30 μl were injected into HPLC/MS/MS apparatus. Each sample was extracted in duplicates. Quantitative determination of total extracted analytics was performed using a HPLC system consisting of two micropumps by Perkin Elmer Series 200, coupled with an API 3000 Triple Quadrupole mass spectrometer (Applied Biosystem Sciex). Elution was achieved with a Phenomenex Luna 3 μ C18(2) 100 A (50 × 2·00 mm) column and by using the following mobile phases: A = water–acetonitrile–formic acid 94·9:5:0·1 (by vol.) and B = acetonitrile–formic acid 99·9:0·1 (v/v); the flow rate was 200 μl/min. The linear gradient for chromatographic separation was: 0–1 min, 4–40 % B; 1–3 min, 40–100 % B; 3–5 min, 100 % B; and 6–10 min, 4 % B. Selected compounds in the native form were detected and quantified through electrospray ionisation MS/MS analysis. After ionisation in negative mode, transitions of parent and product ions specific for each compound were tracked in multiple reaction monitoring mode. For each compound, all MS parameters (declustering potential, focusing potential, collision energy, collision cell exit potential), set as previously described[12,29] and then optimised through direct infusion experiments, are reported in Table 2, together with limits of detection and quantification. When analytical standards were not available, compounds were identified comparing molecular weight and fragmentation patterns with those reported in the literature[12,29]. Epigallocatechin was quantified using the calibration curve built with epicatechin; hippuric, dihydroferulic and hydroxybenzoic acids were quantified using the calibration curve built

Table 2. MS parameters, negative ions and transition product ions analysed by HPLC/MS/MS

Compound	M−H	Product ions	DP	FP	CE	CXP 1	CXP 2	LOD (ng/ml)	LOQ (ng/ml)
Procyanidin B_2	577	289; 425; 407	−45	−300	−35; −25; −31	−7		2·5	5·0
(Epi)catechin	289	245	−40	−300	−21	−7		5·0	10·0
EGC*†	305	179	−40	−375	−30	−7		–	–
Protocatechuic acid	153	109	−45	−400	−21	−10		0·5	1·0
Vanillic acid	167	152; 108; 123	−45	−250	−22; −26	−9	−11	2·5	5·0
Ferulic acid	192·8	133·9; 177·9	−35	−250	−22; −17	−10		0·5	1·0
Chlorogenic acid	353	191	−35	−250	−21	−8		0·5	1·0
Caffeic acid	179	135	−49	−350	−35	−8		0·5	1·0
Hippuric acid*‡	178	134; 77	−45	−350	−20	−7		–	–
Homovanillic acid	180·8	136·7; 122	−50	−350	−10; −18	−7		25·0	100·0
Hydroxybenzoic acid*‡	137	93	−50	−350	−25	−7		–	–
Coumaric acid	163	119·1	−40	−350	−23	−5		2·5	5·0
Di-HCA	181·1	109; 137	−50	−300	−25; −14	−9		25	50
Di-HFA*‡	195	136	−40	−350	−20	−6		–	–
DHPA	167	123·1	−30	−250	−11	−7		0·5	1·0
HPA	151	107; 78·9	−35	−250	−16; −25	−7		3·0	5·0
HPP	164·9	121; 105·9; 76·7	−25	−250	−10; −20; −10	−7		25·0	100·0
DHPV*§	207	163; 122	−35	−350	−25	−7		–	–

CXP, collision cell exit potential; DP, declustering potential; FP, focusing potential; CE, collision energy; LOD, limit of detection; LOQ, limit of quantification; EGC, epigallocatechin; Di-HCA, dihydrocaffeic acid; Di-HFA, dihydroferulic acid; DHPA, 3,4-dihydroxyphenylacetic acid; HPA, 4-hydroxyphenylacetic acid; HPP, 3-(4-hydroxyphenyl)propionic acid; DHPV, 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone.
* Identified based on molecular weight and specific fragmentation patterns, as reported by Fogliano *et al.*[12] and Urpi-Sarda *et al.*[29].
† Reference calibration curve was epicatechin.
‡ Reference calibration curve was ferulic acid.
§ Reference calibration curve was HPA.

with ferulic acid; and dihydroxyphenyl-γ-valerolactone was quantified using the calibration curve built with hydroxyphenylacetic acid.

Statistical analysis

Statistical analysis was performed using the statistical package SPSS for Windows (version 15; SPSS Inc.). The results from HPLC/MS/MS analysis were analysed and expressed as the absolute changes from the baseline to reduce possible effects of inter-subject fasting variability. The AUC for each compound from baseline to 6 h after first cream portion consumption in the case of serum samples and to 0–6 and 6–24 h for urine samples were estimated using the linear trapezoidal rule. As data were normally distributed and presented homogeneous variance (Levene test), they were analysed by one-way ANOVA for repeated measures; the subjective time curves for all measured compounds were compared and tested for the effect of treatment and of time as factors. For all tests, following a significant main effect in the ANOVA, individual means were compared using the Bonferroni test ($P < 0·05$). Results were considered significant at $P < 0·05$. All values were reported as means with their standard errors.

Results

Sensory analysis of creams

The products enriched with 2 and 2·5 % (w/w) of polyphenol extracts obtained an overall acceptability score of 4·6 (SEM 1·3) and 4·6 (SEM 1·1) (2·0 % polyphenols) and 4·12 (SEM 1·0) and 4·2 (SEM 1·2) (2·5 % polyphenols) for free cocoa polyphenol and encapsulated extract, respectively. These low scores, compared to the one attributed to the CC (7·5 (SEM 1·3)), were conditioned by the bitterness perception for the creams enriched with 2·0 and 2·5 % of the free extract (3·5 (SEM 1·2) and 2·9 (SEM 1·3), respectively) and the granularity for those containing the same amounts of encapsulated extract (3·7 (SEM 1·2) and 3·0 (SEM 1·3), respectively). The 1·5 %-enriched creams obtained a mean score for total acceptability of approximately 6·5 (6·4 (SEM 1·3) and 6·6 (SEM 1·2), when contained free cocoa polyphenol or encapsulated cocoa polyphenol extract, respectively), while hedonic for bitterness was even scored slightly higher for EPC (6·0 (SEM 0·3)) than for FPC (4·8 (SEM 0·2)) and, on the contrary, granularity being scored 5·0 (SEM 0·2) and 6·3 (SEM 0·3), respectively (Fig. 1).

Thus, 1·5 %-enriched creams were selected as the final products to be tested in the bioavailability study and will be

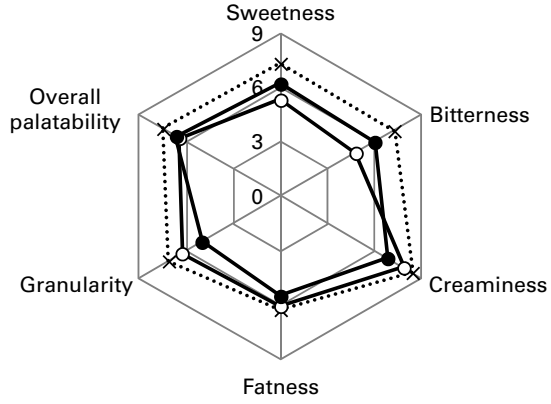

Fig. 1. Hedonic profile of control cream (-×-), and experimental creams enriched with 1·5 % of free (FP, -○-) and encapsulated (EP, -●-) cocoa polyphenol extract. Values are mean scores (n 30) anchored by 0 (extremely disliking) to 9 (extremely liking).

here-after indicated as FPC (the one containing 1·5 % free cocoa polyphenol extract) and EPC (the one containing 1·5 % encapsulated cocoa polyphenol extract).

Bioavailability study

Serum. Fig. 3 shows the mean serum concentration–time curves of total flavanols and phenolic acids found in subjects following consumption of experimental cocoa-nut creams.

None of monitored flavanols (i.e. (epi)catechin, epigallocatechin and procyanidin B_1) was found in serum at baseline. Only (epi)catechin was found in serum of subjects after consumption of FPC and EPC, while it was absent after CC consumption. In particular, (epi)catechin reached a concentration peak (C_{max} = 4·1 (SEM 2·3) nmol/l) 1 h after FPC consumption and it slowly returned to baseline value at 6 h post-consumption. After EPC consumption, the increase of serum (epi)catechin from baseline was significantly lower (C_{max} = 0·9 (SEM 0·6) nmol/l) than after FPC and was found only within the first hour after consumption. Accordingly, AUC_{0-6} of (epi)catechin after FPC was 13·9-fold higher than that found after EPC (7·4 (SEM 4·4) *v.* 0·5 (SEM 0·3) nmol/l × h, respectively; $P<0·05$).

Serum concentration of total phenolic acids at baseline was always about 135·2 (SEM 19·1) nmol/l, without the differences among treatments; after consumption of the creams, it was always higher than baseline. At 30 min after FPC consumption, serum phenolic acid concentration was higher than that found after EPC and CC (198·3 (SEM 101·0) *v.* 37·2 (SEM 17·2) and 24·8 (SEM 5·2) nmol/l, respectively), while the latter cream guaranteed a much higher concentration of phenolic acids (approximately 150 nmol/l) at both 6 and 24 h after consumption compared to the cocoa polyphenol-enriched creams (approximately 25 nmol/l).

The phenolic acids retrieved in serum samples and the relative amounts are reported in Table 3.

No significant difference was recorded in serum total phenolic acid concentrations following CC and FPC consumption, while a significantly lower amount was found after EPC consumption (726·8 (SEM 73·44) *v.* 1954·26 (SEM 236·33) and 1459·37 (SEM 137·63) nmol, respectively).

Urine. Fig. 4 shows the mean urinary excretion–time curves of total catechins and phenolic acids found in subjects following consumption of experimental cocoa-nut creams.

(Epi)catechins and procyanidins were never found in the urine of subjects at baseline, while they were retrieved after consumption of all the creams. In particular, within 4 h from CC consumption, their total concentration reached a plateau value that was maintained for up to 8 h, returned to baseline value at 10 h and was again higher than baseline at 24 h. After FPC consumption, only one concentration peak within the 2 h from consumption was found, while after EPC, catechin and procyanidin excretion was always negligible. However, only EPC always elicited a lower excretion of total flavanols than CC and FPC, while urinary excretion of total flavanols after FPC was significantly reduced compared to that after CC, only after the consumption of the second cream portion (AUC_{6-24} being 9·1 (SEM 6·3) and 62·0 (SEM 42·0) nmol/l × h, respectively).

According to serum data, the concentration of phenolic acids that were retrieved from baseline urine samples (approximately 25·3 (SEM 2·0) μmol/l, with no differences between treatments) and after cream consumption was approximately 1000-fold higher than that of total flavanols. Moreover, EPC elicited the lowest excretion, both over the first 6 h and over the 24 h after consumption, while a significantly higher excretion of phenolic acids following FPC than CC consumption was recorded only within 6 h after consumption (see Table 4). Apart from those found in serum samples, urinary phenolic acids also comprised of ferulic acid, dihydroferulic acid, protocatechuic acid, coumaric acid, caffeic acid and dihydrocaffeic acid.

Faeces. Flavanols were never found in faeces collected at baseline, and their amount after consumption of EPC (150·97 (SEM 54·65) nmol) was higher than after consumption of FPC (27·98 (SEM 13·97) nmol) and CC (4·27 (SEM 4·52) nmol) (Table 5).

Table 3. Amount (nmol) of serum-free native flavanols and phenolic acids over the time intervals 0–6 h following consumption of the three cocoa creams

(Mean values with their standard errors (*n* 12))

	CC		FPC		EPC	
	Mean	SEM	Mean	SEM	Mean	SEM
Flavanols (nmol)						
(Epi)catechin	ND		22·07*	2·62	1·59*†	0·22
Phenolic acids (nmol)						
Vanillic acid	32·57	2·29	7·53*	0·86	15·31*†	1·51
Chlorogenic acid	5·48	0·67	13·93*	1·59	4·19*	0·53
Hippuric acid	1386	196·1	994·7*	90·89	318·7*†	42·32
Hydroxybenzoic acid	480·2	32·64	318·8*	27·64	332·9*†	24·13
DHPV	16·87	0·82	115·2*	15·36	29·18*†	2·47
HPA	16·78	1·82	3·04*	0·43	5·63*	0·67
HPP	15·82	2·01	6·12*	0·87	20·92*	1·81
Total	1954	236·3	1459	137·6	726·8*†	73·44

CC, control cream; FPC, cream enriched with 1·5 % (w/w) of the free cocoa polyphenol extract; EPC, cream enriched with 1·5 % (w/w) of the encapsulated cocoa polyphenol extract; ND, not determined; DHPV, 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone; HPA, 4-hydroxyphenylacetic acid; HPP, 3-(4-hydroxyphenyl) propionic acid.

* Mean values were significantly different from that of CC ($P<0·05$; Bonferroni test).

† Mean values were significantly different from that of FPC ($P<0·05$; Bonferroni test).

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Table 4. Amount (nmol) of parental flavanols and phenolic acids excreted in urine collected over the time intervals 0–6 and 6–24 h following consumption of the three nut-cocoa creams

(Mean values with their standard errors (*n* 12))

	CC				FPC				EPC			
	0–6 h		6–24 h		0–6 h		6–24 h		0–6 h		6–24 h	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Flavanols (nmol)												
(Epi)catechin	5·26	3·72	11·75	7·16	4·82	3·41	2·16*	1·53				
EGC	0·02	0·01	0·50	0·35					0·19	0·14	2·00†	1·42
Procyanidin	0·39	0·24	1·17	0·82	0·85	0·47	1·83	1·29			2·73	1·80
Total	5·68	3·97	13·42	8·33	5·67	3·88	4·00	2·82	0·19	0·14	4·73	3·22
Phenolic acids (nmol)												
Protocatechuic acid	42·0	15·2	420·4	139·3	22·9	9·0	548·2	180·0	9·6*	4·2	693·7	252·2
Ferulic acid	15·2	5·3	1340·4	533·6	67·9*	38·2	91·6	51·2	19·1	9·2	1024·1	315·9
Vanillic acid	6·3	2·9	338·4	110·4	34·4*	11·2	452·2	192·5	16·3	10·2	195·9	84·1
Caffeic acid	1·4	0·6	99·0	29·7	2·5	0·9	74·3	18·7	0·5	0·3	21·3	7·7
Coumaric acid	1·2	0·6	23·9	13·0	3·1	1·4	34·4	12·4	1·4	0·4	38·5	12·4
Chlorogenic acid	0·1	0·0	1·9	0·5	0·2	0·0	1·4	0·6	0·4	0·2	2·9	0·9
Hippuric acid	2279	1411	98 571	45 317	8562*	3854	44 321	20 004	341·0*†	241·1	4128	2919
Homovanillic acid	515·8	160·7	48 861	17 674	2023*	802·9	48 489	16 071	223·4	156·4	32 110	7995
DHPA	122·1	72·7	6205	2881	199·5	73·6	10 911	3986	117·2	48·6	3467	1139
Di-HCA	40·6	14·5	2106	896·2	94·6	31·3	2417	749·1	34·4†	11·5	1915	566·3
Hydroxybenzoic acid	22·2	12·3	470·7	156·2	31·1	19·8	269·0	189·2	42·4	19·5	1202	536·6
Di-HFA	10·1	4·5	449·7	318·0	302·1*	177·0	313·7	163·3	60·7*†	34·3	104·9	51·2
HPP	3·4	2·1	30·2	10·4	1·1	0·6	3·3*	1·5	0·1	0·0	2·2*	1·6
HPA	2·6	1·2	87·6	42·5	64·2*	37·7	78·0	40·0	24·3*	10·5	11·4	7·2
DHPV	0·3	0·1	9·2	3·0	0·4	0·2	3·1	1·4	0·9	0·3	3·2	1·5
Total	3062	1703	159 015	68 124	11 409*	5058	108 007	41 661	891·7*†	546·7	44 921	13 890

CC, control cream; FPC, cream enriched with 1·5 % (w/w) of the free cocoa polyphenol extract; EPC, cream enriched with 1·5 % (w/w) of the encapsulated cocoa polyphenol extract; EGC, epigallocatechin; DHPA, 3,4-dihydroxyphenylacetic acid; Di-HCA, dihydrocaffeic acid; Di-HFA, dihydroferulic acid; HPP, 3-(4-hydroxyphenyl)propionic acid; HPA, 4-hydroxyphenylacetic acid; DHPV, dihydroxyphenyl-γ-valerolactone.

*Mean values were significantly different from that of CC ($P<0·05$; Bonferroni test).

†Mean values were significantly different from that of FPC ($P<0·05$; Bonferroni test).

Table 5. Amount (nmol) of parental flavanols and phenolic acids excreted in faeces collected the day after consumption of the three nut-cocoa creams

(Mean values with their standard errors (*n* 12))

	CC		FPC		EPC	
	Mean	SEM	Mean	SEM	Mean	SEM
Flavanols (nmol)						
(Epi)catechin	ND		10·25	5·13	31·54	12·44
EGC	4·27	4·52	ND		0·52	0·30
Procyanidin	ND		17·73*	8·84	118·9*†	41·91
Total	4·27	4·52	27·98	13·97	151·0*†	54·65
Phenolic acids (nmol)						
Ferulic acid	42·58	6·33	ND		ND	
PCA	25·13	6·31	4·69*	0·00	9·65*	0·11
Vanillic acid	8·46	1·91	ND		ND	
Coumaric acid	8·01	1·25	ND		4·56	2·34
Caffeic acid	1·67	0·00	2·67*	0·00	ND	
Chlorogenic acid	0·86	0·43	0·05*	0·01	0·04	0·00
Homovanillic acid	322·2	0·00	ND		ND	
Di-HCA	141·3	38·61	ND		ND	
Di-HFA	37·75	27·94	ND		ND	
HPP	53·44	16·28	ND		ND	
DHPV	ND		0·70	0·12	0·41	0·23
Total	641·4	99·07	8·11*	0·13	14·66*	2·68

CC, control cream; FPC, cream enriched with 1·5 % (w/w) of the free cocoa polyphenol extract; EPC, cream enriched with 1·5 % (w/w) of the encapsulated cocoa polyphenol extract; ND, not determined; EGC, epigallocatechin; PCA, protocatechuic acid; Di-HCA, dihydrocaffeic acid; Di-HFA, dihydroferulic acid; HPP, 3-(4-hydroxyphenyl)propionic acid; DHPV, dihydroxyphenyl-γ-valerolactone.

*Mean values were significantly different from that of CC ($P<0·05$; Bonferroni test).

†Mean values were significantly different from that of FPC ($P<0·05$; Bonferroni test).

On the contrary, the amount of phenolic acids retrieved in faeces at 24 h following CC consumption (641·43 (SEM 99·07) nmol) was the only one to be higher than that found at baseline (1767·64 (SEM 594·27) nmol, without differences among treatments) and after FPC (8·11 (SEM 0·13) nmol) and EPC (14·66 (SEM 2·68) nmol) consumption.

The bioavailability data have been summarised in Table 6 to gain the complete picture of the distribution of the ingested bioactive compounds.

Discussion

Enrichment of foods with catechin and epicatechin can be difficult due to their bitter taste and astringency in the mouth. Encapsulating flavanols with a coating material inaccessible by salivary enzymes, such as HACS, can overcome this problem. The cocoa-nut cream enriched with microencapsulated cocoa flavanols (EPC) used in the present study showed a slightly higher hedonic for bitterness than the cream added with the ingredient in the free form (FCP; see Fig. 1).

Testing the bioavailability of the encapsulated bioactive compounds from enriched foods is fundamental to foresee their *in vivo* bioactivity. To our knowledge, the present study is the first study evaluating cocoa flavanol and phenolic acid bioavailability from cocoa-nut creams in human subjects. The study was designed to follow the fate of parental and free cocoa polyphenols after the consumption of one portion of three cocoa-nut creams (33 g) differing in amount (CC *v.* FPC) and form (FPC *v.* EPC) of contained cocoa polyphenols and of a total of 99 g cream (three portions) distributed throughout the day (one portion at each daily meal, i.e. at breakfast, lunch and dinner; see Fig. 2). Thus, the comparison between CC and FPC allowed us to evaluate the effect of dose on bioavailability of free parental flavanols and of phenolic acids contained in the creams, while comparison between FPC and EPC allowed us to investigate the effect of microencapsulation of the bioactive compounds.

Data indicated that flavanol absorption at 6 h after the consumption of cocoa-nut creams was dose-dependent, as previously reported after cocoa and chocolate consumption[8,15–20]. In fact, as summarised in Table 6, after consumption of one CC portion, flavanols were not detected in the bloodstream, while consumption of FPC, containing an almost doubled amount of free monomers, determined (epi)catechin presence in the serum. The time-course values of (epi)catechin concentration in serum and urine following FPC consumption (Figs. 3(a) and 4(a)) demonstrated a rapid absorption ($t_{max} = 1$ h) and excretion (within 2 h) upon consumption. The rapid absorption and serum clearance of parental (epi)catechin after cocoa-nut cream (t_{max} at 1 h and absence of compounds at 6 h) was consistent with literature data on the bioavailability of epicatechin from cocoa and chocolate, thus confirming the occurrence of gastric absorption and a rapid plasma clearance (within 6 h from consumption) of compounds[30–32]. This feature was corroborated by data obtained after consumption of EPC, which elicited a 10-fold lower amount of serum (epi)catechin than FPC. In fact, as about 50 % (195·3 μmol of a total 385·3 μmol/100 g of cream)

Table 6. Summary of bioavailability of cocoa polyphenols from the three cocoa-nut creams‡

	CC			FPC			EPC		
	Total flavanols	Total phenolic acids	Total polyphenols	Total flavanols	Total phenolic acids	Total polyphenols	Total flavanols	Total phenolic acids	Total polyphenols
Blood									
0–6 h (μmol)	0·000	1·977	1·977	0·022*	1·459	1·481	0·002	0·744*†	0·745*†
% Dose one portion	0·000	49·4	2·808	0·020	15·6	1·043	0·001	7·9	0·525
Urine									
0–6 h (μmol)	0·006	3·062	3·068	0·006	11·41*	11·41*	0·000	0·892*†	0·892*†
% Dose one portion	0·010	76·6	4·358	0·005	121·8	8·033	0·000	9·5	0·628
6–24 h (μmol)	0·013	159·0	159·03	0·004	108·0	108·0	0·005	44·92	44·93
0–24 h (μmol)	0·019	162·1	162·1	0·010	119·4	119·4	0·005	45·81*†	45·82*†
% Dose three portions	0·011	1351	76·75	0·003	425·0	28·01	0·0015	163·0	10·75
Faeces									
0–24 h (μmol)	0·004	0·641	0·646	0·028	0·008*	0·036*	0·151†	0·015*	0·134*
% Dose three portions	0·002	5·3	0·306	0·008	0·03	0·008	0·045	0·05	0·031

CC, control cream; FPC, cream enriched with 1·5 % (w/w) of the free cocoa polyphenol extract; EPC, cream enriched with 1·5 % (w/w) of the encapsulated cocoa polyphenol extract.
* Values were significantly different from that of CC ($P<0.05$; Bonferroni test).
† Values were significantly different from that of FPC ($P<0.05$; Bonferroni test).
‡ Subjects ingested on three different occasions three portions (33 g each)/d of CC (providing a total of approximately 190 μmol/d flavanols and acids 12 μmol/d total phenolic), FPC (providing a total of 385 μmol/d flavanols and 28 μmol/d total phenolic acids) or EPC (providing a total of 385 μmol/d flavanols and 28 μmol/d total phenolic acids).

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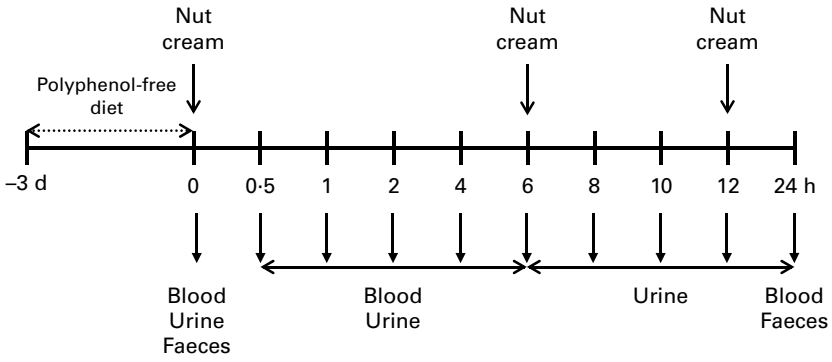

Fig. 2. Study design. Each subject followed the time schedule for each type of cocoa-nut cream by a cross-over randomised design. After a 1-week wash-out period during which subjects returned to their habitual diet, they switched to the 3-d polyphenol-free diet and were randomised for another treatment. A total of three portions (33 g each) of the cocoa-nut cream were consumed upon each treatment.

of cocoa flavanols in EPC were encapsulated by a coating material resistant to gastric digestion (HACS), their gastric absorption was reduced compared to FPC[33]. Moreover, we hypothesised that the higher amount of dietary fibre in EPC (+7·2 %, due to the HACS coating) might slow gastric emptying rate[34], thus blunting free epicatechin absorption compared to CC. A slower arrival of total flavanols (the half deriving from cocoa as in CC plus the half encapsulated) in the intestine after consumption of EPC might have also caused an increased formation of conjugated metabolites at the level of intestinal mucosa or liver, as a consequence of a lower amount of substrate per unitary time than after ingestion of CC and FPC. This hypothesis justified the missed detection in urine of the monitored cocoa native and free flavanols and the much lower procyanidin concentrations found after EPC consumption compared to CC and FPC consumption

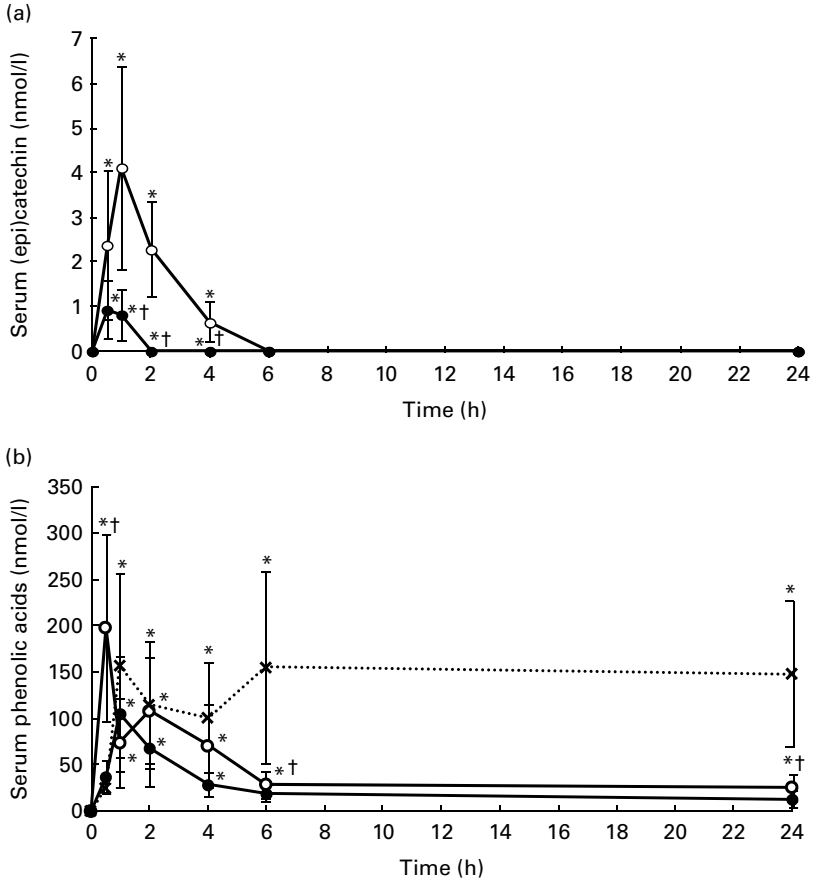

Fig. 3. Serum concentration–time curves of (a) (epi)catechin and (b) total phenolic acids over 24 h following consumption of the three types of cream (CC, control cream; FPC, free cocoa polyphenol cream; EPC, encapsulated cocoa polyphenol cream). (a) -○-, FPC; -●-, EPC. (b) -○-, FPC; -●-, EPC; ···×···, CC. Values are means, with their standard errors represented by vertical bars (n 12). * Mean values were significantly different from that of time 0 ($P<0·05$; Bonferroni test). † Mean values were significantly different from that of CC ($P<0·05$; Bonferroni test).

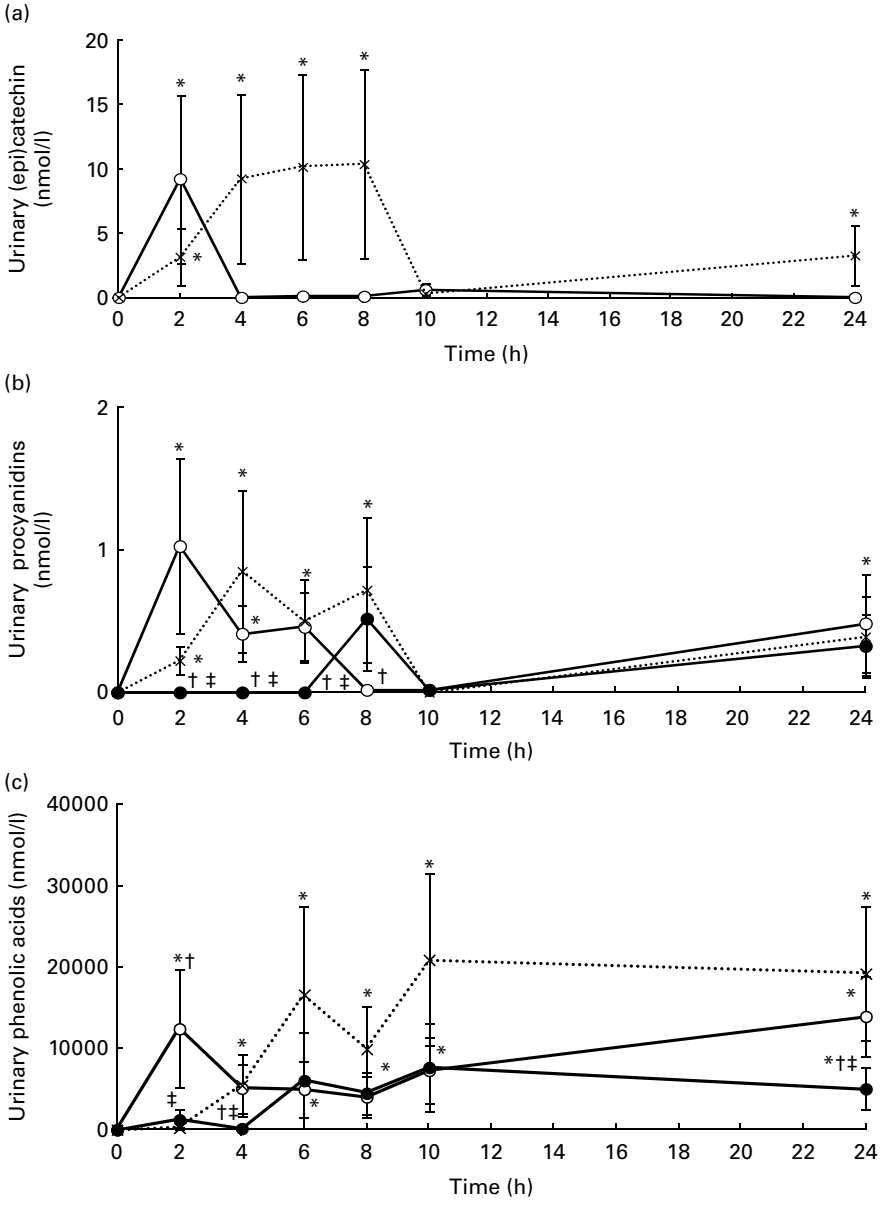

Fig. 4. Urinary excretions of (a) (epi)catechin, (b) procyanidins and (c) total phenolic acids over 24 h following consumption of the three types of cream (CC, control cream; FPC, free cocoa polyphenol cream; EPC, encapsulated cocoa polyphenol cream). Values are means, with their standard errors represented by vertical bars (n 12). (a) -○-, FPC; ···×···, CC. (b) -○-, FPC; -●-, EPC; ···×···, CC. (c) -○-, FPC; -●-, EPC; ···×···, CC. * Mean values were significantly different from that of time 0 ($P<0{\cdot}05$; Bonferroni test). † Mean values were significantly different from that of CC ($P<0{\cdot}05$; Bonferroni test).

(see Fig. 4(a) and (b)). Encapsulation of polyphenols might influence gastric emptying and in turn modify metabolism of the part of flavanols present in EPC that were not encapsulated.

Looking at the time-course values of phenolic acids in serum and in urine, a double concentration peak was found: the first at 30 min–1 h and the second at 4–6 h after consumption. They could account for the absorption of cocoanut cream parental phenolic acids from the stomach and for the absorption of compounds delivered in the intestine by the ring scission of procyanidins or flavones and/or by hydrolysis from cocoa fibres, as previously demonstrated or suggested *in vitro* [35,12]. In fact, Ortega *et al.*[35] demonstrated an increased amount of phenolic acids (mainly hydroxybenzoic, syringic and chlorogenic acids) in the bioaccessible fraction deriving from both gastric and duodenal digestion of cocoa liquor and cocoa powder, compared to the amount present in the food matrices. Accordingly, in a previous work, we showed that pancreatin digestion of water-insoluble cocoa dietary fibre led to a soluble fraction (correspondent to the bioaccessible moiety), exerting the same antioxidant capacity of pepsin fraction despite a reduced concentration of catechins, and thus suggesting that other antioxidant compounds might form in the medium at intestinal-simulated conditions[12].

Anyway, the total flavanols found in the parental form in blood and urine within 6 h from consumption, compared to

the dose ingested, were 0·010 % after CC, 0·025 % after FPC and 0·002 % after EPC. Percentages below 0·1 % of the ingested dose for native free flavanols were expected, as it is known that flavanols are mainly metabolised by the liver and intestine. In fact, authors reporting plasma concentration, of flavanols from 257 nmol/l up to 6–12 μmol/l, 1–2 h after consumption of 40–100 g of commercial cocoa or high-flavanol cocoa or chocolate, always analysed samples after treatment with glucuronidase, thus summing both free and metabolised compounds[9,18,19,30,32,36]. As we did not perform glucuronidase treatment on samples, we measured the compounds present in biological samples in the free form. Only in the study by Schroeter *et al.*[22], both parent compounds and their metabolite concentrations in plasma at 2 h after high-flavanol cocoa consumption were shown to be approximately 300 and 1400 nM, respectively. Thus, in that case, after ingestion of 604 μmol of flavanol monomers in cocoa, a concentration ratio between epicatechins and glucuronides of 1:4 could be calculated. Indeed, in the present study, after consumption of cocoa-nut creams containing 190–385 μmol of total flavanols and 12–28 μmol of phenolic acids per 100 g of cream, serum maximum concentration of (epi)catechin ranged between 0 and 4 nM, while concentration of total phenolic acids was between 100 and 200 nM within 2 h after consumption (see Fig. 3(b)). Thus, within 2 h after consumption of cocoa-nut creams, the concentration ratio between (epi)catechin and phenolic acids in serum was 1:50, much higher than that achievable for glucuronidated products.

Phenolic acids in serum, over a time-window shortly following cocoa or chocolate consumption, have never been evaluated in previous studies. Rios *et al.*[13] quantified phenolic acids in urine collected over 0–48 h after cocoa consumption. They reported that among the eleven aromatic acids retrieved (3,4-dihydroxyphenylpropionic acid, *m*-hydroxyphenylpropionic, ferulic acid, 3,4-dihydroxyphenylacetic acid, *m*-hydroxyphenylacetic acid, phenylacetic acid, vanillic acid, *m*-hydroxybenzoic acid, *p*-hydroxybenzoic acid, *p*-hydroxyhippuric acid and hippuric acid) only vanillic acid showed a peak excretion shortly after chocolate consumption (0–3 h), probably deriving from oxidation of vanillin present in the chocolate, while the other phenolic acids increased starting from 6 h after consumption. However, Urpi-Sarda *et al.*[37] successively showed that, although phenolic acids were most abundant in urine collected after 6 h from consumption, concentrations higher than baseline values were retrieved in the 0–6 h time interval for 3,4-dihydroxyphenylacetic acid, five hydroxybenzoic acids and two hydroxycinnamic acids; moreover, they found that when the cocoa beverage was prepared with milk, compared to water, vanillic acid was more abundant and majorly excreted over the first 6 h after consumption[37].

The different matrices tested (chocolate by Rios *et al.*[13]; Urpi-Sarda *et al.*[37] cocoa beverage prepared with water or milk and cocoa-nut creams in the present study) might account for the different results obtained by the studies. However, looking at 0–6 h urinary excretion of phenolic acids following CC and FPC consumption, the present data indicated a dose-dependence from ingested flavanol monomers and phenolic acids excreted. This feature was reinforced by the data recorded after EPC consumption: when cocoa polyphenols were partly encapsulated, phenolic acids were markedly reduced over 0–6 h time intervals, while an increased rate of excretion after 6 h was recorded. These results suggested that flavanols, other than cocoa dietary fibres, might contribute to the phenolic acid formation in the short term and confirmed the major role of gut microflora on phenolic acid formation in the long term[8,10–14]. In fact, the higher amount of flavanols in the faeces collected after consumption of EPC, than in those after FPC, together with an equivalent amount of phenolic acids indicated that the encapsulation allowed the delivery of bioactive compounds in the lower gut and the successive metabolism by local microflora (see Figs. 3(b) and 4(b), 24 h time point).

Despite the fact that FPC caused the highest phenolic acid excretion over the first 6 h after consumption, the amount of phenolic acids excreted in 24-h urine following its consumption did not differ by that recorded after CC, both being consistent with that observed by Rios *et al.*[13] after consumption of 80 g chocolate. It may be possible that a different concentration ratio of monomers (not absorbed and/or degraded in the upper gastro-intestinal tract) and oligomers plus polymers (naturally reaching the gut) in the gut after consumption of the three creams might differently influence microflora metabolism and absorption of metabolites over the 24 h.

All in all, the present data demonstrated that parental cocoa flavanols are absorbed by cocoa-nut creams in a dose-dependent manner and phenolic acids are the major metabolites in the short term, being at a concentration ratio of 50:1 *v.* (epi)-catechin. Encapsulation of cocoa polyphenols with HACS caused a reduced 24-h bioavailability of these compounds. On the other hand, encapsulation effectively masked bitter taste and allowed delivering of flavanol monomers into the gut. From the nutritional point of view, encapsulated cocoa polyphenols may be considered as a functional prebiotic ingredient, as evidenced in a recent human study[38]. Further studies should be performed to test this hypothesis and to evaluate the *in vivo* efficacy of this ingredient towards specific pathologies and functions.

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