Dietary flavonoids increase plasma very long-chain (n-3) fatty acids in rats.

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The Journal of Nutrition
Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interaction

Dietary Flavonoids Increase Plasma Very Long-Chain (n-3) Fatty Acids in Rats[1,2]

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Abstract

Flavonoids probably contribute to the health benefits associated with the consumption of fruit and vegetables. However, the mechanisms by which they exert their effects are not fully elucidated. PUFA of the (n-3) series also have health benefits. Epidemiological and clinical studies have suggested that wine flavonoids may interact with the metabolism of (n-3) PUFA and increase their blood and cell levels. The present studies in rats were designed to assess whether flavonoids actually increase plasma levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the main very long-chain (n-3) PUFA. Rats were fed a corn-derived anthocyanin (ACN)-rich (ACN-rich) or ACN-free diet with constant intakes of plant and marine (n-3) PUFA for 8 wk (Expt. 1). Plasma fatty acids were measured by GC. The ACN-rich diet contained ~0.24 ± 0.01 mg of ACN/g pellets. There were no significant differences between groups in the main saturated, monounsaturated, and (n-6) fatty acids. In contrast, plasma EPA and DHA were greater in the ACN-rich diet group than in the ACN-free diet group ($P < 0.05$). We obtained similar results in 2 subsequent experiments in which rats were administered palm oil (80 μL/d) and consumed the ACN-rich or ACN-free diet (Expt. 2) or were supplemented with fish oil (60 mg/d, providing 35 mg DHA and 12 mg EPA) and consumed the ACN-rich or ACN-free diet (Expt. 3). In both experiments, plasma EPA and DHA were significantly greater in the ACN-rich diet group. These studies demonstrate that the consumption of flavonoids increases plasma very long-chain (n-3) PUFA levels. These data confirm previous clinical and epidemiological studies and provide new insights into the health benefits of flavonoids. J. Nutr. 141: 37–41, 2011.

Introduction

Flavonoids are plant-derived bioactive compounds that include a large group of molecules commonly found in human foods and beverages such as berries, apples, onions, cocoa, tea, or wine (1). Epidemiological studies have suggested beneficial health effects of the chronic consumption of flavonoid-rich foods (2–4). Although the health effects of flavonoids have been mostly attributed to their antioxidant properties (5), the exact mechanisms by which they exert their health effects have not been fully elucidated so far.

PUFA of the (n-3) series are also associated with major health benefits, in particular the very long-chain eicosapentaenoic acid (EPA)[6] and docosahexaenoic acid (DHA), also called marine (n-3) PUFA, because they are present in large amounts in fatty fish (6,7). We have recently reported increased concentrations of EPA and DHA in the blood and cells of wine drinkers (8,9). These studies have indicated that components of wine other than ethanol, such as flavonoids, are probably responsible for these effects (8,9). If the effect of flavonoids on EPA and DHA is confirmed, the consequences may be considerable in terms of aquaculture and sea farming as well as human and animal nutrition and medicine. However, although the "fish-like" effect of moderate wine drinking has been clearly demonstrated (8), the interaction between flavonoids and (n-3) PUFA in the absence of ethanol has not yet been investigated.

The present study was therefore designed to assess whether the chronic consumption of plant-derived flavonoids such as anthocyanin (ACN) actually increases blood EPA and DHA levels in rats. Using a well-controlled experimental model (10), we assessed the changes in plasma fatty acid composition in rats fed an ACN-rich or ACN-free diet. Because the whole diet, especially fat intake, may play an important role in the interactions between ACN and (n-3) PUFA metabolism, we also analyzed the effect of ACN in rats exposed to diets enriched in either saturated fats (palm oil) or fish oil.

Materials and Methods

A total of 90 male Wistar rats (1 mo old, baseline body weight 75–100 g) were purchased from Charles River Laboratories. The rats were fed a standard diet (A04, SAFE) while acclimating and before being distributed

[1] Supported by the 6th Framework Program FLORA contract no. FOOD-CT-01730 from the European Community.
[2] Author disclosures: M-C. Toufektsian, P. Salen, F. Laporte, C. Tonelli, and M. de Lorgeril, no conflicts of interest.
[7] 1 kcal = 4.187 kJ.
[6] Abbreviations used: ACN, anthocyanin; ACN-free, anthocyanin-free diet; ACN-rich, anthocyanin-rich diet; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.
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Manuscript received June 04, 2010. Initial review completed July 02, 2010. Revision accepted September 30, 2010.
First published online November 10, 2010; doi:10.3945/jn.110.127225.

into 3 experimental groups. The animals were cared for according to 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. The rats were housed under conditions of constant temperature, humidity, and a standard light-dark cycle (12 h/12 h). The rats consumed ad libitum food and tap water.

Food preparation for ACN supply. Rodent food pellets enriched (or not) in ACN were prepared as previously described (10) using nongenetically modified corn lines selected from germplasm collections. Briefly, the ACR corn genotype carried the R-r allele, conferring high ACN accumulation and purple pigmentation to the aleurone of the seed, whereas the r-Δ902 genotype (referred to herein as r1) carried an interstitial deletion involving a region of the long arm of chromosome 10 containing the r1 locus (10). To obtain ears with a high kernel yield, the ACR and r1 genotypes were crossed with a commercial hybrid stock and the F1 progeny seed was used to produce 2 synthetic populations differing only in their r1 constitution (10). Special diets were prepared by replacing the corn content (20% of the total) of a standard pellet formula (A04, SAFE) with corn seed powder obtained from the r1 or ACR synthetic populations to produce the ACN-rich and ACN-free diets, respectively. Both diets had the same energy content, with macronutrient concentrations of 67% carbohydrate, 23% protein, and 10% lipids (SAFE). Both the ACN-rich and ACN-free diets were similar in terms of fatty acid composition, as detailed in **Table 1**.

Experimental protocol. Three experiments were conducted successively using 3 different groups of rats. *Expt. 1:* To test the effects of ACN in rats fed a standard diet, the rats were randomized into 2 experimental groups (n = 15/group) for an 8-wk feeding trial during which they received either the ACN-free or the ACN-rich diet. *Expt. 2:* To test the effects of ACN in rats fed a saturated fat-rich diet, they were fed either the ACN-free or the ACN-rich diet in combination with a daily oral administration of palm oil (80 μL/d, Marka) in both groups for 8 wk (n = 15/group). In addition to the standard diet, palm oil provided 37 mg/d of SFA, 28 mg/d of monounsaturated fatty acids, and 7 mg/d of PUFA. The palm oil contained no EPA or DHA. *Expt. 3:* To test the effects of ACN in rats fed an (n-3) PUFA-rich diet, they were fed either the ACN-free or the ACN-rich diet in combination with daily oral administration of fish oil (60 mg/d; IsodisNatura) in both groups supplying ~35 mg/d DHA and 12 mg/d EPA for 8 wk (n = 15/group).

During the 3 experiments, body weight and food consumption were recorded weekly. After 8 wk in all experiments, rats were anesthetized and heparinized (100 IU/100 g body weight). Plasma was obtained after centrifugation (5 min, 1000 × g, 4°C). Samples were stored at –80°C for subsequent fatty acid analyses.

TABLE 1 Fatty acid composition of the ACN-free and ACN-rich diets

Fatty acid	*mg/100 g food*
14:0	20
16:0	520
18:0	50
20:0	10
18:1(n-9)	400
16:1(n-7)	20
18:2(n-6)	1310
20:4(n-6)	0
18:3(n-3)	110
20:5(n-3)	20
22:5(n-3)	10
22:6(n-3)	30
Total SFA	620
Total MUFA[1]	510
Total PUFA	1480
Total (n-3)	170
Total (n-6)	1310

[1] MUFA, total monounsaturated fatty acids.

Lipid and fatty acid analyses. Plasma lipids were extracted in hexane/isopropanol as described previously (11). Briefly, methylated fatty acids were extracted with hexane, separated, and quantified by GC using a 6850 Series gas chromatograph system (Agilent Technologies). 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 acid content. Total cholesterol, HDL cholesterol, and triglycerides were measured using standard clinical methodology on a Synchron Clinical System LX20 (Beckman Coulter).

Statistical analysis. The data are expressed as means ± SEM. The 3 experimental protocols were not conducted at the same period of time and comparisons were made only between groups within each protocol. Differences between groups were evaluated using unpaired Student's t test. $P < 0.05$ was considered significant.

Results

ACN content of the food pellets. As previously reported (10), HPLC analyses showed that ACN were detected in the ACN-rich seeds but were entirely absent from the ACN-free maize seeds. The same ACN remained in the food pellets. Quantitative analyses indicated that the ACN-rich diet contained ~0.24 ± 0.01 mg of ACN/g of food pellets.

Body weight and food consumption. In Expt. 1, body weight (data not shown) and daily food consumption did not differ between the ACN-free (22.0 ± 0.4 g) and ACN-rich (21.8 ± 0.3 g) diet groups. In rats supplemented with palm oil (Expt. 2), body weight (data not shown) and daily food intake did not differ between the ACN-free+palm oil (23.4 ± 0.8 g) and the ACN-rich+palm oil (23.3 ± 1.1 g) groups. In Expt. 3, body weight (data not shown) and daily food consumption did not differ between the rats receiving the ACN-free+fish oil (21.8 ± 0.6 g) and the ACN-rich+fish oil (22.0 ± 0.3 g) diet.

Plasma lipid concentrations. Cholesterol and triglyceride concentrations did not significantly differ in the rats in Expt. 1 fed the ACN-free and the ACN-rich diets (**Table 2**). Similarly, levels of the main SFA, oleic acid, arachidonic acid, total levels of saturated and monounsaturated fatty acids, and total (n-6) PUFA did not differ between the ACN-rich and ACN-free groups. In contrast, total (n-3) PUFA and the (n-3):(n-6) fatty acid ratio were significantly greater in rats fed the ACN-rich diet ($P < 0.01$). Plasma EPA and DHA concentrations were greater in rats fed the ACN-rich diet than in those fed the ACN-free diet ($P < 0.05$).

In Expt. 2, in which both groups received palm oil, the plasma cholesterol, triglycerides, and saturated, monounsaturated, total (n-6), and total (n-3) fatty acid concentrations did not differ between the rats receiving the ACN-free and the ACN-rich diet (**Table 3**). However, the plasma (n-3):(n-6) fatty acid ratio and EPA and DHA concentrations were greater in rats fed the ACN-rich diet than in rats fed the ACN-free diet ($P < 0.05$).

In Expt. 3, in which both groups received fish oil, the plasma cholesterol, triglycerides, and saturated, monounsaturated, and total (n-6) fatty acid concentrations did not differ between the rats receiving the ACN-free and the ACN-rich diet (**Table 4**). However, total (n-3) fatty acid concentrations, the (n-3):(n-6) fatty acid ratio, and EPA and DHA concentrations were greater in rats fed the ACN-rich diet than in rats fed the ACN-free diet ($P < 0.05$).

TABLE 2 Plasma lipids and fatty acid concentrations in rats fed ACN-free or ACN-rich diets for 8 wk (Expt. 1)[1]

	ACN-free	ACN-rich
Lipids, *mmol/L*		
Total cholesterol	1.06 ± 0.18	1.29 ± 0.05
HDL cholesterol	0.95 ± 0.05	1.01 ± 0.05
Triglycerides	1.52 ± 0.31	1.17 ± 0.29
Fatty acids, *% of total fatty acids*		
Myristic	0.8 ± 0.1	0.7 ± 0.1
Palmitic	21.9 ± 0.6	20.6 ± 0.7
Stearic	6.3 ± 0.4	6.0 ± 0.4
Oleic	12.8 ± 0.7	11.9 ± 1.1
Linoleic	23.4 ± 0.9	23.8 ± 0.5
α-Linolenic	0.7 ± 0.1	0.8 ± 0.1
Arachidonic	16.1 ± 1.6	17.5 ± 1.7
EPA	0.9 ± 0.03	1.2 ± 0.09**
Docosapentaenoic	0.6 ± 0.05	0.7 ± 0.05
DHA	3.8 ± 0.2	4.5 ± 0.2*
Total SFA	29.1 ± 0.6	27.4 ± 0.6
Total MUFA	23.5 ± 1.4	22.4 ± 1.8
Total PUFA	47.2 ± 1.6	50.0 ± 2.1
Total (n-3)	6.1 ± 0.2	7.2 ± 0.2**
Total (n-6)	41.1 ± 1.4	42.8 ± 1.9
(n-3):(n-6) fatty acids	0.15 ± 0.0	0.17 ± 0.0**

[1] Values are means ± SEM, $n = 15$ per group. Asterisks indicate difference from ACN-free: *$P < 0.05$, **$P < 0.01$.

TABLE 3 Plasma lipids and fatty acid concentrations in rats fed a ACN-free or ACN-rich diet and supplemented with palm oil for 8 wk (Expt. 2)[1]

	ACN-free+palm oil	ACN-rich+palm oil
Lipids, *mmol/L*		
Total cholesterol	1.01 ± 0.05	1.08 ± 0.05
HDL cholesterol	0.82 ± 0.03	0.90 ± 0.05
Triglycerides	0.63 ± 0.10	0.64 ± 0.08
Fatty acids, *% of total fatty acids*		
Myristic	1.0 ± 0.1	1.1 ± 0.1
Palmitic	22.6 ± 0.3	22.9 ± 0.6
Stearic	7.7 ± 0.4	8.0 ± 0.7
Oleic	11.3 ± 0.7	11.2 ± 0.6
Linoleic	20.3 ± 0.4	19.7 ± 0.7
α-Linolenic	0.5 ± 0.1	0.4 ± 0.0
Arachidonic	18.8 ± 1.1	17.1 ± 1.1
EPA	1.3 ± 0.07	1.6 ± 0.09*
Docosapentaenoic	0.9 ± 0.05	0.9 ± 0.05
DHA	4.9 ± 0.1	5.4 ± 0.2*
Total SFA	31.4 ± 0.6	32.0 ± 0.9
Total MUFA	19.9 ± 1.1	20.5 ± 1.3
Total PUFA	48.5 ± 1.2	46.8 ± 1.6
Total (n-3)	7.6 ± 0.2	8.3 ± 0.4
Total (n-6)	40.9 ± 1.1	38.5 ± 1.4
(n-3):(n-6) fatty acids	0.19 ± 0.0	0.22 ± 0.0*

[1] Values are means ± SEM, $n = 15$ per group. *Different from ACN-free, $P < 0.05$.

Discussion

For the first time, to our knowledge, in well-controlled experimental conditions, the present study shows that dietary plant-derived ACN modulate the metabolism of (n-3) PUFA and increase plasma concentrations of the very long-chain (n-3) PUFA, EPA and DHA, also called marine (n-3) fatty acids. This was also observed in rats consuming an ACN-rich diet combined with either saturated fat (palm oil, Expt. 2) or fish oil (Expt. 3). In the 3 experiments, EPA increased more (41, 25, and 24%) than DHA (16, 10, and 16%), although the increase was significant for both fatty acids. The different increases for EPA and DHA were not unexpected, because the synthesis of DHA involves additional steps, (double) carbonyl chain elongation, desaturation, and β-oxidation, compared with that of EPA from the plant precursor, α-linolenic acid (12). The different increases in EPA and DHA suggest, but do not definitely demonstrate, that ACN may exert their effects through the conversion pathway of α-linolenic acid into the very long-chain EPA and DHA. An alternative mechanism, increased intestinal absorption of EPA and DHA, would (at least in theory) induce a parallel increase in EPA, DHA, and some other fatty acids, including (n-6) PUFA. However, this did not happen. Also, in case of increased intestinal absorption of EPA and DHA in the presence of dietary ACN, we would have observed the strongest EPA and DHA increases among rats receiving fish oil, because more EPA and DHA were available in the intestinal tract of these rats. Again, this was not the case.

The different increases in EPA and DHA levels were also not unexpected because of the quite short (8 wk) duration of the feeding periods in this study. In fact, we speculate that the stimulation of a more complex pathway (the synthesis of DHA from α-linolenic acid) would require more time to see large effects on DHA than the stimulation of the shortest pathway from α-linolenic acid to EPA.

Further studies with longer feeding periods are therefore required to monitor the rate and cofactors of this newly described process and to study the accumulation of EPA and DHA in specific cells and tissues, including RBC, leukocytes, brain, heart, and kidney.

Clinical implications. ACN are a large group of water-soluble plant pigments that belong to the flavonoid family (1,13). Epidemiological, clinical, and experimental evidence suggests that dietary flavonoids, including flavones, flavonols, and ACN, may exert beneficial health effects (4,10,14–16). For instance, we previously demonstrated that the regular consumption of ACN protects rats' hearts against both ex vivo and in vivo ischemia-reperfusion injury (10). In these cardiac experiments, as in the present study, corn-derived ACN were included into the standard diet to produce an ACN-rich diet and isogenic corn seeds free of ACN were added to the control (ACN-free) diet (10). The main ACN identified in the ACN-rich corn and also in the corresponding ACN-rich pellets, cyanidin-glucoside, cyanidin-malonylglucoside, cyanidin-dimalonylglucoside, and pelargonidin-malonylglucoside, were detected only in the urine samples of rats fed the ACN-rich diet (10). This food model therefore allowed studying the specific effects of the ACN identified in the ACN-rich corn selected for our studies. Because the corn used in the control group is isogenic to the ACN-rich corn, this experimental model almost totally excludes that the effects associated with consumption of ACN-rich corn, e.g. the metabolism of (n-3) PUFA and myocardial ischemia-reperfusion injury, were caused by some unidentified factors or cofactors (other than ACN) present in ACN-rich but not in ACN-free corn.

In this study in rats, we observed a marked increase in plasma EPA and DHA, fatty acids known to be protective against heart disease complications (6,7). Moderate wine consumption, also known to be cardioprotective (17,18), has been associated with

TABLE 4 Plasma lipids and fatty acid concentrations in rats fed a ACN-free or ACN-rich diet and supplemented with fish oil for 8 wk (Expt. 3)[1]

	ACN-free+fish oil	ACN-rich+fish oil
Lipids, *mmol/L*		
Total cholesterol	0.95 ± 0.03	0.90 ± 0.05
HDL cholesterol	0.80 ± 0.03	0.72 ± 0.05
Triglycerides	0.41 ± 0.0	0.51 ± 0.06
Fatty acids, *% of total fatty acids*		
Myristic	0.9 ± 0.1	0.8 ± 0.1
Palmitic	22.4 ± 0.3	21.9 ± 0.7
Stearic	7.9 ± 0.3	7.9 ± 0.5
Oleic	9.3 ± 0.3	10.1 ± 0.4
Linoleic	21.9 ± 0.6	20.8 ± 0.7
α-Linolenic	0.4 ± 0.0	0.4 ± 0.0
Arachidonic	14.4 ± 0.5	12.9 ± 0.9
EPA	4.1 ± 0.2	5.1 ± 0.4*
Docosapentaenoic	1.4 ± 0.1	1.6 ± 0.1*
DHA	7.7 ± 0.3	9.0 ± 0.3**
Total SFA	31.3 ± 0.5	30.7 ± 1.0
Total MUFA	16.8 ± 0.6	17.7 ± 0.9
Total PUFA	51.6 ± 1.0	51.4 ± 1.6
Total (n-3)	13.6 ± 0.5	16.1 ± 0.6**
Total (n-6)	38.0 ± 0.9	35.4 ± 1.6
(n-3):(n-6) fatty acids	0.36 ± 0.02	0.47 ± 0.36**

[1] Values are means ± SEM. Asterisks indicate difference from ACN-free: *$P < 0.05$, **$P < 0.01$.

increased levels of EPA and DHA in patients with heart disease, a phenomenon referred to as a fish-like effect of moderate wine drinking (8). Moreover, in the IMMIDIET cohort, a significant association between ethanol intake and EPA and DHA levels was shown among wine drinkers, whereas no significant association was found among beer or spirits drinkers (9). These data strongly suggested that some components of wine other than ethanol (e.g. ACN) were mainly responsible for the association between wine drinking and (n-3) PUFA.

Thus, our present experimental data confirm that, independently from their consumption through alcoholic beverages (i.e. in the absence of ethanol), food-derived ACN interfere with the metabolism of (n-3) PUFA and induce their accumulation in blood. Whereas the health-promoting effects of flavonoids were originally attributed mostly to their antioxidant properties, there is increasing evidence that many of their biological effects are related to their ability to modulate mammalian cell signaling pathways (19). The interactions between ACN and (n-3) PUFA in this study provide totally new insights into the potential mechanisms of protection afforded by dietary flavonoids.

The next question is whether the changes observed in this study are biologically or clinically important. Although rat data should be prudently extrapolated to the human situation, and vice versa, the present consensus about long-chain marine (n-3) fatty acids is that small changes result in large clinical effects, especially in populations with low consumption of both plant and marine (n-3) fatty acids. Thus, a 40% increase in plasma EPA associated with a 16% increase in DHA in this study is not anecdotal. In human studies, similar and even smaller changes in marine (n-3) have been associated with significant reduction of the risk of coronary heart disease complications (8,20).

Finally, it should be noted that the average intake of ACN in Western populations is estimated to be 12 mg/d (10), equivalent to a daily intake of 6 μg/(kcal·d)[7] assuming a daily energy intake of 2000 kcal. Because we calculated that rats fed the ACN-rich diet received ~0.08 mg/(kcal·d) ACN (10), they received ~13-fold more ACN than most people consuming a standard Western-type diet (10). In contrast, many typical Mediterranean foods and beverages (fruits, vegetables, and wine, for instance) are rich in ACN and the ACN content of the traditional Mediterranean diet is much higher than that of the Western diet, which might partly explain why the Mediterranean diet is cardioprotective (21).

Potential mechanisms. Beside the above-discussed possibility that ACN increase the intestinal absorption of EPA and DHA, the main hypothesis regarding the effect of ACN on (n-3) PUFA is that they may accelerate the synthesis of EPA and DHA from their precursor α-linolenic acid, the main (n-3) PUFA in the diet of both rats and humans (12,22). The dietary intakes of α-linolenic acid did not differ in rats fed the ACN-free or ACN-rich diet in our 3 experiments, which again suggests that increased EPA and DHA levels resulted from the activation of their biosynthesis from α-linolenic acid. Two desaturases, Δ6 and Δ5, are required for EPA formation from α-linolenic acid, with Δ6 desaturase being the rate-limiting step in the pathway (12). Whereas (n-3) and (n-6) PUFA are in competition for desaturation and elongation, Δ6 desaturase is thought to preferentially use (n-3) PUFA (12,23), which may explain why we did not detect any change in the levels of (n-6) PUFA, in particular arachidonic acid, in our 3 experiments. Both microsomal enzymes are regulated by genetics, nutrition, and hormonal status (24,25). Whether flavonoids influence Δ6 or Δ5 desaturase or both requires further studies. It is noteworthy that the metabolic benefits induced by several classes of flavonoids have been linked at least partly to a PPARα-dependent mechanism that also interferes with the PUFA elongation-desaturation pathway (26–28). For example, PPARα activators, such as the cholesterol-lowering drugs fibrates, have been shown to upregulate hepatic Δ6 desaturase expression and activity (29). However, we have shown in a randomized trial in patients that fenofibrate actually decreases rather than increases EPA and DHA levels (30). At the same time, fenofibrate significantly increases arachidonic acid levels, which was not the case with ACN in the present study. It is therefore unlikely that ACN may increase EPA and DHA levels through a PPARα-dependent mechanism. Further studies are required to determine the exact mechanism by which ACN consumption modulates plasma EPA and DHA concentration increases.

Compared with rats fed the ACN-free diet plus palm oil or fish oil, the rats fed the ACN-rich diet plus palm oil or fish oil had higher plasma EPA and DHA levels in the present study. These data confirm that the effect of ACN on EPA and DHA levels is relatively independent from other fat supplies. Xiang et al. (31) have shown that high dietary intake of saturated fat increases the expression of both Δ5 and Δ6 desaturase genes in peripheral blood mononuclear cells in humans, suggesting that both (n-3) and (n-6) PUFA biosynthesis may be modulated by saturated fat. However, in the rats receiving palm oil in our study, ACN specifically increased (n-3) PUFA levels without an effect on (n-6) PUFA levels. This suggests that the mechanisms by which ACN interfere with the metabolism of PUFA are different from those involved in the saturated fat stimulation described by Xiang et al. (31). In our study, fish oil induced a marked accumulation of plasma EPA and DHA in both the ACN-rich and ACN-free groups. This was expected. In that specific nutritional condition, however, EPA and DHA increased significantly more in the ACN-rich than in the ACN-free group.

Limitations of the study. As discussed above, the present experiments were performed in rats, so our data have yet to be confirmed in humans. It is noteworthy, however, that previous studies (8,9) have suggested that a comparable phenomenon occurs in humans. In addition, our experiments were conducted in male rats exclusively, which raises a gender issue. Gender differences in the efficiency of (n-3) PUFA bioconversion have been reported and might reflect differential hormonal regulation of PUFA metabolism (12). In the present study, gender-related differences were not evaluated and further studies are needed to examine whether hormonal status may significantly modulate the interactions between ACN and (n-3) PUFA. Also, our nutritional protocols were conducted in young rats (from 1 to 3 mo old). However, the impact of ACN consumption on PUFA levels may change with age both in rats and humans. Further studies are also needed to examine this important issue.

In conclusion, our data show that dietary flavonoids, and more specifically ACN consumption, interact with the metabolism of (n-3) PUFA and increase blood EPA and DHA levels. The effect was observed for various dietary conditions, including fat sources. Although the exact mechanisms involved in these interactions are not yet understood, our results provide new insights into the cellular pathways of the health benefits afforded by dietary flavonoids.

Acknowledgments

We thank Katia Petroni and Roberto Pilu (from the Dipartimento di Scienze Biomolecolari e Biotecnologie in Milano) for their help in providing the ACN-rich corn line. M.C.T., P.S., and M.d.L. designed, conducted, and analyzed the research; P.S. and M.d.L. wrote the paper; F.L. was in charge of the fatty acid measurements and had primary responsibility for final content of the paper; C.T. provided the ACN-rich corn line and had primary responsibility for final content of the paper. All authors read and approved the final manuscript.

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