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Potential favourable health effects of some dietary uncommon fatty acids

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Abstract – In addition to the major fatty acids widely studied, our diet contains many bioactive fatty acids less frequently investigated such as n-3 docosapentaenoic acid (n-3 DPA), natural trans fatty acids, conjugated fatty acids (CLAs), furan fatty acids (FuFAs), branched chain fatty acids (BCFAs) and fatty acid esters of hydroxyl fatty acids (FAHFAs). Many of them may have beneficial health effects, particularly in the prevention of cardiovascular diseases, inflammation and metabolic disorders such as diabetes. This review aims to give a brief overview of the current knowledge on these lipids. Thus, information about biosynthesis, food and tissue content, daily intake, biological and potential health effects of these fatty acids is provided.

Keywords: bioactive lipids / biosynthesis / food content / dietary intake / biological and health effects

Résumé – Effets potentiellement bénéfiques sur la santé de certains acides gras peu courants de l’alimentation. Parallèlement aux acides gras prépondérants qui sont largement étudiés, notre alimentation contient de nombreux acides gras bioactifs moins fréquemment considérés tels que l’acide docosapentaénoïque n-3 (n-3 DPA), des acides gras trans naturels, des acides gras conjugués (CLAs), des acides gras furaniques (FuFAs), des acides gras à chaîne ramifiée (BCFAs) et des esters d’acides gras hydroxylés (FAHFAs). Nombreux d’entre eux peuvent avoir des effets bénéfiques sur la santé, notamment dans la prévention des maladies cardiovasculaires, de l’inflammation et des troubles métaboliques tels que le diabète. Cette revue vise à donner un bref aperçu des connaissances actuelles sur ces lipides. Ainsi, des informations sur la biosynthèse, les teneurs tissulaires et dans les aliments, l’apport alimentaire quotidien, les effets biologiques et les effets santé potentiels de ces acides gras sont rapportés.

Mots clés : lipides bioactifs / biosynthèse / composition des aliments / apport alimentaire / effets biologiques et sur la santé

Food plays an important role not only for an optimal growth and development but also in the maintenance of a good health. While the role of nutrients and micronutrients and the needs of the organism in these components are widely studied, there are many molecules in our diet whose roles are yet to be investigated more extensively. These include many bioactive lipids with potential beneficial health effects such as n-3 docosapentaenoic acid (n-3 DPA), natural trans fatty acids, conjugated fatty acids (CLAs), furan fatty acids (FuFAs), branched chain fatty acids (BCFAs) and fatty acid esters of hydroxylated fatty acid esters (FAHFAs). This review aims to give a brief overview of the current knowledge regarding these lipids. We present their structure and their biosynthesis (Fig. 1 for precursors and structure of these lipids), their content in food and their daily intake (Tab. 1), their tissue content, their biological and potential health effects. Some dietary lipids can be toxic (cyclopropene-containing lipids, mono-unsaturated long-chain fatty acids as erucic acid, trans-unsaturated fatty acids from industrial hydrogenated fats, and lipid peroxides) (Gurr et al., 2002). However, except trans fatty acids of industrial origin, they are not discussed in this article.

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The n-3 docosapentaenoic acid DPA

1.1 Structure and biosynthesis

n-3 DPA (C22:5 n-3) belongs to the n-3 polyunsaturated fatty acid (PUFA) family. It is also called clupanodonic acid. It is an intermediate between eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) in the conversion pathway of n-3 PUFA from α-linolenic acid (ALA, C18:3 n-3). n-3 DPA as a reservoir is metabolized into DHA, and further retro-conversed back to EPA (Guo et al., 2020).

1.2 Food content and daily intake

n-3 DPA is present in numerous foods in notable quantities, in particular in seafood (1 to 5% of total fatty acids in fish, i.e. up to 1/3 of EPA or DHA levels taken individually) as well as in human breast milk (0.2% of total fatty acids) (Drouin, 2018). For example, fish oils such as menhaden or sardine oil contain in percentage of total fatty acids: 10–13% EPA, 2–5% DPA and 9–11% DHA. Atlantic salmon contains 0.3 g of EPA, 0.3 g of DPA and 1.1 g of DHA per 100 g. (Kaur et al., 2016).

The estimated average consumption of n-3 DPA is between 10 and 106 mg/d in Western countries and Japan (Richter et al., 2019). n-3 DPA may represent a significant proportion of the total long-chain n-3 fatty acid intake, depending on the population, up to 30% of average total long-chain n-3 fatty acid intake in some cases (Richter et al., 2019). However, the digestibility of n-3 DPA in rodents (digestibility reflecting net absorption in the digestive tract after enzymatic hydrolysis by digestive enzymes and microflora of the gastrointestinal tract) is lower than that of EPA and DHA (Drouin et al., 2019a).

1.3 Tissue content and biological functions

The majority of the tissues present n-3 DPA levels in the order of 5% compared to DHA levels (Ghasemi Fard et al., 2021). In the brain, n-3 DPA is the second n-3 PUFA found, although at levels 70 times lower than DHA (Drouin et al., 2019b).
n-3 DPA is a source of EPA and to a lesser extent DHA in major metabolic tissues (liver, heart, lung, spleen and kidney), two fatty acids with numerous known health benefits. Moreover, n-3 DPA is the precursor of many major lipid mediators (protectins, resolvins, maresins, isoprostanooids), involved in the pro-resolution of inflammation, with specific effects compared to other n-3 PUFAs (Ghasemi Fard et al., 2021).

1.4 Health effects

Low availability of n-3 DPA in sufficient quantity, at high purity and at an affordable price (Drouin, 2018; Drouin et al., 2019b) has limited its studies in rodents and humans. Thus, only 11 studies in animals and 2 studies in humans with n-3 DPA in pure form have been reported (Ghasemi Fard et al., 2021). The effects of n-3 DPA on lipid parameters associated with the prevention of cardiovascular diseases are the most documented (anti-inflammatory properties, inhibition of cytokine synthesis, reduction of thrombosis and inhibition of atherosclerosis, ...) (von Schacky and Harris, 2018). The health effects of n-3 DPA could be both independent and shared with EPA and DHA (Richter et al., 2019). It is important to note that the n-3 DPA could contribute to increasing the n-3 fatty acid status, as n-3 DPA is more present in meat than EPA or DHA and while the sources of fatty fish are limited; therefore, n-3 DPA could be helpful to maintain a suitable n-6/n-3 ratio which is the indicator of a preventive diet for the control of non-communicable diseases (Drouin et al., 2019b). Drouin et al. recently published a comprehensive review on n-3 DPA (Drouin et al., 2019b).

2 The trans fatty acids

2.1 Structure and biosynthesis

Trans fatty acids are fatty acids that have at least one double bond in the trans configuration while most of the naturally occurring unsaturated fatty acids contain cis-double bonds. Naturally occurring trans fatty acids are mostly monounsaturated fatty acids, mainly trans C18:1 n-7 (vaccenic acid), trans-C16:1 n-7 and all isomers of oleic acid (18:1 n-9) (Leray, 2013). There are also di-unsaturated trans fatty acids, derived from linoleic acid (18:2 n-6) or tri-unsaturated trans fatty acids, derived from linolenic acid (18:3 n-3). Conjugated linoleic acids (two conjugated double bonds, one of which is in a trans configuration) are discussed in Section 3.

Naturally occurring trans fatty acids come from a bacterial isomerisation of fatty acids in the digestive tract of ruminants (Leray, 2013). The eukaryotes are unable to synthesise them, however, it is possible that they can be synthesised by the action of intestinal microbiota on dietary fatty acids. Trans fatty acids of industrial origin are formed during the partial hydrogenation of vegetable or fish oils. Thermal treatments (frying, cooking, ...) can also produce trans fatty acids of (poly)unsaturated oils and fats.

2.2 Food content and daily intake

Trans fatty acids of natural origin are found in dairy products and beef and sheep meats. Butter contains 3 to 7 g/100 g of natural trans fatty acids, cheeses from 1.3 to 2 g/100 g, whole milks around 0.15 g/100 g, vegetable oils between 0.5 and 2 g/100 g, beef and sheep meat from 0.1 to 0.5 g/100 g (Leray, 2013). Trans fatty acids from industrial process are used by the food industry as stabilizers and preservatives. Thus, they are found in many processed food products such as pastries, pizzas, quiches (Afssa, 2005).

According to the results of the INCA2 survey, the average and 95th percentile intake of the total trans fatty acids in the French population was estimated at 1–1.5% of total energy intake, regardless of age and sex (Afssa, 2009). More than half is of natural origin (0.5–0.9%), thus below the ANSES recommendations to limit the total trans fatty acid intake to less than 2% of the total energy intake (Afssa, 2009).

2.3 Tissue content and biological functions

In the 2000s, the total amount of trans fatty acids was 2.32±0.50% of the total fatty acids in adipose tissue of French women (Boue et al., 2000) and mean adipose tissue levels were lower in many European countries than in the USA (Arab, 2003).

The trans configuration impacts the physicochemical and functional properties of monounsaturated fatty acids. It makes them closer to the properties of the corresponding saturated fatty acids. Thus, high amounts of trans fatty acids may decrease the membrane fluidity and increase the oxidative stress (Leray, 2013), and may induce inflammation and apoptosis of the cells (Qiu et al., 2018).

2.4 Health effects

A number of epidemiological studies have shown a relationship between trans fatty acid intake and cardiovascular diseases. Controlled feeding studies suggest that dietary trans fatty acids raise serum cholesterol concentrations to a very similar extent as saturated fatty acids (Gurt et al., 2002; Leray, 2013). Numerous studies show that the risks of cardiovascular diseases with dietary trans fatty acids are attributable to industrial trans fatty acid (Oteng and Kersten, 2020). In contrast, no increase in cardiovascular risk has been observed with the consumption of naturally occurring trans fatty acids at the current consumption levels (Guillocheau et al., 2019). Studies on cells, rodents and humans suggest physiological benefits on inflammation, type 2 diabetes and obesity (Guillocheau et al., 2019), without knowing whether these naturally occurring trans fatty acids act directly or through their metabolites (Guillocheau et al., 2019). Guillocheau et al. recently published a comprehensive review on natural trans fatty acids (Guillocheau et al., 2019).

3 The conjugated linoleic acid CLAs

3.1 Structure and biosynthesis

Conjugated linoleic acid (CLA) is a collective term for a mixture of positional and geometrical isomers of linoleic acid (LA,C18:2 n-6) containing conjugated double bounds. Some linoleic acid isomers have conjugated doubles with one of them (at least) in a trans configuration (Leray, 2013).
3.2 Food content and daily intake

Of the possible isomers of CLA, about 20 have been identified in foods (Leray, 2013). Beef meat contains up to 120 mg/100 g of CLA and lamb meat about 80 mg/100 g. The main isomer present in milk fat is the rumenic acid, which accounts for 80% to 90% of the total CLA. Rumenic acid represents up to 700 mg/100 g in butter and up to 100 to 250 mg/100 g in cheese. Women's milk contains the same amount as cow's milk (10 mg/100 g). CLA, including rumenic acid, can also be found after heating vegetable oils and in certain food products. In fact, it is possible to obtain CLA through the partial hydrogenation of linoleic acid or by thermal treatments, and thus to find up to 0.5 g of CLA for 100 g of products in certain food products (industrial pasta, cookies) (Leray, 2013).

The intake of CLA from a typical diet is estimated at several 100 mg/d in various countries (Parodi, 2003). In the 2000s, mean daily intake of rumenic acid was 250 mg/d to 320 mg/d in female students in Germany (Fremann et al., 2002), mean daily intake of CLA was 176 mg total CLA/d for men and 104 mg for women in the USA and was estimated to be almost 100 mg in the UK (Ritzenthaler et al., 2001).

3.3 Tissue content and biological functions

To our knowledge, no data are found in the literature on CLA tissue content in human. It was demonstrated in vitro and in vivo in animal models that CLA plays a major role in lipid metabolism, especially as regards the oxidative cellular system. In fact, in conjugated fatty acids, the electrons become delocalized over conjugated double bonds, conferring to CLA unusual chemical properties (Gurr et al., 2002). In addition to its role on lipid metabolism and lipid peroxidation, the impact of CLA on energy expenditure, insulin metabolism and inflammation were also observed (Lehnen et al., 2015; Wang et al., 2020a).

3.4 Health effects

Some animal studies show that CLA (and also CLNA, CEPA, CDHA) may have some beneficial health effects such as reduction of body fat, improved insulin resistance, anti-thrombogenic and anti-carcinogenic effects, reduction of atherosclerosis, improved lipid profile, modulation of the immune system and stimulation of bone mineralization (Wang et al., 2020a). The most studied CLA supplementation effect is its capacity to alter the body composition, promoting an increase in lean mass and reduction of the fatty mass (Lehnen et al., 2015). However, in humans, the clinical evidence appears to be insufficient and not unanimous regarding the health effects of CLA (Ritzenthaler et al., 2001; Lehnen et al., 2015). Of the different isomers of CLA, rumenic acid has been reported to be the most bioactive CLA (Belury, 1995). Lehnen et al. recently published a comprehensive review on CLAs (Lehnen et al., 2015).

4 The furan fatty acids FuFAs

4.1 Structure and biosynthesis

The furan fatty acids (FuFAs) are fatty acids with a furan ring. To date, thirty different structures have been identified (Glass et al., 1974; Wang et al., 2020b). The most common FuFAs are methylated or dimethylated forms. However, non-methylated furans have been also described (Yurawecz et al., 1995).

Methylated FuFAs are formed from polyunsaturated fatty acids, in particular linoleic acid (Batna et al., 1993) and non-methylated FuFAs are formed from conjugated dienes, in particular CLA (Yurawecz et al., 1995). Currently, the biosynthetic pathway of FuFAs is not completely established and might depend on the species considered (plant, bacteria, animal, ...).

4.2 Food content and daily intake

Fish are an important source of FuFAs, with 1 to 4% of total fatty acids in the form of FuFAs (Vetter et al., 2012). Butter and dairy products contain 5 to 50 mg/100 g FuFAs (Vetter et al., 2012; Wendlinger and Vetter, 2014). FuFAs have been found in wheat, rice, potatoes, cabbage, orange, lemon, raspberries, with levels ranging from 1 to 350 µg/g dry matter (Hannemann et al., 1989). Soybeans contain FuFAs at levels of 30 to 300 µg/g (Guth and Grosch, 1991; Wu et al., 1997) while the levels are not quantifiable in olives, sesame, nuts, grape seeds and sunflower (Wahl et al., 1994).

Few data exist on the ingested amount of FuFAs. The estimated average consumption of FuFAs was estimated in Germany in 2014, and observed to be about 10 to 25 mg/day (6.6 to 16.5 mg via fish, 0.7–4.8 mg via milk fat, 1.4 to 2.5 mg via soybean oil, 0.2–0.5 mg via rapeseed oil and 0.008 mg via olive oil) (Wendlinger and Vetter, 2014).

4.3 Tissue content and biological functions

To our knowledge, no data are found in the literature on FuFAs tissue content in human. FuFAs possess antioxidant properties due to the presence of the furan ring (Okada et al., 1990, 1996; Masuchi Buscato et al., 2020). They also have anti-microbial (Knechtle et al., 2014; Dasagrandhi et al., 2016; Kimura et al., 2018) and anti-inflammatory (Wakimoto et al., 2011; Khan et al., 2018; Lauvai et al., 2019) properties. FuFAs may thus participate to the anti-inflammatory effects of fish oils and fish-based diets.

4.4 Health effects

FuFAs present in fish may be involved in the beneficial effects of fish consumption on cardiovascular disease (Spiteller, 2005). Several in vitro studies support this hypothesis
5. The branched chain fatty acids BCFAs

5.1 Structure and biosynthesis

BCFAs are saturated fatty acids with one or more methyl groups in the linear carbon chain. There are two distinct series of BCFAs: the iso-series where the terminal group is \( \text{CH}_3 \) and the anteiso-series where the terminal group is \( \text{CH}_3-\text{CH}_2-\text{CH}_3 \) (Gurr et al., 2002). However, branch points can also be found in other positions. More than 50 BCFAs have been identified in ruminant-derived fats (Taormina et al., 2020). Monomethyl BCFAs are the most abundant. Among multimethyl BCFAs, phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) and pristanic acid (2,6,10,14-tetramethylpentadecanoic acid) are predominant although in lesser amounts compared to monomethyl structures (Leray, 2013; Taormina et al., 2020).

In ruminants, BCFAs are synthesized by microorganisms in the rumen, from dietary branched-chain amino acids such as valine, leucine and isoleucine (Taormina et al., 2020). Wallace et al. (Wallace et al., 2018) demonstrated also that BCFAs were synthesized de novo in adipose tissues from branched-chain amino acids catalyzed in mitochondria, and then exported by carnitine acetyltransferase to the cytosol, where they were elongated by fatty acid synthase.

5.2 Food content and daily intake

BCFAs occur widely but mainly at low concentrations in animal fat and some marine oils (Gurr et al., 2002). BCFAs are present in the milk and tissues of ruminants consumed by humans (beef, sheep, goat). In cow’s milk, the concentration of phytanic acid ranges from 0.16 to 0.59 g/100 g of lipids and that of pristanic acid from 0.03 to 0.09 g/100 g of lipids (Leray, 2013). In some wild fishes, BCFAs were only 1% ± 0.5% (mean ± SD) of the total fatty acids, contributing only a small amount of BCFAs per serving to the diet. Consuming a standardized portion (70 g) of wild freshwater fish contributes to only small amounts of BCFAs (for instance 2.5–24.2 mg, in the American diet) (Wang et al., 2016). Asian food, fermented soy known as natto and fermented shrimp paste have high BCFA levels, 1.71 ± 0.17% and 3.18 ± 0.14% BCFAs, respectively (Wang, 2017), relative to total fatty acids.

Few data exist on the ingested amount of BCFAs. In the USA in 2011, the consumption of milk, cheese and beef contributed to a daily dietary intake of about 400 mg of branched fatty acids (Ran-Ressler et al., 2011). Consumption of chocolate contributed to about 6 mg BCFA/day (Ran-Ressler et al., 2014).

5.3 Tissue content and biological functions

In mammalian tissues, BCFAs rarely constitute more than 1–2% of the total fatty acid pool (Pakiet et al., 2020). BCFAs are present in the gut from a very early age and throughout the human life cycle. BCFAs are major components of the lipids of Gram-positive bacteria (such as Bacillus and Lactobacillus). They play an important regulatory role in fluidity and permeability of bacterial membrane (Taormina et al., 2020). They have a positive influence on the development of commensal bacteria from birth, and on intestinal metabolism (Leray, 2013).

5.4 Health effects

BCFAs may contribute to the positive health effects attributed to dairy product consumption. Several in vivo studies show protective effects against inflammation, cancers and metabolic disorders (Ran-Ressler et al., 2014; Taormina et al., 2020). In an animal model, BCFAs play a beneficial role against inflammation in the premature intestine, modulate the microbiota and increase the expression of anti-inflammatory cytokines (Taormina et al., 2020). To date, no data concerning the metabolic effects were reported in humans. However it was suggested that BCFAs may favourably influence insulin sensitivity, energy and glucose metabolism in human (Taormina et al., 2020). Taormina et al. recently published a comprehensive review on BCFA (Taormina et al., 2020).

6 The branched fatty acid esters of hydroxy fatty acids FAHFA

6.1 Structure and biosynthesis

FAHFA are fatty acid esters of hydroxy fatty acids. As multiple combinations of fatty acids (FA) and hydroxylated fatty acids (HFA) are possible, there are hundreds of FAHFA (Yore et al., 2014; Kuda et al., 2016). Almost 50 families of FAHFA have been identified, the esters of palmitic acid and hydroxy stearic acid (PAHSA) being the most studied. In each family of branched FAHFA, several positional isomers are possible, with more than 300 regiosomers identified, and for each isomer, there are also 2 possible configurations (Kuda et al., 2018).

Branched FAHFA are synthesized in vivo (Yore et al., 2014) or can be obtained exogenously from food. To date, only PAHSA biosynthesis pathway in adipocytes was elucidated, involving esterification of hydroxy fatty acids with acyl-CoA fatty acids by an acyltransferase (Kuda et al., 2018), and storage in adipocyte as FAHFA-containing triacylglycerols (Tan et al., 2019).

6.2 Food content and daily intake

Numerous branched FAHFA families have been detected in food of plant origin (fruits, vegetables and cereals) (Zhu et al., 2018; Liberati-Cizmek et al., 2019) and of animal origin (egg, chicken, beef, caribou, moose) (Yore et al., 2014; Pham et al., 2019). Abundance of each FAHFA varies according to the type of food considered. Total FAHFA range from 45 to 320 ng/g in fresh food. Branched FAHFA were also detected in caribou meat and moose at very high doses (50 µg/g).
compared to other food sources. Branched FAHFAs are present in breast milk, although at very low concentrations (Kuda et al., 2018).

To our knowledge, no data are available on the amount of FAHFAs ingested daily. Moreover, absorption and bioavailability of dietary FAHAs are unknown.

6.3 Tissue content and biological functions

FAHFAs are present in blood and in many tissues in rodent and humans. PAHSA content is around 100 ng/g in white adipose tissue, 150 ng/g in brown adipose tissue, and 10–20 ng in liver, kidney and pancreas (Yore et al., 2014). In lung, kidney, thymus, liver and heart FAHFAs content is rather in pg/g (Zhu et al., 2017). Short-chain FAHFAs are in a concentration range from 0.84 to 57 pmoles/mg in the large intestine (Gowda et al., 2020b, 2020c).

Only a few FAHFAs have been studied. They modulate favourably insulin sensitivity and glucose metabolism. In particular, 5- and 9-PAHSA have been reported to improve glucose metabolism and insulin signalling (Yore et al., 2014; Moraes-Vieira et al., 2016; Smith and Kahn, 2016; Syed et al., 2018). 9-PAHPA or 9-OAHPA increased insulin sensitivity, but without modifying glucose tolerance, and increased basal metabolism, both in healthy mice and in obese mice with lower insulin sensitivity (Benlebna et al., 2020a, 2020b). Moreover, 9-PAHPA or 9-OAHPA induced a switch toward a more oxidative contractile phenotype of skeletal muscle, suggesting a muscular origin of the increase in insulin sensitivity observed (Benlebna et al., 2020c). Surprisingly, 9-PAHPA or 9-OAHPA induced hepatic steatosis and fibrosis in some healthy mice but not in obese mice, likely because both FAHFAs had insulin-sensitized the healthy liver so much that de novo lipogenesis promoted steatosis/fibrosis (Benlebna et al., 2020a, 2020b). FAHFAs activate GPR120 and GPR40 and increase GLP-1 secretion (Yore et al., 2014; Hammarstedt et al., 2018; Kimura et al., 2020). The FAHFAs studied to date have anti-inflammatory effects, as demonstrated both in vitro and in vivo in chronic and acute inflammation models (Yore et al., 2014; Kuda et al., 2016; Lee et al., 2016; Kolar et al., 2019). At least some FAHFAs, notably from omega-3 fatty acid derived-FAHFAs family, may have antioxidant effects (Gowda et al., 2020a).

6.4 Health effects

Metabolic dysfunction in adipose tissue of healthy moderately overweight humans is associated with reduced levels of PAHSAs in the same tissue (Hammarstedt et al., 2012; Hammarstedt et al., 2018). In addition, serum PAHSAs levels are reduced in obese patients and in diabetics (Yore et al., 2014; Moraes-Vieira et al., 2016). Thus, beneficial effects of PAHSAs were suggested in human in various metabolic disorders such as type 1 and type 2 diabetes, and in chronic inflammation (Brejchova et al., 2020). Other beneficial health effects have been also suggested, in particular against some cancers (Rodriguez et al., 2019). It is important to note that these health effects of FAHFAs have been demonstrated with pharmacological doses in animal models or linked to circulating FAHFAs levels, but not associated to diet normal content. Several comprehensive reviews on branched FAHFAs have been recently published (Brejchova et al., 2020; Benlebna et al., 2021).

7 Conclusion

Human diet contains many uncommon fatty acids with bioactive properties such as n-3 DPA, natural trans fatty acids, CLAs, FuFAs, BCFAs and FAHFAs. Many of them may have favourable effects on health, in particular on prevention of cardiovascular diseases, inflammation and metabolic disorders such as diabetes. It is interesting to note that many of these lipids are found mainly in seafood and in dairy products. As bacteria are involved in the synthesis of some of these fatty acids, the role of intestinal microbiota in their metabolism in humans deserves to be explored. As not only food intake but also bioavailability is important to provide adequate nutrients status, and as bioavailability is still unknown for some of these uncommon fatty acids, this parameter needs to be investigated to better understand their health effects.

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Conflicts of interest. The authors declare that they have no conflicts of interest in relation to this article.

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