food & nutrition research

COACTION PUBLISHING

ORIGINAL ARTICLE

Wheat aleurone polyphenols increase plasma eicosapentaenoic acid in rats

Fayçal Ounnas[1,2,3]*, Florence Privé[1,2,3], Patricia Salen[1], Florence Hazane-Puch[4], François Laporte[4], Eric Fontaine[2,3], Daniele Del Rio[5], Luca Calani[5], Camilla Melegari[6], Marta Angela Bianchi[6], Christine Demeilliers[2,3#] and Michel de Lorgeril[1#]

[1]TIMC-IMAG CNRS UMR 5525, Laboratoire PRETA, Cœur et Nutrition, Université Joseph Fourier, Grenoble, France; [2]Laboratory of Fundamental and Applied Bioenergetics (LBFA), Université Grenoble Alpes, Grenoble, France; [3]Inserm, U1055, Grenoble, France; [4]Unité Biochimie Hormonale et Nutritionnelle, Département de Biochimie, Pharmacologie et Toxicologie, Centre Hospitalier et Universitaire de Grenoble, Grenoble, France; [5]LS9 Interlab Group, Laboratory of Phytochemicals in Physiology, Department of Food Science, University of Parma, Medical School, Parma, Italy; [6]Barilla G. R., Fratelli Spa, Parma, Italy

Abstract

Methods: These studies were designed to assess whether wheat polyphenols (mainly ferulic acid [FA]) increased the very-long-chain omega-3 fatty acids (VLC n-3) [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] in rats. Wheat aleurone (WA) was used as a dietary source of wheat polyphenols. Two experiments were performed; in the first one, the rats were fed WA or control pellets (CP) in presence of linseed oil (LO) to provide alpha-linolenic acid (ALA), the precursor of VLC n-3. In the second one, the rats were fed WA or CP in presence of control oil (CO) without ALA. The concentrations of phenolic acid metabolites in urine were also investigated.

Results: The urinary concentration of conjugated FA increased with WA ingestion ($p < 0.05$). Plasma EPA increased by 25% ($p < 0.05$) with WA in the CO group but not in the LO group. In contrast, there was no effect of WA on plasma DHA and omega-6 fatty acids (n-6). Finally, both n-3 and n-6 in the liver remained unchanged by the WA.

Conclusion: These results suggest that WA consumption has a significant effect on EPA in plasma without affecting n-6. Subsequent studies are required to examine whether these effects may explain partly the health benefits associated with whole wheat consumption.

Keywords: *polyphenols*; *wheat aleurone*; *very-long-chain omega-3 fatty acids*; *plasma*; *liver*; *rats*

Responsible Editor: Asim Duttaroy, Oslo University, Norway.

To access the supplementary material to this article please see Supplementary files under Article Tools online

Received: 10 April 2014; Revised: 27 June 2014; Accepted: 1 July 2014; Published: 19 August 2014

Clinical and epidemiological studies suggest that whole-grain consumption is associated with beneficial health effects (1–4). The mechanisms of these effects are not fully identified. They may be partly attributed to the presence of polyphenols in wheat aleurone (WA), the inner layer of the bran. Beyond their antioxidant properties, certain polyphenols increase the endogenous synthesis of the very-long-chain omega-3 fatty acids (VLC n-3) [EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid)] with no significant effect on omega-6 fatty acids (n-6) (5–7). VLC n-3 are known to be health protective (8, 9). Our previous studies in rats demonstrated that the anthocyanins present in black corn were responsible for this effect (7) and raised the question of whether other polyphenols, including those present in whole wheat, may have a similar effect. Ferulic acid (FA) is the most abundant polyphenol in whole wheat and represents about 90% of the total polyphenol content of wheat bran (10, 11). The hypothesis tested in this study was that the FA-rich WA increases VLC n-3 status.

#CD and MdeL contributed equally to this work as senior investigators.

The concentrations of phenolic acid metabolites in urine were also investigated in order to evaluate the absorption of these bioactive compounds.

Materials and methods

The rats were cared for in accordance with the European Council Directive 86/609/EEC on the care and use of laboratory animals (OJ L 358). The protocols were performed under license from the French Ministry of Agriculture (license no. A380727) and approved by the local animal ethics committee (license n°113_ LBFA-FO-01).

Animals and experimental design

Forty-eight male Wistar rats (Charles River Laboratories, baseline body weight 75–100 g) were fed a standard diet (A04, SAFE Diets, France). The animals were acclimated 1 week before being distributed into the four experimental groups (Fig. 1). They were housed in individual cages under conditions of constant temperature, humidity, and a standard light-dark cycle (12 h/12 h). Tap water and standard diet were provided *ad libitum*.

In experiment 1 (Exp. 1), the rats ($n = 12$/group) received daily, in addition to the standard diet, either control pellets (CP) with control oil (CO) or WA pellets with CO. The CO was palm oil which is free from alpha-linolenic acid (ALA), the precursor of VLC n-3 fatty acids, and served as an isoenergetic control matrix (Fig. 1).

In experiment 2 (Exp. 2), the rats ($n = 12$/group) received daily, in addition to the standard diet, either CP with linseed oil (LO) or WA pellets with LO. LO was used to provide ALA (18:3n-3) (Fig. 1).

Both CO and LO were provided by gavage and brought 9 kcal/g. The amount of fat provided by the oils was adjusted to the recommendations of the American Institute of Nutrition Rodent Diets-93 (12). Thus, the quantity of linseed oil used for gavage was 200 µL/day corresponding to 92 mg/day of ALA. The WA pellets were prepared by mixing 2 g of the WA layer flour containing 26.7 mg/g dry weight of total FA with 2.5 mL water and 12.5 mg commercial white sugar. The aleurone fraction was provided by Barilla G. e R. F.lli, Parma (Italy), and obtained as follows: durum wheat (*Triticum turgidum L. subsp. Durum*) kernels went through three subsequent steps of debranning. The material obtained was micronized and separated by a turbo separator into inner and outer parts of the aleurone layer. The outer part of the aleurone, characterized by small particles size (340–400 nm), was used for this study.

The WA pellets provided, in addition to the standard diet, a total of 53.4 mg of FA per day and per rat. The CP were prepared by mixing 1 g of standard A04 ground in flour with 2.5 mL water and 12.5 mg refined commercial white sugar. Mass density of 1 g of the flour of standard A04 was equivalent to 2 g of the WA layer flour. The CP provided, in addition to the standard diet, a total of 8.4 mg of FA per day and per rat. The WA and CP were prepared daily. Table 1 shows the lipid composition of the WA and A04 used in the studies.

In all the experiments body weights and food consumption were recorded weekly. At the end of Exp. 1 and 2, blood was collected on fasted rats and plasma was stored at −80°C. Immediately after blood collection, the rats were euthanized and their liver sampled, weighed, frozen in liquid nitrogen, and stored at −80°C until analysis. Finally, the urinary concentration of phenolic acids and their metabolites was estimated in the urine samples collected overnight during the last week of treatment of the animals in Exp. 1.

Fatty acid analyses

Plasma and liver lipids were extracted in hexane/isopropanol as described (5–7). Briefly, methylated fatty acids were extracted with hexane, separated, and quantified by GC using a 6850 Series gas chromatograph system (Agilent Technologies, Palo Alto, CA, USA). Methyl ester peaks were identified by comparing their retention time to those of a standard mixture. Saturated, mono-, and poly-unsaturated fatty acid levels were expressed as a percentage of total fatty acids. Total cholesterol and triglycerides were measured using standard methodology on a Synchron Clinical System LX20 (Beckman Coulter, Brea, CA, USA).

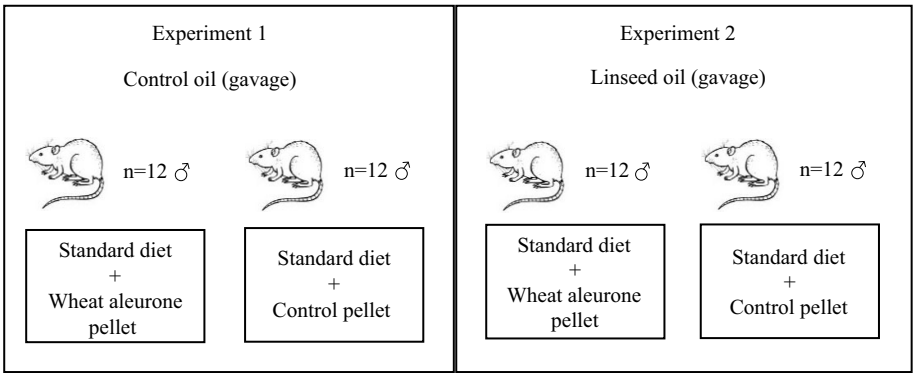

Fig. 1. Study design.

Citation: Food & Nutrition Research 2014, **58**: 24604 - http://dx.doi.org/10.3402/fnr.v58.24604

Table 1. Macronutrient and fatty acid composition of A04 diet and wheat aleurone

	A04 diet (SAFE)	Wheat aleurone
	g/100 g	
Proteins	16.1	12.6
Total carbohydrates	59.9	61.8
Lipids	3.1	8.7
Fatty acids	mg/100 g lipid	
Saturated fatty acids	620	955
C18:1n-9	400	1,211
C18:2n-6	1,310	3,240
C18:3n-3	110	326
C20:4n-6	0.0	0.6
C20:5n-3(eicosapentaenoic acid)	20	12
C22:5n-3	10	11
C22:6n-3(docosahexaenoic acid)	30	1.2

Polyphenols (phenolic acids) and metabolite analyses

Phenolic acids in CP and WA, as well as phenolic metabolites in rat urine, were analyzed using an Accela UHPLC 1250 equipped with a linear ion-trap-mass spectrometer (LTQ XL, Thermo Fisher Scientific Inc., San Jose, CA, USA) fitted with a heated-electrospray ionization probe (H-ESI-II; Thermo Fisher Scientific Inc.). Separations were performed using a BlueOrchid-1.8 C18 column (50×2 mm) (Knauer, Berlin, Germany). Pure helium gas (99.999%) was used for collision-induced dissociation (CID). The HPLC-MS^n conditions used for the detection are available in the Supplementary file.

Extraction of polyphenols (phenolic acids) compounds from the pellets (A04 and WA)

For the extraction of the free phenolic fraction, 500 mg of sample were mixed with 6 mL of water. The sample was mixed for 2 min, left under agitation for 30 min at room temperature, and centrifuged at $9{,}200 \times g$ for 10 min. Finally, the supernatant was recovered and stored at -20°C until the UHPLC-MS^n analysis. For the extraction of the bound phenolic fraction, the extraction residue was further digested with 1.5 mL of 2 mol/L sodium hydroxide at room temperature for 1 h. After alkaline hydrolysis, the pH of the mixture was adjusted to 3 by adding 1.35 mL of 3 mol/L citric acid. The samples were extracted with 6 mL of ethyl acetate and treated as the water extract. The ethyl acetate extracts were evaporated to dryness and the residue was dissolved in methanol. Bound fraction extracts were kept at -20°C in the dark prior to UHPLC-MS^n analysis. The HPLC-MS^n conditions used for the detection are available in the Supplementary file.

Urinary phenolic acid metabolites

Urine samples of rats treated with CP or WA were filtered through a 0.45 µm nylon filter before UHPLC-MS^n analysis. The HPLC-MS^n conditions used for the detection are available in the Supplementary file.

Statistical analysis

Statistics were realized using the software Minitab version 15 (GrimmerSoft, Paris, France). The difference between each fatty acid was compared with unpaired Student's *t*-test. The difference between each phenolic acid metabolites concentration excreted in urine after the consumption of the WP or CP were compared by non-parametric Mann–Whitney test.

Results

Body weight and food consumption

In Exp. 1, body weight after 12 weeks (373 ± 26 g vs. 367 ± 13 g) and food intake (23.8 ± 2.6 g/day vs. 22.1 ± 1.4 g/day) were not different in the two groups. In Exp. 2, body weight after 12 weeks (376 ± 23 g vs. 378 ± 21 g) and food intake (24.1 ± 1.0 g/day vs. 23.3 ± 1.3 g/day) were not different in the two groups.

Phenolic acids content in WA and CP pellets

Several phenolic acids have been identified and quantified in the WA and CP pellets. The sum of phenolic acids was 28 mg/g for WA and 10 mg/g for CP, mostly in bound form (Table 2). FA was by far the most abundant compound as monomeric and dimeric forms.

Urinary concentration of polyphenols (phenolic acids) metabolites

Several phenolic acid metabolites have been identified in the urines of rats receiving either CP or WA (Table 3). Six metabolites of FA were present in conjugated forms (as sulfate and glucuronide derivatives). The rats fed WA excreted more FA-*O*-sulfate compared to controls ($p < 0.05$) whereas no significant difference was found for the other major metabolites of other phenolic acids, notably hippuric acid and enterolactone.

Plasma lipid concentrations

In Exp. 1 with CO, cholesterol and triglycerides were not different in the two groups (Table 4). Regarding plasma fatty acids, only EPA was significantly higher among rats receiving WA compared with CP (Table 4). In Exp. 2 with LO; cholesterol, triglycerides, and fatty acid composition were not different in the two groups (Table 4). Liver fatty acids were not different in rats receiving WA or CP, regardless of which oil used for the treatment (data not shown).

Discussion

The main aims of this study were to test whether WA polyphenols may influence the metabolism of VLC n-3 as shown with some other polyphenols in animals and humans (5–7). We actually show that WA had significant

Table 2. Phenolic acid content in WA and CP[a]

	WA, $n=3$		CP, $n=3$	
	Free	Bound	Free	Bound
Compounds, mg/g of diet				
p-Hydroxybenzoic acid	0.12 ± 0.02	ND	ND	ND
p-Coumaric acid	0.29 ± 0.02	0.70 ± 0.07	0.07 ± 0.01	1.1 ± 0.06
Caffeic acid	ND	0.04 ± 0.00	ND	0.07 ± 0.00
Trans-ferulic acid	0.31 ± 0.06	8.9 ± 0.63	0.06 ± 0.01	3.3 ± 0.34
Ferulic acid isomer	ND	2.8 ± 0.44	ND	1.1 ± 0.25
Sinapic acid	ND	0.17 ± 0.02	ND	0.11 ± 0.03
Sinapic acid isomer	ND	0.04 ± 0.01	ND	0.04 ± 0.00
Dimeric ferulic acids	ND	13.5 ± 2.2	ND	3.6 ± 0.23
Trimeric ferulic acids	ND	1.2 ± 0.00	ND	0.33 ± 0.03

[a]Each value is mean ± SD.
WA = wheat aleurone pellets, CP = control pellets, ND = not detected.

effects on VLC n-3 metabolism. However, only EPA and not DHA increased whereas n-6 was not modified. Finally, liver fatty acids were not modified by WA; and supplementation with the ALA-rich LO abolished the effect of WA on plasma EPA, as if a high intake of ALA, the precursor of EPA in the omega-3 fatty acids (n-3) pathway, saturates the VLC n-3 synthesis pathway and may hide the effect of WA.

The significant concentration of FA-*O*-sulfate in the urine of rats fed with WA compared to the control confirmed the significant amount of FA provided by this treatment. However, the individual levels of the FA metabolites in urine presented huge variability between the rats fed with WA. This result can be explained by the low bioavailability of these compounds and also the metabolism occurs in the gastrointestinal tract. Indeed previous studies performed with rats fed with cereal-based meals or wheat bran reported a low bioavailability of FA, ranging from 2 to 5% (13, 14).

In Exp. 1, plasma EPA increased by 25% compared to controls which confirms that some polyphenols can be involved in the metabolism of VLC n-3 in the absence of effect on n-6. Indeed, previous studies in humans suggested that wine polyphenols may increase plasma and tissue levels of EPA and DHA (5, 6). This was confirmed in a study reporting increased concentrations of EPA and DHA in rats after ingestion of anthocyanin-rich black corn (7). The lack of DHA increase in the present study was not totally unexpected. Compared with the synthesis of EPA from ALA, the synthesis of DHA from EPA involves additional steps, carbonyl chain elongation, desaturation, and beta-oxidation. It is theoretically conceivable that WA polyphenols stimulated the synthesis of EPA from ALA but not the synthesis of DHA from EPA as the synthesis of DHA from EPA is partly hormone-dependent (15, 16) contrary to the synthesis of EPA from ALA. Subsequent studies should test whether polyphenols with estrogen-like activity (lignans, for instance) may stimulate

Table 3. Urinary concentration of main phenolic acid metabolites in rats fed WA and CP[a]

	WA, $n=5$	CP, $n=5$	p
Compounds, μmol/L			
Hydroxyphenylpropionic acid-like	36.95 ± 29.2	13.40 ± 2.1	NS
Hippuric acid	2,031 ± 506.9	1,361 ± 258.8	NS
Hydroxyphenylpropionic acid-*O*-sulfate	52.23 ± 25.1	27.95 ± 3.3	NS
Ferulic acid-*O*-sulfate	**481.94 ± 318.5**	**69.28 ± 27.2**	**0.04**
Dihydroferulic acid-*O*-sulfate	16.04 ± 7.8	5.70 ± 2.7	NS
Enterolactone-*O*-glucuronide	43.83 ± 30.3	13.13 ± 5.1	NS

[a]Values are means ± SD, $n=5$ per group.
NS = non-significant ($p>0.05$).
WA = wheat aleurone pellets, CP = control pellets.
Hydroxyphenylpropionic acid-like, 3′-Hydroxyphenylpropionic acid, Methoxyhydroxybenzoic acid-O-sulfate and Ferulic acid-O-glucuronide were also analysed but not reported in the table.

Citation: Food & Nutrition Research 2014, **58**: 24604 - http://dx.doi.org/10.3402/fnr.v58.24604

Table 4. Effect of WA on plasma lipids and fatty acids in rats receiving the control or the linseed oils[a]

	Experiment 1		Experiment 2	
	CP, $n=12$	WA, $n=12$	CP, $n=12$	WA, $n=12$
Lipids, mmol/L				
Total cholesterol	0.53±0.07	0.55±0.13	0.57±0.10	0.50±0.06
Triglycerides	0.84±0.30	0.74±0.15	0.90±0.29	0.83±0.25
Fatty acids,% of total fatty acids				
C14:0	0.27±0.06	0.27±0.05	0.28±0.08	0.28±0.11
C16:0	18.8±1.5	18.7±1.7	17.7±1.1	17.5±1.5
C18:0	7.4±0.93	6.7±0.80	6.9±0.90	6.6±1.2
C18:1n-9	9.6±2.4	10.0±2.7	9.3±2.45	8.7±1.81
C18:2n-6	23.1±1.4	24.1±1.4	26.0±1.1	26.5±1.1
C18:3n-3	0.75±0.12	0.85±0.22	2.8±0.49	2.9±0.49
C20:4n-6	26.8±5.1	25.6±4.9	21.1±4.4	21.2±4.2
C20:5n-3 (EPA)	**1.6±0.28**	**2.0±0.35***	4.0±0.69	4.1±0.70
C22:4n-6	0.29±0.04	0.25±0.04	0.18±0.03	0.16±0.03
C22:5n-3	0.78±0.14	0.79±0.09	1.3±0.32	1.4±0.33
C22:6n-3 (DHA)	4.8±0.41	4.6±0.42	4.8±0.75	4.8±0.67
Total	100.0	100.0	100.0	100.0

[a]Values are means±SD, $n=12$ per group.
*$p<0.05$. WA = wheat aleurone pellets, CP = control pellets.

the two main steps of the n-3 pathway, EPA from ALA and DHA from EPA.

We found no difference between groups in the liver fatty acids. These results were quite surprising because the liver is thought to play a central role in the synthesis of EPA (and eventually DHA) from ALA. The difference between liver and plasma in the effect of WA polyphenols on VLC n-3 suggests a mechanism other than stimulation of the n-3 pathway mainly in the liver. For instance, the implication of the gut microbiota can be suspected in accordance with a recent study in growing gilts fed flax-seed containing diet (17). The authors showed that the ileal flow of EPA and DHA exceeded their dietary intakes indicating net appearance of these fatty acids in the upper gut, and suggesting that the enteric microbiota could actually elongate and desaturate ALA. Subsequent studies are required to confirm that hypothesis.

In Exp. 2, we tested whether increased ALA (under the form of LO) intake can stimulate the synthesis of EPA compared with a CO not containing any ALA. Contrary to our expectations, WA had no effect on EPA in presence of dietary ALA.

Two explanations could be proposed. If the gut microbiota is really involved in the synthesis of EPA from ALA, it could be speculated that high ALA intake may have inhibited the growth of some specific bacteria in the gut (18) and therefore prevent EPA increase. Another possibility would be that too much substrate, that is, too much ALA in the diet, could have saturated and inhibited the whole n-3 pathway and again prevent EPA increase. Several concordant mechanisms could also be considered in future studies.

Subsequent studies are needed to evaluate the dose-effect, or whether there is a threshold effect, in the relation between WA and the n-3 pathway. Also it is not clear whether longer exposition to WA polyphenols may influence the effects on VLC n-3. The low bioavailability of FA provided by aleurone can be also a limitation to this study. Finally, the biological mechanisms involved in the effect of the polyphenols on VLC n-3 are not elucidated yet. More investigations on the PUFA gene expression pathways and transcription factors are needed, and the role of the gut microbiota is still unclear.

In conclusion, our data show that WA and probably the polyphenols present in WA interferes with the metabolism of VLC n-3 without affecting the metabolism of n-6. Although the exact mechanisms are not clearly identified, our results provide new insights into the health benefits of polyphenol-rich whole grains.

Acknowledgements

The authors acknowledge C. Tellier for care of the animals, and M. Tripier-Champ and Y. Maffren for the technical assistance.

Conflict of interest and funding

This project has been funded by Barilla G. R. Fratelli Spa and supported in part by the 7th Framework Program ATHENA (Grant agreement no.: 245121) from the European Community. Ounnas, Privé, Hazane-Puch,

Laporte, Del Rio, Calani and Demeilliers have no conflicts of interest. Salen and de Lorgeril disclose receiving research grant (through Grenoble University School of Medicine) from the Barilla G&R F.lli Company. Melegari and Bianchi are employees of Barilla G. R. Fratelli Spa.

References

1. Harris K, Kris-Etherton PM. Effects of whole grains on coronary heart disease risk. Curr Atheroscler Rep 2010; 12: 368–76.
2. Ye EQ, Chacko SA, Chou EL, Kugizaki M, Liu S. Greater whole-grain intake is associated with lower risk of type 2 diabetes, cardiovascular disease, and weight gain. J Nutr 2012; 142: 1304–13.
3. McKeown NM, Meigs JB, Liu S, Wilson PW, Jacques PF. Whole-grain intake is favorably associated with metabolic risk factors for type 2 diabetes and cardiovascular disease in the Framingham offspring study. Am J Clin Nutr 2002; 76: 390–8.
4. Aune D, Chan DS, Lau R, Vieira R, Greenwood DC, Kampman E, et al. Dietary fibre, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of prospective studies. BMJ 2011; 343: d6617.
5. di Giuseppe R, de Lorgeril M, Salen P, Laporte F, Di Castelnuovo A, Krogh V, et al. Alcohol consumption and n-3 polyunsaturated fatty acids in healthy men and women from 3 European populations. Am J Clin Nutr 2009; 89: 354–62.
6. de Lorgeril M, Salen P, Martin JL, Boucher F, de Leiris J. Interactions of wine drinking with omega-3 fatty acids in patients with coronary heart disease: a fish-like effect of moderate wine drinking. Am Heart J 2008; 155: 175–81.
7. Toufektsian MC, Salen P, Tonelli C, de Lorgeril M. Dietary flavonoids increase plasma very long-chain (n-3) fatty acids in rats. J Nutr 2011; 141: 37–41.
8. Leaf A, Kang JX, Xiao YF, Billman GE. Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 2003; 107: 2646–52.
9. de Lorgeril M, Salen P, Defaye P, Rabaeus M. Recent findings on the health effects of omega-3 fatty acids and statins, and their interactions: do statins inhibit omega-3? BMC Med 2013; 11: 5.
10. Lempereur I, Rouau X, Abecassis J. Genetic and agronomic variation in arabinoxylan and ferulic acid contents of durum wheat (*Triticum durumL.*) grain and its milling fractions. J Cereal Sci 1997; 25: 103–10.
11. Sosulski F, Krygier K, Hogge L. Free, esterified, and insoluble-bound phenolic acids. Composition of phenolic acids in cereal and potato flours. J Agric Food Chem 1982; 30: 337–40.
12. Reeves PG, Nielsen FH, Fahey GC, Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993; 123: 1939–51.
13. Adam A, Crespy V, Levrat-Verny MA, Leenhardt F, Leuillet M, Demigné C, et al. The bioavailability of ferulic acid is governed primarily by the food matrix rather than its metabolism in intestine and liver in rats. J Nutr 2002; 132: 1962–8.
14. Rondini L, Peyrat-Maillard MN, Marsset-Baglieri A, Fromentin G, Durand P, et al. Bound ferulic acid from bran is more bioavailable than the free compound in rat. J Agric Food Chem 2004; 52: 4338–43.
15. Burdge GC, Calder PC. Conversion of α-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. Reprod Nutr Dev 2005; 45: 581–97.
16. Giltay EJ, Gooren LJ, Toorians AW, Katan MB, Zock PL. Docosahexaenoic acid concentrations are higher in women than in men because of estrogenic effects. Am J Clin Nutr 2004; 80: 1167–74.
17. Martínez-Ramírez HR, Kramer JK, de Lange CF. Ileal flows and apparent ileal digestibility of fatty acids in growing gilts fed flaxseed containing diets. J Anim Sci 2013; 91: 2729–39.
18. Laparra JM, Sanz Y. Interactions of gut microbiota with functional food components and nutraceuticals. Pharmacol Res 2010; 61: 219–25.

*Fayçal Ounnas
TIMC-IMAG, CNRS UMR 5525
Laboratoire PRETA, Cœur et Nutrition
Université Joseph Fourier
FR-38041 Grenoble, France
Email: ounnasf@ujf-grenoble.fr

Citation: Food & Nutrition Research 2014, **58**: 24604 - http://dx.doi.org/10.3402/fnr.v58.24604