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Review

Conjugated linoleic acids in milk fat: Variation and physiological effects

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Abstract

Much attention has been directed toward conjugated linoleic acid (CLA) since the discovery of its anticarcinogenic properties two decades ago. Many other biological activities have been reported over the past few years confirming that individual CLA isomers present in milk fat have a high health promoting potential. Its possible use in functional dairy products explains the increasing interest of the food industry in CLA research. Recent advances in the analytical methodology offer new possibilities to study the individual effects of the various isomers in biological systems. The aim of this review is to summarize the current knowledge in CLA research including the formation of CLA in cows, analysis of CLA isomers, factors influencing the CLA content in milk, processing of CLA-enriched milk and dairy products, as well as aspects concerning nutrition and health.

Keywords: CLA; Milk fat; Variation; Formation

Contents

1. Introduction

In 1979, Pariza, Ashoor, Chu, and Lund (1979) reported that grilled ground beef contained both bacterial mutagens and a substance that inhibited mutagenesis. The finding of

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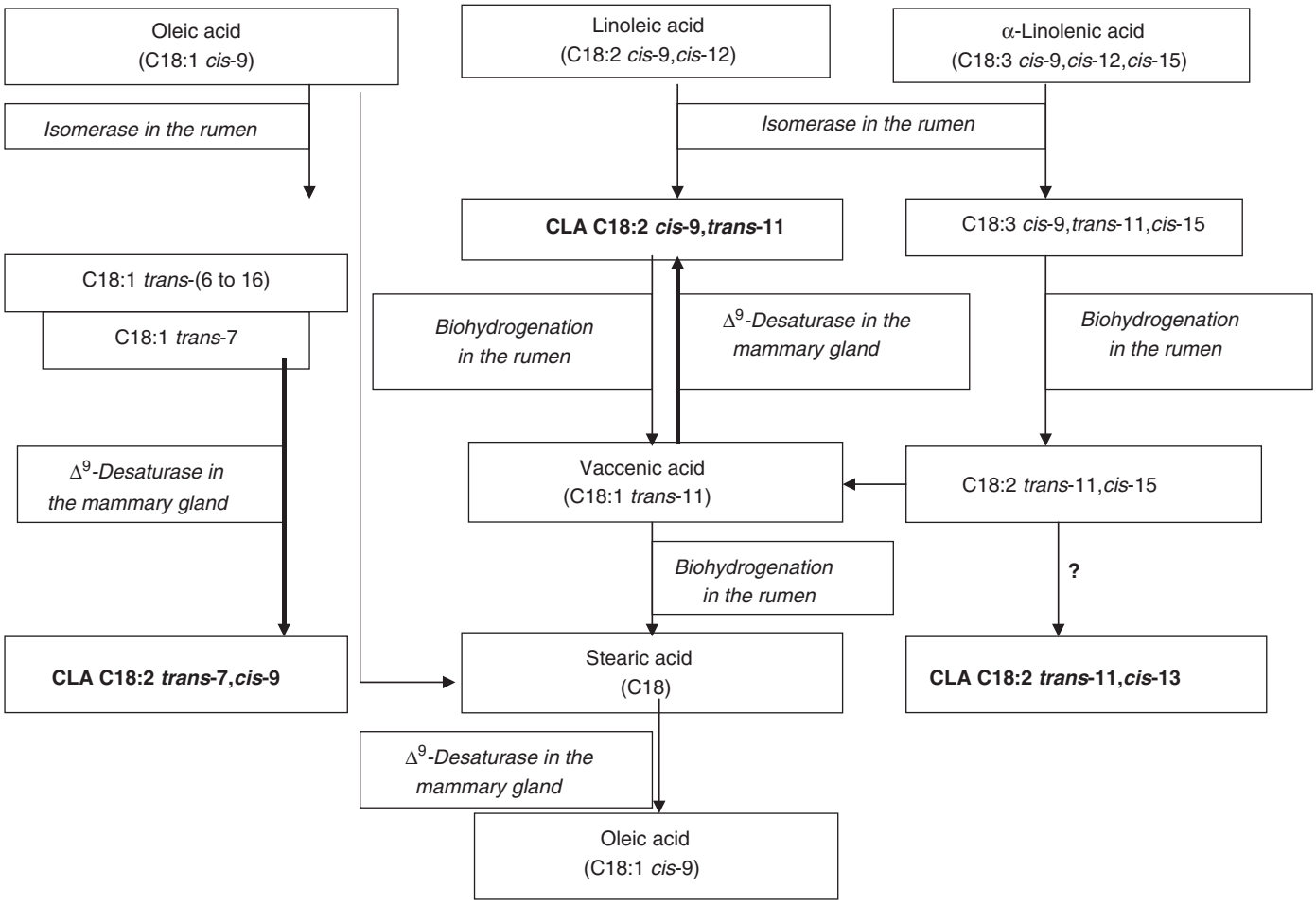

Fig. 1. Known metabolic pathways for the formation of CLA isomers.

mutagens in grilled beef was confirmatory, but evidence of a mutagenesis inhibitor was a novel discovery that had not been previously reported. That study concluded with a speculative prediction: "…it may also be found that the mutagenic inhibitory activity inhibits carcinogenesis". Subsequently, this speculation was indeed the case (Pariza & Hargraves, 1985) and the new anticarcinogen was identified as conjugated linoleic acid (CLA) (Ha, Grimm, & Pariza, 1987). The term CLA consists of a collection of positional and geometrical isomers of octadecadienoic acid, with conjugated double bonds ranging from 6,8 to 12,14. For every positional isomer, four geometric pairs of isomers are possible (i.e., *cis*,*trans*; *trans*,*cis*; *cis*,*cis*; and *trans*,*trans*). The term CLA therefore includes a total of 28 positional and geometrical isomers.

2. Metabolism in the cow

The presence of CLA in milk fat from ruminants relates to the isomerization and biohydrogenation of unsaturated fatty acids (FAs) by rumen bacteria as well as the Δ^9–desaturase activity in the mammary gland (Fig. 1). The *cis*-9,*trans*-11 CLA comprises 75–90% of total CLA and is derived from linoleic acid and α-linolenic acid (Bauman, Corl, & Peterson, 2003). Linoleic acid (*cis*-9,*cis*-12 18:2) is first isomerized to the CLA *cis*-9,*trans*-11 by *cis*-12,*trans*-11 isomerase and then hydrogenated by *Butyrivibrio fibrisolvens* to vaccenic acid (VA, *trans*-11 18:1) in the rumen (Kepler & Tove, 1967). These initial steps occur rapidly. A strong positive correlation between the *trans* isomers of 18:1 (VA, *trans*-13–14, *trans*-15, and *trans*-16) in milk fat and the level of linoleic acid in the diet was first found by Loor, Bandara, and Herbein (2002). The hydrogenation of VA to stearic acid appears to involve a different group of organisms and occurs at a slow rate (Griinari, Chouinard, & Bauman, 1997; Harfoot & Hazelwood, 1997). For this reason, VA typically accumulates in the rumen. This main *trans* FA is responsible for the formation of the CLA isomer *cis*-9,*trans*-11, which occurs by desaturation (Δ^9-desaturase) of the ruminally derived VA in the mammary gland (Griinari et al., 2000; Piperova et al., 2002). The pathway for the formation of the CLA *cis*-9,*trans*-11 from α-linolenic acid (*cis*-9,*cis*-12,*cis*-15 18:3) in the rumen involves an initial isomerization to a conjugated triene (*cis*-9,*trans*-11,*cis*-15 18:3), followed by reduction of double bonds at carbons 9, 15, and 11 to yield the *trans*-11,*cis*-15 18:2, *trans*-11 18:1, and 18:0 FA, respectively, but not *cis*-9,*trans*-11 CLA, as intermediates

(Wilde & Dawson, 1966). Kraft, Collomb, Möckel, Sieber, and Jahreis (2003) hypothesized that α-linolenic acid is the indirect precursor of another CLA (*trans*-11,*cis*-13). The pathway from *trans*-11,*cis*-15 FA to the *trans*-11,*cis*-13 CLA isomer is as yet unclear.

In the rumen oleic acid from fodder is either not hydrogenated (Morris, 1970), is isomerized to *trans* 18:1 FAs with double bonds at positions 6–16 of the carbon chain, or is hydrogenated directly to stearic acid (Mosley, Powell, Riley, & Jenkins, 2002). Through the use of two different inhibitors of Δ^9-desaturase, Corl et al. (2002) demonstrated that the *trans*-7,*cis*-9 CLA in milk fat originated almost exclusively *via* endogenous synthesis by Δ^9-desaturase with ruminally derived *trans*-7 FAs; consistent with this result, the CLA isomer *trans*-7,*cis*-9 was not present in the ruminal fluid and was present in only small quantities in the duodenal flow (Piperova et al., 2002).

Other individual CLA isomers found in ruminant fat make up a very small portion of total CLA and are derived from rumen output. These isomers (>12) are at a low concentration when present, generally representing <0.5% of the total CLA in ruminant fat (Bauman et al., 2003).

3. Analysis of the CLA isomers

In analyzing CLA, it is important to separate and quantify the geometrical and positional isomers, avoiding additional isomerization during any derivatization steps. There is now a substantial body of work to confirm that acid-catalyzed transesterification can cause general isomerization with an increase in the relative proportions of *trans*,*trans* CLA isomers, and other unwanted side reactions (Christie, Sébédio, & Juanéda, 2001; Shantha, Decker, & Hennig, 1993; Yurawecz, Kramer, & Ku, 1999). Using sodium methoxide, or potassium hydroxide in methanol, glycerolipids of milk fat are rapidly transesterified without isomerization. Free CLA must be methylated by acid catalyzed procedures. Mild boron trifluoride–methanol or sulfuric acid (1%–methanol reagents) can be used provided that scrupulous attention is paid to detail (Christie et al., 2001); in particular, freshly prepared reagent and the minimum reaction time are essential. Yurawecz et al. (1999) reviewed techniques which have been developed to minimize negative effects of using acid methylation.

For the optimum resolution of CLA isomers by GC, long columns (100–120 m) are required (Dobson, 2003). GC using a 100 m CP Sil 88 column, for example, clearly resolves five distinguishing peaks in the CLA region of the chromatogram of milk fat: (*cis*-9,*trans*-11- + *trans*-8,*cis*-10- + *trans*-7,*cis*-9-CLA), (*trans*-11,*cis*-13- + *cis*-9,*cis*-11-CLA), *trans*-10,*cis*-12 CLA, *trans*-11,*trans*-13-CLA, and *trans*-9,*cis*-11-CLA (Kramer et al., 2004). The important *cis*,*trans*-isomers usually elute in a region of the chromatogram that is free of other FAs. On the other hand, naturally occurring 21:0 or 20:2 FAs elute in the same part of the chromatogram as the *cis*,*cis*- and the *trans*,*trans*-isomers (Roach et al., 2000). In such cases GC-MS with selected ion monitoring may be useful to identify non-conjugated FAs that co-elute with those of interest. Unequivocal identification of specific CLA isomers presents more of a problem. GC-MS is one of the first techniques that should be considered, and applications to CLA have been reviewed elsewhere (Dobson, 2003; Roach, 1999, 2001). With appropriate derivatives, it is possible to identify and locate the positions of the double bonds in CLA (but not their *cis*,*trans* configuration). For example, the CLA adducts of the 4-methyl-1,2,4-triazoline-3,5-dione (MTAD) have excellent mass spectrometric properties that enable location of the conjugated double bonds (Dobson, 1998). Gas chromatography linked to Fourier transform infrared spectrometry (GC-FTIR) is a powerful technique for determining the *cis*,*trans* configuration of double bonds in FAs (Mossoba et al., 1999). In the context of CLA, it

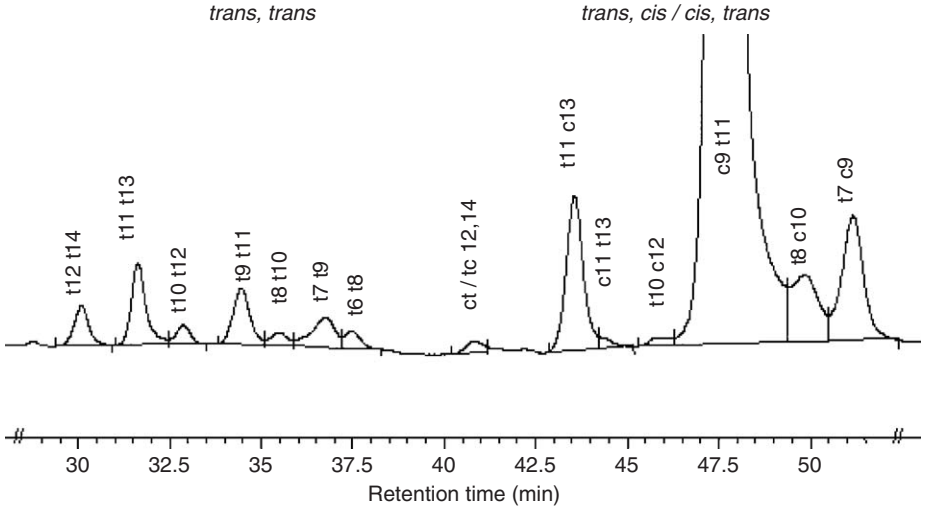

Fig. 2. Silver-ion-HPLC (Ag^+-HPLC) separation of CLA methyl esters of alpine cows' milk fat using three columns in series (Collomb, Sieber, et al., 2004). CLA isomer *trans*-6,*trans*-8 tentatively assigned according to Rickert et al. (1999).

enables the identification of *cis,trans*-, *cis,cis*- and *trans,trans*-isomers. Although it does not distinguish between *cis,trans*- and *trans,cis*-isomers, assignments can be made with reasonable certainty when GC retention data are taken into account. Nevertheless, the use of all these methods is time-consuming and laborious. Chemical ionization tandem mass spectrometry (CIMS-MS) is considered to be a more rapid technique. Michaud et al. (2003) reported a convenient mass spectrometry-based strategy to establish double bond geometry by analysis of collisional dissociation products of *cis*,*trans* and *trans*,*cis* CLAs, as methyl esters, and to distinguish CLAs from homoallylic (methylene interrupted) FA in a single-stage mass spectrum.

In addition, silver-ion high-performance liquid chromatography (Ag-HPLC) can provide separations of CLA not attainable by other means (Adlof, 2003; Kramer et al., 1999). Columns packed with 5–10 μm Nucleosil SA^{TM} (phenylsulfonic acid group bonded to a silica substrate in which the sulfonic acid protons have been exchanged with silver ions) are mostly used. A mobile phase of hexane containing a small amount of acetonitrile to separate the methyl ester derivatives, using the UV absorbance of the conjugated double bonds (234 nm) for detection and quantification constitutes the most used technique. *Trans*, *trans*-isomers elute first, followed by *cis*,*trans*/*trans*,*cis* then *cis*,*cis* (Delmonte, Kataoka, Corl, Bauman, & Yurawecz, 2005). As an example, Fig. 2 illustrates a separation of the most important CLA isomers (*trans*,*trans*; *cis*,*trans*/*trans*, *cis*) of a milk fat (Collomb, Sieber, & Bütikofer, 2004).

To obtain reproducible results between Ag-HPLC systems, potential sources of errors should be addressed. These include (i) batch-to-batch variations in silver loadings of the lipids columns, (ii) differences in instrument configuration (number of solvent pumps, mixing chambers, valves), (iii) changes in elution volumes and elution orders with sample size, solvent composition and even storage times, (iv) lack of internal standards and (v) control of column temperature (Adlof, 2003; Kramer et al., 1999, 2004).

4. Variation of CLA content in milk fat

Cows' milk fat is the richest natural common source of CLA. Levels ranging from 2 to 37 mg g^{-1} fat have been recorded (Parodi, 1999; Stanton, Murphy, McGrath, & Devery, 2003) and recently *cis*-9,*trans*-11 CLA contents of 53.7 mg g^{-1} of FA (Shingfield et al., 2006) and 51.5 mg g^{-1} of total FA (Bell, Griinari, & Kennelly, 2006) were reported (Table 1). This large range in CLA values can be attributed to a number of factors. Diet is the most significant factor affecting the CLA content of milk fat. High values often occur with the feeding of fresh pasture (Chilliard, Ferlay, & Doreau, 2001; Chilliard, Ferlay, Mansbridge, & Doreau, 2000; Collomb, Bütikofer, Sieber, Bosset, & Jeangros, 2001; Collomb et al., 2002a; Dhiman et al., 1999; Lock & Bauman, 2004; Stanton et al., 2003). However, much higher CLA levels were found when suitable total mixed rations (TMRs) including safflower or fish oil were fed or when monensin, an antibiotic food additive, was used in combination with such TMR (Bell et al., 2006; Lynch et al., 2005; Shingfield et al., 2006). Breed (Kelsey, Corl, Collier, & Bauman, 2003; Lawless et al., 1999) and lactation number or age (Stanton et al., 1997) can have a small influence on CLA levels. However, cows can exhibit large individual variation in CLA levels (Kelly, Berry, et al., 1998; Kelsey et al., 2003; MacGibbon, van der Does, Fong, Robinson, & Thomson, 2001; Peterson, Kelsey, & Bauman, 2002). Up to a three-fold individual variation in the CLA content of milk was obtained although all animals were fed the same diet and milk was sampled the same day (Kelsey et al., 2003). When a series of two different dietary treatments was applied to a group of cows, the individual animals maintained the same hierarchy in terms of milk fat CLA content (Peterson et al., 2002). Tissue Δ^9-desaturase activity, and the viability of certain rumen microflora responsible for aspects of isomerization and biohydrogenation may be contributing factors (Bauman et al., 2003; Parodi, 2003), whereas production variables such as days in milk, milk yield, milk fat content, and milk fat yield had little or no effect on the CLA content of milk (Kelsey et al., 2003; Lock, Bauman, & Garnsworthy, 2005). Seasonal effects on milk CLA content have also been reported, with the trend that content is greatest when fresh pasture is plentiful, and decreases throughout the growing season (Banni et al., 1996; Jahreis, Fritsche, & Steinhart, 1997; Lock & Garnsworthy, 2003; Riel, 1963). The reason for this observation remains unclear (Lock & Garnsworthy, 2003).

4.1. Pasture feeding

A number of studies have confirmed that pasture feeding can increase in the short term milk fat CLA concentrations in lactating dairy cows when changed from indoor winter feeding and that milk fat CLA content increases with increasing proportions of pasture in the diet (Dhiman, Arnand, Satter, & Pariza, 1999; Kelly, Kolver, Bauman, van Amburgh, & Muller, 1998; Stanton et al., 1997) (Table 1). The CLA-enriching effect of pasture has been attributed to the effects on biohydrogenation and the provision of α-linolenic acid as a lipid substrate for the formation of VA in the rumen and its subsequent desaturation to *cis*-9,*trans*-11 CLA in the mammary gland (Bauman et al., 2003). Cows receiving all of their daily feed as pasture produced higher milk fat CLA content (22.1 mg g^{-1} fat) than cows receiving only one-third (8.9 mg g^{-1} fat) or two-thirds (14.3 mg g^{-1} fat) of their daily diet as pasture (Dhiman, Arnand, et al., 1999). The pasture consumed in this study consisted of *Poa pratensis*, *Elytrigia repens*, *Bromus inermis* and *Trifolium repens*. The remainder of the diet consisted of a supplement containing alfalfa hay, corn, and roasted soybean.

Table 1
Examples of studies on dietary manipulation of milk CLA concentrations

Dietary treatment	CLA content		CLA[a]	Reference
	mg g^{-1}	as		
Pasture				
33% pasture	8.9	FAME[b]	c9	Dhiman, Arnand, Satter, & Pariza (1999)
66% pasture	14.3			
100% pasture	22.1			
100% pasture, lowlands	8.7	Fat	c9	Collomb, Bütkofer, Sieber, Jeangros, & Bosset (2002a)
100% pasture, mountains	16.1			
100% pasture, highlands	23.6			
Plant oils				
Control (51% forage, 49% grain)	3.9	FAME	c9	Dhiman et al. (2000)
3.6% soybean oil	21.0			
2.2% linseed oil	15.8			
4.4% linseed oil	16.3			
Control (55% forage, 45% grain)	5.09	FAME	c9	Dhiman et al. (2000)
0.5% soybean oil	7.5			
1.0% soybean oil	7.6			
2.0% soybean oil	14.5			
4.0% soybean oil	20.8			
1.0% linseed oil	7.3			
Control	4.0/3.3	FAME	Σ/c9	AbuGhazaleh, Schingoethe, Hippen, & Whitlock (2002)
2.5% soybean oil	9.1/7.9			
Low (35:65 concentrate:forage)	8.2/6.2	FAME	Σ/c9	Loor, Ferlay, Ollier, and Chilliard (2005)
Low with linseed oil (3% DM)	18.2/13.4			
High (65:35 concentrate:forage)	10.4/8.1			
High with linseed (3% DM)	30.0/25.4			
Peanut oil	13.3	Fat	c9	Kelly, Berry et al. (1998)
Sunflower oil	24.4			
Linseed oil	16.7			
Control	3.5	Fat		Chouinard et al. (2001)
4% Ca salts of FAs from canola oil	13.2			
4% Ca salts of FAs from soybean oil	22.5			
4% Ca salts of FAs from linseed oil	19.5			
Control (ground soybeans)	3.1			
Extruded soybeans (120, 130, 140 °C)	19.9			
Hay ad libitum + 15 kg fodder beet	4.7	Fat	Σ	Collomb, Sollberger et al. (2004)
+ 1.0 kg ground rapeseed	6.8			
+ 1.0 kg ground sunflowerseed	8.8			
+ 1.4 kg ground sunflowerseed	17.6			
+ 1.0 kg ground linseed	6.3			
+ 1.4 kg ground linseed	9.9			
Control	9	FAME	c9	Gonthier et al. (2005)
12.6% DM raw flaxseed	14			
12.6% DM micronized flaxseed	14			
12.6% DM extruded flaxseed	19			
Control (60% forage, 40% concentr.)	4.5	Fat	c9	Bell et al. (2006)
Control + 24 ppm monensin	5.2			
6% DM safflower oil	33.6			
+ 24 ppm monensin	51.5			
Control (60% forage, 40% concentr.)	6.8			
6% DM safflower oil	41.2			
+ 150 IU vitamin Ekg DM^{-1}	34.8			
+ 24 ppm monensin	45.5			
+ vitE + monensin	47.5			
6% DM flaxseed oil + vit.E	28.0			
Marine oils				
0% fish meal	3.9	FAME	c9	AbuGhazaleh, Schingoethe, & Hippen (2001)
25% fish meal	4.4			
50% fish meal	4.6			
100% fish meal	7.2			
Control	6.9	Fat	c9	Baer et al. (2001)
2% fish oil	24.3			

Table 1 (*continued*)

Dietary treatment	CLA content		CLA[a]	Reference
	mg g^{-1}	as		
Control	~6	Fat		Chouinard et al. (2001)
Fish oil 200 mL d^{-1}	~18			
Fish oil 400 mL d^{-1}	~17			
Control	7.1/6.0	FAME	Σ/c9	Donovan et al. (2000)
1% fish oil	17.1/15.8			
2% fish oil	25.3/22.3			
3% fish oil	21.2/19.0			
Control	5.6/3.9	FAME	Σ/c9	Shingfield et al. (2003)
+250 g fish oil d^{-1}	18.5/16.6			
Plant and marine oils				
Control	4.0/3.3	FAME	Σ/c9	AbuGhazaleh et al. (2002)
0.5% fish oil	5.6/4.7			
0.5% fish oil + 2% soybean oil	15.9/13.9			
Control	3.3	FAME	c9	AbuGhazaleh, Schingoethe, Hippen & Kalscheur (2004)
0.5% fish oil + 2% soybean oil	11.6			
Control (corn-based TMR)	5.2	FAME	c9	Lynch et al. (2005)
1% fish oil + 2% soybean oil	47.4			
Control (44% forage, 56% concentr.)	6.1/5.6	FAME	Σ/c9	Allred et al. (2006)
2.7% Ca-salts of palm and fish oil	12.7/12.0			
+ 5% extruded soybean	14.4/13.6			
+ 0.75% soybean oil	18.2/17.4			
Control	5.0	FAME	Σ (c9)	Shingfield et al. (2006)
45 g DM^{-1} fish oil + sunflower oil (1:2)	34.7 (53.7/23.5)[c]			

[a]Σ = total CLA; c9 = *cis*-9,*trans*-11 CLA.
[b]FAME = fatty acid methyl esters.
[c]On day 5 and 15, resp.

With rising altitude, which is accompanied by a decrease in the proportion of grasses and a corresponding increase in dicotyledonous species, there was an increase in CLA levels from lowlands (mean value 8.7 mg g^{-1} fat) to mountains (16.1 mg g^{-1} fat) and highlands (23.6 mg g^{-1} fat) (Collomb et al., 2002a; Collomb, Sieber et al., 2004). In the milk fat from cows grazing at the three altitudes, the *cis*-9,*trans*-11 (7.8, 14.1 and 21.3 mg g^{-1} fat, respectively) and *trans*-11,*cis*-13 CLA (0.4, 0.8, and 1.8 mg g^{-1} fat, respectively) isomers were the most abundant among the *cis,trans*/*trans,cis* isomers (Collomb, Sieber, et al., 2004). Compared with the lowlands, the concentration of the *trans*-11,*cis*-13 CLA increased by 88% in milk fat from the mountains and by 310% in milk fat from the highlands. Normally, the *trans*-7,*cis*-9 is the second-most predominant CLA isomer in ruminant fat (Bauman et al., 2003). This isomer represents as much as 40% of the total CLA under special conditions (Piperova et al., 2000). By contrast, in milk fat from cows grazing at high altitude, the second-most important CLA isomer was the *trans*-11,*cis*-13 CLA (Collomb, Sieber, et al., 2004; Kraft et al., 2003). Therefore, the CLA isomer *trans*-11,*cis*-13, could be a useful indicator of milk products of alpine origin. In the same study, the FAs in milk fat were correlated with botanical families and individual plant species. The percentage of three species [*Leontodon hispidus*, *Lotus corniculatus* (and *alpina*), and *Trifolium pratense*] correlated positively with the concentrations of CLA, monounsaturated *trans* 18:1 FAs and polyunsaturated FAs in milk fat (Collomb, Bütikofer, Sieber, Jeangros, & Bosset, 2002b).

4.2. Plant oils

Plant oils from different oilseeds have quite different FA compositions and accordingly would be expected to have different effects on milk fat CLA concentrations (Stanton et al., 2003) (Table 1). Comparisons between different types of plant oils suggest that those rich in linoleic acid increase CLA concentration most effectively (Collomb, Sieber, et al., 2004; Collomb, Sollberger, et al., 2004; Dhiman et al., 2000; Kelly, Berry, et al., 1998; Stanton et al., 2003). Nevertheless, Lock and Garnsworthy (2002) showed that the feeding of linolenic acid can result in comparable increases in the CLA content of milk compared with linoleic acid. Different dietary oil treatments (peanut oil, high in oleic acid; sunflower oil, high in linoleic acid; linseed oil and flaxseed, high in α-linolenic acid) have been shown to exert different degrees of enrichment of milk fat with CLA. Feeding sunflower oil (53 g kg^{-1} of diet dry matter (DM)) for 2 weeks resulted in a CLA concentration in milk fat of 24.4 mg g^{-1} fat which was significantly greater than that achieved with similar inclusions of peanut oil (13.3 mg g^{-1} fat) or linseed oil (16.7 mg g^{-1} fat) (Kelly, Berry, et al., 1998). In milk fat of cows fed a high concentrate diet supplemented with linseed oil the total conjugated 18:2 FAs were higher than without

supplemental oil or low concentrate without and with linseed oil (Loor et al., 2005). Feeding flaxseed increased CLA content, but the effects of raw vs. micronized vs. extruded flaxseed showed no significant differences (Gonthier et al., 2005). Dhiman et al. (2000) demonstrated that feeding soybean oil, also rich in linoleic acid, was more effective in increasing the CLA content of the milk fat than feeding linseed oil. A Swiss study (Collomb, Sieber et al., 2004, Collomb, Sollberger, et al., 2004) evaluated variations in the distribution of CLA isomers in milk fat from cows fed either a control diet consisting of hay ad libitum and 15 kg fodder beets or the control diet supplemented with ground oilseeds containing a high concentration of either oleic (rapeseed), linoleic (sunflowerseed) or α-linolenic acid (linseed). Compared with the control diet, the concentration of the main CLA isomer *cis*-9,*trans*-11, increased significantly by 34% on the 1 kg rapeseed diet, by 19% on the 1 kg linseed diet, by 83% on the 1.4 kg linseed diet, by 83% on the 1 kg sunflowerseed diet and by 280% on the 1.4 kg sunflowerseed diet. For the last diet, a 33% increase in the daily intake of linoleic acid (from 281 to 375 g; α-linolenic acid = 1 g) increased the CLA *cis*-9,*trans*-11 content by 107% [from 7.5 to 15.5 mg g^{-1} fat (total CLA content: 17.6 mg g^{-1} fat)]. This effect was firstly observed by Dhiman et al. (2000) and also confirmed by Secchiari et al. (2003) in milk from cows fed a soybean oil supplement or full-fat extruded soybeans. In the Swiss study (Collomb, Sieber, et al., 2004), the authors also found strong positive correlations ($P \leqslant 0.001$) between the daily intakes of (i) oleic acid or linoleic acid and the concentration of the CLA isomer *trans*-7,*cis*-9 in milk fat (correlation = 0.57); (ii) linoleic acid and the concentrations of the CLA isomers *trans*-10,*trans*-12 (0.78), *trans*-9,*trans*-11 (0.58), *trans*-8,*trans*-10 (0.60), *trans*-7,*trans*-9 (0.47), *trans*-10,*cis*-12 (0.89), *cis*-9,*trans*-11 (0.81), *trans*-8,*cis*-10 (0.85), and *trans*-7,*cis*-9 (0.74); and (iii) α-linolenic acid and the CLA isomers *trans*-12,*trans*-14 (0.88), *trans*-11,*trans*-13 (0.89), *cis,trans*/*trans*,*cis*-12,14 (0.88), *trans*-11,*cis*-13 (0.76), and *cis*-11,*trans*-13 (0.74). These FAs (oleic-, linoleic-, and α-linolenic acids) are probably the main indirect precursors of the above mentioned CLA isomers. The calcium salt of oils also increased the CLA content in milk fat. Feeding Ca salts of FAs from soybean oil caused the highest level of CLA (22 mg g^{-1} FAME) compared with canola and linseed oil (Chouinard et al., 2001).

4.3. Marine oils

In general, studies have shown that equivalent amounts of dietary fish oils are more effective than plant oils at increasing milk fat CLA content (Chilliard et al., 2000; 2001). The mechanism of CLA enrichment is unclear, and although biohydrogenation of long-chain PUFAs is unlikely to yield CLA or VA directly, increased ruminal and milk VA has been observed (Chilliard et al., 2000). Feeding of fish oil increased rumen output of VA as seen in in vivo studies (Shingfield et al., 2003) and the reduction of VA to 18:0 was inhibited by docosahexaenoic acid (22:6 n-3) according to in vitro culture studies (AbuGhazaleh & Jenkins, 2004). The content of VA in milk fat increased rapidly, but was transient and declines after 5 days of fish oil feeding. This decrease was associated with progressive increases in *trans*-10 18:1 concentrations (Shingfield et al., 2006). Replacing soybean meal with fish meal resulted in a milk CLA content which increased from 3.9 mg g^{-1} FAME with increasing concentration of fish oil to 7.2 mg g^{-1} FAME (AbuGhazaleh et al., 2001). High concentrations of CLA and VA in milk were obtained using 2% of the DM as fish oil (Baer et al., 2001; Donovan et al., 2000), with no additional effect on these FAs obtained by increasing the dietary fish oil concentration to 3% of DM (Donovan et al., 2000).

4.4. Combination of plant and marine oils

There are several recent publications addressing the effects of mixtures of plant and marine oils on CLA content in milk fat (AbuGhazaleh et al., 2004; AbuGhazaleh et al., 2002; Allred et al., 2006; Lynch et al., 2005; Shingfield et al., 2006) (Table 1). AbuGhazaleh et al. (2002) and AbuGhazaleh et al. (2004) obtained a three- to four-fold increase in CLA content by feeding 0.5% fish oil and 2% soybean oil. A corn-based TMR supplemented with 1% fish oil and 2% soybean oil combined with the selection of cows with naturally high CLA content even led to a VA + CLA content of more than 16% of total fatty acids and to a *cis*-9,*trans*-11 CLA content of 47.4 mg g^{-1} of FAs in VA-CLA-enriched milk compared with 5.2 mg g^{-1} of FAs in the control (Lynch et al., 2005). Shingfield et al. (2006) reported that feeding of fish oil and sunflower oil supplement (45 g kg^{-1} of DM, 1:2) elevated rapidly the *cis*-9,*trans*-11 CLA content, reached a maximum with 53.7 mg g^{-1} of FAs on day 5 and declined to 23.5 mg g^{-1} of FA by day 15. Due to time-dependent modifications in biohydrogenation, *trans*-11 18:1 decreased in milk fat and *trans*-10 18:1 increased progressively which was explained as an adaptation to the oils in the diet. The combination of palm and fish oil with extruded soybean or soybean oil (Allred et al., 2006) was as effective as fish oil and soybean oil in the study of AbuGhazaleh et al. (2002; 2004).

5. Technological effects

The influence of manufacturing conditions on the content of CLA in dairy products has been studied by many authors. However, there are relatively few studies on the effects of processing on CLA-enriched milk. A modified FA profile can influence several of the physical and chemical properties of dairy products. It is well known that a high content of unsaturated FAs increases the risk of oxidation and off-flavors. However, studies on modified milk do not indicate major concern in this respect even when the concentration of CLA is increased many-fold by dietary means as compared with conventional milk.

Feeding of extruded soybeans, fish oil or their combination did not have any adverse effect on the sensory properties of pasteurized milk (Ramaswamy et al., 2001). Avramis, Wang, McBride, Wright, and Hill (2003) studied the quality of dairy products manufactured from modified milk produced by feeding a fish meal supplement. They found no difference between fish meal milk and control pasteurized and UHT milk (2% fat) in terms of color, flavor and flavor stability. Enriched milk had a reduced fat globule size, which was in agreement with the studies of Jones et al. (2005) showing smaller fat globules in enriched UHT milk compared with control UHT milk. Even exposure to oxidation by light does not seem to exert adverse effects. Baer et al. (2001) observed no significant difference in flavor characteristics between pasteurized milk from cows fed a fish oil diet and a control diet after storage even when the milk was subjected to oxidation by sunlight. However, for milks with added copper oxidative flavor was more pronounced in modified milk after 2 days of storage. According to Lynch et al. (2005) the FA composition and sensory quality of pasteurized and homogenized 2% fat milk with a high level of CLA and VA (over 16%) remained stable over 14 days' shelflife even when exposed to light. The milk was produced by feeding cows with supplementary soybean oil and fish oil.

Lacasse, Kennelly, Delbecchi, and Ahnadi (2002), in contrast, reported that a taste panel easily detected deterioration of pasteurized and homogenized milk from cows fed protected (3%) and unprotected (3.7%) fish oil. However, the supplementation of fish oil in their study was rather high compared with other studies. In another study, the addition of 1% or 2% CLA (synthetic) in milk resulted in a grassy or vegetable oil flavor in pasteurized milk (Campbell, Drake, & Larick, 2003). Further, a significant decrease in CLA content was documented due to HTST pasteurization. A recent study by Herzallah, Humeid, and Al Ismail (2005) found that conventional pasteurization at different temperatures and boiling of milk had no significant effect on the CLA content of milk (not enriched). UHT heating and microwave treatment, on the other hand, caused a significant decrease in CLA content of milk.

Many studies have shown that a modification of FA content by unsaturated fat supplementation yields softer butter (Avramis et al., 2003; Baer et al., 2001; Gonzalez, Duncan, O'Keefe, Sumner, & Herbein, 2003; Ramaswamy et al., 2001; Ryhänen et al., 2005). The churning time of cream with a modified FA composition has been reported to be longer than normal (Avramis et al., 2003; Gonzalez et al., 2003). This may be due to the higher content of unsaturated fat and smaller fat globule size in modified milk (Avramis et al., 2003). No significant differences in the flavor of CLA-enriched butter have been documented (Baer et al., 2001; Ramaswamy et al., 2001). The storage stability of butter from modified milk seems to be good. The free FA and peroxidase values have been reported to remain within the expected ranges (Baer et al., 2001; Ramaswamy et al., 2001; Ryhänen et al., 2005). The potential of fat globule fractionation or dry fractionation of milk fat for influencing the content and distribution of CLA seems to be limited (Michalski, Briard, & Juaneda, 2005; O'Shea, Devery, Lawless, Keogh, & Stanton, 2000). Another method to produce CLA-enriched milk fat was published by Romero, Rizvi, Kelly, and Bauman (2000), who utilized carbon dioxide extraction to enhance CLA concentration in one of the fractions of milk fat.

Processing of milk to cheese appears to have no effect on the final content of CLA in cheeses; its content is primarily dependent on the CLA level of the unprocessed milk. Milk from cows fed extruded oilseed was used to produce Mozzarella cheese with no alterations in CLA content (Dhiman, Helmink, McMahon, Fife, & Pariza, 1999). Similar processing of milk from cows receiving a grass silage supplement with a cereal-based concentrate to Edam cheese showed no effect on the CLA content (Ryhänen et al., 2005). This is in accordance with studies reporting that processing parameters have negligible effects on the CLA content of cheeses made from unmodified milk, such as processed cheese (Luna, de la Fuente, & Juarez, 2005), Emmental cheese (Gnädig et al., 2004), Mozzarella, Gouda and Cheddar (Shantha, Ram, O'Leary, Hicks, & Decker, 1995), and Swedish hard cheeses Greve and Herrgårdsost (Jiang, Björck, & Fondén, 1997).

On the other hand, the properties of cheeses produced from CLA-enriched milk have been shown to differ from control cheeses. Avramis et al. (2003) found that Cheddar cheese made from milk of cows fed supplemental fish oil ripened faster after the first 3 months of ripening and developed a more desirable texture and Cheddar flavor. Cheeses from CLA-enriched milk seem to be softer than normal. Jones et al. (2005) manufactured cheeses from milk obtained from cows fed fish oil. The experimental cheeses had a CLA content over seven times higher and were significantly softer than the control. Edam cheeses made from CLA-enriched milk were also found to have a softer texture than control cheeses (Ryhänen et al., 2005). The softer texture of CLA-enriched cheese was probable due to the higher content of unsaturated FAs of CLA-enriched milk. CLA-enriched milk has been shown to have a reduced fat globule and casein micelle size as well as altered protein distribution of the casein micelles, which may affect its cheese making properties (Avramis et al., 2003).

Luna, Fontecha, Juarez, and de la Fuente (2005), by contrast, reported that the organoleptic characteristics of cheeses made from CLA-enriched milk from ewes fed linseed supplements did not differ from control cheeses. Neither did the total content nor isomer profile of CLA change during ripening. In another study, Cheddar cheese from cows grazing on pasture had a CLA content three times higher than cheeses manufactured from milk of cows fed conserved forage and grain. Open and trained panel evaluations showed no differences in the sensory characteristics among treatments and the authors suggest that the consumer acceptability of CLA cheese is similar to products with a low level of CLA (Khanal et al., 2005).

Possibilities to increase the CLA content of dairy products with microbial cultures have been studied by many authors and recently reviewed by Sieber, Collomb, Aeschlimann, Jelen, and Eyer (2004). Dairy starter bacteria strains which are able to convert linoleic acid to CLA in vitro have been identified, such as propionibacteria (Jiang, Björck, & Fondén, 1998), lactic acid bacteria (Kim & Liu, 2002; Lin, Lin, & Lee, 1999) and bifidobacteria (Coakley et al., 2003; Oh et al., 2003; Song et al., 2005). The conversion has been suggested to result from the action of the isomerase enzyme (Lin, 2006; Lin, Lin, & Wang, 2002, 2003). However, the contribution of dairy starter bacteria in increasing CLA in dairy products seems to be minor (Sieber et al., 2004). A potential approach to raising the CLA content in dairy products is the microbial conversion of free linoleic acid to CLA. In a recent study, Das, Holland, Crow, Bennett, and Manderson (2005) showed that yeast lipase and propionibacteria together in wash-curd, dry-salted cheese were not able to increase the CLA content although free linoleic acid was present. Studies by Lin (2003) in turn showed that the production of CLA in set yogurt prepared with mixed cultures comprising *Lactobacillus acidophilus* and yogurt bacteria was significantly enhanced by the addition of linoleic acid (0.1%). Recently, Lin, Hung, and Cheng (2005) reported that *Lb. delbrueckii* ssp. *bulgaricus* immobilized with polyacrylamide at pH 7 was effective in promoting CLA formation, indicating the potential to improve CLA production through immobilization of lactic acid bacteria.

6. Nutritional and health aspects

6.1. Dietary intake of CLA by humans

The average total CLA intake estimated so far ranges between 95 and 440 mg, and differs from country to country. Data from Germany (Fremann, Linseisen, & Wolfram, 2002; Fritsche & Steinhart, 1998; Jahreis, 1997), Sweden (Jiang, Wolk, & Vessby, 1999), European Union (Wolff & Precht, 2002), United States (Ritzenthaler et al., 2001), and Canada (Ens, Ma, Cole, Field, & Clandinin, 2001) are available with calculations of the human daily CLA intake (Table 2). However, average intake also varies considerably between subjects and on a day-to-day basis due to varying food consumption habits and differing CLA values in food. Dairy products contain VA (0.4–4.0% of total FAs), which can be desaturated to *cis*-9,*trans*-11 CLA in human tissue. A conversion rate of on average 19% was measured by Turpeinen et al. (2002), representing a significant contribution to the amount of *cis*-9,*trans*-11 CLA available to the body. Therefore, total contribution of milk fat to body CLA status is likely to be about 1.5 times the CLA content because of endogenous synthesis from VA (Turpeinen et al., 2002). Optimal dietary intake remains to be established. Hypotheses based on extrapolations from animal studies and on calculations from epidemiologic findings range between 95 mg and 3.5 g d^{-1} (Enser et al., 1999; Ha, Grimm, & Pariza, 1989; Ip, Singh, Thompson, & Scimeca, 1994; Parrish et al., 2003; Watkins & Li, 2003). However, all recommendations not based on experimental human data should be taken with caution.

6.2. Physiological effects of CLA

The different CLA isomers do not necessarily exhibit the same biological effects (Martin & Valeille, 2002). Above all the isomers *cis*-9,*trans*-11 and *trans*-10,*cis*-12 are currently under investigation and pleiotropic effects have been documented that are attributed to one or the other or both CLA-isomers. In milk fat, *cis*-9,*trans*-11 CLA amounts to 75–90% of total CLA, whereas *trans*-10,*cis*-12 CLA constitutes a minor isomer. Therefore, this section addresses mainly physiological effects attributed to *cis*-9,*trans*-11 CLA. However, in most human studies synthetic

Table 2
Human daily CLA intake with food

Country	Method	Remarks	Daily dietary intake in mg m/f[a]	Reference
Germany	1-Week dietary records	based on consummation data of the German "Nationale Verzehrsstudie"	310	Jahreis (1997)
Germany	1-Week dietary records	*cis*-9,*trans*-11, based on German nutrition survey	440/360	Fritsche and Steinhart (1998)
Germany	1-Week dietary records	52 female students	–/320	Fremann et al. (2002)
Sweden	1-Week dietary records + 24 h recall interviews	123 men, *cis*-9,*trans*-11	160/–	Jiang et al. (1999)
EU	Milk consumption in 15 countries per person and year	Intake from dairy products only	140–380	Wolff and Precht (2002)
Canada	1-Week dietary records	22 females and males, *cis*-9,*trans*-11	15–174	Ens et al. (2001)
US	3-Day dietary records	93 adults, total CLA (*cis*-9,*trans*-11)	210/150 (190/140)	Ritzenthaler et al. (2001)

[a]m, male; f, female.

CLA supplements were used. These are surpassing usual dietary intake by far and do not reflect natural isomer composition, making it difficult to judge whether dairy products may exert the same effects.

Strong evidence from animal trials supports an influence of CLA on body composition, i.e. lowering of body weight and fat mass and a relative increase in lean body mass (see review by Roche, Noone, Nugent, & Gibney, 2001). Results from human trials do not support any weight loss-inducing effect of CLA but indicate a body fat-lowering effect associated with an increase in lean body mass (as reviewed in Larsen, Toubro, & Astrup, 2003; Martin & Valeille, 2002). CLA supplementation for 24 months in healthy, overweight humans decreased body fat mass, mainly during the first 6 months (Gaullier et al., 2005). However, findings suggest that *trans*-10,*cis*-12 CLA is responsible for the effects on body composition, whereas *cis*-9,*trans*-11 is neutral (Martin & Valeille, 2002; Terpstra, 2004).

Several studies in animals and humans have found an antidiabetic effect of CLA and suggested the *trans*-10,*cis*-12 isomer to be responsible for decreasing glucose levels and increased insulin sensitivity (Khanal, 2004). Contradictory to this, other studies documented opposite effects namely the promotion of insulin resistance by the *trans*-10,*cis*-12 isomer (Moloney, Yeow, Mullen, Nolan, & Roche, 2004; see reviews by Khanal, 2004; Wang & Jones, 2004). The *cis*-9,*trans*-11 isomer seems without positive or negative effect (Martin & Valeille, 2002). Further studies are therefore necessary to clarify this issue and find the underlying mechanism.

Another controversial issue is the antiatherogenic effect of CLA. In rodents dietary CLA supplementation significantly lowered serum cholesterol and triacylglycerol concentrations but the results were not consistent (Lock, Horne, Bauman, & Salter, 2005; reviewed by Terpstra, 2004). Feeding CLA to rabbits in various amounts resulted in a dose-dependent reduction in the severity of cholesterol-induced atherosclerotic lesions in the aorta (Kritchevsky, Tepper, Wright, & Czarnecki, 2002; Kritchevsky, Tepper, Wright, Tso, & Czarnecki, 2000). However, earlier studies with cholesterol-fed rabbits did not show any effect on fatty streak lesions (Lee, Kritchevsky, & Pariza, 1994) and feeding C57BL/6 mice an atherogenic diet the addition of CLA even resulted in an increase of aortic fatty streaks (Munday, Thompson, & James, 1999). With the exception of one study, results from human studies investigating CLA influence on body composition and blood lipids showed that CLA had no significant effects on plasma total, LDL-, and HDL-cholesterol as well as triacylglycerol concentrations when compared with control group effects (overview of studies in Terpstra, 2004). Tricon et al. (2004b) compared the effects of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA on blood lipids and suggested that *cis*-9,*trans*-11 is the isomer with positive effects. Moloney et al. (2004) found significantly lower plasma fibrinogen concentrations after CLA supplementation compared with control supplementation in humans with type 2 diabetes mellitus, whereas serum interleukin-6 and plasma C-reactive protein concentrations were not altered by either group. To date, no human studies have been conducted either on plaque initiation or regression. Summarizing the above results, antiatherogenic effects seem not definitive.

Considerable in vitro experiments and animal trials have been done with regard to CLA inhibition of carcinogenesis (see reviews by Banni, Heys, & Wahle, 2003; Belury, 2002; Ip, Masso-Welch, & Ip, 2003; Lee & Lee, 2005; Parodi, 2004) but no human data are available so far. However, epidemiologically there seems to be a connection between CLA and the incidence of breast cancer in humans although positive as well as negative relations have been described (Aro et al., 2000; Voorrips et al., 2002). In a recent publication, the results of a cohort study suggest that high intakes of high-fat dairy foods and CLA may reduce the risk of colorectal cancer (Larsson, Bergkvist, & Wolk, 2005). So far it has been assumed that CLA is involved in various steps in all three stages of carcinogenesis (initiation, promotion, progression) and that the effect differs according to CLA isomer, type and site of the cell/organ and stage of carcinogenesis (see review by Lee & Lee, 2005). CLA are included in the phospholipids of membranes and replace other polyunsaturated fatty acids. Thus, cell metabolism and signal transduction may be influenced in several ways: modulation of cell proliferation and apoptosis, regulation of gene expression, influence on eicosanoid synthesis and metabolism, and antioxidative mechanisms (Kraft & Jahreis, 2004). However, clinical studies are needed to provide information on possible anticancerogenic effects of CLA in humans. In this context it has to be mentioned that rat studies revealed a contribution of VA to the anticarcinogenic effects due to its desaturation to *cis*-9,*trans*-11 CLA (Corl, Barbano, Bauman, & Ip, 2003; Ip et al., 1999; Lock, Corl, Barbano, Bauman, & Ip, 2004).

The influence on eicosanoid synthesis may have another implication: a modification of the immune system. In this regard it was shown that CLA modulates the immune system and prevents immune induced wasting in animals. In humans a beneficial effect in certain types of allergic or inflammatory responses was proposed due to CLA induced reductions in TNF-α and IFN-γ, increases in IL-1β and IL-10 additionally to parallel increases of IgA and IgM and a decrease of IgE (O'Shea, Bassaganya-Riera, & Mohede, 2004). Another study found raised protective antibody levels after hepatitis B vaccination in healthy men given a CLA concentrate compared with the control group (Albers et al., 2003). In contrast, in young healthy women alterations of immune status after an influenza vaccination was not documented (Kelley et al., 2000). A recently published study (Tricon et al., 2004a) found *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA to decrease mitogen-induced T lymphocyte activation in a dose-dependent manner in healthy humans with both isomers showing similar impacts.

So far only animal studies are available concerning the influence of CLA on bone metabolism. It remains to be established whether CLA may exhibit beneficial effects in humans and the related mechanisms (Watkins, Li, Lippman, Reinwald, & Seifert, 2004).

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