Concentrations of n-3 and n-6 fatty acids in Dutch bovine milk fat and their contribution to human dietary intake

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ABSTRACT

Weekly samples representative of Dutch milk were analyzed for concentrations of n-3 and n-6 fatty acids (FA). Concentrations of the n-3 FA α -linolenic acid (ALA), eicosatetraenoic acid, eicosapentaenoic acid, and docosapentaenoic acid were 0.495 ± 0.027 , 0.041 ± 0.004 , 0.067 ± 0.005 , and 0.086 ± 0.008 g per 100 g of fat, respectively, whereas docosahexaenoic acid was absent or present in concentrations lower than 0.020 g per 100 g of fat. Concentrations of the n-6 FA linoleic acid (LeA), γ -linoleic acid, dihomo- γ -linoleic acid, and arachidonic acid were 1.428 ± 0.068 , 0.070 ± 0.007 , 0.066 ± 0.004 , and 0.089 ± 0.004 g per 100 g of fat, respectively; adrenic acid was present in concentrations lower than 0.020 g per 100 g of fat, whereas docosapentaenoic acid was absent in all samples. The concentrations of ALA and LeA were significantly higher in spring and summer, compared with autumn and winter. The concentrations of all other ALA- and LeA-derived n-3 and n-6 FA were not significantly different between seasons. The contribution of milk fat to the daily intake of eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid was calculated for human consumption levels in different countries. Milk fat contributed between 10.7 and 14.1% to the daily intake of eicosapentaenoic acid and between 23.5 and 34.2% to the intake of docosapentaenoic acid; whereas docosahexaenoic acid contribution was marginal. Arachidonic acid from milk fat contributed between 10.5 and 18.8% to the human intake of n-6 FA.

Key words: n-3 fatty acid, n-6 fatty acid, milk fat, dietary intake

INTRODUCTION

Bovine milk fat mainly consists (~98%) of triglycerides, which contain a large variety of different FA. More

than 400 different FA have been identified in bovine milk but most of the FA are present in relative low concentrations (Jensen, 2002). Only 12 to 14 FA are present in concentrations higher than 1% of the milk fat (Walstra et al., 2006; Heck et al., 2009). Two of the minor long-chain FA present in milk, α -linolenic acid (ALA; 18:3n-3) and linoleic acid (LeA; 18:2n-6) cannot be synthesized by cows because mammals are missing the desaturases that are needed to introduce double bonds at the Δ 12 and Δ 15 positions of FA (Arterburn et al., 2006). Therefore, ALA and LeA must be obtained from plant materials in the diet (Ratnayake and Galli, 2009). Mammals convert ALA and LeA metabolically into 2 different series of very long chain n-3 and n-6 FA by a single set of desaturases and elongases (Sprecher, 2002). These FA are classified into the n-3 and n-6 families based on the position of the first double bond from the methyl end group in the FA chain.

α -Linolenic acid is the precursor of very long chain n-3 PUFA such as eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). Figure 1 shows the n-3 pathway (Sprecher, 2000); starting from ALA, the chain length increases and extra double bonds are inserted at the carboxyl end of the FA molecules by the action of elongases and desaturases, respectively. The n-6 FA linoleic acid is converted into arachidonic acid (ARA; 20:4n-6) and finally into docosapentaenoic acid (DPA n-6; 22:5n-6) by the same set of elongases and desaturases (Figure 1). The metabolic pathway for conversion of ALA into EPA by cows is supposed to be similar to the human pathway (Hagemeister et al., 1991). The genes *FADS1* and *FADS2*, encoding for Δ^5 - and Δ^6 -desaturase, respectively, are expressed in the bovine mammary gland (Bionaz and Loor, 2008).

Dietary intake of n-3 and n-6 FA determines in large part the composition of cell membranes (Burdge, 2006; Simopoulos, 2008). In addition, EPA and ARA are metabolized into a group of signaling molecules, which are collectively referred to as eicosanoids. These low-abundance metabolites, as well as DHA-derived docosanoids, have a broad range of biological activities (Ratnayake and Galli, 2009). Long-chain n-3 FA

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Figure 1. Metabolic pathways of n-3 and n-6 FA. Adapted with permission from Schmitz and Ecker (2008).

are substrates for production of less-inflammatory and, in some cases, antiinflammatory eicosanoids, whereas ARA is the precursor of proinflammatory and prothrombic eicosanoids (Calder, 2006).

Humans have the ability to metabolize ALA into EPA and DHA. The relative contribution of ALA for meeting the dietary demands of EPA and DHA is unknown (Bakewell et al., 2006) but this metabolic pathway does not provide adequate levels of EPA and DHA for optimal human health and nutrition (Anderson and Ma, 2009). To meet the recommended dietary intake, consumption of around 450 mg of EPA + DHA per day is recommended in most countries, especially for prevention of coronary heart disease (Kris-Etherton et al., 2009).

The main source for EPA and DHA in human diets is fatty fish. In the absence of fatty fish consumption, the intake of n-3 FA is less than 100 mg/d (Calder, 2006), which is far below the recommended daily intake. Milk

and dairy products are considered not to contribute significantly to dietary intake of n-3 FA (Astorg et al., 2004; Meyer et al., 2003; Sioen et al., 2006). However, in cow feeding trials, these FA are often reported to be present in milk from control diets but the low concentrations are considered as negligible for n-3 FA intake by humans (Lock and Bauman, 2004; Woods and Fearon, 2009; O'Donnell et al., 2010). Contrary to these results, a recent study suggested that milk fat may be a source for dietary intake of EPA, reporting that dairy products supply 10.7 to 11.3% of EPA in the diets of meat eaters and 18.4 to 26.3% of EPA in the diets of vegetarians (Welch et al., 2010).

In the present study, accurate concentrations of FA were obtained by analyses of composite samples that were representative of Dutch milk composition. These weekly samples also provide a reliable overview of the seasonal variation in milk fat composition (Heck et al., 2009). However, the study of Heck et al. (2009) did

not report the variation in concentrations of very long chain n-3 and n-6 FA. The objectives of the present study were to determine which FA belonging to the n-3 pathway or n-6 pathway can be identified and quantified in bovine milk fat to determine the variability of n-3 and n-6 FA throughout the year, and to calculate the contributions of EPA, DPA, DHA, and ARA to the dietary intake by humans.

MATERIALS AND METHODS

The samples used were bovine milk samples that are collected weekly by the Dutch milk control station (Qlip, Zutphen, the Netherlands) as a representative Dutch milk sample. Every week from March 2011 to February 2012, milk samples of 50 L each were collected from the bulk tanks (average size around 200,000 L) of 20 large dairy plants situated in different regions of the Netherlands. Samples were pooled and conserved with 0.03% sodium azide. Fifty-two subsamples of the pooled milk were stored at 4°C and analyzed for FA composition (samples of wk 22 and 23 have not been analyzed) within 1 wk after sample collection. The distribution of the weeks over the seasons is according to previous research (Heck et al., 2009): spring = wk 12 to 24, summer = wk 25 to 37, autumn = wk 38 to 50, and winter = wk 51 to 11.

Milk fat was extracted from the pooled milk samples and FA methyl esters were prepared from the fat fractions as described in International Organization for Standardization (ISO) Standard 15884:2002 (ISO, 2002a). Methyl esters were analyzed according to ISO Standard 15885:2002 (ISO, 2002b) on a Trace GC Ultra chromatograph (Thermo Electron Corp., Madison, WI), using a Varian Fame Select column (100 m × 0.25-mm i.d.; Varian Inc., Palo Alto, CA). The initial temperature was held at 70°C for 1 min, raised to 225°C at 3°C/min, and held at 225°C for 5 min. A volume of 1 µL was injected. Each peak was identified and quantified using pure methyl ester standard (Sigma-Aldrich; Larodan). The FA were expressed as a proportion of total fat weight and expressed as grams per 100 g of fat. The FA containing 24 C atoms could not be quantified because pure methyl esters of these FA are not available. Stearidonic acid (SDA; 18:4n-3) could not be identified because it co-eluted with *trans*-10,*cis*-11 C18:2.

Dietary intake of n-3 and n-6 PUFA provided by milk fat was calculated as follows:

$$\text{FA (\%)} = \frac{\text{milk fat consumption} \times [\text{FA}]}{\text{milk fat consumption} \times [\text{FA}] + \text{intake of FA from other food sources}}.$$

Milk fat consumption (g/d) and intake of each n-3 and n-6 FA (g/d) from nondairy food sources were derived from the literature (Meyer et al., 2003; Astorg et al., 2004; Sioen et al., 2006), whereas concentrations of n-3 and n-6 FA (g/100 g of fat) from milk fat were obtained from this study.

The n-3 and n-6 FA concentrations in different seasons were statistically evaluated by one-way ANOVA. Duncan's multiple range test was applied for detection of significant differences between mean FA concentrations.

RESULTS AND DISCUSSION

Average concentrations of ALA and LeA in milk fat were 0.495 ± 0.068 and 1.428 ± 0.068 g/100 g of fat (Table 1), which are within the ranges previously reported (Jensen, 2002). The LeA:ALA ratio of 2.88 falls within the recommended range, from 1:1 to 4:1, that is considered to be important for human homeostasis and normal development (Simopoulos, 2008). The concentrations of ALA and LeA throughout the year in the current study are close to those reported in the literature (Heck et al., 2009). In spring and summer, ALA and LeA concentrations were significantly higher than in autumn and winter. For cows, the main sources of ALA and LeA are grass and maize, respectively (Chilliard et al., 2001). In the outdoor season, grazing will increase the intake of ALA, whereas the intake of LeA from maize silage is lower compared with the indoor season (Chilliard et al., 2001; Heck et al., 2009). A large part of dietary ALA and LeA is biohydrogenated by rumen bacteria (Chilliard et al., 2007); ALA and LeA that escape rumen biohydrogenation will probably, as in other mammals, be β -oxidized (Cunnane et al., 2003), stored in body tissues, or incorporated in milk fat.

Linolenic acid and linoleic acid are metabolized to 2 distinct families of very long chain n-3 and n-6 FA. In contrast to ALA and LeA, concentrations of very long chain n-3 and n-6 FA in bovine milk fat are not reported in review articles. For validation of our results, concentrations of very long chain n-3 and n-6 FA were derived from control diets of cow feeding experiments in the past 10 yr. In general, control diets represent common diets without supplemental fat sources that may have affected milk fat content and composition. The number of cows in most of these trials was between 4 and 24, which implies a larger variability in results compared with our study. Literature values on concentrations of n-3 and n-6 FA in control diets of cows are summarized in Table 2 (n-3 FA) and in Table 3 (n-6 FA). Average concentrations of n-3 PUFA (Table 1) for Dutch milk fat are within the ranges obtained from the literature

Table 1. Fat contents and concentrations (mean $\pm$ SD) of n PUFA (g/100 g of fat) in Dutch bovine raw milk during the different seasons of 2011

Item	Season				
	Winter (n = 13)	Spring (n = 11)	Summer (n = 13)	Autumn (n = 13)	2011 Average (n = 50)
Fat content (%)	4.502 $\pm$ 0.060	4.364 $\pm$ 0.116	4.209 $\pm$ 0.020	4.359 $\pm$ 0.095	4.358 $\pm$ 0.131
n-3 PUFA					
α -Linolenic acid (ALA)	0.479 $\pm$ 0.012 ^a	0.513 $\pm$ 0.035 ^b	0.511 $\pm$ 0.023 ^b	0.481 $\pm$ 0.017 ^a	0.495 $\pm$ 0.027
Stearidonic acid (SDA)	0.000	0.000	0.000	0.000	0.000
Eicosatetraenoic acid (ETA)	0.040 $\pm$ 0.005	0.041 $\pm$ 0.003	0.041 $\pm$ 0.004	0.042 $\pm$ 0.006	0.041 $\pm$ 0.004
Eicosapentaenoic acid (EPA)	0.065 $\pm$ 0.004	0.068 $\pm$ 0.008	0.069 $\pm$ 0.003	0.069 $\pm$ 0.004	0.067 $\pm$ 0.005
Docosapentaenoic acid (DPA)	0.088 $\pm$ 0.013	0.085 $\pm$ 0.005	0.084 $\pm$ 0.004	0.086 $\pm$ 0.005	0.086 $\pm$ 0.008
Tetracosapentaenoic acid (TPA)	ND ¹	ND	ND	ND	ND
Tetracosahexaenoic acid (THA)	ND	ND	ND	ND	ND
Docosahexaenoic acid (DHA)	<0.02	<0.02	<0.02	<0.02	<0.02
n-6 PUFA					
Linoleic acid (LeA)	1.391 $\pm$ 0.023 ^a	1.453 $\pm$ 0.055 ^b	1.465 $\pm$ 0.037 ^b	1.406 $\pm$ 0.103 ^a	1.428 $\pm$ 0.068
γ -Linoleic acid (GLA)	0.066 $\pm$ 0.010	0.073 $\pm$ 0.005	0.071 $\pm$ 0.006	0.070 $\pm$ 0.006	0.070 $\pm$ 0.007
Dihomo- γ -linoleic acid (DGLA)	0.068 $\pm$ 0.003	0.065 $\pm$ 0.003	0.065 $\pm$ 0.005	0.065 $\pm$ 0.006	0.066 $\pm$ 0.004
Arachidonic acid (ARA)	0.088 $\pm$ 0.003	0.091 $\pm$ 0.003	0.088 $\pm$ 0.005	0.088 $\pm$ 0.004	0.089 $\pm$ 0.004
Adrenic acid (AdA)	<0.02	<0.02	<0.02	<0.02	<0.02
Tetracosatetraenoic acid (TSA)	ND	ND	ND	ND	ND
Tetracosapentaenoic acid (TPA)	ND	ND	ND	ND	ND
Docosapentaenoic acid (DPA)	0.000	0.000	0.000	0.000	0.000

^{a,b}Means within a row with no common superscript differ significantly ($P < 0.01$) by Duncan's multiple range test.

¹ND = not determined.

(Table 2). In line with the results of average Dutch bovine milk (Table 1), the EPA concentration in milk fat with control diets in various experiments reported in the literature is much higher (0.058 g/100 g of fat) than that of DHA (0.018 g/100 g of fat). However, in control diets of the feeding trials, concentrations higher than 0.04 g of DHA per 100 g of fat were obtained. This might be caused by the use of 60-m GC columns instead of the 100-m columns that are nowadays commonly in use. With 60-m columns, co-elution of DHA and DPA n-3 may occur; subsequently, DPA n-3 may be incorrectly assigned as DHA when no standard of the latter has been used for peak identification. Without the results obtained by short GC columns, DHA is absent or present in low concentrations (<0.04 g/100 g of fat) in samples of individual cows of the control diets.

For n-6 PUFA (Table 1) our results for dihomom- γ -linoleic acid (DGLA) and ARA average concentrations are lower than those reported in the literature (Table 3). Although ALA and LeA concentrations were significantly higher in spring and summer, the average concentrations of ALA- and LeA-derived PUFA were not significantly different between the seasons (Table 1). The low conversion rates of ALA and LeA and the small differences in concentrations between seasons may result in small, undetectable, changes in levels of very long-chain PUFA.

In the first step of the n-3 and n-6 pathways (Figure 1), Δ^6 -desaturase converts ALA into SDA (18:4n-3) and

LeA into γ -linoleic acid (**GLA**; 18:3n-6). In milk fat, SDA was absent or present in low concentrations (<0.02 g/100 g of fat), whereas the average GLA concentration was 0.070 g/100 g of fat. The low concentrations, or even absence, of SDA is remarkable because the affinity of Δ^6 -desaturase is higher for n-3 than for n-6 FA (Sprecher, 2002). Desaturation of ALA into SDA is, therefore, expected to occur at a higher rate than the conversion of the n-6 fatty acid LeA into GLA. Product inhibition of Δ^6 -desaturase by high levels of LeA (Emken et al., 1994) is unlikely to occur due to the low LeA:ALA ratio (about 3; see Table 1). The low concentrations of SDA may be caused by rapid metabolism of SDA into eicosatetraenoic acid (**ETA**; 20:4n-3). The rapid conversion of SDA without significant accumulation of ETA has been reported to occur in human blood lipids and mononuclear cells (Miles et al., 2004).

Table 1 shows that the average concentration of ETA was 0.041 $\pm$ 0.004 mg/100 g of fat, which is relatively low compared with average EPA and DPA n-3 (22:5n-3) concentrations of 0.067 $\pm$ 0.005 and 0.086 $\pm$ 0.008 g/100 g of fat, respectively. Desaturation of ETA causes the formation of EPA, which is subsequently elongated to form DPA n-3 (Figure 1). In humans, the main products formed out of ALA are EPA and DPA n-3; the latter may have beneficial biological effects (Kaur et al., 2011) and may also serve as a substrate for metabolic retro-conversion to EPA (Russo, 2009). However, the absorption levels of DPA n-3 and the conversion rate to EPA have never been reported. In the generally accepted n-3

Table 2. Concentrations of n-3 PUFA in milk fat obtained from control diets of feeding trials reported in the literature

n-3 PUFA	Average concentration (g/100 g of fat)	Range (g/100 g of total FA)	Number of trials	References
α -Linolenic acid (ALA)	0.48	0.05–0.99	29	AbuGhazaleh et al. (2002); Abu-Ghazaleh et al. (2002); Allred et al., 2006; Amorcho et al. (2009); Baer et al. (2001); Bernal-Santos et al. (2010); Boeckaert et al. (2008); Caroprese et al. (2010); C��rtes et al. (2010); Couvreur et al. (2006); Jacobs et al. (2011); Jones et al. (2005); Kliem et al. (2008); Kupczyński et al. (2011); La Terra et al. (2010); Lacasse et al. (2002); Leiber et al. (2005); Loor et al. (2005); Moallem (2009); Nelson and Martini (2009); Palmquist and Grinari (2006); Rego et al. (2005, 2009); Shingfield et al. (2003, 2006); Sterk et al. (2011, 2012); Whitlock et al. (2006); Zachut et al. (2010)
Stearidonic acid (SDA)	0.03	0.00–0.09	5	Bernal-Santos et al. (2010); Jones et al. (2005); Moallem (2009); Shingfield et al. (2006); Zachut et al. (2010)
Eicosatetraenoic acid (ETA)	0.06	0.01–0.12	6	Bernal-Santos et al. (2010); C��rtes et al. (2010); Jones et al. (2005); Nelson and Martini (2009); Shingfield et al. (2003, 2006)
Eicosapentaenoic acid (EPA)	0.05	0.00–0.12	24	AbuGhazaleh et al. (2002); Abu-Ghazaleh et al. (2002); Allred et al. (2006); Amorcho et al. (2009); Baer et al. (2001); Bernal-Santos et al. (2010); Caroprese et al. (2010); Jacobs et al. (2011); Jones et al. (2005); Kliem et al. (2008); Kupczyński et al. (2011); La Terra et al. (2010); Lacasse et al. (2002); Leiber et al. (2005); Loor et al. (2005); Moallem (2009); Nelson and Martini (2009); Palmquist and Grinari (2006); Rego et al. (2005, 2009); Shingfield et al. (2003, 2006); Whitlock et al. (2006); Zachut et al. (2010)
Docosapentaenoic acid (DPA n-3)	0.07	0.00–0.15	15	Baer et al. (2001); Bernal-Santos et al. (2010); Couvreur et al. (2006); Jacobs et al. (2011); Jones et al. (2005); Kliem et al. (2008); Lacasse et al. (2002); Leiber et al. (2005); Loor et al. (2005); Moallem (2009); Shingfield et al. (2003, 2006); Zachut et al. (2010)
Docosahexaenoic acid (DHA)	0.02	0.00–0.08	20	AbuGhazaleh et al. (2002); Abu-Ghazaleh et al. (2002); Allred et al. (2006); Amorcho et al. (2009); Baer et al. (2001); Bernal-Santos et al. (2010); Caroprese et al. (2010); Casta��eda-Guti��rrez et al. (2007); Jacobs et al. (2011); Jones et al. (2005); Kupczyński et al. (2011); La Terra et al. (2010); Lacasse et al. (2002); Leiber et al. (2005); Loor et al. (2005); Nelson and Martini (2009); Palmquist and Grinari (2006); Shingfield et al. (2003, 2006); Whitlock et al. (2006)

Table 3. Concentrations of n-6 PUFA in milk fat obtained from control diets reported in the literature

n-6 PUFA	Average concentration (g/100 g of fat)	Range (g/100 g of total FA)	Number of trials	References
Linoleic acid (LA)	2.50	1.12–4.28	25	Abu-Ghazaleh et al. (2002); Amorcho et al. (2009); Baer et al. (2001); Bernal-Santos et al. (2010); Boeckaert et al. (2008); Cortés et al. (2010); Couvreur et al. (2006); Jacobs et al. (2011); Jones et al. (2005); Kupczyński et al. (2011); La Terra et al. (2010); Lacasse et al. (2002); Leiber et al. (2005); Loo et al. (2005); Moallem (2009); Nelson and Martini (2009); Palmquist and Grinari (2006); Rego et al. (2005, 2009); Shingfield et al. (2003, 2006); Sterk et al. (2011, 2012); Whitlock et al. (2006); Zachut et al. (2010)
γ -Linoleic acid (GLA)	0.06	0.02–0.18	16	AbuGhazaleh et al. (2002); Allred et al. (2006); Baer et al. (2001); Bernal-Santos et al. (2010); Cortés et al. (2010); Jacobs et al. (2011); Jones et al. (2005); Kupczyński et al. (2011); La Terra et al. (2010); Lacasse et al. (2002); Leiber et al. (2005); Nelson and Martini (2009); Shingfield et al. (2006); Whitlock et al. (2006); Zachut et al. (2010)
Dihomo- γ -linoleic acid (DGLA)	0.11	0.07–0.18	15	AbuGhazaleh et al. (2002); Allred et al. (2006); Caroprese et al. (2010); Cortés et al. (2010); Couvreur et al. (2006); Jacobs et al. (2011); Jones et al. (2005); Kliem et al. (2008); Leiber et al. (2005); Loo et al. (2005); Moallem (2009); Nelson and Martini (2009); Rego et al. (2009); Shingfield et al. (2006); Zachut et al. (2010)
Arachidonic acid (ARA)	0.11	0.00–0.22	17	Bernal-Santos et al. (2010); Caroprese et al. (2010); Cortés et al. (2010); Couvreur et al. (2006); Jacobs et al. (2011); Jones et al. (2005); Kliem et al. (2008); Kupczyński et al. (2011); La Terra et al. (2010); Loo et al. (2005); Moallem (2009); Palmquist and Grinari (2006); Rego et al. (2009); Shingfield et al. (2003, 2006); Sterk et al. (2012); Zachut et al. (2010)
Adrenic acid (AdA)	0.02	0.00–0.03	5	Jacobs et al. (2011); Kliem et al. (2008); Shingfield et al. (2003, 2006); Zachut et al. (2010)
Docosapentaenoic acid (DPA n-6)	0.02	0.00–0.08	5	Couvreur et al. (2006); Jones et al. (2005); Shingfield et al. (2003, 2006); Sterk et al. (2012)

pathway (Figure 1), DPA n-3 is further converted into tetracosapentaenoic acid (TPA; 24:5n-3), tetracosahexaenoic acid (THA; 24:6n-3), and finally DHA.

The conversion of DPA n-3 is the rate-limiting step for the conversion of ALA to DHA (Arterburn et al., 2006). Unfortunately, the intermediates tetracosapentaenoic and tetracosahexaenoic acid could not be determined in our study, which makes it impossible to determine the actual rate-limiting step. Concentrations of DHA were lower than 0.020 g per 100 g of fat in all samples, which is in agreement with the low conversion rates from ALA (<0.1%) that are frequently reported in the literature (Emken et al., 1994; Goyens et al., 2006; Williams and Burdge, 2006).

Our study confirmed that milk fat is not an important source for DHA intake, but the average EPA concentration of 0.067 ± 0.005 g per 100 g of milk fat (Table 1) implies that milk fat does contribute to human intake of this n-3 FA. Studies on n-3 and n-6 PUFA intake from different food sources report milk fat intakes of 13.7 (Meyer et al., 2003), 15.3 (Sioen et al., 2006), and 26.7 (Astorg et al., 2004) g/d. The intakes of EPA from milk fat are 14.1 (Meyer et al., 2003), 11.6 (Sioen et al., 2006), and 10.7% (Astorg et al., 2004). Based on these daily milk fat consumptions (Table 4), and the average concentration of 0.067 g of EPA per 100 g of fat (Table 1), it may be concluded that a substantial part of the EPA intake by humans comes from milk fat. However, the recommend levels of total EPA and DHA cannot be met by increased milk fat consumption because DHA is absent in milk and the intake of milk fat would have to be at an unrealistically high level.

The intakes of DPA n-3, based on the results of this study, are between 23.5 and 34.2% (Table 4). Therefore, milk is an important source of DPA n-3, together with meat, poultry, and eggs (Astorg et al., 2004). To our knowledge the contribution of different food sources to dietary intake of ETA has never been reported; therefore, ETA was excluded from Table 4. In addition, the contributions of ETA and DPA n-3 to dietary intake must be considered as being unimportant because n-3 fatty acid intake recommendations only consider EPA and DHA. The contribution of ARA to the daily intake was found to be higher than the data from the literature (Table 4).

Milk fat intakes are underestimated because not only dairy products contribute to intake but also milk fat as an ingredient in food products such as chocolate, cookies, puff pastry, and ice cream are sources of milk fat in the human diet. Therefore, the contribution of milk fat to the intake of n-3 and n-6 PUFA may be higher than the percentages in Table 4.

In the present analyses, we used the average FA content of Dutch raw bovine milk. Milk FA compo-

Table 4. Contribution (%) of milk fat to dietary intake of very long chain n-3 and n-6 PUFA¹

Item	Reference		
	Meyer et al. (2003)	Sioen et al. (2006)	Astorg et al. (2004)
Milk fat intake (g/d)	13.7	15.3	26.7
n-3 PUFA			
α -Linolenic acid (ALA)	6.1 (11.4)	5.3 (6.6)	15.7 (24.64)
Eicosatetraenoic acid (EPA)	14.1 (0)	11.6 (0)	10.7 (0)
Docosapentaenoic acid (DPA n-3)	31.2 (0)	34.2 (0)	23.5 (0)
Docosahexaenoic acid (DHA)	0.0 (0)	0.0 (0)	0.0 (0)
n-6 PUFA			
Linoleic acid (LeA)	1.8 (3.18)	1.8 (1.9)	3.6 (5.05)
Dihomo- γ -linoleic acid (DGLA)	19.0 (0)	—	—
Arachidonic acid (ARA)	18.8 (0.12)	19.7 (5.9)	10.5 (1.06)

¹Values in parentheses = contribution of milk fat reported by Meyer et al. (2003), Sioen et al. (2006), or Astorg et al. (2004).

sition is not constant and feeding trials have shown that concentrations of very long chain PUFA may increase significantly due to variation in the diets of dairy cows. For example, the EPA concentration may be increased from 0.06 to 0.10 g per 100 g of fat upon supplementation of the dairy cow diet with linseed or linseed oil (Zachut et al., 2010; Sterk et al., 2012); DHA concentration (determined using a 100-m column) may be at least 1 g/100 g of milk FA when microalgae are included in the cow diet (Boeckaert et al., 2008). In these situations, the contribution of milk fat to daily intake of EPA + DHA may well exceed 50%. Together with the contribution of other food sources mentioned in the literature (Meyer et al., 2003; Astorg et al., 2004; Sioen et al., 2006), consumption of milk fat would cover the recommended value of 450 mg/d for EPA + DHA intake.

The daily intakes of n-3 and n-6 PUFA (Table 4) are based on data of raw milk and not on dairy products. Limited information is available on the stability of native n-3 and n-6 PUFA during processing of raw milk into dairy products and their stability during storage. Human milk FA composition, including PUFA of both the n-3 and n-6 series is not changed after pasteurization, whereas sterilization decreases ARA slightly (Fidler et al., 2001). Fatty acid composition including ALA, LeA, EPA, DPA n-3, and DHA, of pasteurized milk, cream, and butter did not differ from the raw milk used to manufacture these dairy products (Baer et al., 2001).

CONCLUSIONS

We conclude that EPA is present in milk fat at a constant level throughout the year and it contributes around 10 to 15% to the daily intake by humans, whereas the contribution of DHA is marginal. Other very long chain n-3 FA that could be quantified in fat from bulk milk are ETA and DPA n-3; however, no

recommended daily intakes exist for these n-3 FA. We recommend an update of the current food composition tables after analyses of milk fat composition by the appropriate analytical methods.

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