Conjugated linoleic acid (CLA): Effect of processing on CLA in cheese and the impact of CLA on the arachidonic acid metabolism

Konjugierte Linolsäureisomere (CLA):
Einfluss des Herstellungsprozesses auf den CLA Gehalt in Käse und die Auswirkung von CLA auf den Arachidonsäurestoffwechsel

Silke Gnädig

Thesis
Dissertation - zur Erlangung des Doktorgrades des Fachbereichs Chemie der Universität Hamburg

---

INRA, Unité de Nutrition Lipidique, Dijon, FRANCE
University of Hamburg, Institute of Biochemistry and Food Chemistry
Departement of Food Chemistry
Hamburg, February 2002

The present work was carried out from February 1999 until September 2001 under the supervision of Prof. Dr. Dr. H. Steinhart and Dr. J.-L. Sébédio at the Institut National de la Recherche Agronomique, Unité de Nutrition Lipidique, Dijon, FRANCE.

The research was funded by a Marie-Curie-Fellowship of the European Union (FAIR-CT98-5071).

1. Referee: Prof. Dr. Dr. H. Steinhart
2. Referee: Prof. Dr. B. Bisping

Members of the jury: Dr. A. Paschke, Prof. Dr. W. Francke,
Prof. Dr. J. Heck, Prof. Dr. D. Geffken, Prof. Dr. J. Thiem,
Dr. W. Kirstein, Dr. H. Schnabblegger

Date of oral presentation: 01.02.2002

für meine Eltern

ACKNOWLEDGMENTS

First, I will gratefully thank Prof. Dr. Dr. H. Steinhart for his constant interest and his support in this work and for accepting it as a thesis of the Institute of Food Chemistry.

I would like to thank Prof. Dr. J.-L. Sébédio offering me the opportunity to improve my knowledge on CLA research, his readiness to discuss and the aid during the three years at the INRA in Dijon.

I thank Prof. Dr. B. Bisping, Dr. A. Paschke and Prof. Dr. W. Francke for their willingness to have accepted the examination of this Ph.D. work.

I wish to thank J.-F. Chamba and E. Perreard, at the ITFF, for preparing the Emmental cheese samples and the long discussions on manufacturing protocols.

I thank R. Rickert for performing analysis of several cheese samples by Ag^+-HPLC.

I thank O. Loreau and J.P. Noël at the CEA for their support in the fabrication of the labeled C18:3 and C20:3 fatty acids.

I would like to thank the European Union and the ITFF for financial support.

Special thanks are going to

- Dr. O. Berdeaux, Dr. L. Bretillon, and Dr. J.-M. Chardigny, for the various scientific discussions and their never-ending support during the different experiments (sometimes late in the evening).
- Dr. J.-P. Sergiel and Dr. C. Alasnier, for their patience in reading and re-reading the manuscript and their fruitful suggestions.
- S. Chappaz, for analyzing various Emmental cheese samples, and his perpetual good mood.
- S. Almanza, M. Genty, S. Grégoire, L. Leclerc and B. Pasquis, for performing divers fatty acid analysis, technical support and their humor.
- P. Juaneda, for his great availability, his interest in analytical problems and the advice in statistical analysis.
- Alexandra, Florent, Niyazi, my fellow combatants of the thesis, for the scientific and not-scientific discussions in our office, their support and their patience in improving my French.
- All colleagues at the INRA- Unité de Nutrition Lipidique
- the INRA-Badminton group, to ensure the physical fitness.

I will thank my parents and all my family for their inexhaustible support.

Lionel, for his support, understanding, optimism and smile.

Emmanuelle, André, Claudia and Dietrich, Markus, Imke, Ines, Kirstin and Rainer, for amusing and serious discussions, and a lot of fun.

TABLE OF CONTENTS

ABBREVATIONS

6-keto $PGF_{1\alpha}$	6-keto-prostaglandin $F_{1\alpha}$
Ag^{+}	Silver ion
ATP	Adenosine 5'-triphosphate
BF_3 / MeOH	Boron trifluoride in Methanol
BHA	Butylhydroxyanisol
BHT	Butylhydroxytoluene
BuLi	Butyllithium
CE	Cholesterolester
CEA	Commissariat à l'Energie Atomique
chap.	Chapter
CLA	Conjugated linoleic acid
CoA	Coenzyme A
DMF	Dimethylformamide
DMOX	4,4-dimethyloxazoline derivative
DMSO	Dimethyl sulfoxide
DNA	Desoxy ribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EIA	Enzyme immuno assay
FABE	Fatty acid butyl ester(s)
FAME	Fatty acid methyl ester(s)
FID	Flame ionization detector
FTIR	Fourier transformed infra red spectroscopy
GC	Gas chromatography
HDL	High density lipoproteins
HEPES	[4-(2-hydroxyethyl)-piperazino]-ethanesulfonic acid
HETE	12-Hydroxyeicosatetranoic acid
HMDS	Hexamethyldisilazane
HMPA	Hexamethylphosphoramide
HPLC	High performance liquid chromatograpy
Ig	Immunoglobulin
ITFF	Institut Technique Français du Fromage
K_f	correlation factor
LDL	Low density lipoproteins
LTB_4	Leucotriene B_4
mRNA	messenger ribonucleic acid
MS	Mass-spectrometry

MTAD	4-methyl-1,2,4-triazoline-3,5-dione
NADPH	Nicotinamide-adenine dinucleotide phosphate (reduced form)
NMR	nuclear magnetic resonance
PDC	pyridinium dichromate
PGD_2	Prostaglandin D_2
PGE_2	Prostaglandin E_2
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PGH_2	Prostaglandin H_2
PGHS	Prostaglandin H-synthase
PGI_2	Prostaglandin I_2
PL	Phospholipids
PPAR	Peroxisome proliferator-activated receptor(s)
PPTs	Pyridinium p-toluenesulfonate
PRP	Platelet rich plasma
PUFA	Polyunsaturated fatty acid(s)
RP	Reversed phase
SCD	Stearoyl-coenzymeA desaturase enzyme
SD	Standard Deviation
TFA	*Trans* fatty acid(s)
TAG	Triacylglycerols
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TPA	12-O-tetradecanoylphorbol-13-acetate
TRIS	Tris-(hydroxy)-methylaminomethane
TXA_2	Thromboxane A_2
TXB_2	Thromboxane B_2
VLDL	Very low density lipoproteins

1 INTRODUCTION

In the early 1980's PARIZA AND HARGRAVES (1985) reported that an extract of grilled ground beef exhibited a mutagenesis inhibitory activity. The active molecules were later identified as conjugated linoleic acids (CLA), a mixture of positional and geometrical isomers of linoleic acid (HA *et al.*, 1987). Since that time, CLA reached a large research interest because of their potential anticarcinogenic activity on mammary, skin, colon and forestomach cancers *in-vivo* (in animals) and *in-vitro* (IP *et al.*, 1994a). Furthermore other beneficial properties were revealed in various animal experiments. It was shown that CLA positively influenced atherosclerosis, modulated the immune-response and showed a capacity to change body composition by reducing fat to lean body mass ratio (BANNI AND MARTIN, 1998; COOK AND PARIZA, 1998; NICOLOSI *et al.*, 1997; PARK *et al.*, 1997).

The major dietary sources of CLA are foods containing ruminant fat. Milk and dairy products have shown the highest amounts. About 75-90% of CLA in ruminant fat is 9c11t-C18:2, also called rumenic acid (KRAMER *et al.*, 1998). It is formed by bioconversion of polyunsaturated fatty acids (PUFA) in the rumen and by Δ9-desaturation of *trans*-vaccenic acid in the mammary gland of the lactating cow (FRITSCHE AND STEINHART, 1998a; GRIINARI *et al.*, 2000). Synthetic mixtures of CLA contain mainly two CLA-isomers in equal amounts, 9c11t-C18:2 and 10t12c-C18:2 (BERDEAUX *et al.*, 1998a).

Regarding the favorable physiological effects of CLA observed in experimental studies, it may be beneficial to enrich them in human nutrition in order to improve human health. In Germany the daily intake of CLA is evaluated to be 0.36g / day for women and 0.44g / day for men in a normal diet (FRITSCHE AND STEINHART, 1998b). This is only about one-fifth of the proposed anticarcinogenic beneficial level of 0.1% CLA of total food in the daily nutrition. This dose of 0.1% CLA was extrapolated to human requirements from the efficient dose found in animal studies. A supplementation with 0.1% CLA by weight in the diet reduced the incidence of mammary carcinogenesis in the rat (IP *et al.*, 1994b).

Until now, the mechanisms of action of CLA still remain unclear. It is suggested that CLA may affect many different metabolic pathways, and individual isomers of CLA would act differently (PARIZA *et al.*, 2000). An important modulation of lipid metabolism may be one of their impacts. One hypothesis suggests the influence of

CLA on the arachidonic acid metabolism, perhaps by competing with linoleic acid. The respective conjugated C18:3 and C20:3 metabolites of CLA were detected in the rat liver (BANNI *et al.*, 1999a; SEBEDIO *et al.*, 1997). This suggests that CLA can also be metabolized into long chain PUFA probably using the same way as linoleic acid. Linoleic acid is metabolized *in-vivo* into arachidonic acid by successive desaturation and chain elongation.

Moreover it was suggested that CLA are able to modify the eicosanoid synthesis (MOYA-CAMARENA AND BELURY, 1999). Eicosanoids, e.g. prostaglandins and leucotrienes, are cyclooxygenase and lipoxygenase products, mainly formed from arachidonic acid. They have numerous physiological effects in the organism. They have been described as hormone-like mediator substances. Eicosanoids, as for example prostaglandin E_2 (PGE_2), prostaglandin I_2 (PGI_2) and thromboxane A_2 (TXA_2) play an important role in the inflammatory process and in the development of arteriosclerotic lesions and thrombosis.

1.1 Structure and formation of CLA

Fatty acids in food are an important source of energy, as their caloric value is about twice as high as that of carbohydrates and proteins. Furthermore PUFA (linoleic acid, α-linolenic acid) have to be included in the diet, as they could not be synthesized in the organism. These essential fatty acids are the precursors of polyunsaturated C20 and C22 long chain fatty acids, which play an important role in membrane structure and functions, and are the precursors of the synthesis of eicosanoids. Physiologically active PUFA normally contain double bonds in *cis* configuration. Fatty acids containing more than one double bond show a methylene group (-CH_2-) inserted within the double bond system. Technological and microbiological treatments often lead to the formation of *trans* isomers, the so called *trans* fatty acids (TFA), for example geometrical isomers of oleic acid or linoleic acid.

The term CLA describes a mixture of positional and geometrical isomers of linoleic acid containing a conjugated double bond system instead of isolated double bonds. Each single double bond can be in *cis* or *trans* configuration. Therefore CLA can be included in the group of TFA. Figure 1 shows the most important CLA isomers, 9c11t-C18:2 and 10t12c-C18:2, compared to their natural homologue linoleic acid.

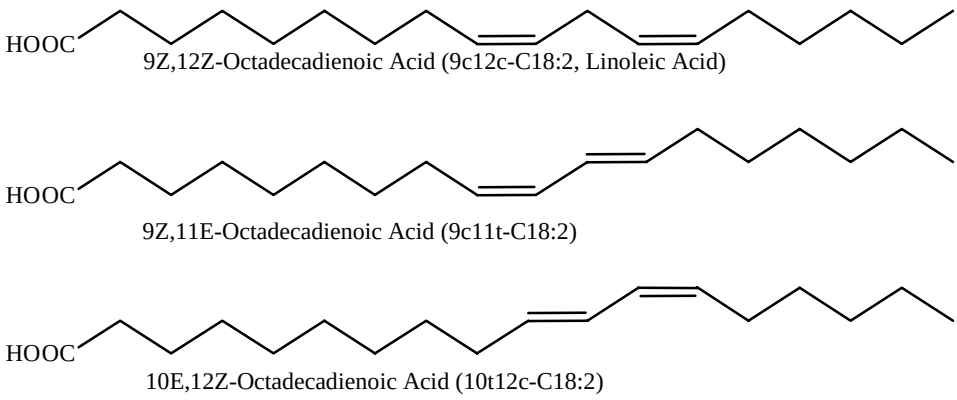


Figure 1 Chemical structure of linoleic acid and CLA (9c11t- / 10t12c-C18:2)

1.1.1 Biosynthesis in ruminants

CLA are naturally formed by biosynthesis in ruminants, using two different pathways (figure 2), which mainly lead to 9c11t-C18:2 (about 75-90% of the totally produced CLA).

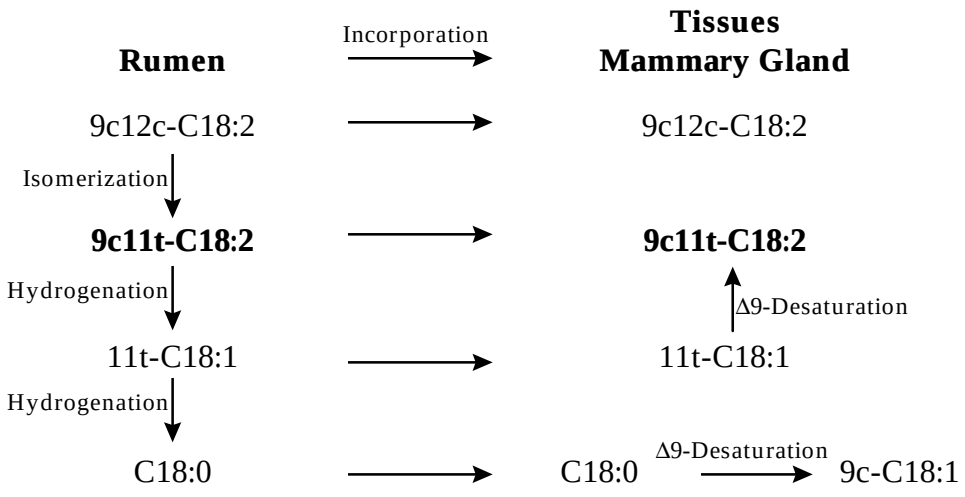


Figure 2 Pathways of CLA biosynthesis (9c11t-C18:2)

A first pathway is the biohydrogenation of ingested dietary unsaturated fatty acids, e.g. linoleic acid, into stearic acid by enzymes of different bacteria present in the rumen (HARFOOT AND HAZELWOOD, 1988). Various TFA appear along this biohydrogenation pathway as intermediates, e.g. 9c11t-C18:2 (the main CLA isomer in milk) and *trans*-vaccenic acid (11t-C18:1). KEPLER AND TOVE (1967) extracted a linoleate isomerase (EC 5.2.1.5) from the rumen bacteria «*Butyrivibrio fibrisolvens*» which is responsible for the isomerization of linoleic acid into 9c11t-C18:2 in a first step. In the following the double bond in position Δ9 is hydrogenated to form *trans*-vaccenic acid. The last step of the bioconversion is the reduction of *trans*-vaccenic acid into stearic acid. This seems to be the rate limiting reaction. Therefore the intermediate products 9c11t-C18:2 and *trans*-vaccenic acid accumulated (KEMP *et al.*, 1975) and they will be absorbed in the intestine and incorporated into different tissues.

In a second pathway CLA is formed by Δ9-desaturation of *trans*-vaccenic acid in adipose tissue and in the mammary gland of the lactating cow (GRIINARI *et al.*, 2000). The endogenous synthesis in the mammary gland was reported to be very important, as about 60% of CLA in milk fat is formed via this pathway in the lactating cow.

1.1.2 Chemical synthesis

The production of large quantities of CLA was carried out by chemical synthesis. The best results were obtained by isomerization of linoleic acid under strong alkali conditions. In contrast to the naturally occurring CLA, chemical synthesis produces complex CLA mixtures containing mainly 9c11t-C18:2 (43-45%) and 10t12c-C18:2 (43-45%) (CHIPAULT AND HAWKINS, 1959). The two major isomers are accompanied by small amounts of other CLA isomers with double bonds in 8,10 or 11,13 positions. In addition to *cis-trans* and *trans-cis* isomers, all-*cis* and all-*trans* CLA isomers are formed (CHRISTIE, 1997). Depending on the exact synthesis procedure the CLA isomer composition varies considerably. It was shown, that the number of isomers depended of the severity of the alkali isomerization conditions in the preparations of CLA (ACKMAN, 1998). It has been demonstrated that the «undesired» isomers arose with the duration of reaction. Limiting the conversion level of linoleic acid into CLA to 70%, the synthetic CLA-mixture contained more than 99% of 9c11t-C18:2 and 10t12c-C18:2 and less than 1% of «undesired» isomers (REANEY *et al.*, 1999).

CLA mixtures of different isomer composition were also obtained in varying solvents (ethylene glycol, glycerol, propylene glycol, *tert*-butanol, water, dimethyl sulfoxide (DMSO), dimethylformamide (DMF)), catalysts (lithium-, sodium- or potassium hydroxide) or reaction vessels.

Usually propylene glycol, glycerol or ethanol / water are used as solvents. Other solvents could lead to residues which are not desired in the final product, because of toxicological aspects, when CLA products are devoted to be used for human nutrition and have to be food-grade.

From the different catalysts, sodium-hydroxide is preferably used. Potassium-hydroxide has similar efficiency of conversion but it is more expensive than sodium-hydroxide. Lithium-hydroxide is the least effective one and it is not used as catalyst (REANEY *et al.*, 1999).

Recently, synthesis procedures were developed to obtain pure CLA isomers. BERDEAUX *et al.* (1998a) described the formation of the two major CLA isomers (9c11t and 10t12c-C18:2) by alkali-isomerization of methyl-linoleate, followed by a fractional crystallization in acetone. The obtained single isomers showed a good isomeric purity (90-97%) and were available in quantities about 70g at once. An alternative method for the production of pure 9c11t-C18:2 was described through the

dehydration of ricinoleic acid. 50-60g batches of 9c11t-C18:2 were prepared with a 70% overall yield (BERDEAUX *et al.*, 1997). The necessity to use expensive reagents (1,5-diazobicyclo(5.4.0)undec-5-ene) for this reaction to eliminate by-products increases the production costs and makes this synthesis process uneconomical (REANEY *et al.*, 1999).

Pure CLA isomers can also be produced by total stereoselective multiple step synthesis, in small quantities up to 1g. The isomeric purity can be higher than 98%. Stereoselective synthesis guarantees the exact chemical structure of the final molecule and limits by-products due to several purification procedures which are done during the synthesis pathway. Examples for the preparation of molecules containing a conjugated diene system are syntheses of pheromones. These synthesis pathways might be adapted to the preparation of CLA isomers (ADLOF, 1999).

A scheme for the synthesis of deuterium-labeled 9c11t-C18:2 involving a combination of acetylenic and Wittig coupling reactions has recently been described by ADLOF (1997). Unfortunately, the final Wittig coupling reaction formed two CLA isomers, 9c11t- and 9t11t-C18:2. An additional step of purification (Reversed phase-high performance liquid chromatograpy (RP-HPLC)) must be carried out to obtain pure single isomers.

Stereoselective synthesis procedures for 9c11c-, 9t11t-, 10t12c-, 7t9c- and 9c11t-C18:2 using comparable pathways have been published by LEHMANN (2001). All isomers were synthesized from commercial reagents via alcin-coupling, and stereo-selective hydrogenation of the first double bond in E or Z configuration. The second double bond was formed selectively in E or Z configuration by Wittig reaction. All synthesized CLA-isomers showed a good isomeric purity without any further purification.

1.1.3 Analysis

As described before, many positional and geometrical CLA isomers are present in natural and synthetically produced materials. Therefore, high quality analytical methods are needed, to determine the exact isomer composition of the complex CLA mixtures in synthetic CLA, foodstuff or biological matrices. Great care has to be taken with the analysis of CLA as they are unstable and very sensitive to isomerization (SHANTHA *et al.*, 1993). Therefore detected CLA levels could be

incorrect because of the use of unsuitable analytical methods in the past (HAMILTON, 2001). It was suggested that any data reported prior to five years has to be critically regarded (CHRISTIE, SCI conference, London, 2001).

To avoid any problems, the CLA containing substance has to be transformed into fatty acids methyl esters (FAME) using mild methylation methods. Acid-catalysts or high temperatures led to isomerization, where *cis-trans* and *trans-cis* isomers are converted into *trans-trans* isomers, and to the formation of methoxy-artefacts (KRAMER *et al.*, 1997; PARK *et al.*, 2001). Also Methoxy-artefacts appeared using the methylation with trimethylsilyl-diazomethane, an often recommended weak methylation method (CHRISTIE *et al.*, 2001). Usually sodium-methoxide, as a weak base, or boron trifluoride in methanol (BF_3 / MeOH) are used.

The CLA contents are currently determined using gas chromatography (GC) coupled to a flame ionization detector (FID). To obtain a good separation of the CLA isomers, high polar capillary cyanopropylsiloxan columns of 50 and 100 m length are used. Although a good separation of the CLA isomers can be reached, some of the positional isomers, e.g. 7t9c-C18:2 and 9c11t-C18:2, coelute and the samples have to be analyzed using other techniques (RICKERT AND STEINHART, 2001).

The utilization of silver ion (Ag^+)-HPLC improved the resolution of the CLA isomers, since two to six columns were coupled in series. The CLA isomers are separated into three groups depending on the configuration of the double bounds, in *trans-trans*, *cis-trans* / *trans-cis* and *cis-cis* respectively. Furthermore Ag^+-HPLC led to a better separation of the CLA with lesser coelution compared to GC (SEHAT *et al.*, 1999).

Exact structural determination of single CLA isomers is possible using different spectroscopic methods. The analysis is often done using GC coupled to mass-spectrometry (MS). Therefore the FAME are transformed into a 4-methyl-1,2,4-triazoline-3,5-dione (MTAD) adduct or a 4,4-dimethyloxazoline derivative (DMOX) (DOBSON, 1997; DOBSON AND CHRISTIE, 1996). The use of these derivatives in GC-MS permits the localization of the double bond position in the fatty acid molecule. To get information about the configuration of the double bond, GC- Fourier-transformed infra-red spectroscopy (FTIR) is applied. The IR-spectra for conjugated *trans-trans*, and *cis-trans* / *trans-cis* isomers exhibited absorption at 990 cm^{-1} and characteristic bands at 988 and 949 cm^{-1}, respectively. However, the differentiation between a *cis-trans* and *trans-cis* diene is not possible by FTIR (MOSSOBA *et al.*, 1999). The positions

and relative intensities of the =C-H stretch bands are highly characteristic and could discriminate between *cis-trans / trans-cis* (3020 and 3002 cm-1), *cis-cis* (3037 and 3005 cm-1) and *trans-trans* (3017 cm-1) conjugated double bond systems (FRITSCHE *et al.*, 1997).

1.2 CLA in human diet

1.2.1 Contents in food

The main dietary sources of CLA in human diet are products of animal origin. CLA contents of various normally consumed foods are presented in table 1.

Table 1 CLA contents in foods, g / 100 g of total fatty acids (GNÄDIG, 1996)

	CLA content		CLA content
Butter	0.63-2.02	Yogurt	0.43-1.12
Milk	0.46-1.78	Cheese	0.50-1.70
Beef	0.67-0.99	Pork	0.15
Lamb	1.62-2.02	Turkey	0.96
Fish	0.04-0.28	Plant Oils	n.d.

Among the different meat products, meat from ruminants shows higher contents of CLA than meat of nonruminant origin. Highest CLA amounts were found in lamb (BANNI *et al.*, 1996). For seafood and poultry, except for turkey, only small CLA contents were reported (CHIN *et al.*, 1992). Dairy products contained higher CLA amounts than other animal products, where the CLA content varied over a wide range. CLA contents up to 30.0mg / g fat were reported (O'SHEA *et al.*, 1998). A recent study by LAVILLONIÈRE *et al.* (1998) revealed variations of the CLA content in cheeses between 5.3 and 15.8mg / g fat, mainly the natural CLA isomer 9c11t-C18:2.

Plant oils or margarine contain only small amounts of CLA (0.1-0.5mg / g fat) (CHIN *et al.*, 1992). CLA are formed as a result of industrial processing - oil refining processes (mainly bleaching and deodorization) and catalytic process of hydrogenation to produce margarine (JUNG AND HA, 1999). Otherwise CLA appear in oils due to high temperature treatments. For example, sunflower oil after its use as frying oil contained CLA amounts up to 0.5g CLA / 100g of oil (JUANEDA *et al.*, 2001). Further analysis of the CLA composition showed that among total occurring CLA, the all-

trans isomers were formed favorably and less than 50% of total CLA was 9c11t-C18:2 (CHIN *et al.*, 1992; JUANEDA *et al.*, 2001).

Processed, canned or infant foods showed CLA amounts comparable to these of unprocessed foods (CHIN *et al.*, 1992). CLA in foods, such as chocolates, pastries, bakeries, originated predominantly from dairy fat, whereby the CLA level in the final foodstuff reflected the proportion of dairy fat in the preparation (FRITSCHE AND STEINHART, 1998b).

1.2.2 Factors affecting CLA content in milk

The variability of the CLA amount in dairy products is explained by great variations of CLA content in the raw milk. Various factors are known to influence the CLA content in milk, such as the food of the ruminant, the season, the animal breeding type, the number of lactation and the stage of lactation (FRITSCHE AND STEINHART, 1998b; JAHREIS *et al.*, 1999; SEBEDIO *et al.*, 1999).

STANTON *et al.* (1997) described that a high number of lactations led to high CLA concentrations in milk fat. An interaction between the lactation stage and the CLA-level was not examined, as the milk-sampling in this study excluded the beginning and the end of the lactation period. But it seems that the lactation stage can also affect the CLA content in milk, as body fat stores of the cow are mobilized at the beginning of lactation (JAHREIS *et al.*, 1999).

The CLA concentration in milk is season dependent. All ruminants showed a decreased CLA level during Winter, with the lowest CLA level found in March, and inversely an increase was observed during Summer, which is positively correlated to the grazing period. Indeed, pasture in Spring time contains higher amounts of PUFA which caused a higher bacterial biohydrogenation in the rumen (JAHREIS *et al.*, 1997). The relationship between pasture feeding and CLA-level was also described by DHIMAN *et al.* (1996). The CLA-level increased from 8.4 to 22.7mg/g milk fat when the pasture level was increased in the diet. To show the influence of different diets on the CLA content in milk, an experiment was carried out to compare three different types of farm management applying three different feeding methods. Cows were fed either with maize silage all over the year (indoor group), with grazing in summer and maize silage in winter (conventional group) or with grazing during summer and clover-alfalfa-grass silage in winter (ecological group). The highest amounts of CLA

in milk were measured in the ecological group, whereas smallest CLA contents were found in the indoor-maize silage group (JAHREIS *et al.*, 1996). The application of ecological feeding conditions rich in pasture would be useful to produce milk naturally high in CLA (JIANG *et al.*, 1996).

Various experiments were carried out to increase the CLA content in milk, by modulating the dietary regimen of the cows. Milk from cows offered a diet supplemented with oils rich in PUFA had up to fivefold higher CLA levels. Enrichment with sunflower, linseed or rapeseed oil caused an increase of the CLA levels (KELLY *et al.*, 1998). A positive correlation was found between the content of linoleic acid in the oil and the increase of CLA in the milk. The same effects were obtained by feeding with extruded oilseeds (DHIMAN *et al.*, 1999). Feeding fish oils which contained large amounts of PUFA, mainly eicosapentaenoic acid and docosahexaenoic acid, resulted in the same effects. The CLA content increased importantly, due to the greater formation of biohydrogenation products in the rumen (DONOVAN *et al.*, 2000; JONES *et al.*, 2000).

Direct feeding of CLA in the regimen and the abomasal infusion of CLA was tested as another possibility to enrich CLA in cow's milk (CHOUINARD *et al.*, 1999). It was found that direct CLA supplementation reduced milk fat yield, suggesting a decrease of the *de-novo* fatty acid synthesis (LOOR AND HERBEIN, 1998). Moreover the quantity of CLA required to induce a substantial reduction of milk fat synthesis was considerably low. For example, the infusion with 0.10g / day of 10t12c-C18:2 (0,05% of diet) resulted in a 44% reduction in milk fat yield (BAUMGARD *et al.*, 2001). It was demonstrated that only the 10t12c-C18:2 inhibited milk fat synthesis, whereas the 9c11t-C18:2 had no effect. The application of this method to enrich CLA in milk seems not to be interesting because of the disadvantageous reduction of milk fat accompanied by the reduction of total CLA in milk.

1.2.3 Influence of food processing

The relationship between the CLA content and different parameters of food processing in dairy products is discussed controversially. Because of their chemical structure containing a conjugated double bond system, CLA have been described to be more sensitive to oxidation or to isomerization during heat treatment than linoleic acid. Therefore research interests were directed to determine the influence of heat-

treatments such as grilling, cooking and frying, on the CLA content, to test the stability in food products and to avoid a CLA decrease by oxidative damage.

Home-made food preparing had no influence on the CLA content as it was shown by SHANTHA *et al.* (1994). They investigated the influence of frying, baking, broiling or microwaving on the CLA content in grilled hamburger beef patties and described that the CLA content remained unchanged. These findings were supported by a recent study which described that only intensive heating for 15min, using temperatures higher than 200°C, led to isomerization of CLA in milk, whereas moderate heating had no effect. Heating at 225°C for 15min decreased 9c11t-C18:2 from 1.71% to 1.07% of total fatty acids (PRECHT *et al.*, 1999).

The influence of storage on the CLA content was tested in butter, yogurt and sour cream. No modification of the CLA content were detected (SHANTHA *et al.*, 1995).

Dairy products often undergo a microbial fermentation during processing. The use of different fermentation cultures, processing temperatures or ripening periods could modulate the CLA level in the final foodstuff (FRITSCHE AND STEINHART, 1998a). These suggestions were confirmed by findings of JIANG *et al.* (1998), who reported the ability of two strains *Propionibacterium* spp. to produce CLA in culture by conversion of linoleic acid into CLA (9c11t-C18:2). More recently six further *Lactobacillus* spp. were identified to be able to convert linoleic acid into CLA (LIN *et al.*, 1999a; LIN, 2000). The use of these strains could be a possibility to enrich yogurts and cheese in CLA during fermentation.

As cheese processing implies an important bacterial fermentation, various studies were carried out on this subject. Only minor changes in the CLA contents of different cheddar cheese varieties were found (3.20mg / g fat *versus* 3.55mg / g fat) (LIN *et al.*, 1999b). These results were in opposite to a Swedish study, testing various hard cheeses. The same CLA amounts in raw material and in the final product were observed (JIANG *et al.*, 1997). Other processing parameters during cheese fabrication were investigated to check if they are able to modify the CLA content. Changes in milling, pH values or addition of butylhydroxyanisol (BHA) or amino acids (lysine, tyrosine), led to a small decrease of the CLA content in the final cheese (2.70mg / g fat *versus* 2.19mg / g fat by addition of tyrosine and reduced pH) (LIN *et al.*, 1998).

Various studies were undertaken to determine the CLA content in cheese spread using different fabrication conditions. Tests compared processing under atmospheric

conditions and under nitrogen as protective gas. It was shown that processing under atmospheric conditions at temperatures of 80-90°C could increase the CLA content in the product. This effect was amplified by the addition of whey proteins as hydrogen donors or antioxidants as butylhydroxytoluene (BHT). Also the use of iron as Fe^{2+}/Fe^{3+} ascorbate as additive seems to be useful to increase the CLA level (SHANTHA *et al.*, 1992; SHANTHA *et al.*, 1995).

In the contrary, the addition of antioxidants (ascorbate or whey protein concentrates) acting as hydrogen donors to beef patties did not influence the CLA content of the samples (SHANTHA AND DECKER, 1995). The controversial results concerning the relationship of antioxidants and hydrogen donors with the CLA content could be related to the foodstuff itself and the presentation of the fatty acids in the food-matrix.

However, the changes in CLA content, reported during processing, are much less important than the seasonal differences in milk.

1.2.4 CLA intake in humans

Increased dairy fat consumption has been shown to be associated with increased CLA in human adipose tissue, plasma and human milk (BRITTON *et al.*, 1992; JIANG *et al.*, 1999; PARK *et al.*, 1999a). As mentioned before the daily intake of CLA in a normal diet in Germany, based on national dietary records, was evaluated to be 0.36g/day for women and 0.44g/day for men (FRITSCHE AND STEINHART, 1998b). HERBEL *et al.* (1998) estimated the daily CLA intake in the same period in the USA was 0.14g/day, which is about half of the quantity in Germany. A recent publication estimated the daily CLA intake in the USA to be 0.21g/day total CLA for man (0.19g/day 9c11t-C18:2) and 0.15g/day total CLA for women (0.14g/day 9c11t-C18:2). The data were collected during a one year period and are based on the analysis of the CLA content of food duplicates (RITZENTHALER *et al.*, 2001). These results reflected in a good manner the presence of CLA in human diet.

Only limited data are available on the effect the CLA-supplementation to the normal diet of humans. However it is important to know if a high CLA supplementation in human could give any side effects (possible toxicological aspects). Compared to animal experiments, all studies which were carried out on humans until now, have to be classified as short-term nutrition studies. The knowledge of the effects on chronic

high CLA-uptake is still missing. In animal experiments, the daily doses of CLA were much higher than the CLA supplementation in the human studies. This could explain the different effects of CLA on the organism. A high CLA proportion in the dietary fat could induce an imbalance between the different fatty acids. Moreover animal experiments were mainly carried out on growing animals, while human studies investigated CLA-supplementation on adults.

1.2.5 Dietary supplements

Dietary supplements as another source of CLA have become available, otherwise called nutraceuticals. These products, which consist of CLA obtained by chemical synthesis, contain an isomer mixture (free fatty acids) introduced in capsules. They are sold as products against obesity and are supposed to be useful in dietary regimen to loose weight. The intake of the described CLA-supplements is recommended up to 3g per day. Also energy-chocolate bars enriched in CLA are on sale in USA, often used by bodybuilders.

Normal foods enriched in CLA, as for example dairy products or margarine are not yet available on the European market, because of the current legislation in force. A foodstuff containing CLA will be classified as a novel food according to the Novel Food Regulation (EC No 258/97), as it is «a food or food ingredient consisting of or isolated from plants and food ingredients isolated from animals» and was not obtained by «traditional propagating or breeding practices and having a history of safe food». The application of such a foodstuff in the European Community is guided by the directives of this law. It has to prove that the «novel food» is not dangerous or disadvantageous for the consumer. The recommended tests are expensive and time consuming. But as a powerful source of CLA in daily nutrition, the creation of such a type of food could be useful. Moreover the CLA content could be guaranteed and would remain stable without variations.

1.3 Physiological properties of CLA

Due to their beneficial physiological effects, mainly demonstrated in rodents, CLA have been the subject of an increasing number of scientific studies in the past years. The main physiological effects are summarized in figure 3 and are discussed in the following paragraphs.

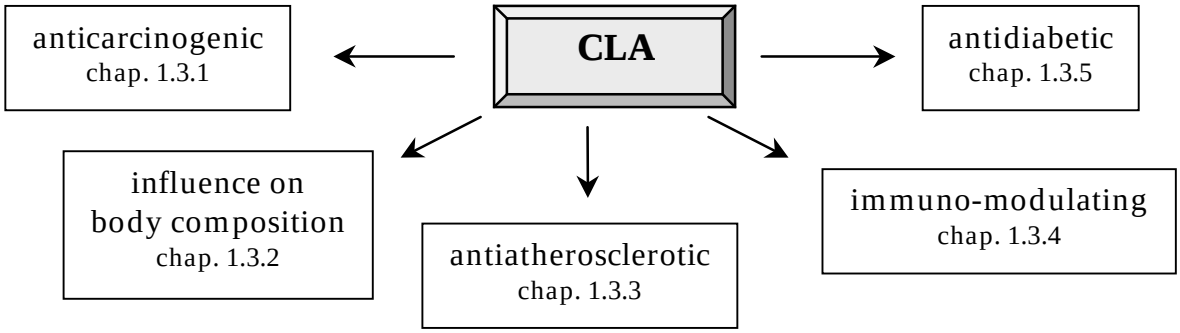


Figure 3 Physiological effects of CLA

1.3.1 Effects on carcinogenesis

CLA have shown anticarcinogenic effects in various cancer models, as chemically induced skin, forestomach and colon cancer, prostate and mammary tumorigenesis. Rats and mice which were fed CLA showed a reduced tumor incidence and tumor progression and also less metastasis than control animals. The protective effects were dose-dependent for dietary levels of CLA up to 1% of diet (BELURY, 1995; WHIGHAM *et al.*, 2000). Anticarcinogenic effects of CLA were also found *in-vitro* on human cancer cell-lines, where cell growth was inhibited by addition of CLA to the growth medium (DURGAM AND FERNANDES, 1997). A recent study using CLA-enriched butter, which contained the natural isomer distribution (about 85% 9c11t-C18:2 of total CLA) showed also anticarcinogenic effects (IP *et al.*, 1999a), suggesting that 9c11t-C18:2 is the active isomer. An inverse correlation between the number of terminal end buds - the primary sites for chemical induction of mammary tumors in the rat - and CLA supplementation was found. This could reduce the incidence of breast cancer (BANNI *et al.*, 1999a).

Current research in this field determined possible mechanisms by which CLA may be acting. Recent studies reported that CLA led to growth inhibition and differentiation of normal rat epithelial cells in primary culture, by reducing desoxy ribonucleic acid (DNA) synthesis and inducing apoptosis (IP *et al.*, 1999b; IP *et al.*, 2000). As another possibility of action, the relationship between CLA and eicosanoid synthesis was often mentioned. Tumor cell growth appeared to be influenced by lipoxygenase and cyclooxygenase products, e.g. PGE_2 (WELSCH, 1987). It was reported that the PGE_2 level is positively related with tumor promotion and

development. In experimental animal studies, dietary CLA led to the reduction of PGE_2 synthesis (LIU AND BELURY, 1998; WHIGHAM *et al.*, 2001).

There may be a link between CLA and peroxisome proliferator-activated receptors (PPAR) with regard to anticancer mechanisms. Experiments on Sprague-Dawley rats and SENCAR mice showed that CLA could be able to activate PPARα and γ (BELURY *et al.*, 1997; MOYA-CAMARENA *et al.*, 1999a; MOYA-CAMARENA *et al.*, 1999b). Hepatic mRNA and protein levels of several enzymes, known to be linked to peroxisome proliferation (acyl Coenzyme A, Cytochrome P450 4A1, Fatty Acid Binding Protein), were found at elevated levels. Using a human cell model only a binding to the PPAR was observed. Regarding these differences between species the influence of CLA on PPAR activation is inconclusive at this moment and has to be further examined (MOYA-CAMARENA AND BELURY, 1999).

1.3.2 Influence on body composition

CLA given as a dietary supplement showed the ability to reduce fat to lean body mass in studies on pigs, mice and rats (OSTROWSKA *et al.*, 1999; PARK *et al.*, 1997; WEST *et al.*, 1998), whereas mice were found to be the most responsive of the tested animals. It was suggested that CLA might be used to reduce fat mass and might be effective against obesity.

Comparing lean rats and obese Zucker rats, the supplementation with CLA reduced retroperitoneal and inguinal fat pad weights only in lean rats (SISK *et al.*, 2001). It seems that the reduction of body fat is due to the 10t12c-C18:2 as recently shown by PARK *et al.* (1999b). Also, the 9c11t-C18:2 was suggested to be associated with a growth stimulation and an improved food efficiency (COOK *et al.*, 1999). Changes in body composition could be affected by reduced fat deposition and increased lipolysis in adipocytes. Enhanced fatty acid β-oxidation was described in muscle cells and adipocytes. Moreover energy expenditure was increased (WEST *et al.*, 1998). Recent data reported that dietary CLA decreased rat fat pad size by reducing adipocyte size and not cell number (AZAIN *et al.*, 2000). It was also suggested, that antiobesity effects may appear by inhibiting proliferation of adipocytes, reducing triacylglycerol content and lipoprotein lipase activity and inducing apoptosis in preadipocytes (EVANS *et al.*, 2000).

A recent study carried out in mice to examine further the mechanisms by which CLA reduce body fat revealed drastic changes in body composition. Mice showed an important reduction of white adipose tissue and a disappearance of brown adipose tissue accompanied by a markedly hepatomegaly and an insulin resistance. The leptin level in blood was decreased. Adipose tissue decrease was due to apoptosis in adipocytes, while Tumor Necrosis Factor α as a marker of induced apoptosis in adipocytes increased about 12-fold. The authors discussed that CLA-supplementation resulted in a state resembling lipoathrophic diabetes (TSUBOYAMA-KASAOKA *et al.*, 2000).

Also human studies on this subject have been carried out and focused therefore on possible effects of CLA on body composition - a decrease of body fat and increase of lean body mass and muscles (WHIGHAM *et al.*, 2000). In some studies CLA was supplemented (3g / day to 7,2g / day) in periods from 28 days to 6 months. No significant reduction of body weight or body fat was observed in these studies. Moreover the lean body mass of the CLA-supplemented group was comparable to that of the control. A current study dealt with body composition and energy expenditure. No difference could be observed between the dietary groups (ZAMBELL *et al.*, 2000).

Leptin is supposed to regulate the lipid metabolism through decreasing food intake and increasing metabolic rate. Moreover it was shown to stimulate lipolysis in adipose tissue explants. So it was hypothesized to mediate the ability of CLA to decrease body fat. Therefore a human study (women) was carried out for 9 weeks, to examine effects of CLA-supplementation on circulating leptin and food intake (MEDINA *et al.*, 2000). The plasma leptin level in the first 7 weeks was significantly decreased by CLA-supplementation but it returned to baseline level in the last two weeks. Neither changes in body composition were observed, nor food intake was altered.

In contrast to the results described above, recent studies on antropometry reported small reduction on body fat induced by CLA mixture. A first study was carried out for 12 weeks on overweight or obese humans. In the CLA group mean body weight was reduced by 1.1kg and body fat decreased not significantly by 0.9kg (BLANKSON *et al.*, 2000). The reduction of mean body weight and body fat mass was also observed for healthy exercising humans, who were administrated 1.8g / day CLA for 12 weeks (GUDMUNDSEN *et al.*, 2001). Another study on healthy human compared the effects of

CLA mixture and purified 10t12c-C18:2 at 3.4g/day during 3 months. A tendency to reduce body fat -preferably abdominal fat - was detected in both groups compared to the control trial. Only 10t12c-C18:2 caused an impairment of glucose concentrations, of peripheral insulin sensitivity and of high density lipoprotein (HDL) levels in serum (VESSBY *et al.*, 2001). In conclusion, in humans the efficiency of CLA to influence body composition or reduce obesity should be further evaluated.

1.3.3 CLA and atherosclerosis

Another effect described by feeding CLA to animals was an inhibition of cholestorol-induced atherosclerosis. CLA decreased significantly total and low density lipoprotein (LDL) cholesterol in rabbits and protected against arterial lipid accumulation (LEE *et al.*, 1994). Recent studies on the same animal model showed a 30% decrease of atherosclerotic lesions (KRITCHEVSKY *et al.*, 2000). Using a hamster-model only 10t12c- and not 9c11t-C18:2 decreased triacylglycerols, total cholesterol and non HDL-cholesterol (DE DECKERE *et al.*, 1999; GAVINO *et al.*, 2000). In the rat model (adult male Sprague-Dawley rats) the feeding of high CLA diets containing 3 and 5 % of CLA mixture in the diet, reductions of LDL and HDL cholesterol were detected (STANGL, 2000). On the contrary, MUNDAY *et al.* (1999) reported an increase of aortic fatty streaks in C57BL/6 mice, but the serum triacylglycerol level was reduced and the HDL to total cholesterol ratio was increased. A 6 week study on female swine showed pro-atherosclerotic effects after feeding a CLA mixture. Very low density lipoprotein (VLDL) and LDL cholesterol were increased by CLA. Moreover the LDL to HDL cholesterol ratio was significantly upregulated (STANGL *et al.*, 1999). In conclusion, the influence of CLA on atherosclerosis is controversial and has to be further investigated, also concerning the evaluation on species-depending differences.

Heart diseases induced by atherosclerosis are identified as the first case of human mortality. Therefore the possible relationship between CLA and atherosclerosis in human has a large research interest. A human study was carried out to look on effects of CLA treatment on plasma lipoproteins and tissue fatty acid composition. In a 63-days study healthy normolipidemic women were supplemented with CLA. Blood cholesterol and lipoprotein levels were not altered by the CLA treatment. 9c11t-C18:2 was incorporated in plasma at only 4,23% of ingested CLA. Contrary to

animal studies, short term nutrition trials with CLA in humans did not change lipoprotein levels and seem to be ineffective in the prevention of atherosclerosis (BENITO *et al.*, 2001a).

But CLA were described to induce an antithrombotic effect. The effects of several CLA isomers on human platelet aggregation were examined *in-vitro* (TRUITT *et al.*, 1999). A CLA mixture was tested in comparison to linoleic acid using platelet aggregation agents as arachidonic acid, collagen and thrombin. CLA was effective to inhibit platelet aggregation. To investigate the influence of CLA on platelet cyclooxygenase and lipoxygenase activities, formation of [^{14}C]-thromboxane B_2 (TXB_2) and [^{14}C]-12hydroxyeicosatetranoic acid (HETE) was measured. All tested CLA-isomers inhibited TXB_2, whether the HETE level was unchanged.

An *in-vivo* experiment on platelet function was carried out in humans. But the daily intake of 3,9g of CLA for 63 days had no influence of *in-vitro* platelet aggregation. The authors concluded that short–term consumption of CLA did not exhibit antithrombotic properties in humans (BENITO *et al.*, 2001b).

1.3.4 Immuno-modulating activities

Some studies indicate that CLA could protect against immune-induced cachexia (growth suppression or weight loss). Usually, an enhanced immunological function leads to a decreased growth. Feeding CLA protected against these catabolic effects of such an immune stimulation (COOK AND PARIZA, 1998). MILLER *at al.* (1994) described that dietary CLA in mice prevent endotoxin-induced growth suppression compared to a control group. The authors hypothesized a relationship between CLA and possible changes in the interleukin-1 level. The capacity of CLA to change interleukin production was later observed by HAYEK *et al.* (1999).

CLA may also influence the allergic reactions-pathways, by alteration of serum immunoglobulins (Ig). IgA, IgG and IgM were increased, whereas IgE was decreased (SUGANO *et al.*, 1998). IgA, IgG and IgM are implicated in the defense metabolism against virus or bacteria. As IgE is related to allergic reactions, its diminution is desired.

The influence of CLA on the immunological function may have implications for human health. A study in humans examined possible changes in immune status,

thereby the number of circulating white blood cells, granulocytes, monocytes, lymphocytes and lymphocyte proliferation were tested. No changes in the investigated parameters were observed (KELLEY *et al.*, 2000). In an other experiment the specific antibody production after Hepatitis B vaccination was tested depending on a CLA treatment. The antibody formation after CLA-treatment (9c11t-C18:2 / 10t12c-C18:2; 50 / 50) was significantly higher (MOHEDE *et al.*, 2001), suggesting that CLA could be able to enhance immune function in humans.

1.3.5 CLA and diabetes

The discussion concerning antidiabetic effects of CLA is controversial. An experiment using Zucker diabetic fatty rats showed a normalization of impaired glucose tolerance and improved hyperinsulinemia induced by CLA. Therefore they could be effective in the prevention and treatment of non-insulin-dependent diabetes mellitus (HOUSEKNECHT *et al.*, 1998). To get information about the active isomer, a second experiment was carried out with the same animal model using CLA mixture and a 9c11t-C18:2 enriched butter (90.5 % 9c11t-C18:2 of total CLA). Only the CLA mixture, not 9c11t-C18:2, reduced improved glucose tolerance. Furthermore an improved insulin action in muscle was observed (RYDER *et al.*, 2001).

A recent study showed inverse effects. C57BL / 6J mice fed a CLA enriched diet developed a state resembling lipoatrophic diabetes, with a marked insulin resistance (hyperinsulinemia) (TSUBOYAMA-KASAOKA *et al.*, 2000). The hyperinsulinemia was reversible by continuous infusion with leptin, suggesting leptin may be antagonistic to the CLA induced effects.

Only few studies were carried out on possible antidiabetic effects of CLA using different animal models. One reason for the opposite results reported above could be a difference in the metabolic pathways of the two animal species.

Type-II-diabetes (diabetes induced by insulin resistance) represents one of the diseases of the affluent society and become more and more important in human. The effects of dietary CLA on insulin resistance in humans are unknown. Therefore a nutritional study was carried out on overweight middle-aged men with insulin resistance syndrome receiving a CLA mixture or 10t12c-C18:2 (RISERUS AND VESSBY, 2001). Glucose metabolism remained unchanged for the CLA-mixture trial. The treatment with pure 10t12c-C18:2 increased the insulin resistance and glycemia. It

can be concluded that the two different CLA treatments showed diverging effects. The influence of CLA on type II-diabetes is not clear, therefore much more results concerning this subject are needed.

1.3.6 Biologically active isomers

There is less knowledge about the active isomer or active isomers. It has been suggested that 9c11t- and 10t12c-C18:2 are the active ones. From all nutritional experiments carried out so far, it is very difficult to determine the active isomer(s), because of the current use of the above described CLA mixtures. It can not be excluded that the isomers will exert synergistic or antagonistic effects. However, feeding of a CLA mixture will complicate data interpretation. Often a correlation of the observed physiological effects with a single isomer are not possible. Moreover studies using different CLA mixtures are not absolutely comparable because of the variability in CLA isomer composition. The recent findings suggest many different action pathways for CLA, and it was shown that different isomers did not lead to the same effects. Today, much more data on action of single isomers is needed in order to understand and evaluate the physiological effects of each of them. Additionally, the use of CLA as free fatty acid in the diet had to be investigated in comparison to CLA as triacylglycerols (TAG), to exclude an influence on intestinal absorption or incorporation of CLA. TAG are the natural nutritional lipid components in food.

1.4 CLA and fatty acid metabolism

1.4.1 Incorporation in tissue

The incorporation of CLA isomers into various biological matrices of animals, e.g. tissues and blood plasma, after CLA administration in animals has been already studied (YURAWECZ *et al.*, 1999). The abundant naturally occurring CLA isomer 9c11t-C18:2 was detected in human plasma lipids, adipose tissue and human milk (FRITSCHE *et al.*, 1997; PARK *et al.*, 1999a). Thereby the incorporated CLA content depends on the dietary habits and could be increased by the intake of food rich in CLA (BRITTON *et al.*, 1992).

The CLA level in tissues was positively correlated with the amount of CLA fed to the animals (IP *et al.*, 1991). The results of a feeding study showed a greater incorporation

of CLA in TAG than in phospholipids (PL) (IP *et al.*, 1996). It was hypothesized that the spatial structure of the molecule containing the conjugated double bond and additionally one double bond in *trans* configuration was the reason for its incorporation behavior. Indeed, the carbon chain of CLA can be superimposed with the three-dimensional structure of oleic acid. CLA is less curved than linoleic acid. As a consequence, the incorporation of CLA seems to be similar to that of oleic acid and occurs preferentially into TAG (BANNI *et al.*, 1999b).

Moreover the incorporation of single CLA isomers was found to be different: The 9c11t-C18:2 was preferably accumulated in liver lipids (BELURY AND KEMPA-STECZKO, 1997; Kramer *et al.*, 1998), whereas the 10t12c-C18:2 seems to be better incorporated into spleen lipids (TUREK *et al.*, 1998). BELURY AND KEMPA-STECZKO (1997) reported that CLA was incorporated at the expense of linoleic acid. Furthermore the incorporation of a CLA mixture into individual liver lipid classes was investigated. The distribution of individual CLA isomers varied little in the different lipid classes. In each lipid class 9c11t-C18:2 was preferably incorporated (KRAMER *et al.*, 1998).

The tissue incorporation of individual CLA isomers is different suggesting either a selective uptake of the individual isomer at high isomer concentration or a rapid metabolism or a discrimination in the uptake at low concentration.

1.4.2 Fatty acid metabolism

- **Conversion of CLA into long chain PUFA**

Linoleic acid and α-linolenic acid are important precursors of several long chain PUFA, which are synthesized *in-vivo* by successive desaturation and chain-elongation (figure 4). Some of these fatty acids are essential because of their metabolism and their subsequent biological activities (SPRECHER *et al.*, 1995).

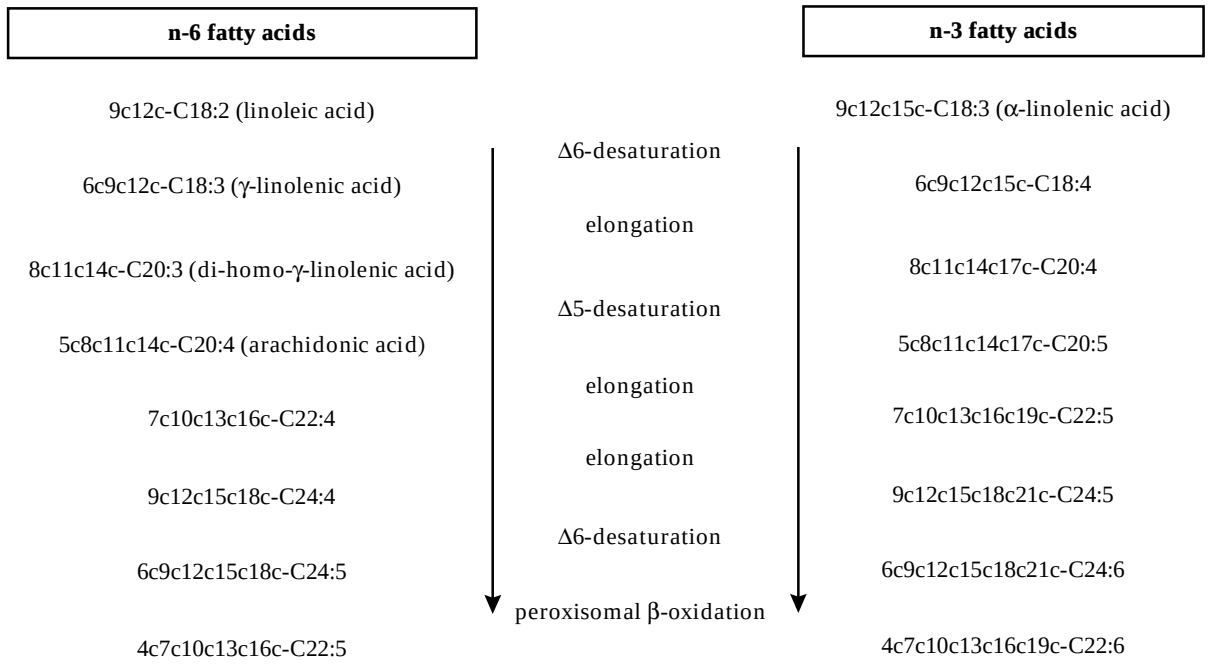


Figure 4 Metabolic pathway of essential fatty acids ((SPRECHER *et al.*, 1995))

One of the most important fatty acids is arachidonic acid, as one of the precursors of the eicosanoids. Arachidonic acid is formed from linoleic acid in three steps. First linoleic is desaturated in position Δ6 to give γ-linolenic acid, which is chain elongated into di-homo-γ-linolenic acid. In the final step di-homo-γ-linolenic acid is Δ5-desaturated to give arachidonic acid.

It was reported that some geometrical isomers of linoleic acid and α-linolenic acid, e.g. 9c12t-C18:2 or 9t12c-C18:2 were desaturated and elongated by the same enzymes as linoleic acid (BERDEAUX *et al.*, 1998b; CHARDIGNY *et al.*, 1997). Regarding the published results, the enzymatic conversion itself and the conversion rate are influenced by the number and the position of the *trans* double bonds in the fatty acid molecule. The examined TFA were as well or less converted in their corresponding desaturation and elongation metabolites than the natural homologues (LOI, 1999).
As mentioned before it was often suggested that CLA may interact with the arachidonic acid metabolism perhaps by competing with linoleic acid. Conjugated C18:3, C20:3 and C20:4 fatty acids, as corresponding metabolites were identified in animals fed CLA.

In a first study SEBEDIO *et al.* (1997) detected conjugated C20:3 and C20:4 fatty acids in the liver and adipose tissue. Male Wistar rats were fed a fat free diet for two weeks and afterwards forced fed with a CLA mixture containing mainly 9c11t-C18:2 and

10t12c-C18:2 for six days while maintaining the fat free diet. Conjugated C20:3 and C20:4 were identified as respective long chain fatty acid metabolites of 9c11t-C18:2 and 10t12c-C18:2. A second experiment was carried out by BANNI *et al.* (1999a) under normal nutritional conditions. Female Sprague Dawley rats were fed a standard diet enriched with CLA mixture for one month. They found conjugated C18:3 and C20:3 in liver and mammary tissue, but no conjugated C20:4 was detected. Recent data on this subject using a CLA enriched butterfat, which contained the natural isomer composition (mainly 9c11t-C18:2), described the formation of conjugated C18:3, C20:3 and C20:4 (BANNI *et al.*, 2001). The incorporation of the metabolites of CLA were found primarily in triacylglycerols. Furthermore the level of conjugated C20:3 fatty acid in the liver was about 4-times higher than those of conjugated C18:3 and C20:4. Similar results were discussed in a feeding study on rats using pure CLA isomers SEBEDIO *et al.* (2001). For 9c11t-C18:2 more conjugated C20:3 than C18:3 were found in the liver, whereas the inverse effect was reported for 10t12c-C18:2. These in-vivo results were confirmed by an *in-vitro* Δ6-desaturase study carried out on rat liver microsomes using radiolabeled CLA in isomeric mixture as substrate (BELURY AND KEMPA-STECZKO, 1997). CLA were converted into C18:3 fatty acids suggesting that the metabolites were the respective Δ6-desaturation products of CLA.

These various results reinforce the hypothesis that CLA could be metabolized *in-vivo* into long chain PUFA using the same pathway as linoleic acid. The hypothetical metabolic pathway of CLA into conjugated C20:4 is shown in figure 5.

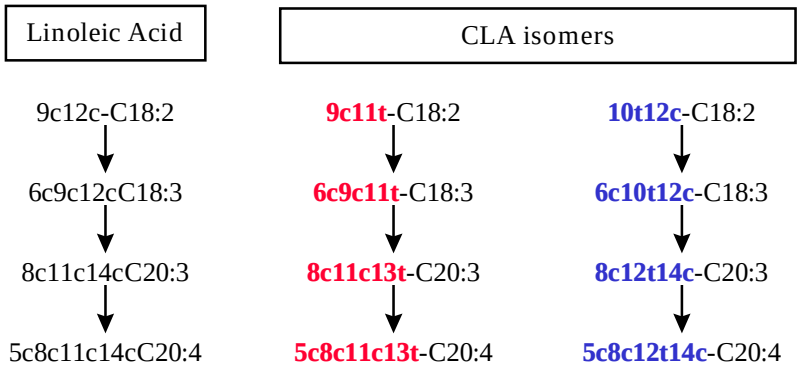


Figure 5 Hypothetical metabolic pathway of 9c11t-C18:2 and 10t12c-C18:2 compared to linoleic acid.

➢ Changes in tissue fatty acid composition induced by CLA

It was suggested that CLA may alter the fatty acid metabolism. A conversion of CLA into conjugated C20:4 fatty acid by successive desaturation and elongation, as hypothesized above, may have consequences especially on the metabolism of essential fatty acids.

It was shown that CLA modified the fatty acid composition in liver of rats and mice. CLA was incorporated into TAG at the expense of linoleic acid, oleic and arachidonic acid were significantly decreased compared to the control. The linoleic acid content was reduced after a CLA containing diet (BELURY AND KEMPA-STECZKO, 1997). A decrease of γ-linolenic acid and di-homo-γ-linolenic acid additionally to arachidonic acid was reported in mammary tissue, but not in the liver after feeding CLA (BANNI *et al.*, 1999a; BANNI *et al.*, 2001).

An important increase in the levels of polyunsaturated C22 fatty acids after supplementation with pure 10t12c-C18:2 in the liver PL fraction was observed by SEBEDIO et al. (2001). Moreover they reported a decrease of 9c-C16:1 and 9c-C18:1 induced by 10t12c-C18:2 suggesting an inhibition of the Δ9-desaturase activity.

In the liver, CLA modified the membrane fatty acid composition by reducing monounsaturated fatty acids resulting from the decreased activity of the stearoyl-coenzymeA desaturase enzyme (SCD) (LEE *et al.*, 1998). A direct inhibition of Δ9-desaturation of C18:0 by 10t12cC18:2 was reported in rat liver microsomes (BRETILLON *et al.*, 1999). CLA supplementation of mice revealed an inhibition of the SCD messenger ribonucleic acid (mRNA) expression (LEE *et al.*, 1998). Further investigation showed that the 10t12c-C18:2 induced these effects (CHOI *et al.*, 2000; PARK *et al.*, 2000). Using a human cell line (Hep2G), the 10t12c-C18:2 inhibited the SCD activity observed by a diminution of the conversion level of C18:0 into 9c-C18:1, but the SCD mRNA expression remained unchanged (CHOI *et al.*, 2001). This suggested human SCD activity is regulated mainly by a posttranslational mechanism.

Regarding these results it might be possible that changes in the fatty acid metabolism after CLA supplementation are mainly induced by 10t12c-C18:2 and not by 9c11t-C18:2.

1.4.3 Modification of the arachidonic acid metabolism

- **influence of CLA on eicosanoid pathway**

Di-homo-γ-linolenic acid, arachidonic acid and eicosapentaenoic acid, mainly derived from essential fatty acids, serve as precursors for a group of bioactive compounds, the eicosanoids. The term eicosanoids combines the classes of prostaglandins, thromboxanes and leukotrienes (figure 6).

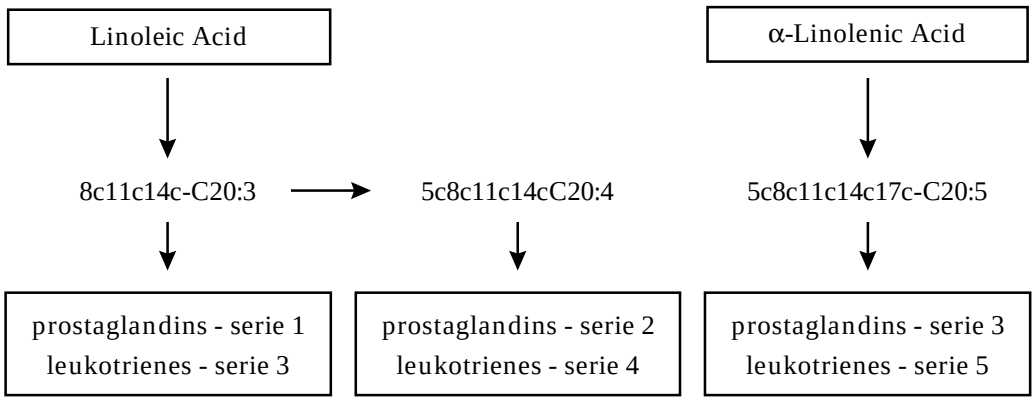


Figure 6 Synthesis of eicosanoids from PUFA

Eicosanoids are produced *via* two different enzymatic pathways, the cyclooxygenase and the lipoxygenase pathway. Prostaglandins and thromboxanes are formed by cyclooxygenation and leukotrienes are formed by lipoxygenation. The formation of prostaglandins and thromboxanes derived from arachidonic acid *via* the cyclooxygenase pathway is shown in figure 7.

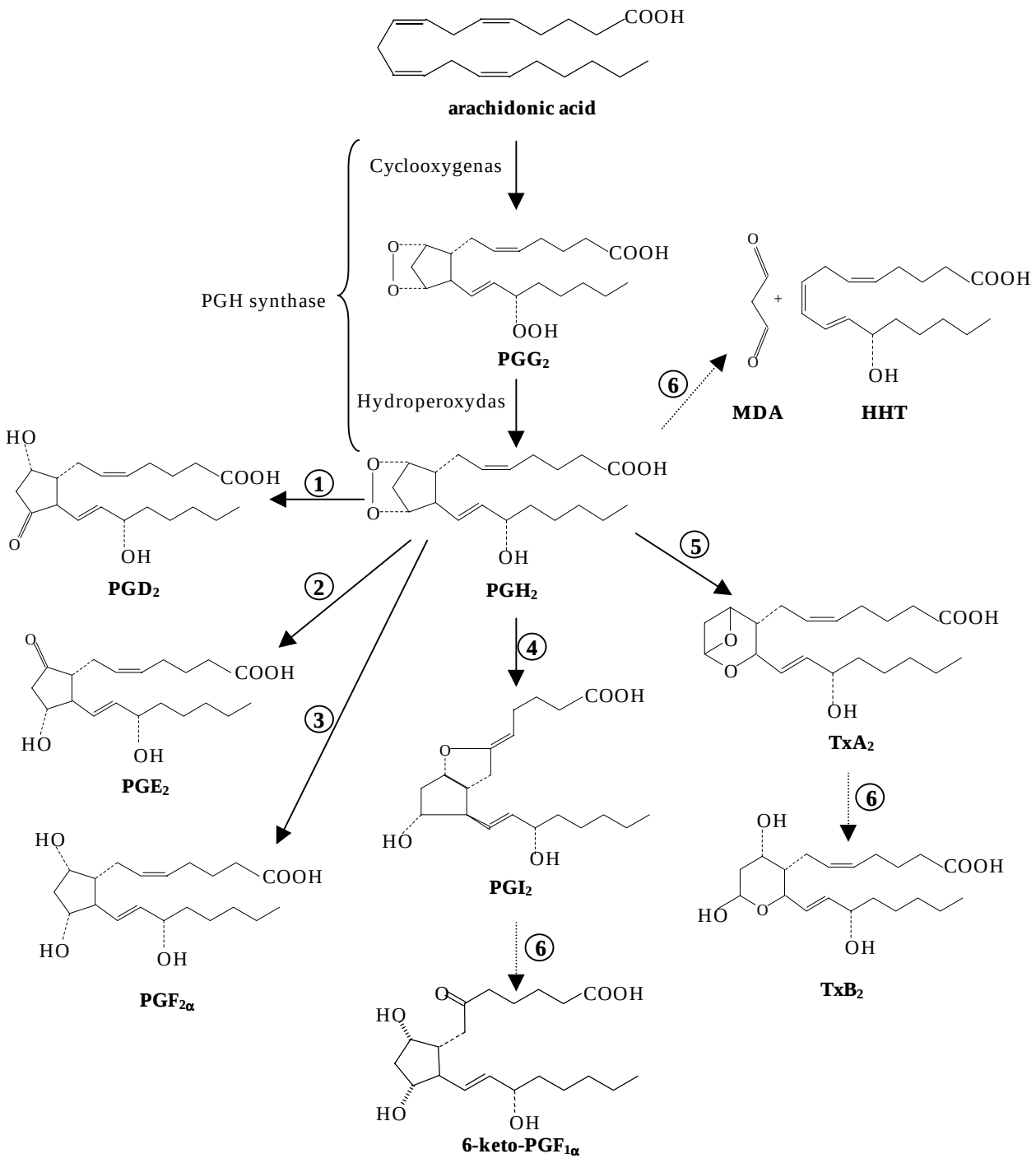


Figure 7 Cyclooxygenation of arachidonic acid

The physiological activities of eicosanoids are of a great diversity. They influence inflammatory process, immune response, thrombosis and platelet function, control the contraction of smooth muscles and the bone metabolism. They act as second messenger of hormones, and also as hormone-like products in the direct

environment of their formation (autocrine effects) and have also influence on the neighbor cells (paracrine effects). As they are desactivated rapidly, their duration of action is limited (KOOLMAN AND RÖHM, 1994).

CLA were able to reduce the production of eicosanoids derived from arachidonic acid. Various studies showed the decrease of PGE_2 release after feeding of CLA to animals. For example, CLA lowered the ex-vivo production of PGE_2 in bone organ culture of rats (tibia and femur) (LI AND WATKINS, 1998). Also the reduction of serum PGE_2 and splenic leucotriene B_4 (LTB_4) in CLA supplemented rat was reported (SUGANO *et al.*, 1997; SUGANO *et al.*, 1998). CLA decreased PGE_2 during a hypersensivity type I reaction. Dietary CLA treatment significantly reduced antigen-induced histamine and PGE_2 release in pigs (WHIGHAM *et al.*, 2001).

Experiments testing the anticarcinogenic activity of CLA revealed the relationship with prostaglandin synthesis, mainly interaction with PGE_2. Results from BELURY *et al.* (1996) demonstrated the efficacy of CLA in reducing 12-O-tetradecanoylphorbol-13-acetate (TPA) induced skin tumors. In a study on cultured keratinocytes, CLA decreased TPA-induced PGE_2-synthesis compared to linoleic acid (LIU AND BELURY, 1998). A recent study showed that dietary CLA significantly decreased PGE_2-synthesis in the epidermis, which suggested that CLA modulated TPA-induced tumor-promotion by a mechanism involving PGE_2 (KAVANAUGH *et al.*, 1999).

To investigate the mechanisms by which CLA reduce prostaglandins, a recent study determined the ability of CLA and specific isomers to alter the activity of a cyclooxygenase enzyme, prostaglandin H synthase (PGHS) (BULGARELLA *et al.*, 2001). Ram seminal vesicle microsomes were incubated with arachidonic acid, which was converted into prostaglandin H_2 (PGH_2). PGHS activity was measured by oxygen-consumption. CLA were added in various concentration as inhibitor. It was shown, that the different isomers of CLA inhibits PGHS differently, where 9c11t-C18:2 was the most effective one and 9c11c-C18:2 was the least. Regarding to these results, CLA seem to modulate prostaglandin synthesis by inhibiting the activity of the central enzyme in the prostaglandin synthesis, the PGHS. As a consequence of the inhibition of PGHS the synthesis of prostaglandin D_2 (PGD_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), PGE_2, PGI_2 and TXA_2 is diminished (BULGARELLA *et al.*, 2001).

- **influence on enzymatic and non-enzymatic lipid peroxidation**

Another indication that CLA might modulate eicosanoid biosynthesis is their influence on lipid peroxidation. CLA seem to be easily oxidized and it has been suggested that they may influence lipid peroxidation reactions *in-vivo*, as CLA-induced cytotoxicity in cancer cell lines was associated with increased lipid peroxidation (O'SHEA *et al.*, 1999). 8-iso $PGF_{2\alpha}$ (a major F_2 isoprostane) and 15-keto-dihydro-$PGF_{2\alpha}$ (a major metabolite of $PGF_{2\alpha}$) are important indicators of lipid peroxidation *in-vivo*. They are formed during non-enzymatic and enzymatic catalyzed oxidation of arachidonic acid, respectively. To investigate the effects of CLA on lipid peroxidation, healthy humans received a daily CLA mixture supplementation during 3 months (BASU *et al.*, 2000a). Non-enzymatic and enzymatic lipid peroxidation was measured by determination of urinary levels of 8-iso $PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$. Both substances were significantly increased in the CLA-treated group: CLA were able to upregulate prostaglandin synthesis, which indicates a modulation of cyclooxygenase-catalyzed arachidonic acid oxidation in humans. The relative increase of the 8-iso $PGF_{2\alpha}$ level was higher than for 15-keto-dihydro-$PGF_{2\alpha}$ suggesting that CLA probably have more effect on the direct non-enzymatic conversion of arachidonic acid. The results were confirmed in a second study using a population of obese volunteers. The supplementary measured urinary levels of 2,3-dinor-thromboxane B_2 as another cyclooxygenase product formed from arachidonic acid remained unchanged, indicating that the production of thromboxane was not affected in humans by nutritional CLA (BASU *et al.*, 2000b).

2 RESEARCH OBJECTIVES

Research on CLA increased sharply over the last two decades. Most experiments focused on the physiological effects induced by CLA, mainly those on cancer and on body composition. However little is known about the metabolism of the CLA and the mechanism(s) by which they may act. In particular the effect of CLA on eicosanoid production should be further investigated, because of their importance as hormone-like molecules in the regulation of important biological functions. Also the action of CLA at the cellular levels has to be elucidated. Further research has to be carried out on the action of CLA in human.

Dairy products are the major source for natural CLA in human nutrition. Many factors (e.g. seasonal, dietary, breeding) were identified in the past to influence the CLA content in milk. But less is known about the behavior of CLA in different foodstuffs, regarding the influence of food processing, storage and culinary utilization on the CLA content. Factors which could increase or decrease the CLA content in the final foodstuff have to be further examined. The stability or the risk of oxidative damage of CLA in the whole food, including the matrix, have to be evaluated.

↳ One objective of this work was to get recent information about the influence of food processing technologies on the CLA content and on the CLA isomer composition. Among the dairy products French Emmental cheese was selected as it is also used cooked. Moreover the manufacturing process of cheese allowed the modification of different fabrication parameters. For this purpose, cheese was prepared in a pilot-scale unit using different temperatures for milling and cooking and different fermentation microorganisms. The research was focused to detect any decrease in CLA by oxidation or an increase induced by microbial fermentation processes. The CLA content and the isomer composition was studied at different processing stages. At least, the final ripened Emmental cheese was used in several culinary models of food to study the influence of cooking and grilling on the CLA content.

↳ The metabolism of CLA and the influence of CLA on the eicosanoid pathway was the other subject of this work. The conversion of CLA into long chain PUFA (conjugated C18:3 and C20:3 fatty acids) was elucidated *via in-vitro* assays using rat liver microsomes, the cellular structure involved in the synthesis of PUFA, which contains desaturation and elongation enzymes. In a first step, it was examined if 9c11t-C18:2 and 10t12c-C18:2 can be desaturated *in-vitro* in position Δ6 into the corresponding conjugated C18:3 metabolites. In the second step the *in-vitro* elongation of the conjugated 18:3 metabolite of 9c11t-C18:2 into conjugated C20:3 was investigated and compared to its natural homologue γ-linolenic acid. In the last step the Δ5-desaturation of the conjugated C20:3 metabolite of CLA (9c11t-18:2) into conjugated C20:4 was compared to that of di-homo-γ-linolenic acid into arachidonic acid. The research was carried out for the metabolites of 9c11t-C18:2, as it is the naturally occurring CLA isomer in food and is consumed regularly by humans. Therefore, its metabolic transformations seem to be very important and are therefore further investigated.

↳ The study on chain-elongation and Δ5-desaturation of the conjugated C18:3 and C20:3 metabolites of 9c11t-C18:2, needed the synthesis of these molecules. Only pure isomers gave the possibility to get more information about the physiological effects of CLA and their metabolites and to clarify the metabolic pathway, especially when radiolabeled products are used. Therefore the stereo-selective synthesis of (8Z,11Z,13E)-eicosatrienoate (8c11c13t-C20:3) and the (6Z,9Z,11E)-octadecatrienoate (6c9c11t-C18:3) was performed in multistep synthesis from commercially available reagents.

↳ The influence of CLA on prostacyclin and thromboxane synthesis *in-vivo* was investigated in order to establish a possible link between dietary CLA and the modification of the eicosanoid pathway. TXA_2 and PGI_2 are important eicosanoids derived from arachidonic acid, which are implicated in platelet function. TXA_2 is produced by platelets, whereas PGI_2 is formed in the endothelial cells of the vascular system. Therefore a study was carried out on rats fed pure single CLA isomers (9c11t-C18:2 and 10t12c-C18:2). The use of isolated isomers allowed to study the specific effect of each isomer on the metabolism. The influence of CLA feeding on TXB_2 (stable metabolite of TXA_2) content in serum and 6-keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2) content in plasma was examined. Furthermore the effect of CLA

on PGI_2 release from the endothelial cells of the aorta was investigated. Therefore the aorta of the rats was incubated and 6-keto-$PGF_{1\alpha}$ contents were measured. The aorta was chosen for incubation as it is the greatest of the blood vessels and easy to withdraw. The determination of the fatty acid profiles of one part of the aorta and platelets should reveal if eventual modifications in eicosanoids can be related to changes in the fatty acid profile. In the same time the analysis of the fatty acid profiles of the aorta, platelets and different fractions of plasma lipids determine the CLA incorporation in the different tissues.

3 MATERIAL AND METHODS

3.1 Influence of processing on the CLA content in cheese

The influence of processing on the CLA content in French Emmental cheese included experiments on the manufacturing of cheese, interactions with the culinary utilization during cooking or grilling, and the fabrication of processed cheese.

3.1.1 Fabrication of cheese samples

- **manufacturing**

The fabrication of French Emmental cheese was carried out in pilot-scale at the ITFF (Institut Technique Français du Fromage, La Roche sur Foron, France). An Emmental cheese using raw (untreated) or heated milk was manufactured according to the protocol, shown in figure 8.

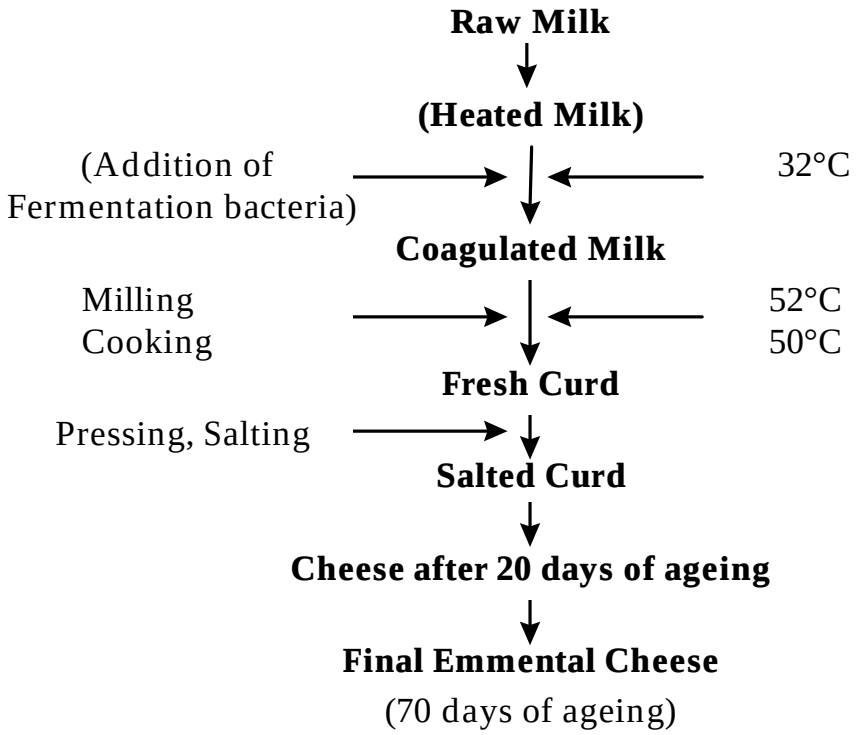


Figure 8 Normal manufacturing protocol for the preparation of French Emmental cheese.

Milk, standardized to 3.6 % fat content, was incubated for 30min at 32°C in the presence of *Streptococcus thermophilus* and *Lactobacillus thermophilus*. Afterwards, the

coagulation was induced by rennet followed by a cutting procedure of coagulated milk. The coagulated milk was milled for 30min at 52°C dipped for about 20-25min and cooked at 50°C. Pressing was carried out for 4h to obtain the fresh curd. The fresh curd was salted and left for ripening at 21°C and 80-82% of humidity for 70 days. For cheeses prepared from heated milk, raw milk was heated at 68°C for 20sec, which eliminated about 65-70 % of the natural bacteria of the milk, so it was necessary to add a *Propionibacterium* sp. as starter culture.

For the experiment two different cheeses using raw and heated milk were produced according to the protocol described above. Samples were taken at the different steps of the fabrication process to determine the CLA-content. Cheeses made from raw and heated milk were processed at two varying temperatures during milling and cooking (milling 48°C/ cooking 48°C and milling 50°C/cooking 50°C) compared to the normal fabrication conditions (milling 52°C/cooking 50°C), to investigate possible changes in the CLA content in the final ripened cheese. All cheeses were prepared in triplicate.

Also two other strains of *Propionibacterium* sp. were used, which possessed different lipolytic activities (high and low lipolytic activity) compared to the normally used *Propionibacterium* sp. Therefore the milk was microfiltrated before utilization to eliminate about 85% of the natural bacteria. These different *Propionibacterium* spp. were used to examine their ability to produce CLA. The study using different *Propionibacterium* spp. was carried out in two repetitions.

Four bulk milk samples, collected in a period of two weeks at the and of April, were used to produce the different cheeses, corresponding to four days of manufacturing. Each day one series of cheese from raw and heated milk, including cheeses prepared at the different milling and cooking temperatures, were prepared. The cheeses using different *Propionibacterium* spp. were manufactured the same day. All samples were prepared over a two week period.

- **preparation of dishes**

To test the influence on the CLA content during culinary utilization, the Emmental cheese was used in different dishes. The preparation of the dishes was carried out at the ITFF-Maison du Goût (Bourg-en-Bresse, France) under standardized repetitive conditions. Three typical French dishes were chosen to determine the stability of CLA in cheese during homemade cooking. The types of dish were chosen to expose

the cheese to moderate or high temperatures for variable duration. All dishes were prepared from the same cheese, grated before use. One sample of the dish was analyzed as raw material, non-cooked, to know the CLA content before processing:

- «purée de pommes de terre gratinée (Gratin)»: mashed potatoes were covered with 9g of grated cheese and grilled at 225°C for 21min.
- «Sauce Béchamel»: Industrial produced Béchamel sauce was mixed with 7.5g of grated cheese. The sauce was slightly heated for 10min up to 78°C until melting of cheese.
- «Fondue Savoyarde»: The grated cheese was melted in white wine for 5min at 98°C.

The study was done using two cheese samples, one manufactured in a pilot-scale unit and one industrially produced Emmental. The different dishes were prepared in triplicates.

➢ **fabrication of cheese spread**

The fabrication of the cheese spread was carried out at the ITFF (La Roche sur Foron, France) under standardized repetitive conditions. Emmental cheese was milled, mixed with melting salts and homogenized. The mixture is melted in vacuum-vessels, additionally heated for 5 or 10 min and packed. The cheese spread was prepared using two different cheeses, one cheese produced in a pilot-scale unit and one industrially fabricated cheese.

Two parameters were studied, the manufacturing time and the processing temperature, as presented in table 2. Samples were fabricated in two repetitions.

Table 2 Sampling of cheese spread

Processing temperature	Additional heating time	number of samples
control (raw material)	-	2
75°C	0min	2
75°C	5min	2
100°C	0min	2
100°C	10min	2

3.1.2 Analysis of the CLA content

- **sample preparation**

Food samples were analyzed by single determination as shown in figure 9. Milk, Sauce Béchamel and cheese spread samples were lyophilized before lipid extraction. All other samples were homogenized by mincing in a kitchen-robot. Lipids were extracted using hexane / diethylether, dried and the solvent was evaporated (SEHAT *et al.*, 1998). Extracted lipids were stored at -80°C until esterification.

An aliquot of extracted lipids was methylated into FAME according to CARREAU AND DUBACQ (1978) with slight modifications. FAME were prepared using a sodium methoxide solution, followed by BF_3 / MeOH. This methylation procedure was carried out at room temperature to avoid isomerization of CLA isomers. FAME were extracted with hexane and stored at -20°C until GC analysis.

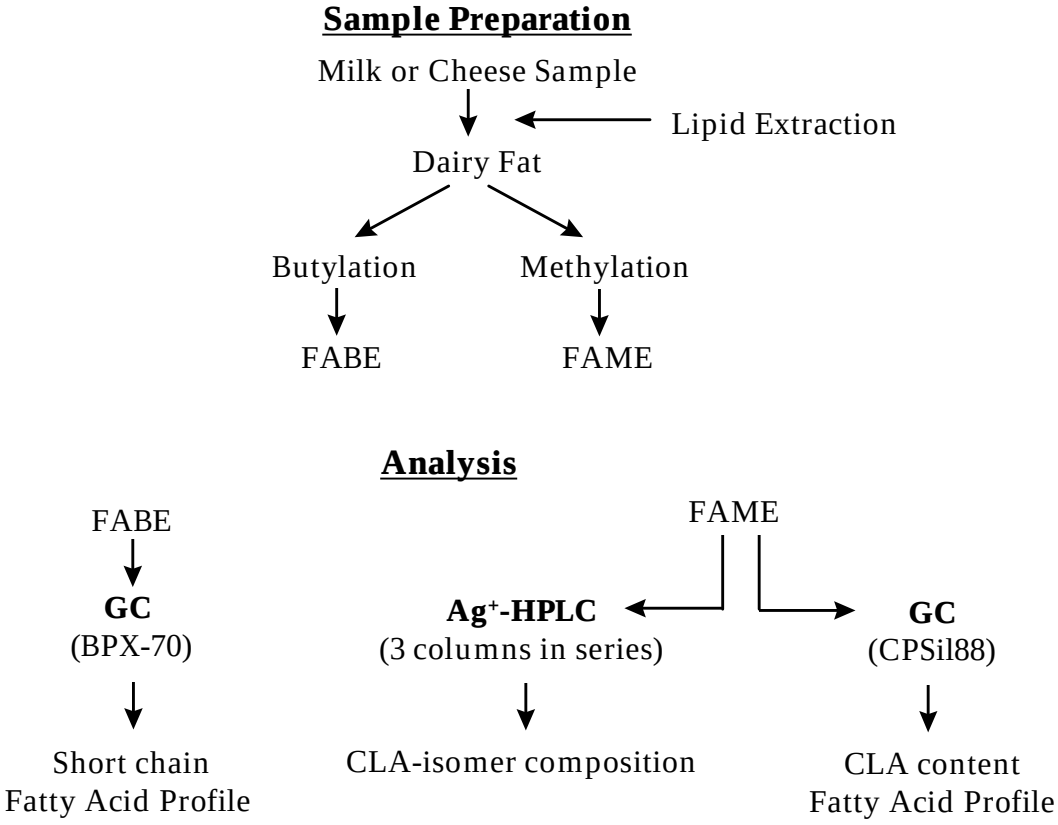


Figure 9 Analysis of CLA in food samples

To determine the complete profile of fatty acid of the samples, another aliquot of the extracted lipids was transformed into fatty acid butyl ester (FABE) using the method of ULBERTH *et al.* (1999). FABE are less volatile than FAME and allow the

determination of short-chain fatty acid (C4:0 up to C10:0). Lipids were butylated at room temperature using a solution of sodium dissolved in butanol. FABE were extracted with hexane and stored at -20°C until GC analysis.
(protocol in the annex, chap. 12.1.1.1-3)

- **analysis of FAME and FABE**

FAME were analyzed by GC. FAME were separated on a high polar capillary column (CPSil™ 88) to determine the fatty acid composition of medium and long chain fatty acids (C14:0 up to C24:0) according to SEHAT *et al.* (1998). Analysis of FABE was carried out by GC using a polar capillary column (BPX-70) to determine the short chain fatty acid profile.

To get further information about the CLA isomer composition of the samples examining the influence of cheese fabrication on the CLA content, FAME were analyzed by Ag^+-HPLC using three columns in series according to RICKERT *et al.* (1999). Ag^+-HPLC was performed at the Institute of Food Chemistry, University of Hamburg.
(analytical procedures in the see annex, chap. 12.1.1.4-5)

- **quantification of CLA contents**

The quantification of the CLA content in the food samples was carried out using C23:0 methyl ester as internal standard using an one point calibration. The internal standard was added before methylation of the aliquot of extracted lipids.
(calculation, see annex, chap. 12.1.1.6)

- **determination of the fatty acid composition**

To obtain the total fatty acid composition of the food samples, integration areas of each fatty acid of FAME and FABE are normalized to the area of C14:0. The results for C4:0 up to C13:0 are taken from FABE to calculate the total fatty acid composition. For all fatty acids of higher chain length, values of FAME are used. Total fatty acid composition was calculated as percent of total fatty acids.
(calculation, see annex, chap. 12.1.1.6)

3.2 *In-vitro* desaturation and Elongation of CLA and CLA metabolites

In-vitro study on the conversion of CLA into conjugated C18:3, C20:3 and C20:4 fatty acids was carried out using microsome preparations freshly isolated from rat liver. The study was divided in three independent experiments, Δ6-desaturation, elongation and Δ5-desaturation.

3.2.1 Radiolabeled compounds

[1-^{14}C]-8c11c14c-C20:3 and [1-^{14}C]-linoleic acid were purchased from New England Nuclear (NEN life sciences, Les Ulis, France). [1-^{14}C]-9c11t- and [1-^{14}C]-10t12c-C18:2 were obtained by total synthesis as reported before (LOREAU *et al.*, 2001). [1-^{14}C]-6c9c12c-C18:3 was a generous gift from the CEA (Commissariat à l'Energie Atomique, Saclay, France). [1-^{14}C]-6c9c11t-C18:3 and the [1-^{14}C]- 8c11c13t-C20:3 were synthesized by total synthesis as described in chapter 5. The radiolabeled fatty acids were diluted with the corresponding unlabeled ones to have a specific activity of 370MBq / mmol.
(for dilution see annex, chap. 12.1.3.1)

3.2.2 Animals and diet

Male Wistar rats of three weeks of age at arrival were housed in individual stainless steel cages in an animal house, maintained at 21±1°C and constant humidity, with a 12 hours light / dark cycle (07:00AM - 07:00PM). Rats were fed *ad libitum* a fat-free semisynthetic diet for two weeks (composition of diet, see annex chap. 12.2.2.1). Fat free diet was given to increase the desaturation activity, to avoid an effect of dietary fatty acids on the substrate conversion by microsomal desaturation or elongation enzymes, and thus to induce an essential fatty acid deficiency (MARCEL *et al.*, 1968; BRENNER, 1981). This was controlled by analyzing the fatty acid composition of microsomes. Therefore, microsomal lipids were extracted by chloroform and methanol and the lipids transmethylated and analyzed as described in chap. 12.1.4.7.

3.2.3 Sacrifice

After an overnight fasting period, rats were killed by concussion and were rapidly exsanguinated. The livers were immediately removed and blotted on filter paper. Livers were hold in ice-cold phosphate buffer solution until homogenization.

3.2.4 Microsome preparation

The microsomes were prepared as described by BERDEAUX *et al.* (1998b). The pooled livers were homogenized and centrifuged to precipitate cellular debris. In a second centrifugation nuclei, mitochondria, lysosomes and peroxisomes were eliminated. A following ultracentrifugation gave a pellet containing the desired microsomes. The microsomes were resuspended in the appropriate buffer and the microsomal proteins were quantified according to the method of LOWRY *et al.* (1951).
(protocol, see annex, chap. 12.1.3.2-3)

3.2.5 Incubation

The incubations were carried out according to BERDEAUX *et al.* (1998b)

- **Δ6-desaturation study**

The Δ6-desaturation study was performed at 37°C. Microsomal protein (5mg) were mixed with an incubation buffer containing the cofactors for the enzymatic reaction (essentially nicotinamide-adenine dinucleotide phosphate in reduced form (NADPH), adenosine 5'-triphosphate (ATP), coenzyme A (CoA)) in an open flask. The substrates [1-^{14}C]-9c11t-C18:2, [1-^{14}C]-10t12c-C18:2 and [1-^{14}C]-linoleic acid were incubated for 15 min with the microsomal suspension at increasing concentrations (30-120 nmol).
(protocol see annex, chap. 12.1.3.4)

- **elongation study**

The elongation studies were carried out at 37°C in an anaerobic medium, under argon as protective gas. The incubation under anaerobic conditions avoid desaturation reactions, which could happen on the elongation product. Also the addition of potassium cyanide to the incubation buffer will block a possible oxygen transport and therefore the desaturation reactions. Microsomal protein (5mg) were mixed with the incubation buffer containing the cofactors (essentially Malonyl-CoA,

NADPH, ATP and CoA). The elongation assay was carried out using [1-^{14}C]-6c9c11t-C18:2 and [1-14C]-γ-linolenic acid as substrate at increasing concentrations (10-100nmol) with the microsomal suspension for 30min.
(protocol see annex, chap. 12.1.3.5)

- **Δ5-desaturation study**

The Δ5-desturation study was carried out using the same experimental conditions as for Δ6-desaturation. The Δ5-desaturation assay used [1-^{14}C]-8c11c13t-C20:3 and [1-^{14}C]-C20:3n-6 as substrate at increasing concentrations (10-100nmol) with microsomal suspension for 15min.
(protocol see annex, chap. 12.1.3.4)

3.2.6 Analysis of the conversion products

The incubations were stopped by addition of potassium-hydroxide in ethanol. The lipids were saponified, extracted and the free fatty acids esterified into FAME using BF_3/MeOH. To avoid isomerization of conjugated fatty acids, the esterification was done at room temperature.
(protocol see annex, chap. 12.1.3.6)
The FAME were analyzed by HPLC on a Nucleosil C18 column coupled to a radiochromatographic Flo-One β detector. The levels of the remaining substrate and its conversion product were determined from their radioactive signal, measured by the detector after mixing the column effluent with a scintillation cocktail. To ensure the results of HPLC analysis, individual samples were also analyzed by GC on a Stabilwax wide bore column connected to a FID and to a radio-GC-detector.
The identification of the conversion products was carried out by GC-MS to determine the molecular weights of unlabeled and labeled conversion products. Moreover the FAME were analyzed by GC on polar column (BPX-70). The conversion products were identified by comparing the retention times to standards, obtained by total synthesis (chap. 5) or isolation from the liver of animals fed CLA, where the chemical structure has already been determined (SEBEDIO *et al.*, 2001).
(analytical procedures see annex, chap. 12.1.3.7-9)

Results were calculated from the radioactive signal of the substrate and the conversion product. The conversion rate was determined as percent of the conversion product of total radioactive signals. Results were expressed as means ± standard deviation (SD) of four determinations.
(calculation, see annex, chap. 12.1.3.10)

3.3 Influence of dietary CLA on PGI_2 and TXB_2 synthesis *in-vivo*

To investigate the interaction of dietary CLA (9c11t-C18:2 and 10t12c-C18:2) with the synthesis of PGI_2 and TXA_2 in young and adult rats, the following parameters were determined.

- circulating PGI_2 (by analysis of 6-keto-$PGF_{1\alpha}$) in plasma
- PGI_2 (by analysis of 6-keto-$PGF_{1\alpha}$) release from isolated rat aorta
- TXA_2 (by analysis of TXB_2) in serum
- fatty acid composition of aorta, platelets and plasma cholesteryl ester (CE), TAG and PL.

3.3.1 Animals and diets

18 male weaning («young») Wistar rats of three weeks of age at arrival and 18 male («adult») Wistar rats of ten weeks of age at arrival were housed in individual stainless steel cages in an animal house, maintained at 21±1°C with a 12 hours light / dark cycle (07:00AM-07:00PM). Young and adult rats were each randomly divided into 3 experimental groups of six animals. They were fed *ad libitum* a semisynthetic diet containing 6% (by weight) of lipids for 3 weeks. The composition of the diet formula is given in annex (chap. 12.2.3.1). 5% basal lipid of the dry diet was made of a mixture of high oleic sunflower oil and linseed oil (98:2, w / w) and according to the dietary group, 1% of basal lipid for «control» group, 1% of 9c11t-C18:2, as triacylglycerol, for the «9c11t» group and 1% of 10t12c-C18:2, as triacylglycerol, for the «10t12c» group were added. The different dietary groups are summarized in table 3.

Table 3 Scheme of dietary groups of the nutritional study

Dietary Group	Age	Experimental Lipid	Number of Animals
Young-control	3 weeks	basal lipid	6
Young-9c11t	3 weeks	9c11t-C18:2 (TAG)	6
Young-10t12c	3 weeks	10t12c-C18:2 (TAG)	6
Adult-control	10 weeks	basal lipid	6
Adult-9c11t	10 weeks	9c11t-C18:2 (TAG)	6
Adult-10t12c	10 weeks	10t12c-C18:2 (TAG)	6

Fresh diet was given every second day, where the food intake was recorded and the health status was observed. Weight was determined twice a week. Rats had free access to tap-water.

3.3.2 Sacrifice and sample collection

After an overnight fasting period at the end of the three-week experimental period, rats were anesthetized by inhaling of Isoflurane.

Blood sampling was carried out according to MAHFOUZ AND KUMMEROW (1999). Arterial blood was withdrawn from the abdominal aorta. 3ml of blood were collected in an ice-cold tube containing ethylenediaminetetraacetic acid (EDTA), as anticoagulant, and indomethacin, as cyclooxygenase inhibitor. Plasma was obtained by immediate centrifugation and stored at -80°C until analysis of 6-keto-$PGF_{1\alpha}$. Another 3ml of blood were allowed to clot at room temperature for 2h, to cause a thromboxane release from blood platelets into serum. Serum was stored at -80°C until quantification of TXB_2.

The remaining blood (3 to 5ml in the young, and 8 to 10ml in the adult) was mixed with EDTA and used for platelet preparation and fatty acid analysis of plasma.
The aorta was rapidly removed, rinsed with tris-(hydroxy)-methylaminomethane (TRIS) buffer and freed from adventitia, while held in ice-cold buffer. Adventitia had to be removed, because of the cell structure containing high contents of lipids, which would interfere with the analysis of the fatty acid composition of the aorta. One part was used for incubation, the other part was stored in chloroform / methanol at -20°C until fatty acid analysis.

3.3.3 Isolation of platelets

Platelets were prepared by centrifugation of blood as shown in figure 10. The centrifugation was carried out at room temperature to avoid an aggregation of platelets. For this reason all manipulations were done using disposable plastic material, because glass induced platelet aggregation.

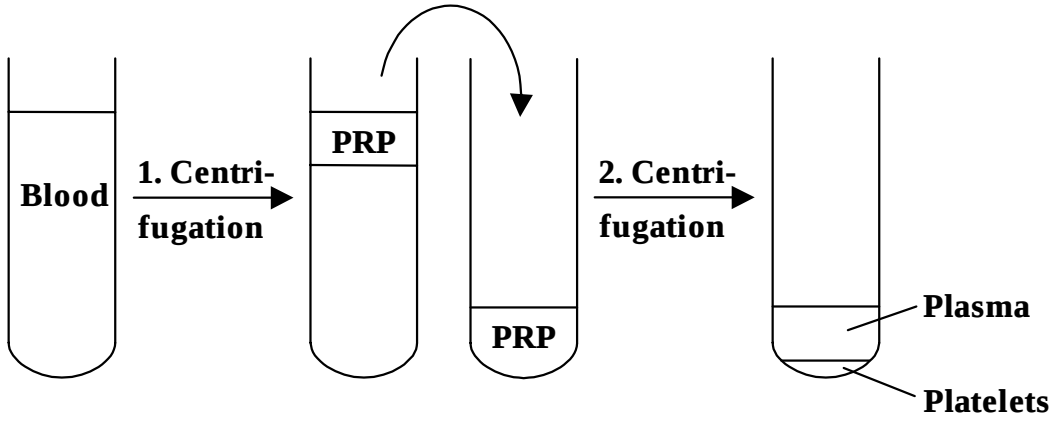


Figure 10 Isolation of platelets

A first centrifugation was carried out at low speed to fractionate the plasma rich in platelet (PRP) from the remaining blood. PRP was withdrawn and was centrifuged a second time at higher speed to pellet platelets. Platelets were rinsed with [4-(2-hydroxyethyl)-piperazino]-ethanesulfonic acid (HEPES) buffer, and after addition of chloroform / methanol and stored at -20°C until analysis to determine the fatty acid composition.
(protocol, see annex, chap. 12.1.4.2)

3.3.4 PGI_2 Release of incubated aorta

Aorta, freed adventitia, was opened longitudinally, and was incubated in a buffer solution at 37°C in a shaking water bath as described by MAHFOUZ AND KUMMEROW (1999). Aliquots of the incubation solution were taken at 30, 60 and 90min to estimate the concentration of 6-keto-PGF1α. At the end of incubation the aortas were removed, dried on a filter paper and weighed.
(protocol, see annex, chap. 12.1.4.1)

3.3.5 Quantification of 6-keto-$PGF_{1\alpha}$ and TXB_2

The content of 6-keto-$PGF_{1\alpha}$ and TXB_2 were determined using commercially available competitive enzyme immuno assay (EIA) test-kits.

The EIA is based on the competition between the analyte (6-keto-$PGF_{1\alpha}$ or TXB_2) and a conjugate of the analyte and acetylcholinesterase (tracer) for a limited number of analyte specific rabbit antiserum binding sites (rabbit-antiserum: specific antibody to 6-keto-$PGF_{1\alpha}$ or TXB_2.) Because the tracer concentration remained constant and the concentration of the analyte varied, the amount of the tracer which bound to the rabbit-antiserum was inversely proportional to the concentration of the test-molecule. The antiserum-tracer or antiserum-analyte-complex bound to a mouse monoclonal anti-rabbit antibody which was previously attached on the well of a EIA-plate. After incubation, the plate was rinsed from unbound reagents and developed by adding a reagent, which reacts with the acetylcholinesterase of the tracer and formed a yellow color. The intensity of this color, determined spectrophotometrically by a microtiter-plate-reader, was inversely proportional to the amount of analyte present during incubation. (see reaction scheme in figure 11).

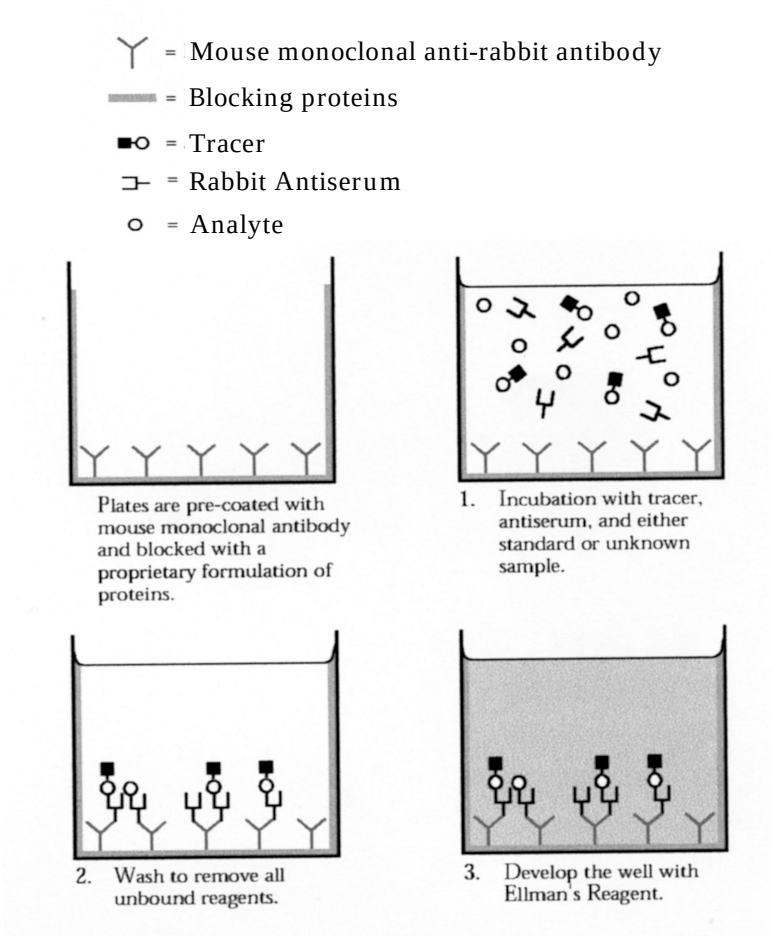


Figure 11 Reaction scheme of the EIA

6-keto-$PGF_{1\alpha}$ samples from the incubation of aorta were used directly after 1:1000 dilution in EIA-buffer for the determination by EIA. The plasmatic level of 6-keto-$PGF_{1\alpha}$was measured after 1:20 dilution by EIA without previous purification. Serum TXB_2 was directly carried out by EIA after 1:1000 dilution in EIA-buffer. Analysis was done from six samples of each group in single determination.

Results were calculated by comparing to a calibration curve done with a standard solution of the analyte (concentration certified by the producer)

(protocol and calculation, see annex, chap. 12.1.4.3-4)

3.3.6 Analysis of the fatty acid composition of aorta, platelets and plasma

- **lipid extraction of aorta and platelets**

Aorta, homogenized before, and platelets were extracted by chloroform / methanol under addition of sodium chloride solution. The water layer was withdrawn and the solvent was evaporated under a stream of nitrogen. The extracted total lipids were dried by addition of ethanol and evaporation under nitrogen. Extracted lipids were stored at -20°C until methylation.
(protocol, see annex, chap. 12.1.4.5)

- **lipid extraction and fractionation of lipid classes of plasma**

The plasma was extracted using chloroform / methanol. The organic layer was evaporated under a stream of nitrogen to obtain the lipid.
Extracted lipids were separated into three lipid classes (CE, TAG and PL) by thin-layer-chromatography (TLC) using a solvent mixture of hexane / diethylether / acetic acid. The corresponding bands were scraped off and diluted in toluene. The samples were stored at -20°C until methylation.
(protocol, see annex, chap. 12.1.4.5-6)

- **methylation of the extracted lipids**

The samples were methylated into FAME using sodium methoxide at 50°C for 5min (EC were heated for 30min). After cooling, samples were neutralized and the FAME were extracted with hexane. FAME were cleaned up using florisil cartridges to eliminate the cholesterol. FAME were diluted in hexane and stored at -20°C until GC-analysis.
(protocol, see annex, chap. 12.1.4.7)

- **fatty acid composition of the samples**

FAME were analyzed by GC. FAME were separated on a highly polar capillary column (CPSil™ 88) to determine the fatty acid composition of the different samples. Results were calculated as percent of total fatty acids.
(analytical procedure, see annex, chap.12.1.4.8)

3.4 Statistical analysis

Statistical analysis was carried out using the SAS-software. A one-way ANOVA was used to assess multiple comparisons. The Student-Newman-Keuls test was performed to demonstrate heterogeneity between more than two groups. The t-test was used for the comparison of two groups.

A p-value less than 0.05 was considered as statistically significant, a p-value less than 0.01 was considered as highly significant.

4 INFLUENCE OF PROCESSING ON THE CLA CONTENT IN CHEESE

4.1 Results

4.1.1 Influence on the CLA content during cheese manufacturing

The CLA content in milk and cheese samples, taken at the different manufacturing steps is shown in table 4. The CLA-content remained unchanged during manufacturing using raw or heated milk.

Table 4 CLA content at different steps of cheese manufacturing (mean [n=3] ± SD)

	CLA content in mg / g fat	
	Raw milk cheese	Heated milk cheese
Raw Milk	8.62 ± 1.92	8.62 ± 1.92
Heated Milk	-	8.51 ± 1.93
Coagulated Milk	8.39 ± 1.24	8.59 ± 1.63
Fresh Curd	8.46 ± 1.31	8.68 ± 1.51
Salted Curd	8.20 ± 1.87	8.55 ± 1.44
Cheese, 20 days of aging	8.73 ± 1.73	8.46 ± 1.50
Final ripened cheese	8.55 ± 1.63	8.38 ± 1.48

CLA contents ranged from 8.62 ± 1.92 mg / g fat in milk to 8.55 ± 1.63 mg / g fat in final cheese fabricated with raw milk and to 8.38 ± 1.48 mg / g fat in the final cheese prepared from heated milk. The results showed a great variation of the CLA content. The complete data is shown in the annex, chap. 12.2.1.1.

The relative CLA content (calculated from the fatty acid composition) was determined for cheese samples prepared from raw milk (data presented in annex, chap. 12.2.1.3). CLA represented 1.06 ± 0.15 % of the total fatty acids in milk. A similar value (1.06 ± 0.13 % of CLA) was found in the final cheese. Furthermore, no changes took place in the total fatty acid composition. At each fabrication step the same fatty acid profile was observed.

The CLA-isomer composition of the cheese samples prepared from raw milk taken at each fabrication step was determined by Ag^+-HPLC. The results of raw milk, fresh curd, cheese after 20 days of aging and final ripened cheese are presented in table 5.

Table 5 CLA isomer composition of raw milk cheese during manufacturing (mean [n=2] ± SD)

	% of total CLA			
	raw milk	fresh curd	cheese, 20 days	final cheese
12t14t	0.63 ± 0.08	0.71 ± 0.22	0.72 ± 0.11	0.74 ± 0.16
11t13t	1.64 ± 0.08	1.79 ± 0.14	1.82 ± 0.12	1.78 ± 0.05
10t12t	0.26 ± 0.08	0.45 ± 0.25	0.55 ± 0.08	0.69 ± 0.03
9t11t	0.73 ± 0.12	0.70 ± 0.04	0.88 ± 0.09	0.85 ± 0.04
8t10t	0.22 ± 0.08	0.40 ± 0.01	0.28 ± 0.11	0.37 ± 0.03
7t9t	0.52 ± 0.01	0.45 ± 0.02	0.54 ± 0.11	0.58 ± 0.17
6t8t	0.20 ± 0.03	0.20 0.02	0.22 ± 0.02	0.29 ± 0.11
12,14 c / t	0.47 ± 0.01	0.30 ± 0.11	0.42 ± 0.07	0.37 ± 0.03
11t13c	2.58 ± 0.08	3.00 ± 0.52	2.91 ± 0.51	2.96 ± 0.50
11c13t	0.17 ± 0.03	0.31 ± 0.02	0.26 ± 0.11	0.31 ± 0.04
10t12c	0.47 ± 0.17	1.27 ± 0.54	0.80 ± 0.28	0.82 ± 0.12
9c11t	86.79 ± 0.19	83.59 ± 2.08	85.26 ± 0.52	84.77 ± 1.07
8t10c	1.62 ± 0.20	1.38 ± 0.34	1.21 ± 0.30	1.24 ± 0.31
7t9c	3.71 ± 0.14	5.45 ± 1.46	4.15 ± 0.41	4.24 ± 0.33
Σ t/t	**4.20 ± 0.16**	**4.70 ± 0.65**	**5.00 ± 0.38**	**5.28 ± 0.05**
Σ c/t	**95.80 ± 0.15**	**95.30 ± 0.65**	**95.00 ± 0.38**	**94.72 ± 0.05**

The CLA isomer composition of the samples consisted mainly of 9c11t-C18:2 (about 85.1 %) accompanied by 7t9c-C18:2 as the second important isomer (about 4.1%), followed by 11t13c-C18:2 (about 2.8 %), 11t13t-C18:2 (about 1.9 %) and 8t10c-C18:2 (about 1.3%). No differences in the content of individual CLA-isomers were found according to the different fabrication steps. Regarding the content of total *trans-trans* isomers, a small but not significant increase from 4.20 ± 0.16 % to 5.28 ± 0.05 % was observed. The complete data are reported in the annex (chap. 12.2.1.2)

4.1.2 Effect of milling and cooking temperatures

Cheeses were fabricated either from raw or heated milk. CLA contents of final ripened cheese processed at normal temperature of milling and cooking and at two lower temperatures were measured. The results were compared to CLA-contents of the corresponding raw milk and heated milk used for the cheese. The results are presented in table 6. No changes in the CLA-content were observed using different temperatures during milling and cooking.

Table 6 CLA-content in cheese using different temperatures during milling and cooking (mean [n=3] ± SD)

	CLA content in mg / g fat	
	Raw milk cheese	Heated milk cheese
Raw milk	8.62 ± 1.92	8.51 ± 1.93
Milling 52°C-Cooking 50°C	8.55 ± 1.63	8.38 ± 1.48
Milling 48°C-Cooking 48°C	8.85 ± 1.80	8.98 ± 2.57
Milling 50°C-Cooking 50°C	8.64 ± 1.69	8.36 ± 1.75

Raw milk cheese prepared at normal temperature during milling and cooking contained 8.55 ± 1.63 mg CLA / g fat compared to 8.85 ± 1.80 mg CLA / g fat for raw milk cheese prepared at the lowest temperature. Cheese prepared with heated milk at normal temperatures contained 8.38 ± 1.48 mg CLA / g fat were found and the lowest fabrication temperature for milling and cooking led to a CLA content of 8.98 ± 2.57 mg / g fat. (complete results, annex, chap. 12.1.1.1)

The fatty acid composition and the relative CLA content were not altered by the changes in the fabrication conditions. The corresponding data are presented in the annex (chap. 12.2.1.3).

Modifications of the milling and cooking temperatures led to slight variations in the CLA isomer composition for cheese samples prepared from raw milk only, as shown in table 7. Samples prepared from heated milk showed no alteration in the CLA isomer composition.

Table 7 CLA isomer composition in cheese prepared of raw milk using different temperatures during milling and cooking (mean [n=2] ± SD)

	% of total CLA			
	Milk	Milling 52°C / Cooking 50°C	Milling 48°C / Cooking 48°C	Milling 50°C / Cooking 50°C
12t14t	0.63 ± 0.08	0.74 ± 0.16	0.81 ± 0.09	0.87 ± 0.04
11t13t	1.64 ± 0.08	1.78 ± 0.05	1.72 ± 0.01	1.94 ± 0.21
10t12t	0.26 ± 0.08	0.69 ± 0.03	0.59 ± 0.02	0.86 ± 0.62
9t11t	0.73 ± 0.12	0.85 ± 0.04	0.98 ± 0.14	0.93 ± 0.40
8t10t	0.22 ± 0.08	0.37 ± 0.03	0.34 ± 0.01	0.42 ± 0.11
7t9t	0.52 ± 0.01	0.58 ± 0.17	0.49 ± 0.16	0.47 ± 0.11
6t8t	0.20 ±0.03	0.29 ± 0.11	0.21 ± 0.06	0.22 ± 0.06
12,14 c / t	0.47 ±0.01	0.37 ± 0.03	0.34 ± 0.08	0.33 ± 0.04
11t13c	2.58 ±0.08	2.96 ± 0.50	2.97 ± 0.37	2.64 ± 0.01
11c13t	0.17 ±0.03	0.31 ± 0.04	0.42 ± 0.16	0.48 ± 0.33
10t12c	0.47 ±0.17	0.82 ± 0.12	1.03 ± 0.22	1.27 ± 0.27
9c11t	86.79 ±0.19	84.77 ± 1.07	84.43 ± 1.53	84.90 ± 1.82
8t10c	1.62 ±0.20	1.24 ± 0.31	1.29 ± 0.63	0.96 ± 0.31
7t9c	3.71 ±0.14	4.24 ± 0.33	4.36 ± 0.74	3.72 ± 0.61
Σ t/t	**4.20 ±0.16**	**5.28 ± 0.05**	**5.14 ± 0.26**	**5.71 ± 1.55**
Σ c/t	**95.80 ±0.15**	**94.72 ± 0.05**	**94.86 ± 0.26**	**94.29 ± 1.55**

The results showed a small increase in the content of the total *trans-trans* CLA isomers from 4.20 ± 0.16 % in milk to 5.28 ± 0.05 % in cheese prepared at the normal fabrication temperatures and 5.14 ± 0.26 % or 5.71 ± 1.55 % for milling and cooking at 48°C / 48°C or 50°C / 50°C, respectively. However, these small changes were not significant. (detailed results, annex, chap. 12.1.1.2)

4.1.3 Effect of different strains of *Propionibacterium* spp.

Two strains of *Propionibacterium* spp. with a high and a low lipolytic activity were used in the fabrication process compared to the normally used *Propionibacterium* sp. As presented in table 8, no difference in the CLA content were detected in final cheeses between the various *Propionibacterium* spp. used.

Table 8 CLA-content in cheese ripened with different strains of *Propionibacterium* spp. (mean [n=2] ± SD)

	CLA content in mg / g fat
Milk	10.01 ± 0.52
Cheese, normal *Propionibacterium* sp.	9.54 ± 0.30
Cheese, *Propionibacterium* sp. high lipolytic	9.98 ± 0.71
Cheese, *Propionibacterium* sp. low lipolytic	9.87 ± 0.65

Cheese fermented with the high lipolytic *Propionibacterium* sp. or the low lipolytic *Propionibacterium* sp. contained 9.98 ± 0.71 mg CLA / g fat and 9.87 ± 0.65 mg CLA / g fat, respectively. The use of the normal *Propionibacterium* sp. for fermentation led to a CLA content of 9.54 ± 0.30 mg / g fat in the final cheese. The complete data are shown in the annex, chap. 12.1.1.1.

Furthermore the different *Propionibacterium* spp. had no influence on the other analytical parameters. Neither changes in the fatty acid profile nor a variation in the analyzed CLA isomer composition of the different cheese samples were found. The complete results are shown in the annex (chap. 12.2.1.2 and chap. 12.2.1.3).

4.1.4 Influence of cooking and grilling

The CLA content remained unchanged during culinary utilization. The CLA content in the cooked sample was compared to that of a non-cooked sample as control. The results of the grilling and cooking experiments of cheese in different dishes are shown in table 9.

Table 9 CLA contents in cooked and grilled dishes (mean ± SD)

	CLA content in mg / g fat	
	cheese, fabricated in pilot-scale	cheese, industrially fabricated
Gratin, raw [n=1]	8.53	7.29
Gratin [n=3]	7.30 ± 0.85	7.40± 0.26
Sauce Béchamel, raw [n=1]	6.37	6.82
Sauce Béchamel [n=3]	5.99 ± 0.11	6.78 ± 0.14
Fondue Savoyarde, raw [n=1]	7.80	9.16
Fondue Savoyarde [n=3]	7.83 ± 0.22	8.70 ± 0.10

Regarding the results of the two different cheeses, the CLA-content before and after cooking was the same. For example, the CLA content in the «Gratin» prepared with industrial cheese was determined as being 7.29 mg/g fat and 7.40 ± 0.26 mg/g fat before and after the exposure of the dish to high temperatures, respectively. For the «Sauce Béchamel» prepared with industrial cheese the CLA content was measured as 6.82 mg/g fat and 6.78 ± 0.14 mg/g fat before and after the exposure of the dish to a moderate temperature, respectively. (results chap. 12.1.1.1)

The determination of the relative CLA content remained unchanged The fatty acid composition in the dishes before and after cooking or grilling showed no differences in the fatty acid profiles (see annex, chap. 12.2.1.3).

4.1.5 Preparation of cheese spread

The CLA content was determined in cheese samples before and after the fabrication of cheese spread. The results, presented in table 10, revealed that longer heating times or processing temperature did not seem to have any influence on the CLA content.

Table 10 CLA content in cheese spread using different processing conditions (mean [n=2] ± SD)

processing temperature	additional heating time	CLA content in mg/g fat
control		8.15 ± 0.04
75°C	0 min	8.37 ± 0.62
75°C	5 min	8.06 ± 0.50
100°C	0 min	8.37 ± 0.48
100°C	10 min	6.95 ± 0.82

A small but not significant decrease of the CLA content was found for the highest processing temperature and the longest manufacturing time. The CLA content decreased from 8.15 ± 0.04 mg/g fat to 6.95 ± 0.82 mg/g fat. The detailed results are presented in the annex, chap. 12.1.1.1.

Various processing conditions during the fabrication of cheese spread seem to influence neither the relative CLA content nor the fatty acid composition. (results in the annex, chap. 12.2.1.3)

4.2 Discussion

The CLA content and CLA isomer composition were determined in cheese produced under different manufacturing conditions and after their use in selected culinary preparations. The studies focused on possible CLA increases induced by microbial fermentation processes during manufacturing or heat treatments as suggested by LIN *et al.* (1999b) and SHANTA *et al.* (1992). Otherwise the possible decrease of the CLA content during ripening of cheese or cooking and grilling of cheese due to oxidative damage was investigated, as the risk was previously evaluated to be similar to that of docosahexanoic acid and to be much higher than linoleic acid by ZHANG AND CHEN (1997).

The CLA content in cheese prepared from raw milk was found to be 8.55 ± 1.63 mg / g fat and for cheese prepared with heated milk at 8.38 ± 1.48 mg / g fat. The great variation observed between the different samples used in this study was possibly due to the sampling period of the milk used for fabrication of the cheeses. In fact, the cheese manufacturing was carried out at the end of April during two weeks, when cows begin to change from indoor to pasture feeding, suggesting a seasonal influence on the CLA content. Therefore the cows changed to a food containing higher contents of polyunsaturated fatty acids, which increased the bioconversion in the rumen and as a consequence, the CLA content was upregulated in the same time (JAHREIS *et al.*, 1996).

These results were comparable to those published in previous studies, where the CLA content in different cheeses varied from 2.82 ± 0.10 mg / g fat in cheddar cheese to 20.8 ± 4.0 mg / g fat in French hard cheese (LAVILLONNIÈRE *et al.*, 1998; LIN *et al.*, 1998). For Swiss cheeses, produced by fermentation with *Propionibacterium* spp., and similar to the investigated French Emmental cheese, CLA contents of 6.7 ± 0.56 mg / g fat and 5.45 ± 0.59 mg / g fat were reported (CHIN *et al.*, 1992; LIN *et al.*, 1995). Swedish hard cheeses using *Propionibacterium* spp. for fermentation contained 7.06 ± 0.33 mg / g fat (JIANG *et al.*, 1997).

4.2.1 Influence of manufacturing and varying processing conditions on CLA content and isomer composition

- **influence on the CLA content**

In a first part of the study the influence of cheese manufacturing was examined on CLA. All samples prepared from raw or heated milk taken at the different fabrication steps contained similar CLA amounts, 8.62 ± 1.92 mg/g fat in the milk sample and 8.38 ± 1.48 mg/g fat in ripened cheese. The samples prepared from heated milk presented similar results and led therefore to the same conclusion. An influence of manufacturing on the CLA content was not observed under the used fabrication conditions. The results are in agreement with a Swedish study investigating the fabrication process and the influence of ripening time on the CLA content in *Propionibacterium* sp. fermented Swedish hard cheese. No changes during manufacturing and ripening were described (JIANG *et al.*, 1997).

Interestingly, a study carried out on cheddar cheese fabrication reported opposite results. The CLA content was increased at the fresh curd stage and after 3 months of aging compared to the raw milk used for fabrication. In that study, the raw milk contained 3.38 ± 0.03 mg CLA/g fat, fresh curd 3.56 ± 0.03 mg CLA/g fat and 3 month aged cheese 3.76 ± 0.03 mg CLA/g fat. The authors explained the raising of the CLA content by enzymatic isomerization reactions and an interaction of proteins as hydrogen donors, with linoleic acid oxidation products, during heating processes under anaerobic conditions. It was proposed that CLA could be formed from linoleic acid by lipid peroxidation, with formation of conjugated dienyl radicals which could then react with hydrogen liberated from protein (LIN *et al.*, 1999b). However, the differences in the CLA content between milk and the final ripened cheese were only about 0.38 mg CLA/g fat which seems negligible, compared to the variations found between different types of cheese described above. Moreover the authors used strong methylation conditions (hydrolysis by alkali at 100°C and methylation with BF_3/MeOH) which can lead to isomerization reactions of CLA, inducing a wrong determination of the CLA content (CHRISTIE *et al.*, 2001). The formation of important concentration of trans-trans CLA isomers is an indicator for isomerization. As the CLA isomer composition was not determined in that study, an interaction with the methylation procedure could not be detected.

Furthermore the manufacturing and ripening conditions of cheddar and Emmental cheese are slightly different, and could explain the discrepancy between the two

studies. Cheddar cheese manufacturing used other starter cultures than Emmental. For cheddar, an additional processing step the «cheddaring» is carried out, where the curd is exposed during a longer time to oxygen than the curd during manufacturing of Emmental (BELITZ *et al.*, 2001). Also the two cheese types are ripened differently. In contrast to Emmental cheese, which is ripened under atmospheric conditions to form a cheese rind, cheddar is ripened in plastic bags to avoid drying.

Earlier results published on this subject always discussed the analysis of commercial cheese samples, but the composition of the corresponding raw material was not determined (HA *et al.*, 1989; LIN *et al.*, 1995). Therefore, observed differences in the CLA content in the samples of these studies were the result of seasonal, dietary regimen and cow-to-cow variations, and not of differences induced by processing conditions (BANNI AND MARTIN, 1998; SHANTHA *et al.*, 1995).

In a second part of the study different temperatures for milling and cooking and their relationship to the CLA content in the final ripened cheese were examined, as research never focused on this subject. Small modifications in the processing temperature could influence the development of the fermenter bacteria, as each bacterium underlies a specific temperature for optimal living (KRÄMER, 1996). The obtained results for the different cheeses prepared from raw and heated milk showed that the CLA content was not altered, indicating that moderate changes in processing temperatures did not change the CLA content.

At last, the influence of different *Propionibacterium* spp. on the CLA content in Emmental cheese was investigated. Current research described a capacity of several fermentation bacteria to produce CLA from linoleic acid by enzymatic isomerization in culture (LIN *et al.*, 1999a; LIN, 2000). Moreover three strains of *Propionibacterium* spp. were identified to form CLA (JIANG *et al.*, 1998). It was suggested that the same effects could be induced during fermentation of dairy products. As a consequence, the use of specific starter cultures could increase the CLA content in the final foodstuff. The use of three different *Propionibacterium* spp. to produce the Emmental cheese under the used processing conditions failed to induce any variations on the CLA content, indicating either that the used strains of *Propionibacterium* spp. were not able to convert linoleic acid to CLA or the changes were too low to be detected.

In conclusion, neither an increase nor a decrease of the CLA content was observed by changing the different processing parameters under the used manufacturing conditions.

- **influence on the CLA isomer composition**

No major influence of processing on the CLA isomer distribution were observed. Small changes were found for the cheeses fabricated from raw milk, where the amount of *trans-trans* isomers seemed to be increased. Only one comparable study was carried out to investigate manufacturing dependent changes in the CLA isomer using three types of cheddar cheese, and no difference was reported (WERNER *et al.*, 1992). As cheddar cheese uses pasteurized milk and a specific starter culture for fabrication, the observed changes on the CLA isomer composition in this work seem to be related to the natural bacteria found in raw milk. It can only be hypothesized that one or some bacteria of the natural flora is able to alter the CLA isomer composition. Further experiments have to be carried out using raw milk and a higher sampling number in order to explain these results.

- **influence on the fatty acid composition**

The determination of the fatty acid composition was carried out to determine changes in the ratio of CLA to other fatty acids and to examine ripening dependent influences on the short chain fatty acids, which are more volatile. No changes in the fatty acid profile were induced by the processing conditions used or added fermentation bacteria, indicating that the cheese rind was closed and did not allow the disappearance of products by volatilization. Otherwise this prevented the oxidation effects, as oxygen could not enter the cheese.

4.2.2 Influence of cooking and grilling on the CLA content

The fabrication of different dishes containing cheese was carried out to evaluate the stability of CLA during cooking and grilling. None of the heating processes used in the preparation of the dishes led to a change in the CLA content or in the relative CLA content determined from the fatty acid profile. This indicates a good thermal stability of CLA in cheese. An additional increase of CLA during the heat-treatments was also not observed. These findings are in agreement with results published by SHANTHA *et al.* (1994). They heated ground beef up to 80 °C in normal culinary preparations. No changes in the CLA content were induced by frying, baking or grilling of the meat.

Comparing the results from this work to experiments on the oxidative stability of CLA (mainly purified CLA standard substances) where CLA was described to be

rapidly oxidized, CLA incorporated in a foodstuff seem to be stable and might be protected from oxidative damage by the food matrix (CHEN *et al.*, 1997; CHEN *et al.*, 2001; YANG *et al.*, 2000).

4.2.3 Influence of processing on the CLA content

No changes of the CLA content or the fatty acid profile were observed by the fabrication of cheese spread under the used processing conditions. This is in opposition to former published results. GARCIA-LOPEZ *et al.* (1994) reported a slight increase of the CLA content from 9.5 mg / g fat to 10.7 mg / g fat during the fabrication of cheese spread. This effect could be related to their used processing conditions, which were not further detailed. So no comparison between the different fabrication conditions can be done to explain the differences in the observations.

Another study investigating the influence of fabrication conditions of cheese spread, reported an increase of the CLA content in cheddar-based cheese spreads from 3.99 ± 0.20 mg / g fat to 4.99 ± 0.20 mg / g fat at a processing temperature of 80°C under atmospheric conditions (SHANTHA *et al.*, 1992). Processing under nitrogen did not increase the CLA content in their study, suggesting that CLA increased during processing via an oxidation process of linoleic acid, as described under 4.2.1. In this work, cheese spread was manufactured under slight vacuum, and the absence of oxygen could explain a missing lipid peroxidation reaction, and consequently no increase in CLA. Using American cheese instead of cheddar cheese for the preparation of cheese spread, the CLA content remained unchanged (SHANTHA *et al.*, 1992). It seems possible that the increase of CLA during processing could be cheese type dependent, because of the differences in the manufacturing of the cheese.

In conclusion, none of the examined processing parameters of cheese manufacturing, culinary utilization of the cheese or the fabrication of cheese spread changed the CLA content in Emmental cheese, indicating that the CLA content of the different dairy products is determined by the milk used for processing. Also CLA seem to be unaffected by oxidative damage in this type of dairy product under the used processing conditions. To obtain dairy products high in CLA, high CLA milk has to be produced.

5 SYNTHESIS OF THE C18:3 AND C20:3 METABOLITES OF 9c11t-C18:2

5.1 Development of the synthesis pathway

As discussed in chapter 1, it was often hypothesized that CLA might act through conjugated C18:3 and C20:3 fatty acids, which are the result of successive desaturation and elongation of CLA. To get exact information about the physiological effects CLA and their metabolites and to clarify the metabolic pathway pure molecules of CLA and their metabolites are needed. The synthesis of pure isomers of CLA, 9c11t- and 10t12c-C18:2, was reported (BERDEAUX *et al.*, 1997; BERDEAUX *et al.*, 1998a; LOREAU *et al.*, 2001), but the syntheses of the metabolites were never done. Consequently, the syntheses of 8c11c13t-C20:3 ((8Z,11Z,13E)-eicosa-8,11,13-trienoic acid) and 6c9c11t-C18:3 ((6Z,9Z,11E)-octadeca-6,9,11-trienoic acid), the metabolites of 9c11t-C18:2 were carried out.

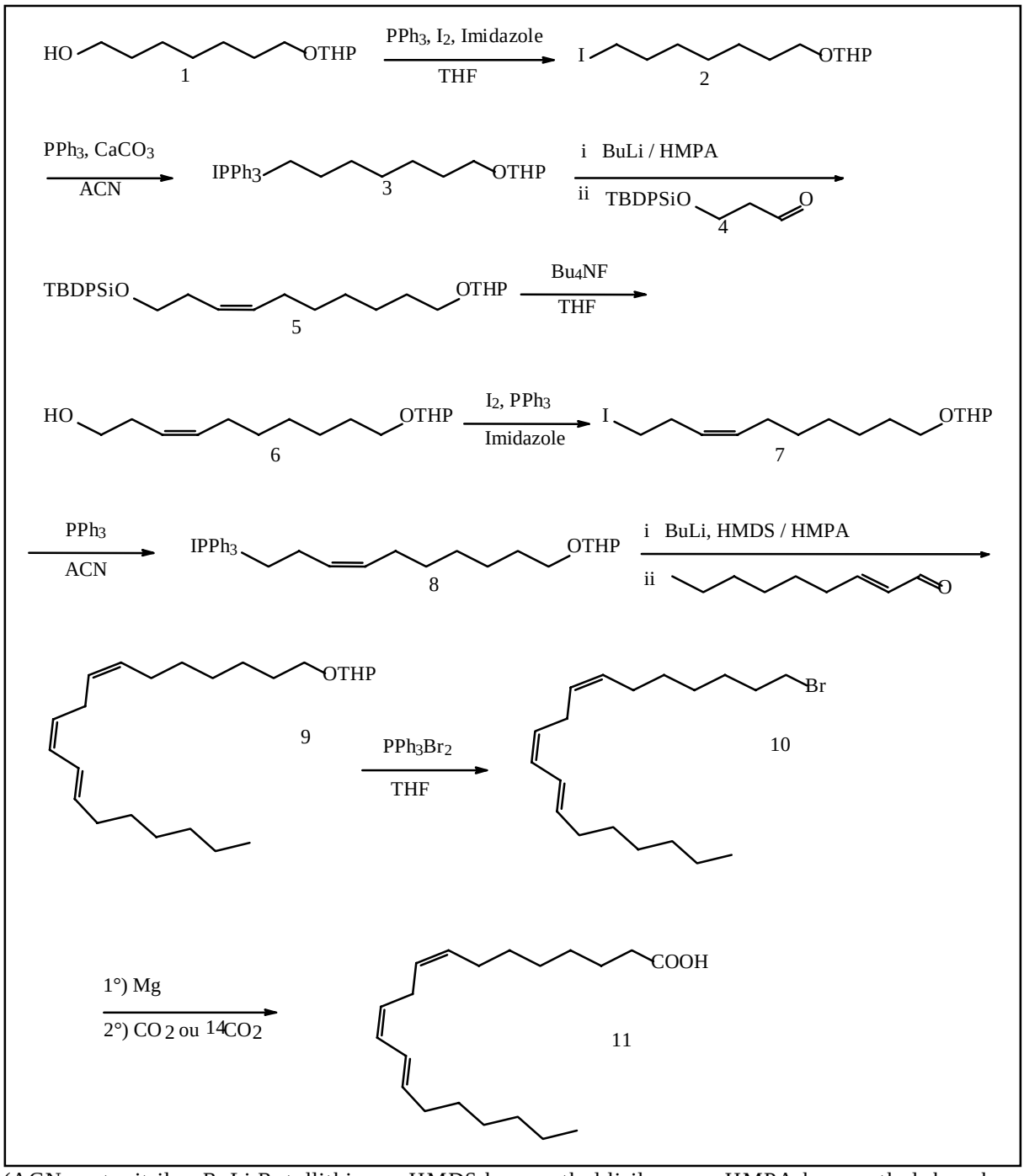
The synthesis of small quantities of pure metabolites up to 1g allowed their use in biological *in-vitro* experiments. The utilization of radiolabeled analogs permits the detection of metabolites produced, so the synthesis of the [1-^{14}C] radiolabeled analogs of (8Z,11Z,13E)-eicosa-8,11,13-trienoic acid and (6Z,9Z,11E)-octadeca-6,9,11-trienoic acid was needed.

The aim of this work was to develop two efficient stereoselective syntheses for (8Z,11Z,13E)-eicosa-8,11,13-trienoic acid and (6Z,9Z,11E)-octadeca-6,9,11-trienoic acid which allowed their preparation up to a gram scale, in high chemical and isomeric purities. Moreover, these syntheses should give the possibility to prepare the corresponding [1-^{14}C] radiolabeled analogs.

Two difficulties had to be considered in the development of a synthesis pathway. The described sensitivity of CLA to oxidation processes and isomerization because of the presence of the conjugated double bond system has to be taken into account. The same will probably be effective for their metabolites containing a double bond system and moreover a third double bond, which could increase the risk of oxidative damage compared to CLA.

The radiolabeling has to be done in the last step, to avoid loss and waste of radioactive material.

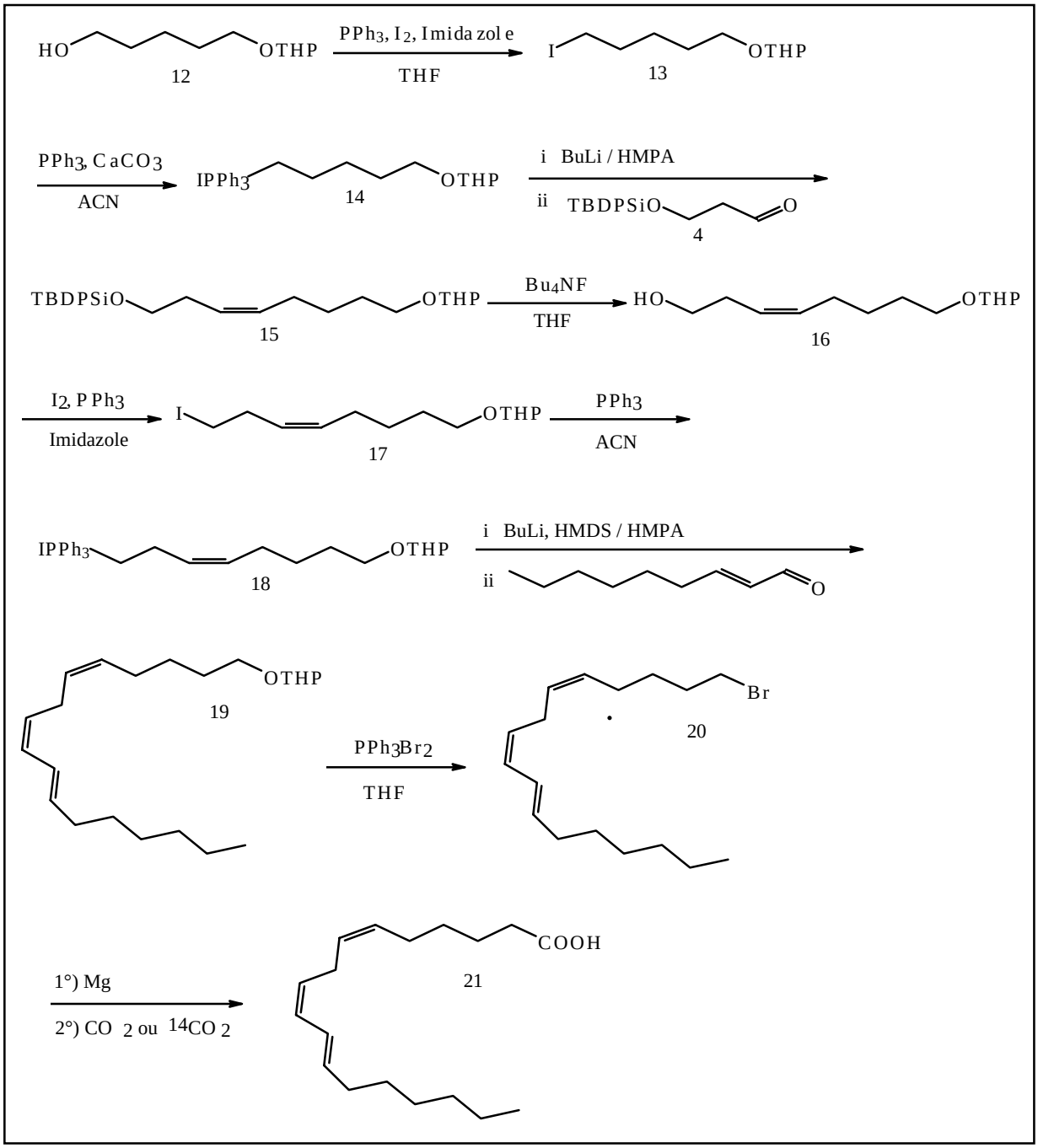
Regarding this background the following synthesis schemes were developed for the synthesis of (8Z,11Z,13E)-eicosa-8,11,13-trienoic acid (figure 12) and (6Z,9Z,11E)-octadeca-6,9,11-trienoic acid (figure 13):



(ACN-acetonitrile; BuLi-Butyllithium; HMDS-hexamethyldisilazane; HMPA-hexamethylphosphoramide; PPh_3-triphenylphosphin)

Figure 12 Synthesis of (8Z,11Z,13E)-eicosa-8,11,13-trienoic acid

To avoid degradation and isomerisation of intermediates and of the final molecule the conjugated double bond system was formed at the end of the synthesis and also just before radiolabeling. Once the conjugated system formed, no heat-treatment higher than 35°C was done. The same reaction pathway was used for the synthesis of (6Z,9Z,11E)-octadeca-6,9,11-trienoic acid starting with pentane-1,5-diol instead of heptane-1,7-diol.



(ACN-acetonitrile; BuLi-Butyllithium; HMDS-hexamethyldisilazane; HMPA-hexamethylphosphoramide; PPh_3-triphenylphosphin)

Figure 13 Synthesis of (6Z,9Z,11E)-octadeca-6,9,11-trienoic acid

The synthesis was carried out in 12 steps from commercially available reagents. The most important steps of the synthesis were two Wittig-reactions which allowed the formation of the two double bonds in *cis* configuration. In the second Wittig reaction 2-trans-nonenal was used as aldehyde, to obtain the *trans* double bond and the conjugated double bond system in the final product. This Wittig reaction was effected just before labeling. The use of CO_2 or $^{14}CO_2$ in the last step furnished the fatty acid as unlabeled or labeled compound. The detailed syntheses procedures are presented in chap. 12.1.2.

During the synthesis the intermediate products as well as the final products were analyzed by GC-FID, GC-MS, GC-FTIR and nuclear magnetic resonance (NMR) to assess the purity of the products. Additionally, the final fatty acids were transformed into DMOX and MTAD derivatives.

Radiolabeling in the last step of the synthesis pathways was carried out at CEA (Saclay, France)

5.2 Results and Discussion

5.2.1 Synthesis of (8Z,11Z,13E)-eicosa-8,11,13-trienoic acid

The synthesis of the eicosatrienoic acid **11** used heptane-1,7-diol as a starting material, which was readily available in large quantities. Heptane-1,7-diol was tetrahydropyranylated in the presence of pyridinium p-toluenesulfonate (PPTs) to give the monoprotected heptane-1,7-diol **1** (DANIELI *et al.*, 1984).

The phosphonium salt **3** was prepared from the monoprotected heptanediol **1** by reaction with iodine in modified Garegg-Samuelsson conditions (BERLAGE *et al.*, 1987; GAREGG AND SAMUELSSON, 1980) to give **2** (79.1% yield) followed by reaction with triphenylphosphine in the presence of calcium carbonate. 1,3-propanediol was monoprotected with tert-butyldiphenylsilyl chloride to give 3-(t-butyldiphenylsilyloxy)propanol (84% yield) which was oxidized to 3-(t-butyldiphenylsilyloxy)propanal **4** with pyridinium dichromate (PDC) in 79.1% yield (ABDEL-BAKY AND GIESE, 1986; HERSCOVICI *et al.*, 1982; MOODY *et al.*, 1992). Highly steroselective Wittig condensation between the ylide of the phosphonium salt **3** and aldehyde **4** in presence of hexamethylphosphoramide (HMPA) (MARYANOFF AND REITZ, 1989) furnished the (3Z)-1-(t-butyldiphenylsilyloxy)-10-(2-

tetrahydropyranyloxy)dec-3-ene **5** in 63% yield. Selective deprotection (desilylation) of compound **5** afforded alcohol **6** (FREEMAN AND KIM, 1992) which was transformed to iodide **7** using modified Garegg-Samuelsson conditions (BERLAGE *et al.*, 1987; GAREGG AND SAMUELSSON, 1980). Displacement of the iodine atom of **7** by triphenylphosphine in refluxing acetonitrile gave the ten-carbon chain unsaturated phosphonium salt **8**. The ylide from **8** was reacted with commercially available (2E)-non-2-enal to give (5Z,8Z,10E)-1-(2-tetrahydropyranyloxy)heptadeca-5,8,10-triene **9** in 51% yield. Therefore, the synthesis of the conjugated double bond system with specific *cis* stereochemistry at the newly formed double bond was carried out using a Wittig reaction. Lithiumhexamethyldisilazide (formed by reaction of BuLi with hexamethyldisilazane (HMDS)) was chosen as a weak base in the presence of HMPA and THF at –78°C according to LABELLE *et al.* (1990), to avoid isomerization of the existing trans-double bond of the (2E)-non-2-enal. Replacement of the tetrahydropyranyloxy function of **9** by a bromine atom was accomplished by triphenylphosphine dibromide (SONNET, 1976) in 77.4% yield. At this stage, no geometrical isomers of compounds **9** and **10** were detected by GC, ^{1}H and ^{13}C NMR.

In previous syntheses of labeled fatty acids (BERDEAUX *et al.*, 1995; EYNARD *et al.*, 1994; VATELE *et al.*, 1994), [1-^{14}C] labeled molecules were prepared by reaction at 80°C of bromo precursors with K^{14}CN and alkaline hydrolysis at 80°C of the resulting nitriles. Unfortunately, in the case of conjugated fatty acids, isomerization of the conjugated system occurred with the heat treatment. Therefore, to avoid degradation and isomerization of the *cis-trans* conjugated double bond system, the carbonation of the corresponding Grignard reagent with ^{14}C-carbon dioxide was chosen for the labeling (CHANNING AND SIMPSON, 1993).

Firstly, the unlabeled fatty acid was prepared. The bromide-compound **10** was transformed into its corresponding nonadecatrienylmagnesium bromide by reaction with magnesium turnings in refluxing anhydrous diethyl ether (90min). The Grignard reagent was carbonated with CO_2 liberated from barium carbonate (HOWTON *et al.*, 1954). (5Z,8Z,10E)-eicosa-8,11,13-trienoic acid **11** was obtained in 51% yield after purification by flash chromatography. GC analysis showed a 95% purity and small quantities of an impurity. The structure of (8Z,11Z,13E)-eicosa-8,11,13-trienoic acid was confirmed by GC-MS and NMR. An aliquot of the product was converted into its DMOX derivative for GC-MS analysis which gave a spectrum with a molecular ion of m/z =359. A mass interval of 12 units (instead of 14) occurred

between m / z 182 (C_7) and 194 (C_8), between m / z 222 (C_{10}) and 234 (C_{11}) and between 248 (C_{12}) and 260 (C_{13}), indicating the presence of a double bond in the position 8 and two conjugated double bonds in positions 11 and 13. The ^{1}H NMR spectrum of free **11** showed four distinct signals for olefinic protons, one multiplet and one double-triplet (δ_H = 5.36 and 5.66 ppm), which correspond to the shifts of the protons of the outer *trans-cis* conjugated diene system (14-H and 11-H), and two doublet-doublet (δ_H = 5.94 and 6.30 ppm), which correspond to the shifts of the protons of the inner *trans-cis* diene system (13-H and 12-H). The 9c-11t-18:2 showed a double-triplet for the proton 11-H at δ_H = 5.36 (BERDEAUX *et al.*, 1998a; LIE KEN JIE *et al.*, 1997). For the 8c-11c-13t-20:3 the same shift was found for the 11-H, but it was overlaid by the shift of the 10H.

Analysis of the impurity showed that it was a geometrical isomer of (8Z,11Z,13E)-eicosa-8,11,13-trienoic acid, the (8Z,11E,13E)-eicosa-8,11,13-trienoic acid. In fact, the DMOX derivative of the impurity and of (8Z,11Z,13E)-eicosa-8,11,13-trienoic acid gave the same spectrum. Moreover, traces of (8Z,11E,13E)-eicosa-8,11,13-trienoic acid were detected by ^{1}H NMR (2 minor multiplets at δ_H = 5.55 for the outer positioned protons (11-H, 14-H) and δ_H =6 ppm for the inner positioned protons (12-H, 13-H). ^{1}H NMR showed a minor triplet at δ_H = 2.77 for the protons 10-H of the methylene carbon which is between the E,E-diene system and the Z-double bond of (8Z,11E,13E)-eicosa-8,11,13-trienoic acid, while the triplet at δ_H = 2.88 corresponded at the protons 10-H of the methylene carbon which is between the E,Z-diene system and the Z-double bond of (8Z,11Z,13E)-octadeca-8,11,13-trienoic acid. These two triplets allowed a quantification of the (8Z,11E,13E)-eicosa-8,11,13-trienoic acid which represented 5% of the mixture, according to the GC and HPLC results.

The synthesis of the [1-^{14}C]-radiolabeled analogue **11'** from the bromo precursor **10** followed the same procedure as that described for the fatty acid, using $^{14}CO_2$ liberated from ^{14}C barium carbonate. Its radiochemical purity was determined by TLC and RP-HPLC as being 94% (specific activity: 31.3mCi / mmol). However, 0.4 mCi of (8Z,11Z,13E)-[1-^{14}C]-eicosa-8,11,13-trienoic acid with a radiochemical purity greater than 99% were obtained by preparative RP-HPLC.

5.2.2 Synthesis of (6Z,9Z,11E)-octadeca-6,9,11-trienoic acid

The octadecatrienoic acid was synthesized in a similar way by using pentane-1,5-diol instead of heptane-1,7-diol as starting material. A highly stereoselective Wittig condensation between the 5-(2-tetrahydropyranyloxy) pentylphosphonium salt **14** and the aldehyde **4** furnished the (3Z)-1-(t-butyldiphenylsilyloxy)-8-(2-tetrahydropyranyloxy)oct-3-ene **15** in 55% yield. After preparation of the phosphonium salt **18** from **15** in 74% yield (3 steps) as described for compound **8**, Wittig condensation of **18** with (2E)-non-2-enal in similar conditions as for the preparation of compounds **9** gave the (5Z,8Z,12E)-1-(2-tetrahydropyranyloxy)heptadeca-5,8,10-triene **19** in 68% yield (no geometric isomers were detected by GC, ^{1}H or ^{13}C NMR). The bromo precursor **20** was obtained in 53% yield by reaction with triphenylphosphine dibromide as described for the preparation of the bromide **10**. Formation of the corresponding heptadecatrienylmagnesium bromide (90min in refluxing diethyl ether) followed by carbonation with CO_2 produced (6Z,9Z,11E)-octadeca-6,9,11-trienoic acid **21**.

After purification by flash chromatography and derivatisation to FAME, GC analysis showed that the (6Z,9Z,11E)-octadeca-6,9,11-trienoic acid had a purity of >88% and it was accompanied by the (6Z,9E,11E)-octadeca-6,9,11-trienoic acid(11%) and small amounts of (6Z,9E,11Z)-octadeca-6,9,11-trienoic acid and (6Z,9Z,11Z)-octadeca-6,9,11-trienoic acid. Direct analysis on the free fatty acids by RP-HPLC confirmed this analysis. Structures of the (6Z,9Z,11E)-octadeca-6,9,11-trienoic acid and its isomers were determined by GC-MS. Therefore, an aliquot of the mixture was converted to its DMOX derivatives. GC-MS analysis of both isomers gave a spectrum with a molecular ion m/z 331, an intense ion at m/z 152 as well as ions of about equal intensity at m/z 166 and m/z 167. A similar type of fragmentation has been reported for unsaturated fatty acids having the first double bond in Δ6 position (DOBSON AND CHRISTIE, 1996). The other two double bonds of the conjugated system in the position 9 and 11 were located by fragments m/z 194 (C_8) and 206 (C_9), and fragments at 220 m/z (C_{10}) and m/z 232 (C_{11}).

The formation of the (6Z,9E,11E)-octadeca-6,9,11-trienoic acid as an important by-product was surprising because the former synthesis of the (8Z,11Z,13E)-eicosa-8,11,13-trienoic acid only gave small amounts of the corresponding *trans-trans* isomer (isomerization rate in the last step was less than 5%). On the contrary, the

isomerisation rate for the (6Z,9Z,11E)-octadeca-6,9,11-trienoic acid was about 2.5 fold higher. The (6Z,9Z,11E)-octadeca-6,9,11-trienoic acid seems to be more sensitive to the heat-treatment, which could be one cause of the high isomerization rate.

To separate the (6Z,9Z,11E)-octadeca-6,9,11-trienoic acid from the (6Z,9E,11E)-octadeca-6,9,11-trienoic acid isomer, HPLC-purification was carried out (BANNI *et al.*, 1994). RP-HPLC allowed the separation of the different geometrical isomers. The purified product showed a 95 % purity. The amounts of (6Z,9E,11Z)-octadeca-6,9,11-trienoic acid and of (6Z,9E,11E)-octadeca-6,9,11-trienoic acid were 2.4 and 2.5% respectively and only traces of (6Z,9Z,11Z)-octadeca-6,9,11-trienoic acid were detected. Structure of the purified (6Z,9Z,11E)-octadeca-6,9,11-trienoic acid was confirmed by NMR.

The synthesis of the [1-^{14}C]-radiolabeled analogue **21** from the heptadecatrienylbromide **20** followed the same procedure of that described for the fatty acid synthesis. As for the unlabeled compound, an important formation of the »*trans-trans*" isomer was detected. RP-HPLC was carried out giving small amounts (0.2mCi) of (6Z,9Z,11E)-[1-^{14}C]-octadeca-6,9,11-trienoic acid with a radiochemical purity of 95%.

6 *IN-VITRO* DESATURATION AND ELONGATION OF CLA AND CLA METABOLITES

6.1 Results

6.1.1 Fatty acid composition of the used rat liver microsomes

The realization of the *in-vitro* studies assumed rat liver microsomes with a high desaturation and elongation activity. As it was demonstrated microsomes isolated from livers of young rats have a high desaturation and elongation activity, therefore 3-week-old weaning Wistar rats were used in this study (BRENNER, 1971). They were fed a fat free diet which further increased the desaturation and elongation activity, by creating an essential fatty acid deficiency (MARCEL *et al.*, 1968; BRENNER, 1981). The index of essential fatty acid deficiency in the microsomes, calculated from the ratio of 5c8c11c-C20:3 / 5c8c11c14c-C20:4 was equal to 0.49. This was in agreement with the criterion required for the characterization of an essential fatty acid deficiency in liver microsomes (MOHRHAUER AND HOLMAN, 1963). The microsomes contained mainly palmitic acid (27.4%), stearic acid (17.2%) and oleic acid (16.4%) accompanied by small quantities of arachidonic acid (9.4%) (fatty acid composition of microsomes given in the annex, chap. 12.2.2.2).

6.1.2 Δ6-Desaturation of 9c11t-C18:2 and 10t12c-C18:2

In a first part of the experiment the Δ6-desaturation of 9c11t-C18:2 and 10t12c-C18:2 was investigated in comparison to their natural homologue, linoleic acid. The assay on the *in-vitro* Δ6-desaturation of 9c11t-C18:2 and 10t12c-C18:2 was carried out to examine whether these CLA isomers were able to be a substrate of the desaturation enzyme and what amount of the substrate could be converted. The Δ6-desaturation is the rate-limiting step in the conversion of linoleic acid to form arachidonic acid (COOK, 1991). It might be hypothesized that the conversion of CLA by this enzyme is essential to form further conjugated C20:4.

Figure 14 illustrates the conversion of [1-^{14}C]-linoleic acid, [1-^{14}C]-9c11t-C18:2 or [1-^{14}C]-10t12c-C18:2 (30-120 nmol) by liver microsomes into their corresponding desaturation metabolites analyzed by radio-HPLC.

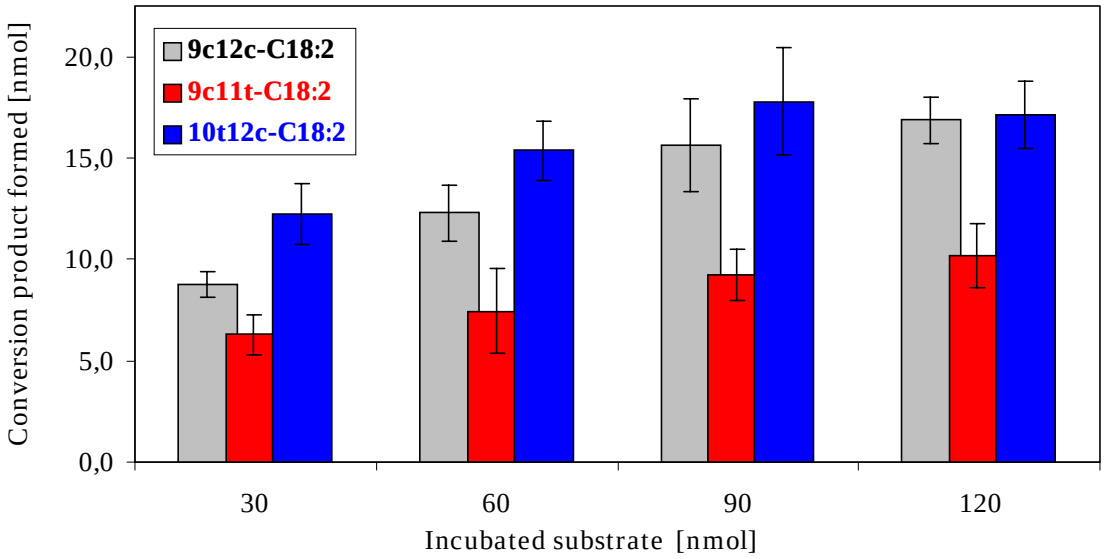


Figure 14 Conversion of [1-^{14}C]-linoleic acid, [1-^{14}C]-9c11t-C18:2 and [1-^{14}C]-10t12c-C18:2 (30-120 nmol) by liver microsomes incubated for 15min with 5mg of microsomal protein

The two investigated CLA isomers were desaturated, as was linoleic acid. The conversion rates of [1-^{14}C]-10t12c-C18:2 were significantly higher ($p < 0.05$) than those of [1-^{14}C]-linoleic acid at substrate concentrations between 30 and 60 nmol (12.2 ± 1.5 *versus* 8.7 ± 0.6 nmol with 30 nmol substrate), suggesting that 10t12c-C18:2 could preferentially be utilized by the Δ6-desaturase at low substrate concentrations. At the highest substrate concentration, 120 nmol, the conversion rates of 10t12cC-18:2 and linoleic acid were similar (17.1 ± 1.7 *versus* 16.9 ± 1.1 nmol). The saturating substrate concentration was reached at 90nmol for the conversion of 10t12c-C18:2, whereas it seemed to be reached only at 120nmol for the conversion of linoleic acid. (Detailed data, chap. 12.2.2.3)

Comparing the CLA isomers, the conversion of 9c11t-C18:2 was significantly ($p < 0.05$) lower than those of 10t12c-C18:2. It was only half of that of 10t12c-C18:2 at all substrate concentrations (6.3 ± 1.0 *versus* 12.2 ± 1.5 nmol using 30 nmol of substrate and 7.4 ± 2.1 *versus* 17.1 ± 1.7 nmol using 120 nmol of substrate), indicating that 10t12c-C18:2 was a better substrate for the Δ6-desaturase than 9c11t-C18:2. The saturating substrate concentration for 9c11t-C18:2 seems to be reached at the highest substrate concentration (120nmol). Under the experimental conditions, used in this work, the saturating substrate concentration was higher for the 9c11t-C18:2 than for 10t12c-C18:2 or linoleic acid. (Detailed data, chap. 12.2.2.3)

The better conversion of 10t12c-C18:2 compared to 9c11t-C18:2 was unexpected, because the double bond in Δ9 position and its *cis*-geometry was hypothesized to be essential for the binding to the enzyme (BRENNER, 1971). This was later confirmed by BERDEAUX *et al.* (1998b), where the geometrical isomer of linoleic acid, 9t12c-C18:2, which contains a *trans* double bond in Δ9 position was only slightly converted by the Δ6-desaturase Regarding the CLA, the hypothesis did not seem to fit. The 10t12c-C18:2 was well converted, although the double bond is shifted in the position Δ10 of the carbon chain.

- **Identification of the conversion products**

It has been demonstrated under similar experimental conditions that the conversion product of linoleic acid was identified as γ-linolenic acid (BERDEAUX *et al.*, 1998b). In contrast, the *in-vitro* desaturation products of 9c11t-C18:2 and 10t12c-C18:2 have to be identified. The use of known reference standards to identify the desaturation products was necessary, as classical analytical methods could not be applied considering the low conversion of the substrates and consequently a very low quantity of the metabolites so formed.

Radio-GC analysis of the methylated lipids extracted from rat liver microsomes incubated with [1-^{14}C]-linoleic acid, [1-^{14}C]-9c11t-C18:2 or [1-^{14}C]-10t12c-C18:2 revealed two labeled compounds, the remaining substrate and the desaturation product. The retention times for the CLA-isomers and their respective desaturation products varied from those of linoleic acid because of the conjugated double bond system. GC-MS analyses detected molecular ions m/z 292 and 294 for the desaturation products, which corresponded to the molecular weight of unlabeled and labeled octadecatrienoic acid methyl ester, respectively.

Finally the desaturation products of 9c11t-C18:2 and 10t12c-C18:2 were analyzed by GC-FID on a BPX-70 column. The metabolite of 9c11t-C18:2 was identified as 6c9c11t-C18:3, by comparing the retention time of the metabolite with that of a 6c9c11t-C18:3 standard obtained by total synthesis as described in chapter 5. The desaturation product formed from 10t12c-C18:2 was clearly identified as 6c10t12c-C18:3 by comparing its retention time with that of an authentic standard, which was isolated from adipose tissue of rats fed 10t12c-C18:2 in diet in an earlier experiment carried out by SEBEDIO *et al.* (2001). The standard was purified by chromatographic methods and characterized by suitable spectroscopic methods.

The two CLA isomers were both substrates for the Δ6-desaturase and were converted into their corresponding C18:3 metabolites. This is in agreement with earlier results published by BELURY AND KEMPA-STECZKO (1997). They observed that a radiolabeled CLA mixture was desaturated into a not further identified C18:3 product at a similar extent as linoleic acid was converted into γ-linolenic acid).

Regarding the results obtained from the present work, it may be possible that the two tested CLA isomers could inhibit the Δ6-desaturation of linoleic acid by competing with the enzyme, as they are both substrates of the Δ6-desaturase. This was earlier published by BRETILLON *et al* (1999) who have shown the *in-vitro* inhibition of the Δ6-desaturation of linoleic acid induced by 9c11t-C18:2 and 10t12c-C18:2.

6.1.3 Elongation of 6c9c11t-C18:3

The elongation of the 6c9c11t-C18:3 was studied in comparison to γ-linolenic acid as its non-conjugated homologue and a normal substrate of the elongation enzyme. Figure 15 illustrates the conversion of [1-^{14}C]-γ-linolenic acid and [1-^{14}C]- 6c9c11t-C18:3 (10-100 nmol) by rat liver microsomes into their corresponding elongation metabolites using radio-HPLC analysis.

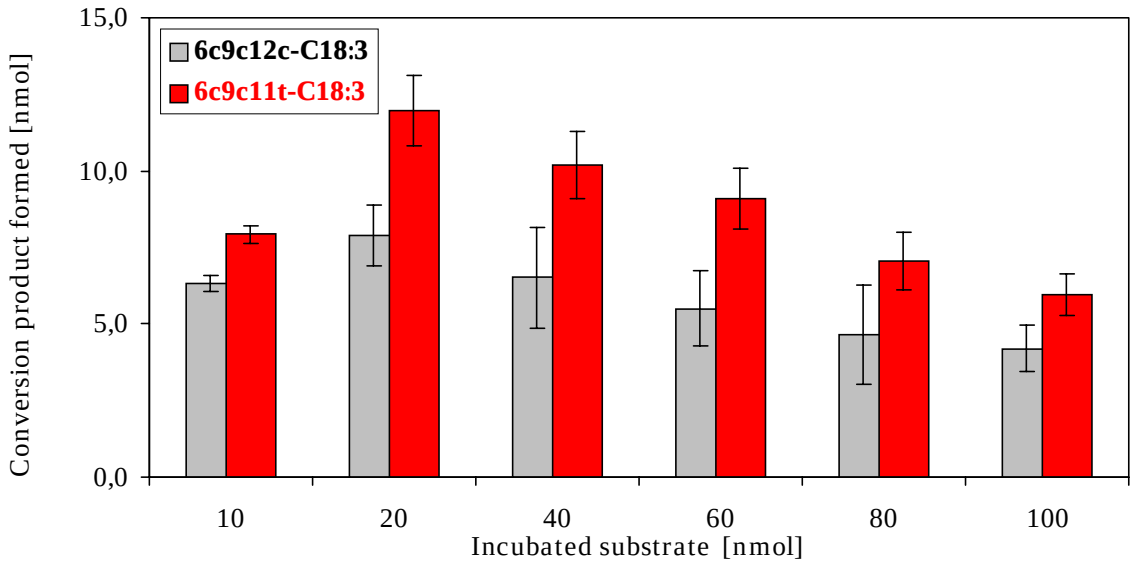


Figure 15 Conversion of [1-^{14}C]-γ-linolenic acid, [1-^{14}C]-6c9c11t-C18:3 (10-100 nmol) by liver microsomes incubated for 30min with 5mg microsomal protein

The 6c9c11t-C18:3 was elongated as γ-linolenic acid and its conversion was about 1,5 fold higher than for the normal homologue, suggesting that 6c9c11t-C18:3 was a

better substrate for the chain elongation enzyme. The conversion rates of [1-^{14}C]-6c9c11t-C18:3 were significantly higher ($p < 0.05$) than those of [1-^{14}C]-γ-linolenic acid at all substrate concentrations (7.9 ± 0.3 *versus* 6.3 ± 0.3 nmol with 10 nmol substrate and 6.0 ± 0.7 *versus* 4.2 ± 0.7 nmol protein with 100 nmol substrate, respectively). The differences in the conversion rates were highly significant for the substrate concentration 10, 20, 40 and 60 nmol (complete data, chap 12.2.2.4). The conversion rates of the two compounds decreased slightly at higher substrate concentrations (above 40 nmol). This might be related to the increase of substrate concentration, which could have inhibited the elongation.

It was hypothesized that the chain-elongation system needs a methylene interrupted *cis*-double bond system vicinal to the carboxylic end of the carbon chain (double bonds in Δ6 and Δ9 position) (TANAKA *et al.*, 1998). This may partly explain the good elongation of the investigated conjugated C18:3 as it contains such a double bond system and a double bond in position Δ11 in trans configuration. It can be hypothesized that the double bond in Δ11t may be a reason for the better elongation.

Some of the samples were additionally analyzed by radio-GC, to exclude the formation of subsequent metabolites of the elongation product during incubation. Radio-HPLC-analysis did not detect this event, because the substrate itself and the possible Δ5-desaturation product coeluted under the used analytical conditions. No Δ5-desaturation product was detected.

- **Identification of the conversion products**

Radio-GC analysis of FAME of liver microsomes incubated in the presence of [1-^{14}C]-6c9c11t-C18:3 and [1-^{14}C]-γ-linolenic acid revealed two labeled compounds, the remaining substrate and the elongation product. The retention times of the [1-^{14}C]-6c9c11t-C18:3 and its respective elongation product varied from those of γ-linolenic acid and the corresponding elongation product because of the conjugated double bonds system present in 6c9c11t-C18:3.

The elongation product of 6c9c11t-C18:3 was analyzed by GC-FID on a BPX-70 column. The metabolite of 6c9c11t-C18:3 was identified as 8c11c13t-C20:3, the corresponding conjugated C20:3 fatty acid, by comparison of the retention time with that of a 8c11c13t-C20:3 standard obtained by total synthesis as described in chap. 5.

6.1.4 Δ5-Desaturation of 8c11c13t-C20:3

Finally the Δ5-desaturation was investigated for 8c11c13t-C20:3 and compared to that of di-homo-γ-linolenic acid, as a normal substrate for the Δ5-desaturase.

Figure 16 illustrates the conversion of [1-^{14}C]-8c11c13t-C20:3 and [1-^{14}C]-8c11c14c-C20:3 (10-100 nmol) by liver microsomes into their corresponding desaturation metabolites.

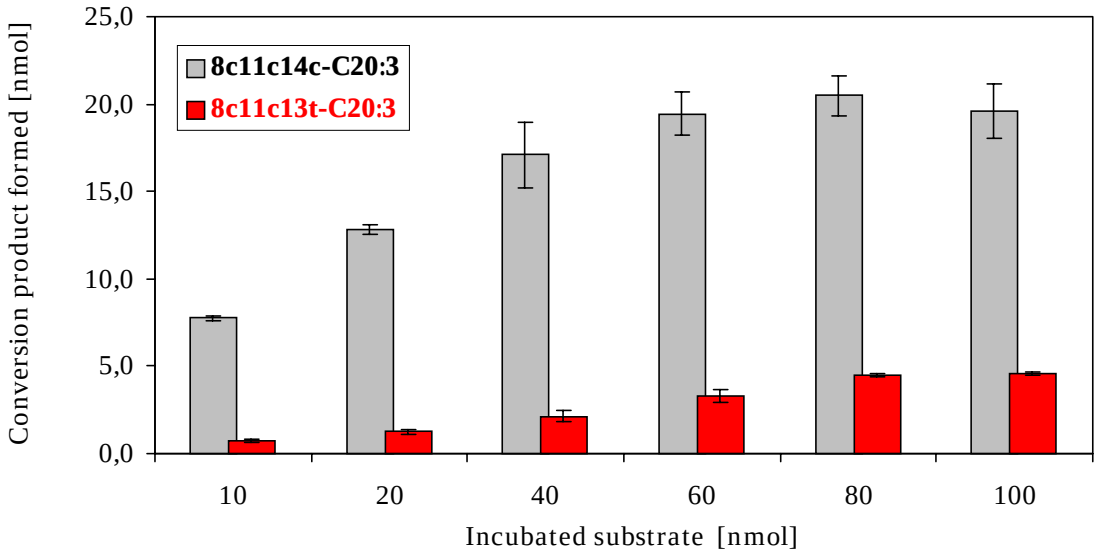


Figure 16 Conversion of [1-^{14}C]-8c11c14c-C20:3 and [1-^{14}C]-8c11c13t-C20:3 (10-100 nmol) by liver microsomes incubated for 15min with 5mg of microsomal protein

8c11c13t-C20:3 was converted into its corresponding Δ5-desaturation product, 5c8c11c13t-C20:4, similarly to di-homo-γ-linolenic acid into arachidonic acid. Comparing the conversion rates of the two incubated substrates, [1-^{14}C]-8c11c13t-C20:3 was significantly less converted than [1-^{14}C]-8c11c14c-C20:3 at all substrate concentrations. At the lowest concentration, [1-^{14}C]-8c11c13t-C20:3 was about ten times less converted than [1-^{14}C]-8c11c14c-C20:3 (0.8 ± 0.1 *versus* 7.8 ± 0.1 nmol with 10 nmol substrate, respectively) and about five times less converted at the highest concentration (4.6 ± 0.1 *versus* 19.6 ± 1.5 nmol with 100 nmol substrate, respectively), indicating that 8c11c13t-C20:3 was a poor substrate for the Δ5-desaturase.

The saturating substrate level was reached for [1-^{14}C]-8c11c14c-C20:3 at 80nmol whereas for [1-^{14}C]-8c11c13t-C20:3 the plateau seems to be reached at 100nmol. The complete data are shown in the annex (chap 12.2.2.5).

- **Identification of the conversion products**

Radio-GC analysis of methylated lipids of liver microsomes incubated in the presence of [1-^{14}C]-8c11c14c-C20:3 or [1-^{14}C]-8c11c13t-C20:3 revealed two labeled compounds, the remaining substrate and the desaturation product. As before for the Δ6-desaturation study and the elongation study the retention times for the conjugated 20:3 fatty acid and its corresponding desaturation product varied from those of 8c11c14c-C20:3 because of the conjugated double bonds.

The desaturation product from 8c11c13t-C20:3 was clearly identified as 5c8c11c13t-C20:4 by GC-FID on a BPX-70 column. The retention time of the desaturation product was compared with an authentic standard previously purified and characterized from adipose tissue of rats fed 9c11t-C18:2 (SÉBÉDIO *et al.*, 2001).

6.2 Discussion: metabolism of CLA isomers

The knowledge on the metabolism of CLA into polyunsaturated conjugated fatty acids was mainly based on results obtained *in-vivo* after CLA supplementation of animals. The different conjugated fatty acids were detected in tissue lipids as the usual intermediates of the enzymatic pathway, whereas the mechanisms have never been investigated. (SEBEDIO *et al.*, 2001; BANNI *et al.*, 2001). The *in-vitro* experiments carried out in this work allow to investigate if CLA are enzymatically converted into polyunsaturated conjugated fatty acids using the proposed pathways similar to the metabolism of linoleic acid into arachidonic acid. The results from this work confirmed that conjugated C18:3, C20:3 and C20:4 fatty acids, identified in the various animals studies, result from successive desaturation and elongation of CLA.

Differences in the metabolism of 9c11t-C18:2 and 10t12c-C18:2 were found. As shown in figure 17, 9c11t-C18:2 seems mainly to be converted into conjugated 20:3 (8c11c13t-C20:3), it was only slightly converted into 6c9c11t-C18:3 by Δ6-desaturation and well elongated into 8c11c13t-C20:3. The conversion of 8c11c13t-C20:3 in the corresponding conjugated C20:4 was considerably low. 10t12-C18:2 was mainly converted into conjugated 6c10t12c-C18:3 fatty acid, as it appeared to be a very good substrate for Δ6-desaturase.

Regarding the conversion of linoleic acid into arachidonic acid, it was suggested that the Δ6-desaturation is the rate limiting step of the enzymatic pathway, therefore only small quantities of the intermediates γ-linolenic acid and di-homo-γ-linolenic acid

were observed (COOK, 1991; SPRECHER, 1977). For 9c11t-C18:2 it seems that the Δ6-desaturation is also a rate limiting step, as its conversion was lower than this of linoleic acid. Moreover the Δ5-desaturation seems to be another rate limiting step as the formation of conjugated C20:4 was very low.

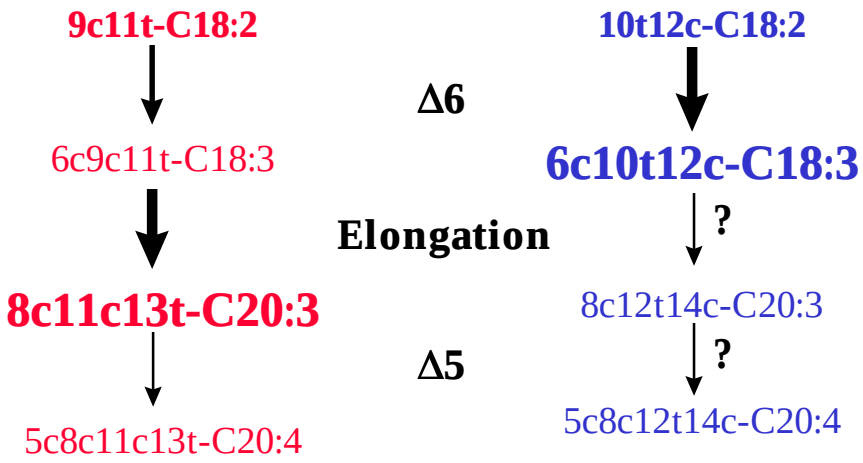


Figure 17 Desaturation and elongation of 9c11t- and 10t12c-C18:2

The results obtained from this work are in agreement with the nutritional studies on rats and mice carried out so far. Recently BANNI *et al.* (2001) reported the occurrence of conjugated C18:3 and C20:3 fatty acids in different lipid classes of the liver after administration of 9c11t-C18:2 to rats. The content of conjugated C20:3 fatty acid was about four-fold higher than that of conjugated C18:3 or C20:4 fatty acid. They measured 0.7 µg / mg lipid of conjugated C18:3, 3.9 µg / mg lipid of conjugated C20:3 and 1.0 µg / mg lipid of conjugated C20:4 (BANNI *et al.*, 2001). Similar results were discussed in a feeding study on rats using pure CLA isomers (SEBEDIO *et al.*, 2001). For 9c11t-C18:2 more conjugated C20:3 than C18:3 were found in the liver, whereas the inverse effect was reported for 10t12c-C18:2. The high level of 6c10t12c-C18:3 combined with low 10t12c-C18:2 level detected in liver *in-vivo* could be explained by the observed *in-vitro* result in this work, showing a good Δ6-desaturation of 10t12c-C18:2, under the condition that conjugated C18:3 is only slightly elongated into conjugated C20:3 and enriched in the tissue.

Comparing the incorporation levels of 10t12c-C18:2 and 9c11t-C18:2 *in-vivo*, the incorporation of 9c11t-C18:2 was about three fold higher in liver TAG (BANNI *et al.*, 2001). Varying incorporation levels of the two CLA isomers may be related to differences in the metabolism or different metabolic rates in enzymatic reactions of

the two isomers. As it was shown in this study the investigated CLA isomers did not act in a comparable way.

To elucidate the metabolic pathway for the 10t12c-C18:2 the respective conjugated C18:3 and C20:3 metabolites should be synthesized. Regarding published *in-vivo* results it seems to be possible that there will be differences in the elongation and Δ5-desaturation activity compared to 9c11t-C18:2. It may be hypothesized that the elongation of the conjugated C18:3 metabolite of 10t12c-C18:2 will lead to different results than these of 9c11t-C18:2, because the chemical structure is quite different. The first (Δ6c) and the second (Δ10t) double bond in the carbon chain are interrupted by two methylene groups. As mentioned above the elongation enzyme seems to be specific to that region and needs a methylene interrupted double bond system (TANAKA *et al.*, 1998)

The *in-vitro* results obtained in this work, on the metabolism 9c11t-C18:2 indicated a low formation of conjugated C20:4 fatty acid. The formation of prostaglandin homologues derived from conjugated C20:4, as it was earlier hypothesized, seems to be unlikely. The modification of the eicosanoid pathway induced by CLA might be related to another mechanism, as e.g. the interaction with enzymes which are directly implicated in the synthesis of prostaglandins.
Di-homo-γ-linolenic acid is the precursor of prostaglandins of the series one. As 9c11t-C18:2 is mainly converted into conjugated C20:3, the formation of prostaglandin homologues derived from conjugated C20:3 may be possible and has to be investigated.

7 INFLUENCE OF DIETARY CLA ON PGI_2 AND TXB_2 SYNTHESIS *IN-VIVO*

7.1 Results

During the experimental 3-week-period animals showed normal behavior and were found in good health. The food intake was regular and similar in all nutritional groups of the young and adult rats. Weight gain was the same in the different dietary groups of young and adult rats (data not shown).

7.1.1 Incorporation of CLA into tissues and plasma

The incorporation of 9c11t- and 10t12c-C18:2 is presented in figure 18.

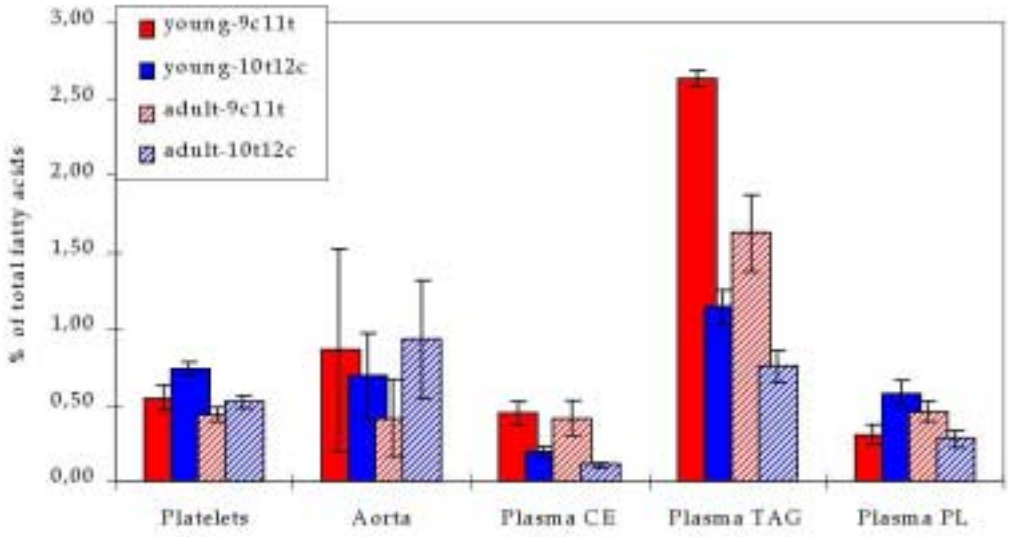


Figure 18 Incorporation of 9c11t- and 10t12c-C18:2 in the different tissues.

The highest CLA incorporation were reported in the TAG-fraction of plasma. 9c11t-C18:2 represented 2.63% and 1.62% of total fatty acids in plasma TAG of young and adult rats, respectively. The incorporation levels of 10t12c-C18:2 in the plasma TAG was significantly lower than those of 9c11t-C18:2 (1.13% and 0.75% for young and adult rats, respectively, $p<0.05$). Moreover age-related changes in the incorporation level were detected among the young and adult rats. Adult rats showed a lower incorporation of CLA. In plasma CE (young and adult), and plasma PL of adult rats, 9c11t-C18:2 was preferably incorporated compared to 10t12c-C18:2 ($p<0.05$). In the contrary, in plasma PL of young rats a higher incorporation of 10t12c-C18:2 was

found ($p<0.05$). In platelet lipids only small changes were found in the incorporation levels. In aorta variations in the incorporation levels of the two CLA isomers were not significantly different, because of great variations of the individual values. (complete results in the annex, chap. 12.2.3.6)

7.1.2 Concentrations of circulating TXB_2 and 6-keto-$PGF_{1\alpha}$

The results for the circulating TXB_2-contents are presented in figure 19.

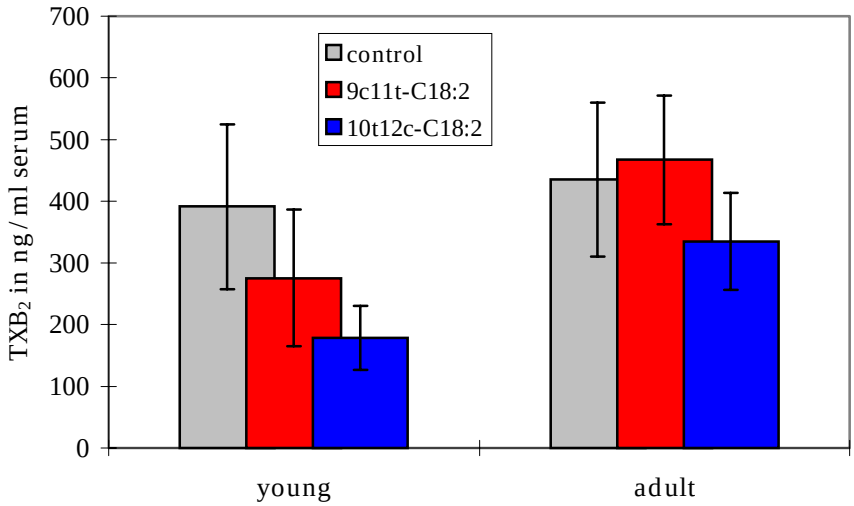


Figure 19 TXB_2 concentrations in serum

In adult rats no significant difference in the serum TXB_2-contents were detected between the three dietary groups. A significant decrease ($p<0.05$) of the TXB_2-level was observed in the young rats between control (391 ± 134ng / ml serum) and 10t12c-C18:2 (179 ± 52ng / ml serum). The TXB_2-content of 9c11t-C18:2 (276 ± 111ng / ml serum) was intermediate between the value observed in the control group and the 10t12c-C18:2 group. (complete results, annex, chap. 12.2.3.3). Comparing the TXB_2-content of young and adult rats fed 10t12c-CLA, a significantly higher TXB_2-level was observed in adult rats (335 ± 79 in adult *versus* 179 ± 52ng / ml serum in young).

The results of the plasma 6-keto-$PGF_{1\alpha}$-levels, are presented in figure 20.

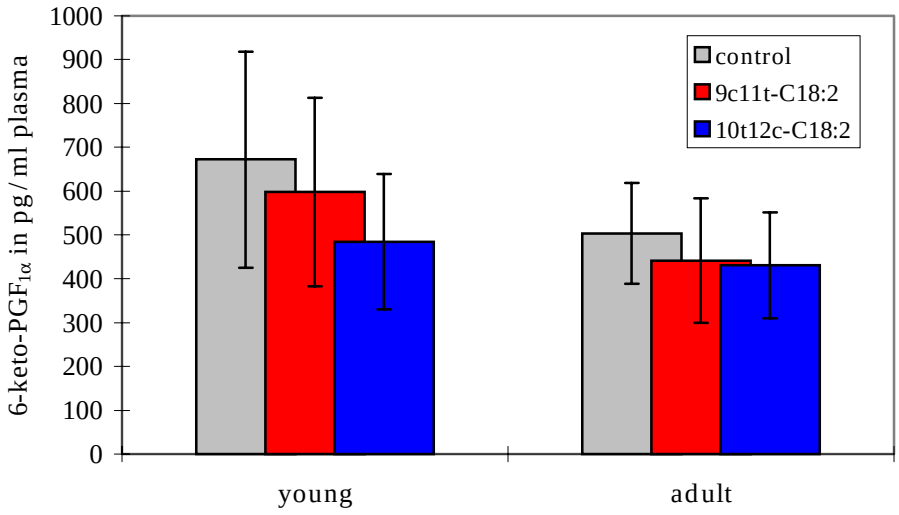


Figure 20 6-keto-$PGF_{1\alpha}$ concentrations in plasma

No significant changes between the different dietary groups and ages were observed. A small but not significant decrease of 6-keto-$PGF_{1\alpha}$ was found in the young rat comparing control and CLA fed rats (control 672 ± 247, 9c11t-C18:2 598 ± 215, 10t12c-C18:2 484 ± 155pg / ml plasma). A not significant decrease was also observed in the adult rats but the differences were lower between the control and the CLA groups. The detailed results are shown in the annex, chap. 12.2.3.4.

7.1.3 Production of PGI_2 from aorta

The results of the PGI_2 release, measured as formation of 6-keto-$PGF_{1\alpha}$, from the incubated aorta are presented in figure 21.

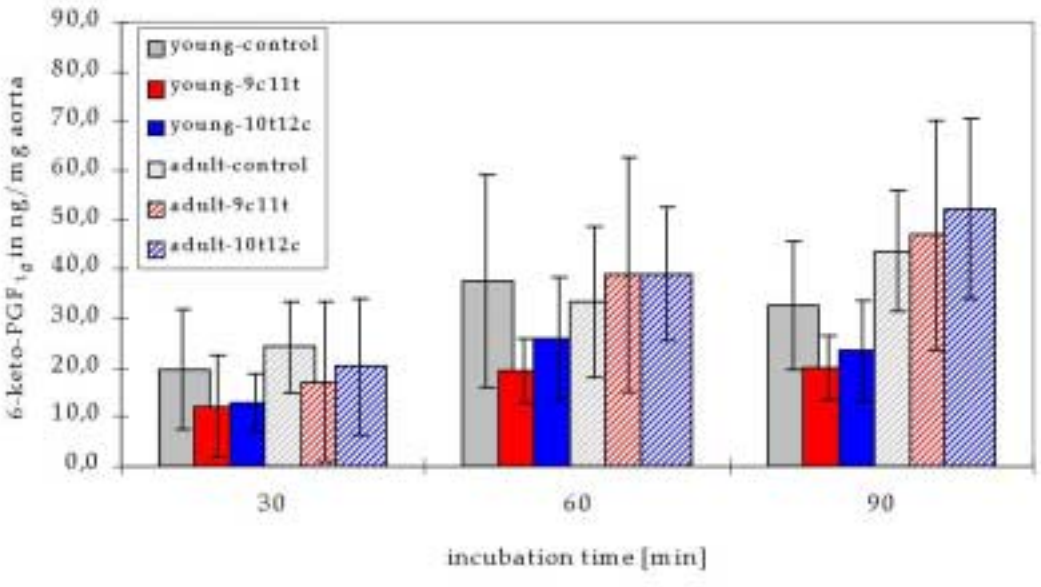


Figure 21 Formation of 6-keto-$PGF_{1\alpha}$ by incubated aorta

The different diets did not influence the production of PGI_2 generated from the incubated aorta. The 6-keto-$PGF_{1\alpha}$ contents measured in the incubation media showed a small, but not significant decrease between control and CLA fed young rats. The 6-keto- $PGF_{1\alpha}$ levels of adult rats were the same in all groups. The complete results are presented in the annex (chap. 12.2.3.5)

7.1.4 Arachidonic acid contents of aorta and platelets

The arachidonic acid contents of aorta and platelet lipids calculated from the fatty acid profile are shown in figure 22.

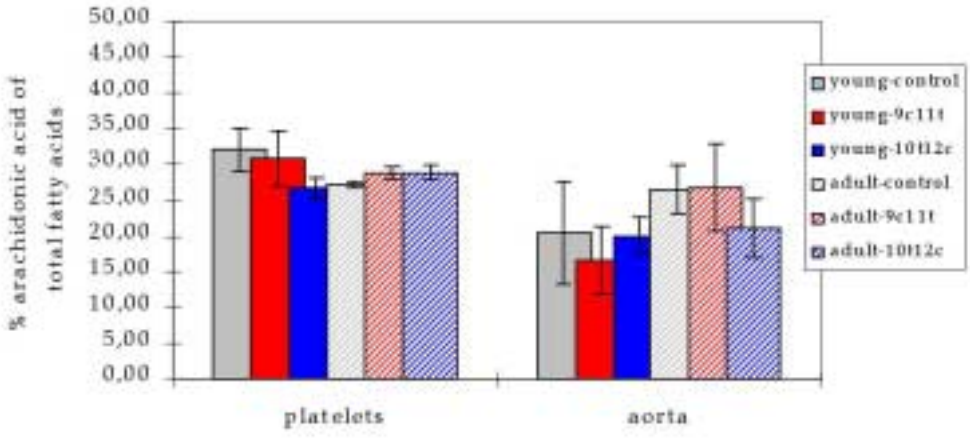


Figure 22 Arachidonic acid contents (% of total fatty acids) in aorta and platelets.

Figure 22 showed that the arachidonic acid contents remained unchanged after feeding of either 9c11-t-C18:2 or 10t12c-C18:2. Also no age-dependent changes were observed. The detailed data are presented in the annex (chap. 12.2.3.6)

7.2 Discussion

The influence of CLA on prostacyclin and thromboxane synthesis *in-vivo* was investigated in order to establish a possible link between dietary CLA and the formation of eicosanoids. TXA_2 and PGI_2 are important eicosanoids derived from arachidonic acid, which are implicated in platelet function and thrombosis. They are measured by their stable metabolites TXB_2 and 6-keto-$PGF_{1\alpha}$. The study was carried out in two ages of the rats to reveal age-related differences between the young and the adult organism.

7.2.1 Incorporation of CLA into tissues and plasma

The incorporation of the two examined CLA isomers was investigated in the implicated tissues (aorta, platelets and plasma). A better incorporation of 9c11t-C18:2 than of 10t12c-C18:2 was found in CE and TAG of plasma for the two ages. Regarding the PL of plasma the growing animals incorporated more 10t12c-C18:2 than 9c11t-C18:2 whereas inverse effects were found for adult rats. In the adult rats the incorporation level of the CLA isomers was inferior to that of growing rats in the plasma lipid classes, suggesting differences in the metabolic state. This could be related to the building of new tissues and formation of membranes in the young rat during the growing phase (BRENNER, 1981).

The incorporation of the two CLA isomers in TAG in plasma was the highest compared to all other analyzed lipid classes, indicating an important relationship with the lipid metabolism. The plasma TAG fraction reflects the ingested nutritional lipids and could explain the high CLA contents.

Regarding the CLA contents of aorta and platelets the CLA isomers were incorporated in similar amounts, but the incorporation level was only about half of that of TAG of plasma. It seems that 10t12c-C18:2 was slightly more incorporated than 9c11t-C18:2.

The results obtained in this study could be compared to former studies investigating the incorporation of CLA into different tissues, which were reviewed by (YURAWECZ *et al.*, 1999). It was shown that the incorporation of the CLA isomers depended on the analyzed tissues. It was described that 9c11t-C18:2 was preferably incorporated in liver (KRAMER *et al.*, 1998; BELURY AND KEMPA-STECZKO, 1997; SEBEDIO *et al.*, 2001), whereas 10t12c-C18:2 was more accumulated than 9c11t-C18:2 in muscle and spleen lipids. Concerning the incorporation of the two CLA isomers in the plasma lipid classes no data are published investigating the incorporation of a synthetic CLA mixture fed in high amounts to animals, into plasma. Studies mainly reported the incorporation of CLA in different lipid classes of liver, where a highest incorporation of CLA was observed in TAG of liver (BANNI *et al.*, 1999; BELURY AND KEMPA-STECZKO, 1997). A more recent publication by SEBEDIO *et al.* (2001) observed the same effects when fed either separated 9c11t-C18:2 and 10t12c-C18:2 or a CLA mixture. The incorporation of 9c11t-C18:2 compared to 10t12c-C18:2 was about three-times higher in TAG of liver.

7.2.2 Dietary CLA and eicosanoid synthesis

The ability of CLA to influence the eicosanoid formation was earlier reported as a decrease of PGE_2-level in mouse keratocytes after feeding a CLA mixture (LIU AND BELURY, 1998). Arachidonic acid is the precursor of PGE_2 and also of many other prostaglandins. Therefore an interaction of CLA with the metabolism of arachidonic acid into eicosanoids is possible and was investigated in this experiment.

In this study, a significant decrease of the TXB_2 content was observed in young rats after feeding 10t12c-C18:2. The feeding of 9c11t-C18:2 to young rats induced only a small not significant diminution of the TXB_2 content. Only small not significant changes were observed in young rats on circulating 6-keto-$PGF_{1\alpha}$ and generation of 6-keto-$PGF_{1\alpha}$ from incubated aorta. No effect was observed in adult rats, suggesting that the observed effects were age-related.

The decrease of the TXB_2 content found in the present study might be induced by different factors, as the decrease of the precursor (arachidonic acid) or a direct interaction of CLA with the enzymes implicated in the formation of TXA_2 from arachidonic acid (PGHS, thromboxane synthase). The obtained results showed only a small not significant decrease of arachidonic acid in platelets in 10t12c-C18:2 fed

young rats, suggesting its probable implication in the diminution of TXB_2, but seems to be not the unique cause.

The relationship between CLA and TXB_2 formation was earlier observed in an *in-vitro* experiment on platelet aggregation (TRUITT *et al.*, 1999). The incubation of isolated human blood platelets with a CLA mixture, 9c11t-C18:2 or 10t12c-C18:2 inhibited the formation of proaggregatory TXA_2. The authors measured a decrease of [^{14}C] TXB_2 produced from exogenously added [^{14}C] arachidonic acid in the presence of CLA. These results suggested an important interaction of CLA with the PGHS or thromboxane synthase.

A recent publication reported a modulation of PGHS activity induced by different CLA isomers (BULGARELLA *et al.*, 2001). 9c11t-C18:2 and 10t12c-C18:2 significantly reduced the activity of the enzyme *in-vitro* in ram seminal vesicle, suggesting an important inhibition of the cyclooxgenase pathway and a diminution of the resulting prostaglandins, e.g. PGE_2, PGI_2, TXA_2. The authors discussed the inhibition of PGHS by CLA as a competitive inhibition.

In conclusion only small changes could be reported on the PGI_2 and TXA_2 metabolism induced by nutritional CLA, which might be related to an interaction of CLA with the enzymes implicated in the formation of TXA_2 and the slight decrease of the precursor. Moreover the observed effects seem to be age-related as the young animal was more sensitive to dietary CLA.

8 CONCLUSION AND PERSPECTIVES

As one part of the present work, the influence of processing on the CLA content in cheese and the following culinary utilization was investigated. Dairy products represent an important source of natural CLA in human nutrition. But until now, only limited data on CLA in different foodstuffs, regarding the influence of food processing, storage and culinary utilization are available. The present study has as an objective to reveal the interaction of cheese processing and the CLA content. Under the examined fabrication and cooking conditions, the CLA content remained unchanged. None of the technological treatments or the use of different fermentation bacteria induced changes neither in the CLA content nor in the CLA isomer composition. The CLA content seems to be totally stable in the Emmental cheese. CLA might be protected in this type of dairy product by the food matrix and its the natural incorporation in food lipids as TAG. In opposite to the earlier described instability of CLA standards, CLA in foodstuffs containing naturally seem to be unaffected by technological treatments and storage and the CLA contents remained unchanged.

Regarding to the use of CLA in functional food the use of synthetic CLA as TAG and not as free fatty acid may lead to good stability of CLA in the foodstuff. CLA in funtional foods are additives. To obtain a better inclusion in the food matrix the CLA addition could be added to the raw material, the mixture will be more homogenous.

In the second part of this work the metabolism of CLA and the interaction of CLA with the eicosanoid pathway was investigated. It was often hypothesized that the ability of CLA to modify the eicosanoid synthesis is one possible way of action and could be the reason for the various beneficial effects. Furthermore CLA were suggested to be metabolized by the same enzymatic pathway as linoleic acid into long chain PUFA and to form homologues of arachidonic acid and prostaglandins containing conjugated double bonds.

The *in-vitro* experiments carried out in this work to investigate the conversion of CLA by desaturation and elongation enzymes suggested that 9c11t-C18:2 was converted mainly into conjugated C20:3, the conversion into conjugated C20:4 was very low. Therefore the formation of superior metabolites derived from conjugated C20:4 of 9c11t-C18:2 *via* cyclooxygenation seems to be less plausible, but has to be further investigated to ensure this hypothesis.

The Δ6-desaturation of 10t12c-C18:2 revealed a good conversion into its corresponding C18:3 metabolite compared to linoleic acid. To investigate the elongation and Δ5-desaturation of the metabolites of 10t12c-C18:2, these compounds have to be synthesized and may be compared to the results of 9c11t-C18:2. As in-vivo experiments reported the accumulation of conjugated C18:3 after feeding 10t12c-C18:2 it might be possible that the conjugated C18:3 metabolite is not further metabolized into a conjugated C20:4. Furthermore the ability of the conjugated C18:3 and C20:3 of the two investigated CLA isomers to inhibit the desaturation and chain elongation of linoleic acid has to be examined.

The effect of CLA on prostacyclin and thromboxane synthesis was investigated to establish a possible link between nutritional CLA and the eicosanoid metabolism. The feeding of CLA to rats only led to changes in the thromboxane synthesis of the young rat induced by 10t12c-C18:2, indicating that prostacyclin and thromboxane were only less modified by CLA *in-vivo.* As earlier published results suggest that CLA may modify thrombosis by influencing platelet function and aggregation, the effects on platelet function *in-vivo* should be further investigated. In this study slight changes in the TXB2 content were found in the young rat only, but not in the adult rat suggesting that the metabolism of arachidonic acid into eicosanoids may only be slightly influenced by CLA in the adult.

With regard on possible health benefits for humans, research has to be increased to ensure that CLA supplementation of the human diet will be advantageous. Furthermore toxicological aspects of a high CLA intake in human have to be investigated. CLA may induce different effects according to age and health status. This has to be taken into account in the future research on CLA.

9 SUMMARY

In the last decade it has been reported that conjugated linoleic acid (CLA) may elicit beneficial effects for human health. Several animal experiments have shown anticarcinogenic and antiatherogenic properties as well as an influence on body fat and energy metabolism. CLA are found naturally in ruminant fats and dairy products.

Because of their chemical structure, CLA have been described to be more sensitive to oxidation processes or heat treatment than their analogues with non-conjugated double bonds. In order to get information on the stability of CLA during food production, the influence of Emmental cheese processing on the CLA content and isomer composition was evaluated as a first part of this work. The content of CLA in the used raw milk was determined to be 8.62±1.92mg/g fat and 8.38mg/g fat in the ripened cheese under normal processing conditions. No changes in the CLA content were observed during the cheese manufacturing process. Neither the use of different fermentation cultures (different strains of *Propionibacterium* spp.) nor changes in processing temperatures influenced the CLA content in the final cheese. Also the CLA isomer composition remained unchanged by processing. The fabrication of cheese spread with Emmental or the cooking or grilling of Emmental did not influence the CLA content. CLA seem to be stable in this type of dairy product under the conditions examined.

The way of action of CLA until now remains unclear. It was often hypothesized that CLA may act by modifying the arachidonic acid metabolism and may influence the formation of different eicosanoids. Consequently, the implication of CLA in the eicosanoid pathway and its metabolism into conjugated long chain fatty acids by successive desaturation and chain-elongation was investigated in a second part of this work.

Therefore the conjugated C18:3 and C20:3 metabolites of 9c11t-C18:2 (major isomer of conjugated linoleic acid) and its radiolabeled analogs were synthesized stereoselectively by a total synthesis in 12 steps with commercially available reagents. The synthesis involved two highly stereoselective Wittig reactions, which permitted the formation of the two double bonds under cis configuration. In the second Wittig reaction (2E)-non-2-enal was used as aldehyde, to obtain the *trans* double bond. To avoid isomerization of this double bond this Wittig reaction was

carried out under modified conditions using a weak base. The reaction scheme allowed in the last step the formation of the fatty acid as unlabeled or ^{14}C labeled fatty acid using a Grignard reaction.

The metabolism of CLA into conjugated C20:4 was studied in *in-vitro* desaturation and elongation experiments on isolated rat liver microsomes. First the Δ6 desaturation of the CLA isomers compared to linoleic acid was investigated. It was shown that 10t12c-C18:2 was desaturated in a similar way than linoleic acid, whereas the conversion of 9c11t-C18:2 was only half of that of 10t12c-C18:2. Further investigation of the chain elongation and Δ5-desaturation of the synthesized conjugated C18:3 and C20:3 metabolites of 9c11t-C18:2 showed that the conjugated C18:3 was better elongated than γ-linolenic acid. 8c11c13t-C20:3 was about 5 fold less desaturated into conjugated C20:4 than di-homo-γ-linolenic acid into arachidonic acid. This suggests that 9c11t-C18:2 will be mainly converted into conjugated C20:3.

An *in-vivo* study on young and adult rats using 9c11t- and 10t12c-C18:2 was carried out to examine an interaction of nutritional CLA with PGI_2 and TXA_2 metabolism. PGI_2 and TXA_2 were analyzed as their stable metabolites 6-keto-$PGF_{1\alpha}$ and TXB_2 in plasma and serum, respectively. A significant decrease of the TXB_2 content was observed in young rats after feeding 10t12c-C18:2. The feeding of 9c11t-C18:2 to young rats induced only a small but not significant decrease of the TXB_2 content. Only small but not significant changes were observed in young rats on circulating 6-keto-$PGF_{1\alpha}$. No effect was observed in adult rats. The reported results might be related to an interaction of CLA with the enzymes implicated in the formation of TXA_2 and the slight decrease of the precursor, and seems to be age-related as the young animal was more sensitive to dietary CLA.

10 ZUSAMMENFASSUNG

Die Forschung auf dem Gebiet der konjugierten Linolsäureisomere (CLA) hat in den letzten 10 Jahren stetig zugenommen, seitdem ihre besonderen physiologischen Eigenschaften beschrieben wurden und eine gesundheitsfördernde Wirkung für den Menschen in Betracht gezogen wird. In zahlreichen Tierexperimenten zeigten CLA ein antikanzerogenes und antiatherogenes Potential, sowie einen begünstigenden Einfluß auf das Verhältnis von Körperfett zu Körperprotein und den Energiestoffwechsel. CLA sind natürlicherweise in Milch und Milchprodukten und in tierischen Fetten, hauptsächlich von Wiederkäuern, zu finden.

Es wurde beschrieben, daß CLA im Vergleich zu Linolsäure eine geringere Stabilität gegenüber Oxidation oder thermischen Prozessen aufweisen. Dies hängt möglicherweise mit ihrer chemischen Struktur insbesondere mit dem Vorhandensein von konjugierten Doppelbindungen im Molekül zusammen. Die Untersuchung der Stabilität von CLA während der Lebensmittelfabrikation stellt den ersten Teil der vorliegenden Arbeit dar. Dazu wurde der Herstellungsprozeß von Emmentaler Käse verfolgt und der Einfluß auf den CLA Gehalt und das CLA Isomerenmuster überprüft. Der CLA Gehalt in der verwendeten Rohmilch betrug 8,62±1,92mg/g Fett und im gereiftem Emmentaler Käse wurden 8.38mg/g Fett gemessen. Eine Veränderung des CLA Gehaltes während des Herstellungsprozesses konnte nicht beobachtet werden. Weder die Verwendung verschiedener *Propionibacterium* spp. noch die Veränderung der Prozeßtemperaturen während der Käseherstellung hatten einen Einfluß auf den CLA Gehalt. Desgleichen blieb das CLA Isomerenmuster unverändert. Die Verwendung des Emmentaler Käses bei Koch- und Bratprozesse sowie die Herstellung von Schmelzkäse veränderte den CLA Gehalt nicht. Somit kann geschlossen werden, daß die untersuchten Herstellungsbedingungen keinen Einfluß auf den CLA Gehalt in dieser Art von Lebensmittel haben.

Bis heute ist der Wirkungsmechanismus von CLA nicht geklärt. Es wurde mehrfach vermutet, daß CLA den Arachidonsäurestoffwechsel und die Bildung verschiedener Eicosanoide beeinflußt. Deshalb wurde im zweiten Teil dieser Arbeit die Einwirkung von CLA auf den Eicosanoidstoffwechsel und ihre Metabolisierung in konjugierte langkettige mehrfach ungesättigte Fettsäure durch Desaturierung und Elongation untersucht.

Dazu wurden die konjugierten C18:3 und C20:3 Metaboliten von 9c11t-C18:2 (als dem CLA Hauptisomer) und ihre radioaktiv-markierten Analoge steroselektiv

synthetisiert. Die Synthesen wurden mit kommerziell erhältlichen Reagenzien in 12 Stufen durchgeführt. Der Syntheseweg beinhaltete zwei hoch stereoselektive Wittig-Reaktionen, durch welche zwei Doppelbindungen in cis-Konformation gebildet wurden. Als Aldehyd wurde in der zweiten Wittig-Reaktion (2E)-Non-2-enal verwendet um die trans-Doppelbindung zu bilden. Zur Verhinderung von Isomerisierungsreaktionen an der neuzubildenen Doppelbindung wurde die zweite Wittig-Reaktion unter veränderten Reaktionsbedingungen mit eine sehr schwachen Base durchgeführt. Das verwendete Reaktionsschema ermöglichte in der letzten Stufe die Bildung der radioaktivmarkiertern oder nicht markierter freien Fettsäure durch Anwendung einer Grignard-Reaktion.

Die Verstoffwechselung von CLA in konjugierte C20:4 wurde in *in-vitro* Desaturierungs- und Elongationsversuchen an isolierten Rattenlebermikrosomen untersucht. Als erstes wurde die Δ6-Desaturierung der CLA Isomere im Vergleich zu Linolsäure untersucht. Hierbei wurde gezeigt, daß 10t12c-C18:2 in vergleichbarer Menge wie Linolsäure umgesetzt wurde, während nur halb soviel 9c11t-C18:2 verstoffwechselt wurde. Die weitere Untersuchung der Elongation und Δ5-Desaturierung an den synthetisierten konjugierten C18:3 und C20:3 Metaboliten ergab, daß 6c9c11t-C18:3 besser umgesetzt wurde als γ-Linolensäure. 8c11c13t-C20:3wurde fünfmal weniger in 5c8c11c13t-C20:4 umgesetzt als di-Homo-γ-Linolensäure in Arachidonsäure. Aus den Ergebnissen kann geschlossen werden, daß 9c11t-C18:2 hauptsächlich in konjugiertes C20:3 metabolisiert wird.

In einer in-vivo Interventionsstudie wurde der Einfluß von mit der Nahrung zugeführtem 9c11t- und 10t12c-C18:2 auf den PGI_2 und TXA_2 Stoffwechsel in jungen und erwachsenen Ratten untersucht. Die Gehalte an PGI_2 in Plasma und TXA_2 in Serum wurden durch Analyse der stabilen Metaboliten 6-keto-$PGF_{1\alpha}$ und TXB_2 ermittelt. Eine signifikante Verminderung des TXB_2 Gehaltes wurde nach Fütterung von 10t12c-C18:2 in der jungen Ratte beobachtet. Die Gabe von 9c11t-C18:2 bewirkte nur eine geringe nicht signifikante Verminderung des TXB_2 Gehaltes in der jungen Ratte. Nur geringe nicht signifikante Veränderungen des 6-keto-$PGF_{1\alpha}$ Gehaltes wurden in der jungen Ratte beobachtet. In der erwachsenen Ratte wurden keine Wirkungen beobachtet. Die beobachteten Ergebnisse lassen eine Wechselwirkung von CLA mit den Enzymen des Thromboxan Stoffwechsels und eine Verringerung des Precursors vermuten. Weiterhin scheinen die Resultate altersabhängig zu sein, da die jungen Ratten stärker auf die CLA Gabe reagierten.

11 RESUME

Au cours des dix dernières années, il a été mis en évidence que l'acide linoléique conjugué possédait des effets bénéfiques pour la santé humaine. De multiples expérimentations animales leur ont attribué des propriétés anti-carcinogènes et anti-athérogènes mais également des effets sur la composition lipidique corporelle et sur le métabolisme énergétique. Les CLA se trouvent naturellement dans les graisses des ruminants et les produits laitiers.

En raison de leur structure chimique, les CLA ont été identifiés comme étant plus sensibles au processus d'oxydation et aux traitements thermiques que leurs analogues sans des doubles liaisons non-conjuguées. Afin d'acquérir plus d'informations sur la stabilité des CLA au cours des processus de fabrication alimentaire, l'influence des différentes étapes de fabrication de l'Emmental sur sa teneur en CLA et sur sa composition en isomères de CLA a été évaluée puis décrite en première partie de thèse. La teneur en CLA du lait cru utilisé et du fromage fermenté élaboré, dans les conditions normales de fabrication, ont été déterminées et représentent respectivement 8,62 ±1,92 mg/g et 8,38 mg/g de la teneur lipidique totale. Aucune variation de cette teneur n'a été observée au cours du processus de fabrication du fromage. L'utilisation de différentes *Propionibacterium* spp pour la fermentation et l'application de diverses températures lors de la fabrication n'ont pas d'influence sur le contenu en CLA du produit final. La composition en isomères de CLA reste également inchangée. La transformation de l'Emmental en fromage à tartiner, sa cuisson ou son utilisation en gratin n'influence pas sa teneur en CLA. Les CLA contenus dans les produits laitiers semblent ne pas être sensibles aux conditions appliquées.

Les mécanismes d'action des CLA sont encore peu connus. Les hypothèses généralement émises suggèrent que les CLA pourraient agir en modifiant le métabolisme de l'acide arachidonique et en influençant la formation des différents eicosanoides. Par conséquent, une étude sur l'implication des CLA dans la voie des eicosanoides et dans l'élongations et la désaturation des acides gras conjugués à longues chaînes a été réalisée dans le cadre de mon travail et sera exposée en deuxième partie de thèse.

Ainsi le C18:3 et le C20:3 conjugués, métabolites du 9c11t-C18:2 (isomère majeur de l'acide linoléique conjugué) et leurs analogues radiomarqués ont été synthétises en 12 étapes avec des réactifs disponibles dans le commerce. La synthèse nécessite deux

réactions de Wittig hautement stéréosélectives qui permettent la formation de deux doubles liaisons de configuration *cis*. Dans la seconde réaction de Wittig, le (2E)-non-2-enal a été utilisé comme aldéhyde afin d'obtenir la double liaison *trans*. Afin d'éviter l'isomérisation de cette double liaison, cette réaction de Wittig a été réalisée dans des conditions modifiées en utilisant une base faible. La dernière étape du schéma réactionnel permet la formation d'acide gras sous forme ^{14}C radiomarqués ou non-marques par réaction de Grignard.

Le métabolisme des CLA au niveau du C20:4 conjugué a été étudié par des réactions de désaturation et d'élongation *in-vitro* réalisée sur des microsomes hépatiques du rat isolés. Tant d'abord, la Δ6 désaturation des isomères de CLA a été comparée à celle de l'acide linoléique. Il a été démontré que le 10t12c-C18:2 était désaturé de la même manière que l'acide linoléique alors que la conversion du 9c11t-C18:2 ne représentait que la moitié de celle observée pour le 10t12c-C18:2. Des études supplémentaires d'élongation et de Δ5 désaturation des métabolites C18:3 et C20:3 du 9c11t-C18:2 synthétisés, montrant que le C18:3 conjugué était davantage élongué que l'acide γ-linoléique. Le 8c11c13t-C20:3 était à peu près 5 fois moins désaturé en C20:4 conjugué que l'acide di-homo-γ-linoléique en acide arachidonique. Ceci suggère que le 9c11t-C18:2 pourrait être principalement converti en C20:3 conjugué.

Une étude *in-vivo* sur des rats jeunes et adultes, testant le 9c11t- et 10t12c-C18:2, a été réalisée afin de rechercher une éventuelle interaction des CLA nutritionnels avec le métabolisme de la PGI_2 et du TXA_2. La PGI_2 et le TXA_2 ont été analysés grâce à leurs métabolites stables, le 6-céto-$PGF_{1\alpha}$ et le TXB_2 contenus dans le plasma et le sérum, respectivement. Une réduction significative de la teneur en TXB_2 a été observée chez les jeunes rats nourris avec le 10t12c-C18:2. Par contre le 9c11t-C18:2 apporté dans le régime alimentaire de jeunes rats n'induit qu'une faible diminution, non significative, de la teneur en TXB_2. Seules de petites variations, non significatives, du 6-céto-$PGF_{1\alpha}$ circulant ont été mises en évidence chez les jeunes rats. Par contre, aucun effet n'a été décelé chez les rats adultes. Les résultats énoncés suggéreraient une interaction des CLA avec les enzymes impliquées dans la formation du TXA_2 et dans la diminution des précurseurs. De plus, il semble que les effets observés des CLA soient corrélés avec l'âge, puisque les animaux jeunes sont plus sensibles aux CLA alimentaires comparés aux rats adultes.

12 ANNEX

12.1 Experimental Part

12.1.1 Influence of processing on the CLA content in cheese

12.1.1.1 Lipid extraction (SEHAT *et al.*, 1998)

The samples of milk, heated milk and coagulated milk, cheese spread and the gratin were lyophilized before extraction. The other samples were minced. A defined quantity of sample was weighed and the lipid content was extracted by Ultraturrax with 5ml hexane / diethyl ether (50:50 v / v) with addition of 1ml of ethanol and 1ml of a saturated solution of sodium chloride. The mixture was centrifuged (1000rpm, 5min, 4°C) to separate the layers. The upper organic layer was withdrawn and the water layer was reextracted twice with 5ml of the hexane / diethyl ether mixture. The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated under vacuum at 30°C and stored under vacuum to determine fat content of the sample until constant weight. The extracted lipids were stored at -80°C until butylation and methylation.

12.1.1.2 Methylation of dairy fats (CARREAU AND DUBACQ, 1978)

A solution of C23:0 methyl ester (c=3.0mg / ml) was prepared as internal standard, and distributed among 15 samples vials in small quantities of 2ml. The vials were stored at -20°C until use.

The lipid extract was diluted with 5ml of hexane and a defined aliquot (containing about 12-15mg lipid) was used for the methylation. 200µl of the internal standard solution was added. 100µl of sodium methoxide (c=1mol / l) was added. The reaction mixture was shaken and was left at room temperature for 5min. 500µl of a solution of BF_3 / MeOH (14%) was added, it was vortexed and was left at room temperature for another 15min. The mixture was diluted by addition of 5ml of distilled water and the FAME were extracted by 2ml of hexane. The samples were centrifuged (2000rpm, 3min) to separate the layers, After withdrawing the upper hexane layer, the reaction mixture was reextracted by another 2ml of hexane. The FAME were analyzed by GC.

12.1.1.3 Butylation of dairy fats (ULBERTH *et al.*, 1999)

An aliquot of extracted lipids diluted in hexane containing about 20mg lipid, was used for the butylation. 50µl of a solution of sodium butoxide (2mol/l, freshly prepared by dilution of sodium in butanol under a nitrogen atmosphere) were added. The mixture was shaken and left at room temperature for 15min. The FABE were neutralized with 200mg $NaHSO_4$ and 5ml of hexane were added. The samples were centrifuged (2000rpm, 3min) to separate the layers and the hexane layer was withdrawn, diluted 1:10 and further used for GC analysis.

12.1.1.4 GC-Analysis of FAME and FABE

- Analysis of FAME

Instrument:	Hewlett Packard 5890, equipped with an autosampler
Injector:	split (split ratio 1:10)
Detector:	FID
Column:	CPSil88, fused silica capillary column, Chrompack, 100m × 0.25mm I.D., 0.25µm film thickness
Carrier gas:	hydrogen, 1ml/min
Temperature program:	70°C (4min), 13°C/min to 175°C (27min), 4°C/min to 215°C (31min)
Operating system:	Borwin-Software

- Analysis of FABE

Instrument:	Hewlett Packard 5890, equipped with an autosampler
Injector:	splitless
Detector:	FID
Column:	BPX-70, fused silica capillary column, SGE, 50m × 0.25mm I.D., film thickness 0.25µm
Carrier gas:	helium, 1ml/min
Temperature program:	60°C (5min), 10°C/min to 120°C (2min), 3°C/min to 220°C (14min)
Operating system:	Borwin-Software

12.1.1.5 Ag^+-HPLC of FAME (RICKERT *et al.*, 1999)

Instrument:	Merck / Hitachi 655A-12 LC, L-5000 controller
Detector:	Photodiode array detector, Waters PDA 996
Column:	3 columns in series
	ChromSpher 5 Lipids, stainless steel, Chrompack,
	250mm × 4.6mm I.D., 5µm particle size
mobile phase :	0.1% acetonitrile in hexane
Flow	1 ml / min
Operating system:	Millenium Software (Waters)

Chromatogram:

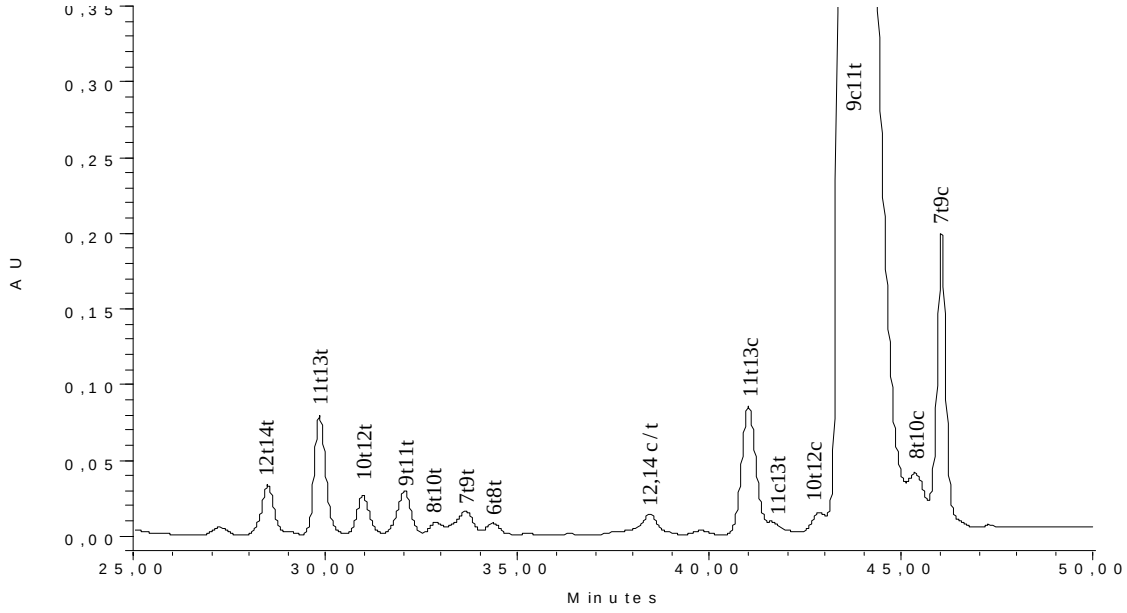


Figure 23 Ag+-HPLC chromatogram of a milk sample.

12.1.1.6 Calculation of results

- Calculation of CLA contents

CLA contents were determined with C23:0 methyl ester as internal standard.

The correlation factor (K_f) was determined by triple injection of a mixture of a standard solution containing 9c11t-C18:2 methyl ester (c=66µg / ml) and C23:0 methyl ester in each serie of sample analysis.

Calculation of K_f

$$K_f = \frac{Area_{(C23:0)} \bullet m_{(9c11t\ -\ C18:2)}}{Area_{(9c11t\ -\ C18:2)} \bullet m_{(C23:0)}}$$

Area: Integration area of 9c11t-C18:2 and C23:0 methyl esters, **m**: mass of the standard substances in the injected solution.

In the following, the CLA content in the samples was determined.

$$c(mg_{(CLA)} / g_{(fat)} = \frac{m_{(C23:0)} \bullet Area_{(CLA)} \bullet 1000}{Area_{(C23:0)} \bullet K_f \bullet m(fat)}$$

$c(mg_{(CLA)}/g_{(fat)})$: CLA concentration in mg/g fat in the analyzed sample, **Area**: Integration area of CLA and C23:0 methyl esters, **m**: mass of internal standard added to the sample before methylation, **m(fat):** quantity of extracted fat of the sample used for the methylation, **1000:** dilution factor.

- Calculation of fatty acid profiles

The total fatty acid profile was determined from integration areas of FAME and FABE. The ratio of each fatty acid of either FAME or FABE and C14:0 methyl ester was calculated (Normalization of fatty acids to C14:0). The fatty acid profile was calculated as % fatty acid of total fatty acid using the following formula:

$$\%\text{fatty acid} = \frac{(Area_{(\text{fatty acid})} \div Area_{(C14:0)}}{\sum \text{normalized fatty acids to C14:0}}$$

Area: Integration area of fatty acid.

- Calculation of CLA isomer composition

The CLA isomer composition was calculated as % of each CLA isomer of total CLA isomers.

12.1.2 Synthesis of the C18:3 and C20:3 metabolites of 9c11t-C18:2

12.1.2.1 Laboratory material

Starting materials and chemical reagents were purchased from Acros Organics or Sigma-Aldrich and [^{14}C]-barium carbonate (55mCi / mmol) from Mayak Production Association. Silica gel (35-70 mesh) was purchased from SDS.
All solvents were purified before use: dichloromethane, HMPA and acetonitrile were distilled from calcium hydride; THF and diethyl ether from sodium benzophenone ketyl.

12.1.2.2 Analytical Procedures

- NMR

NMR spectra were obtained on a Bruker FT-NMR spectrometer operating at 500 MHz for ^{1}H and 125.8 MHz for ^{13}C. Unless otherwise stated, spectra were recorded in $CDCl_3$, and chemical shifts were reported (in ppm) downfield from tetramethylsilane (TMS) (δ).

- Analytical GC

GC analyses was performed using two different instruments.

1. Instrument:	Carlo Erba HRGC 5300 Mega Series
Injector:	on column
Detector:	FID
Column:	DB5, fused silica capillary column, J & W Scientific, 8m × 0.25mm I.D., 0.25µm film thickness
Carrier gas:	helium, 1ml / min
Temperature program:	60°C to 250°C at 20°C / min for 60min
Operating system:	Borwin-Software
2. Instrument:	Packard 438A
Injector:	„Ross" injector
Detector:	FID
Column:	HP1 fused silica capillary, Hewlett Packard, 30m × 0.25mm I.D., 0.25µm film thickness
Carrier gas:	helium 1ml / min
Temperature program:	60°C to 200°C at 10°C / min for 60min
Operating system:	Borwin-Software

- GC-MS

Instrument:	Hewlett-Packard 5890 GC coupled to Hewlett-Packard 5970 MS instrument
Injector:	Splitless
Detector:	electron impact mode at 70eV
1. Column:	DB5, fused silica capillary column, J & W Scientific, 30m × 0.25mm I.D., 0.25µm film thickness
Temperature program:	60°C to 190°C at 20°C / min for 60min
2. Column:	BPX-70, fused silica capillary column, SGE, 30m × 0.25mm I.D., film thickness 0.25µm
Temperature program:	60°C to 170°C at 20°C / min for 60min
Carrier gas:	helium, 1ml / min
Operating system:	HPChem-Station-Software

- GC-FTIR

Instrument:	Hewlett Packard 5890 GC, coupled to a FTIR (FTS 60A, Bio-Rad). Connection by a Digibal tracer® direct-deposition interface
Injector:	Splitless
Column:	BPX-70, fused silica capillary column, SGE, 30m × 0.25mm I.D., film thickness 0.25µm
Temperature program:	60°C to 200°C at 20°C / min for 60min
Carrier gas:	helium 1 ml / min
Operating system:	Bio-Rad-Software

- Thin layer chromatography (TLC)

TLC was performed on a 0.25 mm pre-coated silica gel plate containing a fluorescent indicator. Spots were visualized using one or more of the following techniques (a) UV; (b) spraying with a solution containing anisaldehyde (Kägi-Miescher reagent: anisaldehyde 12,5ml; acetic acid 5ml; sulfuric acid 17ml; 95% ethanol 450ml); (c) iodine vapor.

- RP-HPLC (BANNI *et al.*, 1994)

Separation of the 6c,9c,11t-18:3 and 6c,9t,11t-18:3 was performed as described by Banni *et al.*, 1994.

Instrument:	Varian 9010-LC
Detector:	Photodiode array detector, JASCO MD-1510, wavelength (234nm and 200nm)
Column:	C-18 Nucleosil, Interchim, 250mm × 10 mm I.D, 5µm particle size
mobile phase :	acetonitrile / water / acetic acid (70:30:0.12, v / v / v)
Flow:	4ml / min
Operating system:	Borwin-Software

- RP-HPLC for labeled compounds

Analytical RP-HPLC

Instrument:	Shimadzu LC-10AS with Shimadzu SCL-10A system controller
Radioactive detector:	Berthold LB 503 monitor (liquid scintillation cocktail, MS2)
Column:	Zorbax SB C18, Chrompack, 250mm × 4.6 mm I.D, 5µm particle size
mobile phase :	Ethanol / water / trifluoroacetic acid (70:30:0.1, v / v / v)
Flow:	1.5ml / min
Operating system:	HPChem-Station

Preparative RP-HPLC - Separation of the radiolabeled 6c,9c,11t-18:3 and 6c,9t,11t-18:3 was performed using the following HPLC conditions.

Instrument:	Gilson model 303, with Gilson manometric module, model 802C
Radioactive detector:	Berthold LB 2040 nuclear spectrometer
Column:	Zorbax SB C18, Chrompack, 250mm × 21.2 mm I.D, 5µm particle size
mobile phase :	Acetonitrile / water / acetic acid (70:30:0.12, v / v / v)
Flow:	10ml / min
Operating system:	HPChem-Station

12.1.2.3 Synthesis of (8Z,11Z,13E)-eicosa-8,11,13-trienoic acid and of (6Z,9Z,11E)-octadeca-6,9,11-trienoic acid and their radiolabeled analogs

- (**0**) 3-(t-butyldiphenylsilyloxy)propan-1-ol

To a mixture of propane-1,3-diol (22.13g, 0.29mol), dimethylaminopyridine (1.28g, 0.01mmol) and triethylamine (9.6g, 0.095mol) in 250ml of CH_2Cl_2 was added dropwise at room temperature t-butyldiphenylchlorosilane (20g, 0.072mol) in 60ml of CH_2Cl_2. After stirring at room temperature for 18 hours, the mixture was diluted with diethyl ether, washed with a saturated solution of sodium chloride, several time with water and then dried over Na_2SO_4. After concentration in vacuo, flash chromatography of the residue on silica gel (petroleum ether / diethyl ether, 60:40, v / v) gave the monoprotected diol (19.02g, 60.5 mmol, 84% yield) as a colorless oil.
TLC: Rf=0.68 (petroleum ether / diethyl ether, 50:50, v / v).
GC-MS: m / z = 257 $[M-57]^+$

- (**4**) 3-(t-butyldiphenylsilyloxy)propanal

To a mixture of PDC (26.95g, 70mmol) and powdered molecular sieves 4Å (90g) in 190ml of CH_2Cl_2, was added dropwise 3-(t-butyldiphenylsilyloxy)propan-1-ol **0** (15g, 47mmol) in 130ml of CH_2Cl_2 at 0°C. The mixture was stirred at room temperature for 45min, diluted with a mixture of petroleum ether / diethyl ether (3:1, v / v) and filtered on a pad of silica gel. After evaporation of solvents, chromatography of the residue on silica gel using petroleum ether / diethyl ether (90:10, v / v) gave the aldehyde **4** as a colorless oil (11.28g, 36mmol, 75.5% yield).
TLC: Rf=0.84 (petroleum ether / diethyl ether, 60:40, v / v).
GC-MS: m / z = 255 $[M-57]^+$

- (**1**) 7-(2-tetrahydropyranyloxy)heptan-1-ol

Heptane-1,7-diol (15.4g, 116mmol) and 2.92 g PPTs were dissolved in 300ml of CH_2Cl_2. The reaction mixture was cooled to 0°C and 10.46ml 3,4-dihydropyran (9.62g, 114mmol) were added. The solution was allowed to warm up to room temperature and then stirred for 4h. The mixture was diluted with diethyl ether washed with a saturated solution of sodium chloride and dried by filtration over Na_2SO_4. The solvent was evaporated under vacuum. The residue was chromatographed on silica gel (petroleum ether / diethyl ether, 60:40, v / v). 7-(2-tetrahydropyranyloxy)heptan-1-ol **1** (14.9g, 68.9mmol) was obtained in 58.9 % yield.

TLC: Rf=0.24 (petroleum ether / diethyl ether, 50:50, v / v).
GC-MS: m / z=101 $[OTHP]^+$, 115 $[M\text{-}OTHP]^+$, 216 $[M]^+$.

- (**2**) 1-Iodo-7-(2-tetrahydropyranyloxy)heptane

To a solution containing triphenylphosphine (20.1g, 76.3mmol), imidazole (10.34g, 152mmol) and 7-(2-tetrahydropyranyloxy)heptan-1-ol **1** (10.67, 50.8mmol) in 140ml of THF, iodine (19.36g, 76.3mmol) was added at –10°C. The solution was allowed to warm up to room temperature and then stirred for 30min. The reaction mixture was diluted with diethyl ether, washed successively with a saturated solution of sodium thiosulfate and a saturated solution of sodium hydrogen carbonate. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was stirred with petroleum ether to precipitate triphenyl phosphine oxide. After filtration and concentration, the residue was chromatographed on silica gel (petroleum ether / diethyl ether, 97:3, v / v) to give **2** (13.1g, 40.2mmol, 79.1% of yield) as a clear oil.
TLC: Rf=0.43 (petroleum ether / diethyl ether, 95:5, v / v).
GC-MS: m / z=101 $[OTHP]^+$, 325 $[M\text{-}H]^+$, 326 $[M]^+$.
GC-FTIR: 2932.7, 2855.5, 1136.1, 1078.3, 1026.6cm^{-1}.
^{1}H NMR ($CDCl_3$): 1.25-1.35 (m, 6H, 3,4,5-H_2), 1.4-1.9 (m, 10H, 2, 6-H_2, 3',4',5'-H_2 (THP)), 3.11 (t, 2H, J=7Hz, 1-H_2) 3.25-3.85 (m, 4H, 7-H_2, 6'-H_2 (THP)), 4.5 (t, 1H, J=3.4Hz, 2'H (THP)).
^{13}C NMR ($CDCl_3$): 98.6 (THP C2'), 67.3 (C7), 62.1 (THP C6'), 33.3 (C2), 30.6 (THP C3'), 30.3 (C6), 29.5 (C3), 28.2 (C4), 25.9 (THP C5'), 25.3 (C5), 19.5 (THP C4'), 7.0 (C1).

- (**3**) [7-(2-tetrahydropyranyloxy)heptyl]triphenylphosphonium iodide

A solution containing triphenylphosphine (19.3g, 73.6mmol), 1-iodo-7-(2-tetrahydropyranyloxy)heptane **2** (12g, 36.7mmol) and calcium carbonate (2g) in 100ml of acetonitrile was heated at 80-85°C for 22hours. After filtration of calcium carbonate and concentration, the oily residue was dissolved in a minimum of CH_2Cl_2 and precipitated by addition of diethyl ether. The phosphonium salt **3**, obtained as a gum (19.9g, 34mmol, 92.6% yield), was dried under vacuum (0.05 mmHg) at room temperature.
TLC: Rf=0.69 (CH_2Cl_2 / methanol, 95:5, v / v).

➢ (**5**) (3Z)-1-(t-butyldiphenylsilyloxy)-10-(2-tetrahydropyranyloxy)dec-3-ene

To a solution of phosphonium salt **3** (15.48, 26.3mmol) in a mixture of THF / HMPA (150ml, 5:1, v / v), BuLi (2.5 M in hexane, 10.52ml, 26.3mmol) was added dropwise at –78°C. After stirring for 1 hour at –78°C, 3-(t-butyldiphenylsilyloxy)propanal **4** (8.61g, 27.5mmol) in 25ml of THF was added slowly, and the temperature was allowed to warm up to 0°C within 90min. After stirring at room temperature for 1hour, the reaction-mixture was diluted with diethyl ether / petroleum ether (1:3, v / v) and filtered on a pad of silica gel. After evaporation of the solvents, the residue was chromatographed on silica gel (petroleum ether / diethyl ether, 95:5, v / v) and afforded 8.31g of **5** as an oil (16.8mmol, 63% of yield).

TLC: Rf=0.42 (petroleum ether / diethyl ether, 95:5, v / v).

GC-MS: m / z = 101 $[OTHP]^+$, 437 $[M-57]^+$.

➢ (**6**) (3Z)-10-(2-tetrahydropyranyloxy)dec-3-en-1-ol (FREEMAN AND KIM, 1992)

To a solution of **5** (8.10g, 16.4mmol) in 60ml of THF, was added at room temperature a 1M solution of tetra-n-butylammonium fluoride in THF (9.75ml, 32.8ml). The resulting solution was stirred for 1 hour, diluted with diethyl ether and washed with water. The aqueous layer was extracted with diethyl ether. The organic layers were combined and dried (Na_2SO_4). The solvent was evaporated in vacuo and the residue was chromatographed on silica gel (petroleum ether / diethyl ether, 60:40, v / v) to give **6** (4.18g, 16mmol, 99% of yield) as a clear oil.

TLC: Rf=0.65 (petroleum ether / diethyl ether, 50:50, v / v).

GC-MS: m / z = 101 $[OTHP]^+$, 255 $[M-1]^+$, 256 $[M]^+$.

GC-FTIR: 3386.0, 3005,3, 2934.9, 2856.4, 1138.9, 1025.4cm^{-1}.

^{1}H NMR ($CDCl_3$): 1.2-1.85 (m, 14H, 6,7,8,9-H_2, 3',4',5'-H_2 (THP)), 1.85-2.40 (m, 5H, O**H**, 2,5-H_2), 3.25-3.9 (m, 6H, 1,10-H_2, 6'-H_2 (THP)) 4.5 (t, 1H, J=3.5Hz, 2'-H (THP)), 5.3-5.55 (m, 2H, **H**C=C**H**).

^{13}C NMR ($CDCl_3$): 133.1 (C4), 125.1 (C3), 98.8 (THP C2'), 67.5 (C10), 62.3 (THP C6'), 62.2 (C1), 30.7 (THP C3', C9), 29.6 (C9), 29.5 (C6), 29.0 (C2), 27.2 (THP C5'), 26.0 (C5), 25.4 (C8), 19.6 (THP C4').

➢ (**7**) (3Z)-1-iodo-10-(2-tetrahydropyranyloxy)dec-3-ene

To a solution containing triphenylphosphine (6.14g, 23.4mmol), imidazole (3.18g, 46.8mmol) and (3Z)-10-(2-tetrahydropyranyloxy)dec-3-en-1-ol **6** (5.94g, 23.4mmol) in 45ml of THF, cooled to –30°C, iodine (5.94g, 23.4mmol) was added. The solution was

allowed to warm up to room temperature and stirred at this temperature for 30min. The reaction mixture was diluted with diethyl ether (250ml), washed successively with a saturated solution of sodium thiosulfate (250ml) and water (2 x 250ml). The organic layer was dried (Na_2SO_4) and evaporated. The residue was stirred with petroleum ether to precipitate triphenylphosphine oxide and filtered. After concentration in vacuum, flash chromatography of the residue on silica gel (petroleum ether / diethyl ether, 95:5, v / v) gave the iodide **7** (4.83g, 13.1 mmol, 84.2% of yield) as an oil.

TLC: Rf=0.55 (petroleum ether / diethyl ether, 95:5, v / v).

GC-MS: m / z = 101 $[OTHP]^+$, 239 $[M-I]^+$, 366 $[M]^+$.

GC-FTIR: 3007.1, 2931.9, 2854.1, 1137.1, 1032.2cm^{-1}.

^1H NMR ($CDCl_3$): 1.2-1.9 (m, 14H, 6,7,8,9-H_2, 3',4',5'-H_2 (THP)), 2.0 (m, 2H, 5-H_2), 2.59 (q, 2H, J=7.3Hz, 2-H_2) 3.1 (t, 2H, J=7.3Hz, 1-H_2), 3.3-3.9 (m, 4H, 10-H_2, 6'-H_2 (THP), 4.55 (t, 1H, J=2.5Hz, 2'-H (THP)), 5.2-5.6 (m, 2H, HC=CH).

- (**8**) (3Z)-[10-(2-tetrahydropyranyloxy)dec-3-enyl]triphenylphosphonium iodide

A solution containing triphenylphosphine (6.87g, 26.2mmol), (3Z)-1-iodo-10-(2-tetrahydropyranyloxy)dec-3-ene **7** (4.82g, 13.1mmol) and calcium carbonate (0.7g) in 36ml of acetonitrile was heated at 75°C for 20 hours. After filtration of calcium carbonate and evaporation of acetonitrile, the oily residue was dissolved in a minimum of CH_2Cl_2 and precipitated by addition of diethyl ether. The phosphonium salt **8** obtained as a gum (7.47g, 11.9mmol, 91% yield) was dried under vacuum (0.05 mmHg) for 12 h at room temperature.

TLC: Rf=0.80 (CH_2Cl_2 / methanol, v / v).

- (**9**) (7Z,10Z,12E)-1-(2-tetrahydrpyranyloxy)nonadeca-7,10,12-triene

At –78°C, 3.66ml (9.15mmol) of a 2.5 M solution of BuLi in hexane was added to a solution of 1.67g (10.3mmol) of HMDS in 32ml of THF and 8ml of HMPA. The reaction temperature was raised to 0°C for 3min, and cooled back to –78°C, then a solution of 6.4g (10.2mmol) of the phosphonium salt **8** in 42ml of THF was added. The reaction mixture turned to orange. After stirring at –78°C for 30min, (2E)-non-2-enal (1.86g, 1.33mmol) in 5ml of THF was added (the orange reaction mixture became yellow). After 45min at –78°C, the cooling bath was removed and the reaction was stirred at room temperature for 90min. The mixture was diluted with diethyl ether / petroleum ether (1:3, v / v) and filtered on a pad of silica gel. After

evaporation of the solvents, the residue was chromatographed on silica gel (petroleum ether / diethyl ether, 98:2, v / v) and afforded 1.9g of **9** as an oil (5.25 mmol, 51% of yield).

TLC: Rf=0.60 (petroleum ether / diethyl ether, 95:5, v / v).

GC-MS: m / z = 101 $[OTHP]^+$, 278 $[M]^+$.

^{1}H NMR ($CDCl_3$): 0.88 (t, 3H, J=7Hz, 19-H_3), 1.18-1.90 (m, 22H, 2,3,4,5,15,16,17,18-H_2, 3',4',5'-H_2 (THP)), 2.0-2.15 (m, 4H, 6,14-H_2), 2.90 (t, 2H, J=7, 9-H_2), 3.33-3.91 (m, 4H, 1-H_2, 6'-H_2 (THP)), 4.57 (t, 1H, J=2.8Hz, 2'-H (THP)), 5.25 (dt, 1H, J_{7-6}=7.6Hz, J_{7-8}=10.6Hz, 7-H), 5.37 (m, 2H, 8,10-H), 5.68 (dt, 1H, J_{13-12}=15Hz, J_{13-14}=7Hz, 13-H), 5.95 (dd, 1H, J_{11-10}=10.9Hz, J_{11-12}=10.9Hz, 11-H), 6.32 (dd, 1H, J_{12-11}=10.9Hz, J_{12-13}=15.0Hz, 12-H).

^{13}C NMR ($CDCl_3$): 135.3 (C13), 130.4 (C7), 128.8 (C11), 127.7 (C12), 127.6 (C10), 125.3 (C8), 98.8 (THP C2'), 67.6 (C1), 62.3 (THP C6'), 32.9 (C14), 31.7 (THP C3'), 30.8 (C2), 29.7 (C17), 29.6 (C15), 29.36(C4), 19.2 (C5), 28.9 (C16), 27.2 (THP C5'), 26.2 (C6), 26.0 (C3), 25.5 (C9), 22.6 (C18), 19.7 (THP C4'), 14.1 (C19).

- (**10**) (7Z,10Z,12E)-1-bromononadeca-7,10,12-triene

9 (2.33g, 6.43mmol) diluted in 20ml of CH_2CL_2, was added at 0°C to a slurry of triphenylphosphine dibromide (5.41g, 12.82mmol) in 20ml of CH_2Cl_2. The mixture was stirred at room temperature for 15min, diluted with diethyl ether, washed with a saturated solution of sodium bhydrogencarbonate, then with water. The organic layer was dried over Na_2SO_4 and concentrated. Flash chromatography of the residue on silica gel (hexane) gave the bromide **10** (1.7g, 4.98mmol, 77.4% yield) as a colorless oil.

TLC: Rf=0.94 (petroleum ether / diethyl ether, 95:5, v / v).

GC-MS: m / z = 262 / 264 $[M-Br]^+$, 340 / 342 $[M]^+$.

^{1}H NMR ($CDCl_3$): 0.85 (t, 3H, J=6.9Hz, 19-H_3), 1.1-1.5 (m, 14H, 3,4,5,15,16,17,18-H_2), 1.8-1.9 (m, 2H, 2-H_2), 2.0-2.15 (m, 4H, 6,14-H_2), 2.90 (t, 2H, J=6.5, 9-H_2), 3.38 (t, 2H, J=6.5, 1-H_2), 5.23 (dt, 1H, J_{7-6}=7.6Hz, J_{7-8}=10.6Hz, 7-H), 5.36 (m, 2H, 8,10-H), 5.67 (dt, 1H, J_{13-12}=15Hz, J_{13-14}=7Hz, 13-H), 5.94 (dd, 1H, J_{11-10}=10.9Hz, J_{11-12}=10.9Hz, 11-H), 6.30 (dd, 1H, J_{12-11}=10.9Hz, J_{12-13}=15.0Hz, 12-H).

^{13}C NMR ($CDCl_3$): 135.3 (C13), 130.1 (C7), 128.8 (C11), 127.8 (C12), 127.6 (C10), 125.3 (C8), 33.9 (C2), 32.9 (C14), 32.8 (C17), 31.7 (C1), 29.4 (C15), 29.33(C5), 28.9 (C16), 28.4 (C4), 28.0 (C3), 27.1 (C6), 26.0 (C9), 22.6 (C18), 14.1 (C19).

- (**11**) (8Z,11Z,13E)-eicosa-8,11,13-trienoic acid

Bromide **10** (670mg, 1.96mmol) dissolved in 1.5ml of anhydrous diethyl ether was added to magnesium turnings (120mg, 4.93mmol) and a crystal of iodine in 3.5ml of anhydrous diethyl ether. The reaction mixture was refluxed for 90min at 35°C. The resulting Grignard compound was carbonated at –20°C with CO_2 (liberated from 500mg (2.5mmol) of barium carbonate by addition of concentrated sulfuric acid) After stirring at –20°C for 2.5h, 10ml of 5% NH_4Cl were added. The mixture was diluted with 50ml of saturated NH_4Cl solution and 50ml of ether. A 1N H_2SO_4 solution was added dropwise until the aqueous layer was acidified to pH 3. After decantation, the ether layer was washed with water, dried (Na_2SO_4), filtered and concentrated. Flash chromatography of the residue on silica gel (hexane / diethyl ether / acetic acid, 90:10:0.1, v / v / v) gave the free fatty acid **11** (328.5mg, 1.07mmol, 55% yield) as an oil.

TLC: Rf=0.25 (hexane / diethyl ether / acetic acid, 90:10:0.1, v / v / v).

^{1}H NMR ($CDCl_3$): 0.85 (t, 3H, J=7Hz, 20-H_3), 1.2-1.45 (m, 14H, 4,5,6,16,17,18,19-H_2), 1.62 (quint, 2H, J=7.5Hz, 3H_2), 2.0-2.15 (m, 4H, 7,15-H_2), 2.34 (t, 2H, J=7.5, 2-H_2), 2.88 (t, 2H, J=6.8, 10-H_2), 5.23 (dt, 1H, J_{8-7}=7.6Hz, J_{8-9}=10.6Hz, 8-H), 5.28 (s, 1H, COOH), 5.36 (m, 2H, 9,11-H), 5.66 (dt, 1H, J_{15-14}=15Hz, J_{15-16}=7Hz, 15-H), 5.94 (dd, 1H, J_{12-11}=10.9Hz, J_{12-13}=10.9Hz, 12-H), 6.30 (dd, 1H, J_{13-12}=10.9Hz, J_{13-14}=15.0Hz, 13-H).

^{13}C NMR ($CDCl_3$): 180.3 (C1), 135.3 (C14), 130.2 (C8), 128.7 (C12), 127.7 (C13), 127.6 (C11), 125.3 (C9), 34.0 (C2), 32.9 (C15), 31.7 (C18), 29.4 (C16), 29.3(C6), 28.9 (C5), 28.9 (C17), 28.8 (C4), 27.1 (C7), 26.0 (C3), 24.6 (C10), 22.6 (C19).

The NMR spectrum of (8Z,11Z,13E)-eicosa-8,11,13-trienoic acid **11** is shown in figure 24.

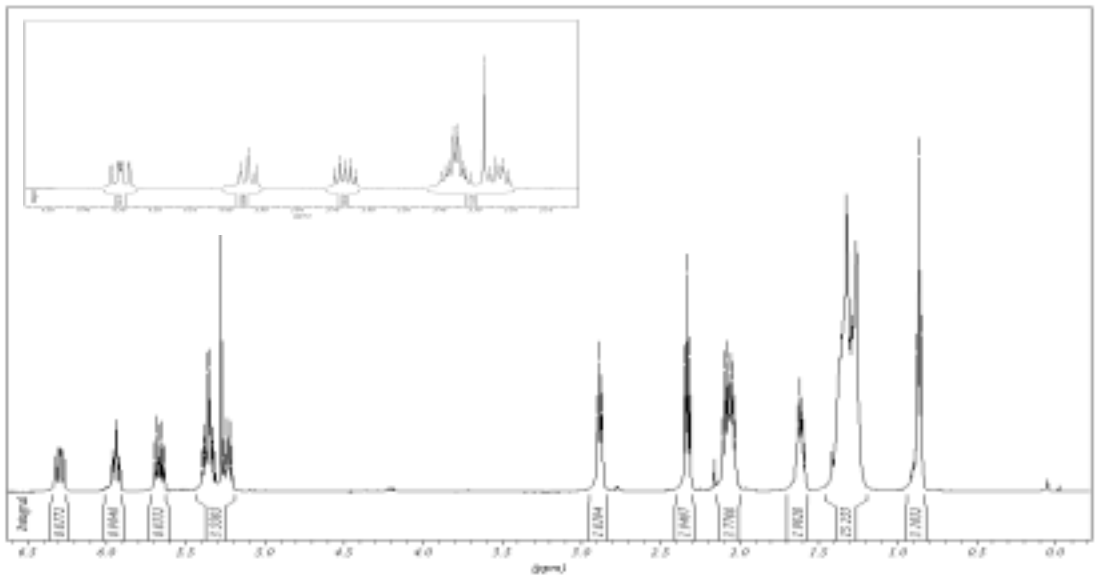


Figure 24 NMR-spectrum of (8Z,11Z,13E)-eicosa-8,11,13-trienoic acid.

- (**11'**) (8Z,11Z,13E)-[1-^{14}C]-eicosa-8,11,13-trienoic acid

(8Z,11Z,13E)-[1-^{14}C]-Eicosa-8,11,13-trienoic acid **11'** (30 mCi; specific activity: 50 mCi / mmol; 0.6 mmol; 60% yield from 10) was obtained from bromide 10 (340 mg; 1 mmol), magnesium turnings (65 mg) and $Ba^{14}CO_3$ (216 mg; 1.1 mmol; 60.5 mCi) according to the procedure described for the preparation of the unlabelled acid **11**. The radiochemical purity of the [1-^{14}C] acid was found to be 93.9% by analytical RP-HPLC.

- (**12**) 7-(2-tetrahydropyranyloxy)pentan-1-ol

7-(2-tetrahydropyranyloxy) pentan-1-ol (7.34g, 44.8mmol) **12** was obtained from pentane-1,5-diol in 50.6% yield using the procedure previously described for the preparation of **1**.

TLC: Rf=0.20 (petroleum ether / diethyl ether, 50:50, v / v).

GC-MS: m / z=101 $[OTHP]^+$, 87 $[M\text{-}OTHP]^+$, 188 $[M]^+$.

- (**13**) 1-iodo-5-(2-tetrahydropyranyloxy)pentane

7.85g (26.34mmol) of 1-iodo-5-(2-tetrahydropyranyloxy)pentane **13** was obtained from 7-(2-tetrahydropyranyloxy) pentan-1-ol **12** in 72.6% yield using the procedure previously described for the preparation of **2**.

TLC: Rf=0.43 (petroleum ether / diethyl ether, 95:5, v / v).

GC-MS: m / z = 101 $[OTHP]^+$, 298 $[M]^+$.

GC-FTIR: 2938, 2863.9, 1135.6, 1030.1cm^{-1}.

^{1}H NMR ($CDCl_3$): 1.45-2.0 (m, 10H, 3,4-H_2, 3',4',5'-H_2 (THP)), 2.4 (m, 2H, 2-H_2), 3.20 (t, 2H, J=7, 1-H_2), 3.70-3.90 (m, 4H, 5-H_2, 6'-H_2 (THP)), 4.57 (t, 1H, J=3.3Hz, 2'-H (THP)).

^{13}C NMR ($CDCl_3$): 98.8 (THP C2'), 67.6 (C5), 62.3 (THP C6'), 33.1 (C2), 31.0 (THP C3'), 28.5 (C4), 27.2 (THP C5'), 25.5 (C3), 19.7 (THP C4'), 7.1 (C1).

- (**14**) [5-(2-tetrahydropyranyloxy)pentyl]triphenylphosphonium iodide

14.03g (25.03mmol) [5-(2-tetrahydropyranyloxy)pentyl]triphenylphosphonium iodide **14** was obtained in 95% yield using the procedure previously described for the preparation of the phophonium salt **3**. The reaction time was 24 hours and temperature was 80-85°C.

TLC: Rf=0.69 (CH_2Cl_2 / methanol, 95:5, v / v).

^{1}H NMR ($CDCl_3$): 1.4-1.85 (m, 12H, 2,3,4-H_2, 3',4',5'-H_2 (THP)), 3.30-3.85 (m, 4H, 5-H_2, 6'-H_2 (THP)), 4.5 (m, 1H, 2'-H (THP)), 5.3 (m, 2H, 1-H), 5.37 (m, 2H, 8,10-H), 7.68-7.88 (m, 15H, -C6H5).

- (**15**) (3Z)-1-(t-butyldiphenylsilyloxy)-8-(2-tetrahydropyranyloxy)oct-3-ene

A Wittig reaction between the ylide of **14** and the aldehyde **4** was effected as described for the preparation of compound **5**. The (3Z)-1-(t-butyldiphenylsilyloxy)-8-(2-tetrahydropyranyloxy)oct-3-ene **15** was obtained in 54.7% yield (4.35g , 9.33mmol).

TLC: Rf=0.44 (petroleum ether / diethyl ether, 90:10, v / v).

^{1}H NMR ($CDCl_3$): 1.05 (s, 9H, -CH3), 1.15-1.9 (m, 10H, 3,4-H_2, 3',4',5'-H_2 (THP)), 2.0 (q, 2H, J=7Hz, 5-H_2), 2.3 (q, 2H, J=6.8, 2-H_2), 3.65 (t, 2H, J=7, 1-H_2), 3.32-3.90 (m, 4H, 8-H_2, 6'-H_2 (THP)), 4.57 (t, 1H, J=3.3Hz, 2'-H (THP)), 5.34-5.50 (m, 2H, -HC=CH-), 7.3-7.75 (m, 10H, -C6H5).

- (**16**) (3Z)-8-(2-tetrahydropyranyloxy)oct-3-en-1-ol

To a solution of the THP ether **15** (4.32g, 9.27mmol) in 60ml of THF, was added at room temperature a 1M solution of tetra-n-butylammonium fluoride in THF (9.75ml, 32.8mmol. The solution was stirred for 90min, diluted with 500ml of diethyl ether and washed with H_2O (3 x 500ml). The water layer was extracted with diethyl ether, and the organic layers were combined and dried (Na_2SO_4). After concentration the residue was chromatographed over silica gel (petroleum ether / diethyl ether, 60:40, v / v) to give **16** (2.07g, 9.1mmol, 98.1% yield) as a clear oil.

TLC: Rf=0.16 (petroleum ether / diethyl ether, 90:10, v / v).

GC-MS: m / z = 101 $[OTHP]^+$, 228 $[M]^+$.

GC-FTIR: 3366.5, 3006.1, 1657.27, 1139.4, 1032.9cm^{-1}.

^{1}H NMR ($CDCl_3$): 1.35-1.9 (m, 11H, 6,7-H_2, 3',4',5'-H_2 (THP), -OH), 2.1 (q, 2H, J=7,3Hz, 5-H_2), 2.32 (q, 2H, J=6.7, 2-H_2), 3.3-3.95 (m, 6H, 1,8-H_2, 6'-H_2 (THP)), 4.55 (t, 1H, J=3.6Hz, 2'-H (THP)), 5.3-5.6 (m, 2H, -HC=CH-).

- (**17**) (3Z)-1-iodo-8-(2-tetrahydropyranyloxy)oct-3-ene

2.58g (7.64mmol) of (3Z)-1-iodo-8-(2-tetrahydropyranyloxy)oct-3-ene **17** was obtained from alcohol **16** in 84% yield using the procedure previously described for the preparation of **7**.

TLC: Rf=0.58 (petroleum ether / diethyl ether, 50:50, v / v).

GC-MS: m / z = 101 $[OTHP]^+$, 211 $[M-I]^+$, 338 $[M]^+$.

GC-FTIR: 2938.7, 2862.9, 1719.6, 1652.1, 1137.1, 1032.5cm^{-1}.

^{1}H NMR ($CDCl_3$): 1.17-1.90 (m, 10H, 3,4-H_2, 3',4',5'-H_2 (THP)), 2.06 (q, 2H, J=7.2Hz, 5-H_2), 2.63 (q, 2H, J=7.3Hz, 2-H_2), 3.13 (t, 2H, J=7.3, 1-H_2), 3.35-3.92 (m, 4H, 8-H_2, 6'-H_2 (THP)), 4.57 (t, 1H, J=3.5Hz, 2'-H (THP)), 5.1-5.6 (m, 2H).

^{13}C NMR ($CDCl_3$): 131.9 (C4), 127.7 (C3), 98.5 (THP C2'), 67.0 (C8), 62.0 (THP C6'), 31.1 (THP C3'), 30.4 (C7), 29.0 (C2), 26.9 (THP C5'), 25.8 (C5), 25.1 (C6), 19.3 (THP C4'), 5.1 (C1).

- (**18**) (3Z)-[8-(2-tetrahydropyranyloxy)oct-3-enyl]triphenylphosphonium iodide

5.61g (9.35mmol) (3Z)-[8-(2-tetrahydropyranyloxy)oct-3-enyl]triphenylphosphonium iodide **18** was obtained in 90% yield using the procedure previously described for the preparation of the phophonium salt **8**. Reaction time was 20 hours.

TLC: Rf=0.53 (CH_2Cl_2 / methanol, 95:5, v / v).

- (**19**) (5Z,8Z,120E)-1-(2-tetrahydrpyranyloxy)heptadeca-5,8,10-triene

A Wittig reaction between the ylide of **18** and (2E)-non-2-enal was effected as described for the preparation of compound **9**. The (5Z,8Z,12E)-1-(2-tetrahydrpyranyloxy)heptadeca-5,8,10-triene **19** was obtained in 67.8% (1.74g , 5.21mmol).

TLC: Rf=0.49 (petroleum ether : diethyl ether, 98:2, v / v).

GC-MS: m / z = 101 $[OTHP]^+$, 334 $[M]^+$.

^{1}H NMR ($CDCl_3$): 0.89 (t, 3H, J=6.7Hz, 17-H_3), 1.20-1.90 (m, 18H, 2,3,13,14,15,16-H_2, 3',4',5'-H_2 (THP)), 2.07-2.18 (m, 4H, 4,12-H_2), 2.91 (t, 2H, J=7, 7-H_2), 3.35-3.90 (m, 4H,

1-H_2, 6'-H_2 (THP)), 4.6 (m, 1H, 2'-H (THP)), 5.25 (dt, 1H, J_{5-4}=7.6Hz, J_{5-6}=10.6Hz, 5-H), 5.37 (m, 2H, 6,8-H), 5.68 (dt, 1H, J_{11-10}=15Hz, J_{11-12}=7Hz, 11-H), 5.95 (dd, 1H, J_{9-8}=10.9Hz, J_{9-10}=10.9Hz, 9-H), 6.35 (dd, 1H, J_{10-9}=10.9Hz, J_{10-11}=15.0Hz, 10-H).

^{13}C NMR ($CDCl_3$): 135.3 (C11), 130.4 (C5), 128.8 (C9), 127.7 (C10), 127.6 (C8), 125.3 (C6), 98.8 (THP C2'), 67.6 (C1), 62.3 (THP C6'), 32.9 (C12), 31.7 (THP C3'), 30.8 (C2), 29.6 (C13), 29.36(C14), (THP C5'), 26.2 (C4), 26.0 (C3), 22.6 (C16), 19.7 (THP C4'), 14.1 (C17).

- (**20**) (5Z,8Z,10E)-1-bromoheptadeca-5,8,10-triene

The tetrahydropyranyl ether **19** was converted to the bromide **20** in 53.3% yield (>97% purity by GC) by the procedure described for the preparation of **10**.

TLC: Rf=0.84 (petroleum ether / diethyl ether, 98:8, v / v).

^{1}H NMR ($CDCl_3$): (t, 3H, J=6.7Hz, 17-H_3), 1.2-1.60 (m, 10H, 3,13,14,15,16-H_2), 1.8-2.2 (m, 6H, 2,4,12-H_2), 2.90 (t, 2H, J=7, 7-H_2), 3.4 (t, 2H, 1-H_2), 5.25 (dt, 1H, J_{5-4}=7.6Hz, J_{5-6}=10.6Hz, 5-H), 5.37 (m, 2H, 6,8-H), 5.68 (dt, 1H, J_{11-10}=15Hz, J_{11-12}=7Hz, 11-H), 5.95 (dd, 1H, J_{9-8}=10.9Hz, J_{9-10}=10.9Hz, 9-H), 6.35 (dd, 1H, J_{10-9}=10.9Hz, J_{10-11}=15.0Hz, 10-H).

^{13}C NMR ($CDCl_3$): 135.2 (C11), 129.0 (C5), 128.8 (C9), 128.4 (C10), 127.3 (C8), 125.0 (C6), 33.8 (C2), 33.0 (C12), 32.0 (C1), 31.6 (C15), 29.0 (C13), 28.9 (C14), 28.0 (C3), 26.1 (C4), 26.0 (C7), 22.5 (C16), 14.0 (C17).

- (**21**) (6Z,9Z,11E)-octadeca-6,9,11-trienoic acid

Bromide **20** (420mg, 1.3mmol) dissolved in 2ml of anhydrous diethyl ether was added to magnesium turning (86mg, 3.5mmol) and a crystal of iodine in 1ml of anhydrous diethyl ether. The reaction was refluxed for 90min at 35°C. The resulting Grignard compounds was carbonated at –20°C with CO_2 (liberated from 2.4g (12.1 mmol) of barium carbonate by addition of concentrated sulfuric acid) After stirring at –20°C for 2h, 7ml of 5% NH_4Cl were added. The mixture was diluted with saturated NH_4CL solution. A 1N H_2SO_4 solution was added dropwise until the aqueous layer was acidified to pH 3. After decantation, the ethereal layer was washed with water, dried over Na_2SO_4, filtered and concentrated. Flash chromatography of the residue on silica gel (hexane / diethyl ether / acetic acid, 80:20:0.1, v / v / v) gave the acid **21** (106mg, 0.38 mmol, 30% yield) as an oil.

TLC: Rf=0.26 (hexane / diethyl ether / acetic acid, 90:10:0.1, v / v / v).

^{1}H NMR ($CDCl_3$): 0.9 (t, 3H, J=7Hz, 18-H_3), 1.2-1.5 (m, 10H, 4,14,15,16,17-H_2), 1.68 (m, 2H, 3H_2), 2.05-2.15 (m, 4H, 7,13-H_2), 2.37 (t, 2H, J=7.5, 2-H_2), 2.9 (t, 2H, J=6.8, 8-H_2),

5.25 (dt, 1H, J_{6-5}=7.6Hz, J_{6-7}=10.6Hz, 6-H), 5.4 (m, 2H, 7,9-H), 5.69 (dt, 1H, J_{13-12}=15Hz, J_{13-14}=7Hz, 13-H), 5.94 (dd, 1H, J_{12-11}=10.9Hz, J_{12-13}=10.9Hz, 12-H), 6.30 (dd, 1H, J_{11-10}=10.9Hz, J_{11-12}=15.0Hz, 11-H). 7.3 (s, 1H, COOH),

- (**21'**)(6Z,9Z,11E)-[1-^{14}C]-octadeca-6,9,11-trienoic acid

A mixture of (6Z,9Z,11E)-[1-^{14}C]-octadeca-6,9,11-trienoic acid and its geometrical isomers (8.5 mCi; 53 mCi / mmol; 0.16 mmol; 19.3% yield from (**20**) was obtained from bromide **20** (260 mg; 0.83 mmol), magnesium turnings (58 mg) and $Ba^{14}CO_3$ (370 mg; 1.88 mmol; 103.4 mCi) according to the procedure described for the preparation of the unlabelled acid **21**. The radiochemical purity of (6Z,9Z,11E)-[1-^{14}C]-octadeca-6,9,11-trienoic acid was found to be 46% by RP-HPLC. 0.2 mCi of (8Z,11Z,13E)-[1-^{14}C]-eicosa-8,11,13-trienoic acid **21'** (radiochemical purity: 95%) were obtained by preparative RP-HPLC.

12.1.2.4 Analysis of the synthesized conjugated fatty acids

- Preparation of fatty acid methyl esters

Free fatty acids were converted to fatty acid methyl esters using BF_3 / MeOH (14%). To free free fatty acids in pentane 500µl BF_3 / MeOH were added. The solution was stirred for 10min at room temperature. The solution was diluted with 2ml of pentane and washed with a saturated solution of sodium bicarbonate. The organic layer was removed and was used for GC analysis.

- Preparation of 4,4-dimethyloxazoline (DMOX) derivatives

FAME (500µg) were converted to their DMOX derivatives by treatment with 2-amino-2-methylpropanol (0.25ml) in a sealed ampoule under nitrogen at 170°C for 8 hours (FAY AND RICHLI, 1991; LUTHRIA AND SPRECHER, 1993; ZHANG *et al.*, 1988). The reaction mixture was cooled, dissolved in 3ml of CH_2Cl_2 and washed twice with 1ml of water. After drying the organic phase, the solvent was removed under a stream of nitrogen and the sample was dissolved in hexane. The sample was applied to a short column of Florisil which was subsequently washed with hexane prior to elute the DMOX derivatives with a mixture of hexane / acetone (96:4, v / v) (DOBSON, 1997).

12.1.3 Desaturation and elongation of CLA and CLA metabolites

12.1.3.1 Dilution of substrates

The radiolabeled fatty acids were diluted with the corresponding unlabeled fatty acids to obtain a final activity of 370mBq/mmol. The radiolabeled fatty acid was withdrawn and the quantity of unlabeled fatty acid was calculated using the specific activity of the radiolabeled fatty acid. The diluted compound was dissolved in ethanol and used for incubation.

As an example the dilution of 9c11t-C18:2 was shown.

The specific activity of the radiolabeled fatty acid was 1968 MBq/mmol. To obtain a radioactivity of 370MBq/mmol in the substrate solution. The radiolabeled fatty acid has to be diluted 5.32-times with unlabeled fatty acid.

To obtain 1.5µmol of diluted substrate (quantity to perform the Δ6-desaturation assay) 0.28µmol radiolabeled 9c11t-C18:2 was mixed with 1.22µmol of unlabeled compound.

12.1.3.2 Isolation of rat liver microsomes (BERDEAUX *et al.*, 1998b)

- Solutions:

Sucrose/Phosphate buffer	$Na_2HPO_4 \bullet 2H_2O$	17,9g
	KH_2PO_4	13.7g
	Sucrose	171,0g

1.8l water were added, the solution was adjusted to pH 7.4 with sodium hydroxide and filled up with water to 2l.

- Protocol:

Livers were removed immediately after sacrifice and sucrose/phosphate buffer was added (about 3 fold quantity of buffer by liver weight), the livers were pooled and homogenized. The homogenate was centrifuged at 400g for 5min at 4°C. The supernatant was separated and centrifuged at 15000 g for 15 min at 4°C. Finally, the supernatant was ultracentrifuged at 105000 g for 60 min at 4°C. The supernatant (cytosol) was withdrawn and the pellet was resuspended into sucrose-phosphate buffer. Aliquots of the suspension were taken to determine the microsomal protein content. The suspension was diluted 0.5 fold with the cytosol. The microsome preparation was stored for 1h at 4°C, the time to perform the quantification of the proteins.

12.1.3.3 Quantification of microsomal protein (LOWRY *et al.*, 1951)

- Solutions:

Tartrate solution	Na / K tartrate	1g
	distilled water	ad 50ml
Copper sulfate solution	$CuSO_4 \bullet 5H_20$	0.5g
	distilled water	ad 50ml
Sodium carbonate solution	Na_2CO_3	10g
	NaOH	2g
	distilled water	ad 500ml
Reagent 1	Tartrate solution	0.5ml
	Copper sulfate solution	0.5ml
	Sodium carbonate solution	50ml
Reagent 2	Folin-reagent	3ml
	distilled water	3ml
Standard solution	Lipid free Albumin	1mg / ml

The photometric determination of the protein content was carried out using a one-point calibration. The standard solution was diluted 1:20 before use. The microsome suspension was diluted 1:500. Distilled water was used as control.

- Protocol:

The determination of the protein content of control, standard and microsme suspensions was carried out in triplicate. 1ml of the sample solution was mixed with 5ml of reagent 1, the solution was shaken and was left 10min in the darkness. 0.5ml of reagent 2 was added and the mixture was reacted for 30min in the dark. The absorption of the sample solutions were measured photometrically at 730nm against the control, and the protein content of the microsome suspension was calculated.

12.1.3.4 Incubation I: Desaturation conditions (BERDEAUX *et al.*, 1998b)

- Solutions:

Phosphate buffer (desaturation)	$Na_2HPO_4 \bullet 2H_2O$	17,9g
	KH_2PO_4	13.7g
	Sucrose	171,0g

450ml distilled water were added, the solution was adjusted to pH 7.4 with sodium hydroxide and filled up with water to 500ml.

Incubation buffer (desaturation)	ATP	225mg
	CoA	44.6mg
	NADPH	114.2mg
	$MgCl_2 \bullet 6H_2O$	102.8mg

adjusted with phosphate buffer (desaturation) to 100ml

- Protocol:

The desaturation studies were performed at 37°C for 15 min in a shaking water bath. Microsomal suspensions containing 5mg protein and 2ml of incubation buffer were incubated in an open flask with the substrate (dissolved in 40µl ethanol) The incubations were stopped by addition of 5ml of potassium hydroxide (12%) in ethanol.

For the Δ6-desaturation assay [1-14C]-9c12c-C18:2, [1-14C]-9c11t-C18:2 or [1-14C]-10t12c-C18:2 were used as substrates at concentrations of 30, 60, 90, and 120 nmol.

For the Δ5-desaturation assay [1-14C]-8c11c14c-C20:3 and [1-14C]-8c11c13t-C20:3 were used as substrates at concentrations of 10, 20, 40, 60, 80 and 100 nmol .

12.1.3.5 Incubation II: Elongation conditions (BERDEAUX *et al.*, 1998b)

- Solutions:

Phosphate buffer (elongation)	$Na_2HPO_4 \bullet 2H_2O$	8.95g
	KH_2PO_4	6.85g
	Sucrose	171,0g

450ml water were added, the solution was adjusted to pH 7.0 with sodium hydroxide and filled up with water to 500ml.

Incubation buffer (elongation)	ATP	367mg
	Malonyl CoA	11.4mg
	NADPH	111mg
	$MgCl_2 \bullet 6H_2O$	67.8mg
	Gluthatione	40.3mg
	KCN	6.53mg
	KF	232.4mg
	CoA	5.12mg
	Nicotinamide	4.1mg
	phosphate buffer (elongation)	80ml

adjusted with distilled water to 200ml

- Protocol:

The elongation study was carried out at 37°C for 30min in a shaking water bath. Incubations flasks were filled with argon as protection gas. 5mg of microsomal protein and 3ml of incubation buffer (elongation) were incubated with the substrate (dissolved in 40µl ethanol). The incubations were stopped by addition of 5ml of potassium hydroxide (12%) in ethanol.
For the elongation assay [1-14C]-6c9c12c-C18:3 and [1-14C]-6c9c11t-C18:3 were used as substrates at concentrations of 10, 20, 40, 60, 80 and 100 nmol .

12.1.3.6 Lipid extraction and methylation

Lipids were saponified by heating for 1h at 75°C. The samples were cooled to room temperature. 3ml of distilled water and 3ml of acetic acid were added to the sample mixture. The lipids were extracted twice, each time with 4ml of hexane. The solvent was evaporated under nitrogen. For methylation, 1ml of BF_3/MeOH (14%) was added, the mixture was shaken and left at room temperature for 15min. To neutralize, 5ml of a saturated solution of sodium hydrogen carbonate was added. The FAME were extracted twice with 2ml of hexane. Hexane was evaporated and the FAME were dissolved in 600µL of acetone for radio-HPLC analysis.

12.1.3.7 Radio-HPLC-Analysis

Instrument:	Waters 600
Detector:	radiochromatographic Flo-One β detector (Series A-100, Radiomatic Instruments); effluent was mixed with a Uniscint BD scintillation cocktail (National Diagnostic) in ratio of 1:1.2 (effluent/scintillation cocktail)
Column:	C-18 Nucleosil, Interchim, 250mm × 4.6 mm I.D, 5µm particle size
mobile phase :	Acetonitrile
Flow:	0.5ml/min
Operating system:	Millenium Software (Waters)

12.1.3.8 Radio-GC-Analysis

Instrument:	Hewlett Packard 5890, equipped with an autosampler
Injector:	splitless
1.Detector:	radio-GC-detector
2.Detector:	FID
Column:	Stabilwax wide bore column, Restek, 60m × 0.53mm I.D., 0.5µm film thickness
Carrier gas:	helium, 1ml / min
Temperature program:	70°C (4min), 13°C / min to 175°C (27min), 4°C / min to 215°C (31min)
Operating system:	Laura software

The output flow from the column was splitted between the FID and the radio-GC-detector in a ratio 10:90.

12.1.3.9 Analytical GC

Instrument:	Hewlett Packard 5890,
Injector:	splitless
Detector:	FID
Column:	BPX-70 capillary column, 50m x 0.25mm I.D., 0.25µm, SGE
Carrier gas:	helium, 1ml / min
Temperature program:	60°C (1min), 10°C / min to 170°C (60min), 20°C / min to 220°C (15min)
Operating system:	Borwin-Software, JMBS Developments

12.1.3.10 GC-MS-Analysis

Instrument:	Hewlett-Packard 6890 gas chromatograph coupled to an HP model 5973
Injector:	splitless
Detector:	electron impact mode at 70eV
1. Column:	HP5, fused silica capillary column, Hewlett Packard, 30m × 0.25mm I.D., 0.25µm film thickness
Temperature program:	60°C to 190°C at 20°C / min for 60min

2. Column:	BPX-70, fused silica capillary column, SGE, 30m × 0.25mm I.D., film thickness 0.25µm
Temperature program:	60°C to 300°C at 18°C / min for 60min
Carrier gas:	helium, 1ml / min
Operating system:	HPChem-Station-Software

12.1.3.11 Calculation of results

Results were calculated as % conversion of substrate into product. From this value the amount of transformed substrate in nmol was calculated.

12.1.4 Influence of dietary CLA on PGI_2 and TXB_2 synthesis *in-vivo*

12.1.4.1 Fractionation of blood (MAHFOUZ AND KUMMEROW, 1999)

- Solutions:

EDTA-54mM	EDTA in distilled water	1.58g ad 100ml (54mmol / l)
EDTA-27mM	EDTA in distilled water	788mg ad 100ml (27mmol / l)
Indomethacin	Indomethacin in ethanol	143mg ad 10ml (40mmol / l)

- Protocol:

After anesthetizing, the blood was withdrawn from the abdominal aorta in a plastic syringe. The blood was distributed among three plastic tubes.

First 3ml of blood were collected on EDTA-54mM (95µl) and Indomethacin (5µl) to obtain plasma by immediate centrifugation (1000g, 10min, 4°C). Plasma was taken and stored at -80°C.

Another 3ml of blood were allowed to clot at room temperature to obtain serum. After 2h it was centrifuged and the serum was stored at -80°C

The remaining blood (about 4ml for young rats, about 8ml for adult rats) was collected on 400µl and 800µl EDTA-27mM for young and adult rats respectively, and used for platelet isolation.

12.1.4.2 Isolation of platelets

- Solutions:

Tyrode-HEPES-buffer	NaCl	800mg
	KCl	20mg
	$NaHCO_3$	100mg
	NaH_2PO_4	5mg
	$MgCl_2 \bullet 6H_2O$	102mg
	Glucose	100mg
	HEPES	119mg
	distilled water	ad 100ml

- Protocol:

Blood was centrifuged (150g, 18min, 25°C) and the PRP was separated into another tube. Platelets were precipitated during a second centrifugation (1000g, 18min, 25°C). The surface of the pellet was rinsed twice with 1.5ml of Tyrode-HEPES-buffer. Platelets were suspended in 2ml of methanol and 4ml of $CHCl_3$ were added. Samples were stored at -20°C until analysis.

12.1.4.3 Incubation of aorta (MAHFOUZ AND KUMMEROW, 1999)

- Solutions:

TRIS wash buffer	TRIS	698mg
(adjusted to pH 7.4)	NaCl	894mg
	distilled water	ad 100ml
TRIS incubation buffer	TRIS	3.49g
(adjusted to pH 8.0)	NaCl	877mg
	distilled water	ad 100ml

- Protocol:

Aorta was held in ice-cold TRIS wash buffer freed from adventitia and 1cm of aorta was taken for the incubation. Aorta was opened longitudinally and incubated in 1ml of TRIS incubation buffer in a shaking water bath at 37°C. Aliquots of 100µl were withdrawn at 30, 60 and 90min.

12.1.4.4 Analysis of 6-keto-$PGF_{1\alpha}$ by EIA

The analysis of 6-keto-$PGF_{1\alpha}$ was performed using a commercially available EIA-test-kit. The assay was performed according to the description given in the instruction booklet.

- Solutions:

EIA-buffer	1 vial of EIA-buffer concentrate diluted with 90ml ultra-pure water
wash-buffer	1 vial of wash buffer concentrate reconstituted to 2l with ultra-pure water adding 1ml of Tween 20 solution
6-keto-$PGF_{1\alpha}$ Tracer	1 vial of tracer concentrate diluted with 6ml of EIA-buffer
6-keto-$PGF_{1\alpha}$ Antiserum	1 vial of antiserum concentrate diluted with 6ml of EIA-buffer
coloration reagent	1 vial of Ellman's reagent reconstituted with 20ml ultra-pure water

- Standard

A standard solution containing 6-keto-$PGF_{1\alpha}$ (c= 54.2ng/ml) was delivered with the test-kit. The standard solution was diluted to obtain a 8 point standard curve from 4.2 pg/ml to 542 pg/ml. The standards were measured in duplicate. The calibration curve is shown in figure 25.

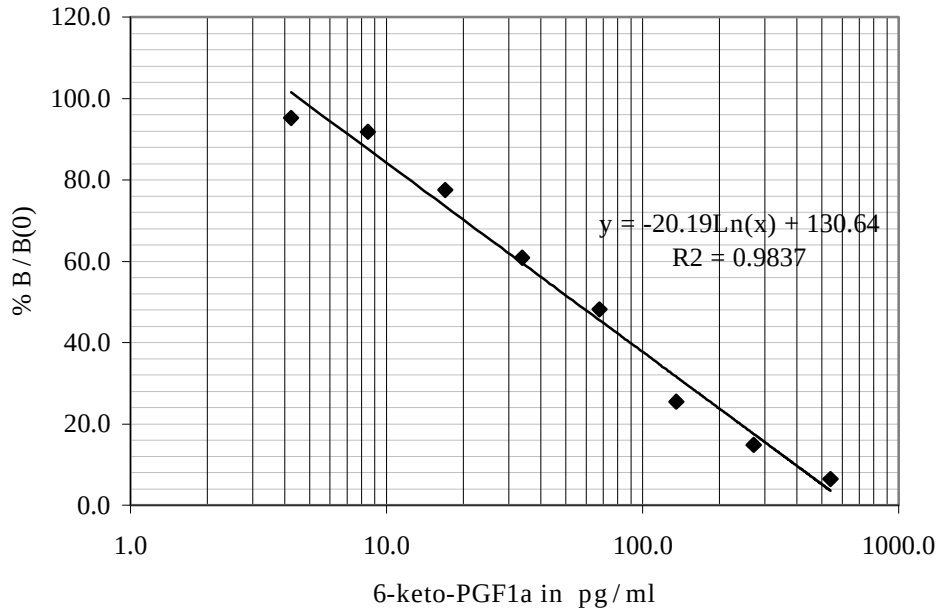


Figure 25 Standard calibration curve for the analysis of 6-keto-$PGF_{1\alpha}$ (B(0): total absorbance from control, B: absorbance of samples)

- Sample preparation:

Samples without further purification were used for analysis after dilution (for plasma samples 1:20, for incubation media samples 1:2000) with the EIA buffer. The samples were analyzed by single determinations.

- Protocol:

A EIA-microtiter-plate (precoated with mouse antibody, included in the test-kit) was used for the assay. In each well of the plate 50µl of EIA-buffer (control), standard or sample-dilution was placed, and 50µl of tracer and 50µl of antiserum were added. The plate was covered with a plastic film and left for incubation during 18h at 4°C. After the incubation period the wells were emptied and rinsed five-times with 250µl of the wash buffer. The wells of the plate were developed by addition of 200µl of the coloration reagent. The plate is recovered with the plastic film, left in the dark for 90min for reaction and is read using a microtiter-plate reader at 405nm. The 6-keto-$PGF_{1\alpha}$ concentration of the samples is calculated by comparing with the calibration curve.

12.1.4.5 Analysis of TXB_2 by EIA

The analysis of TXB_2 was performed using a commercially available EIA-test-kit. The assay was performed according to the description given in the instruction booklet.

- Solutions:

EIA-buffer	1 vial of EIA-buffer concentrate diluted with 90ml ultra-pure water
wash-buffer	1 vial of wash buffer concentrate reconstituted to 2l with ultra-pure water adding 1ml of Tween 20 solution
TXB_2Tracer	1 vial of tracer concentrate diluted with 6ml of EIA-buffer
TXB_2Antiserum	1 vial of antiserum concentrate diluted with 6ml of EIA-buffer

- Standard:

A standard solution containing TXB_2 (c= 94.3ng / ml) was delivered in the test-kit. The standard solution was diluted to obtain a 8 point standard curve from 7.4 pg / ml to 943 pg / ml. The standards were measured in duplicate. The calibration curve is shown in figure 26.

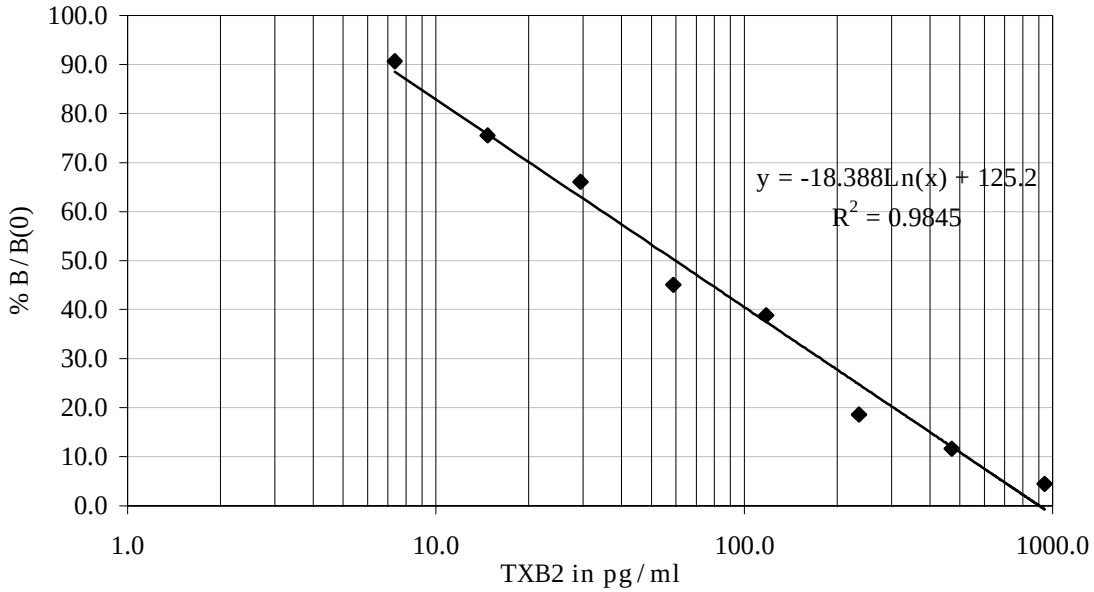


Figure 26 Standard calibration curve for the analysis of TXB_2 (with B(0) total absorbance from control, B absorbance of samples)

- Sample preparation:

Samples without further purification were used for analysis after dilution of the serum (1:2000) with the EIA buffer. The samples were analyzed by single determinations.

- Protocol:

A EIA-microtiter-plate (precoated with mouse antibody, included in the test-kit) was used for the assay. In each well of the plate 50µl of EIA-buffer (control), standard or sample-dilution was placed, and 50µl of tracer and 50µl of antiserum were added. The plate was covered with a plastic film and left for incubation during 18h at 20°C. After the incubation period the wells were emptied and rinsed five-times with 250µl of the wash buffer. The wells of the plate were developed by addition of 200µl of the coloration reagent. The plate is recovered with the plastic film, left in the dark for 60min for reaction and is read using a microtiter-plate reader at 405nm.

The TXB_2 concentration of the samples is calculated by comparing with the calibration curve.

12.1.4.6 Lipid extraction of aorta, platelets and plasma

- Extraction of lipids from aorta

The aorta was cut in small pieces and homogenized in 4ml of $CHCl_3$/methanol (2:1, v/v) and 800µl of a solution of sodium chloride (0.73%) were added to extract the aortic lipids. The samples were mixed and centrifuged (2000rpm, 5min, 4°C). The organic layer containing the lipids was withdrawn and the solvent evaporated under nitrogen. The extracted lipids were dissolved in 1ml of toluene and stored at -20°C until methylation.

- Extraction of lipids from platelets

To the solution of platelets in 6ml of $CHCl_3$/methanol (2:1, v/v) 1.2ml of a solution of sodium chloride (0.73%) was added. The samples were mixed and centrifuged (2000rpm, 5min, 4°C). The organic layer containing the lipids was withdrawn and the solvent evaporated under nitrogen. The extracted lipids were dissolved in 1ml of toluene and stored at -20°C until methylation.

- Extraction of lipids from plasma

2ml of plasma were extracted with 20ml of $CHCl_3$/methanol (1:1, v/v) 1. The samples were mixed and centrifuged (2000rpm, 5min, 4°C). The organic layer containing the lipids was withdrawn and 10ml of $CHCl_3$ and 3ml of a solution of sodium chloride (98mg NaCl, 0.1ml of H_2SO_4 to 100ml with distilled water) were added. The water layer was eliminated and the solvent evaporated under nitrogen. The extracted lipids were dissolved in 100µl of $CHCl_3$ and stored at -20°C until fractionation of plasma in lipid classes.

12.1.4.7 Fractionation of plasma lipid classes

The fractionation of plasma lipid classes was carried out by TLC. The extracted lipids were spotted on a silica gel TLC. The plate was developed in hexane/diethyl ether/acetic acid (80:20:1, v/v/v) to separate EC, TAG and PL. The spots were visualized using 2,7-dichlor-fluorescein reagent. Bands containing EC, TAG and PL were scraped off and dissolved in 1ml of toluene. The samples were stored at -20°C until methylation.

12.1.4.8 Methylation

2ml of sodium methoxide (0.5mol/l) were added to the extracted lipids (dissolved in 1ml of toluene) to methylate them into FAME. The mixture was heated at 50°C for 5min (EC were heated for 30min at 50°C). The solution was neutralized by addition of 100µl of acetic acid and 5ml of distilled water were added. The FAME were extracted with 5ml of hexane. To separate the layers it was centrifuged and the hexane layer was withdrawn, purified over a flourisil cartridge to eliminate the containing cholesterol. FAME were eluted by 5ml of hexane/diethyl ether (95:5 v/v), the solvent evaporated, dissolved in hexane and used for GC analysis.

12.1.4.9 GC analysis of FAME from tissues and plasma

Instrument:	Hewlett Packard 5890, equipped with an autosampler
Injector:	splitless
Detector:	FID
Column:	CPSil88, fused silica capillary column, Chrompack, 100m × 0.25mm I.D., 0.25µm film thickness
Carrier gas:	hydrogen, 1ml/min
Temperature program:	60°C (1min), 10°C/min to 200°C (65min), 20°C/min to 220°C (10min)
Operating system:	Borwin-Software

The fatty acid profile was calculated as % fatty acid of total fatty acids.

12.2 Experimental Data

12.2.1 Influence of processing on the CLA content in cheese

12.2.1.1 CLA contents of the analyzed samples

Table 11 Cheese manufacturing, raw milk cheese

	CLA in mg / g fat				
	Sample 1	Sample 2	Sample 3	**Mean**	*SD*
Raw Milk	7.19	7.87	10.80	**8.62**	*1.92*
Coagulated Milk	7.15	8.39	9.64	**8.39**	*1.24*
Fresh Curd	7.49	7.94	9.95	**8.46**	*1.31*
Salted Curd	6.48	7.91	10.20	**8.20**	*1.87*
Cheese, 20 days of aging	7.33	8.18	10.67	**8.73**	*1.73*
Final cheese	7.32	7.92	10.40	**8.55**	*1.63*

Table 12 Cheese manufacturing, heated milk cheese

	CLA in mg / g fat				
	Sample 1	Sample 2	Sample 3	**Mean**	*SD*
Raw Milk	7.19	7.87	10.80	**8.62**	*1.92*
Heated Milk	6.47	8.73	10.32	**8.51**	*1.93*
Coagulated Milk	7.33	8.00	10.43	**8.59**	*1.63*
Fresh Curd	7.22	8.60	10.23	**8.68**	*1.51*
Salted Curd	7.26	8.28	10.11	**8.55**	*1.44*
Cheese, 20 days of aging	7.13	8.14	10.09	**8.46**	*1.50*
Final cheese	7.16	7.97	10.03	**8.38**	*1.48*

Table 13 Milling and cooking temperatures, raw milk cheese

	CLA in mg / g fat				
	Sample 1	Sample 2	Sample 3	**Mean**	*SD*
Milk	7.19	7.87	10.80	**8.62**	*1.92*
Milling 52°C / Cooking 50°C	7.32	7.92	10.40	**8.55**	*1.63*
Milling 48°C / Cooking 48°C	7.40	8.28	10.87	**8.85**	*1.80*
Milling 50°C / Cooking 50°C	7.15	8.30	10.48	**8.64**	*1.69*

Table 14 Milling and cooking temperatures, heated milk cheese

	CLA in mg / g fat				
	Sample 1	Sample 2	Sample 3	**Mean**	*SD*
Milk	6.47	8.73	10.32	**8.51**	*1.93*
Milling 52°C / Cooking 50°C	7.16	7.97	10.03	**8.38**	*1.48*
Milling 48°C / Cooking 48°C	7.64	7.36	11.94	**8.98**	*2.57*
Milling 50°C / Cooking 50°C	7.01	7.73	10.34	**8.36**	*1.75*

Table 15 Influence of different strains of *Propionibacterium* spp.

	CLA in mg / g fat			
	Sample 1	Sample 2	**Mean**	*SD*
Milk	9.64	10.38	**10.01**	*0.52*
Cheese, normally used *Propionibacterium* sp.	9.75	9.32	**9.54**	*0.30*
Cheese, *Propionibacterium* sp., high lipolytic	9.48	10.48	**9.98**	*0.71*
Cheese, *Propionibacterium* sp., low lipolytic	10.33	9.41	**9.87**	*0.65*

Table 16 Influence of grilling and cooking, cheese fabricated in pilot-scale

	CLA in mg / g fat				
	Sample 1	Sample 2	Sample 3	**Mean**	*SD*
Gratin, raw [n=1]	8.53			**8.53**	
Gratin, grilled [n=3]	8.28	6.80	6.81	**7.30**	*0.85*
Sauce Béchamel, raw [n=1]	6.37			**6.37**	
Sauce Béchamel,cooked [n=3]	5.93	5.92	6.12	**5.99**	*0.11*
Fondue savoyarde, raw [n=1]	7.80			**7.80**	
Fondue savoyarde, cooked [n=3]	7.79	7.63	8.06	**7.83**	*0.22*

Table 17 Influence of grilling and cooking, cheese industrially fabricated

	CLA in mg / g fat				
	Sample 1	Sample 2	Sample 3	**Mean**	*SD*
Gratin, raw [n=1]	7.29			**7.29**	
Gratin, grilled [n=3]	7.55	7.10	7.56	**7.40**	*0.26*
Sauce Béchamel, raw [n=1]	6.82			**6.82**	
Sauce Béchamel,cooked [n=3]	6.72	6.67	6.94	**6.78**	*0.14*
Fondue savoyarde, raw [n=1]	9.16			**9.16**	
Fondue savoyarde, cooked [n=3]	8.71	8.79	8.60	**8.70**	*0.10*

Table 18 Preparation of cheese spread

processing	additional	CLA in mg / g fat			
temperature	heating time	Sample 1	Sample 2	**Mean**	*SD*
control		8.18	8.12	**8.15**	*0.04*
75°C	0 min	8.81	7.93	**8.37**	*0.62*
75°C	5 min	8.42	7.71	**8.06**	*0.50*
100°C	0 min	8.03	8.71	**8.37**	*0.48*
100°C	10 min	6.37	7.53	**6.95**	*0.82*

12.2.1.2 CLA isomer composition

Table 19 Cheese manufacturing, raw milk cheese, CLA isomer composition [in % of total CLA isomers]

	raw milk [n=2]		coagulated milk [n=2]		fresh curd [n=2]		salted curd [n=2]		cheese, 20 days [n=2]		final cheese [n=2]	
CLA isomer	**Mean**	*SD*	**Mean**	*SD*	**Mean**	*SD*	**Mean**	*SD*	**Mean**	*SD*	**Mean**	*SD*
12t14t	**0.63**	*0.08*	**0.85**	*0.05*	**0.71**	*0.22*	**0.79**	*0.21*	**0.72**	*0.11*	**0.74**	*0.16*
11t13t	**1.64**	*0.08*	**1.88**	*0.06*	**1.79**	*0.14*	**2.54**	*0.48*	**1.82**	*0.12*	**1.78**	*0.05*
10t12t	**0.26**	*0.08*	**0.33**	*0.05*	**0.45**	*0.25*	**0.67**	*0.38*	**0.55**	*0.08*	**0.69**	*0.03*
9t11t	**0.73**	*0.12*	**0.91**	*0.02*	**0.70**	*0.04*	**1.05**	*0.30*	**0.88**	*0.09*	**0.85**	*0.04*
8t10t	**0.22**	*0.08*	**0.16**	*0.08*	**0.40**	*0.01*	**0.51**	*0.18*	**0.28**	*0.11*	**0.37**	*0.03*
7t9t	**0.52**	*0.00*	**0.52**	*0.09*	**0.45**	*0.02*	**0.55**	*0.15*	**0.54**	*0.11*	**0.58**	*0.17*
6t8t	**0.20**	*0.03*	**0.16**	*0.07*	**0.20**	*0.02*	**0.25**	*0.07*	**0.22**	*0.02*	**0.29**	*0.11*
12,14 c/t	**0.47**	*0.01*	**0.43**	*0.05*	**0.30**	*0.11*	**0.34**	*0.13*	**0.42**	*0.07*	**0.37**	*0.03*
11t13c	**2.58**	*0.08*	**2.77**	*0.29*	**3.00**	*0.52*	**2.63**	*0.22*	**2.91**	*0.51*	**2.96**	*0.50*
11c13t	**0.17**	*0.03*	**0.17**	*0.04*	**0.31**	*0.02*	**0.32**	*0.04*	**0.26**	*0.11*	**0.31**	*0.04*
10t12c	**0.47**	*0.17*	**0.55**	*0.31*	**1.27**	*0.54*	**1.16**	*0.32*	**0.80**	*0.28*	**0.82**	*0.12*
9c11t	**86.79**	*0.19*	**86.04**	*0.25*	**83.59**	*2.08*	**84.34**	*1.27*	**85.26**	*0.52*	**84.77**	*1.07*
8t10c	**1.62**	*0.20*	**1.65**	*0.04*	**1.38**	*0.34*	**1.14**	*0.73*	**1.21**	*0.30*	**1.24**	*0.31*
7t9c	**3.71**	*0.14*	**3.59**	*0.39*	**5.45**	*1.46*	**3.69**	*0.62*	**4.15**	*0.41*	**4.24**	*0.33*
Sum t/t	**4.20**	*0.16*	**4.80**	*0.09*	**4.70**	*0.65*	**6.37**	*0.37*	**5.00**	*0.38*	**5.28**	*0.05*
Sum c/t	**95.80**	*0.15*	**95.21**	*0.09*	**95.30**	*0.65*	**93.63**	*0.37*	**95.00**	*0.38*	**94.72**	*0.05*

Table 20 Milling and cooking temperatures, raw milk cheese,CLA isomer composition [in % of total CLA isomers]

	Raw milk cheese							
	raw milk		Milling 52°C Cooking 50°C		Milling 48°C Cooking 48°C		Milling 50°C Cooking 50°C	
CLA isomer	**mean [n=2]**	*SD*	**mean [n=2]**	*SD*	**mean [n=2]**	*SD*	**mean [n=2]**	*SD*
12t14t	**0.63**	*0.08*	**0.74**	*0.16*	**0.81**	*0.09*	**0.87**	*0.04*
11t13t	**1.64**	*0.08*	**1.78**	*0.05*	**1.72**	*0.00*	**1.94**	*0.21*
10t12t	**0.26**	*0.08*	**0.69**	*0.03*	**0.59**	*0.02*	**0.86**	*0.62*
9t11t	**0.73**	*0.12*	**0.85**	*0.04*	**0.98**	*0.14*	**0.93**	*0.40*
8t10t	**0.22**	*0.08*	**0.37**	*0.03*	**0.34**	*0.01*	**0.42**	*0.11*
7t9t	**0.52**	*0.00*	**0.58**	*0.17*	**0.49**	*0.16*	**0.47**	*0.11*
6t8t	**0.20**	*0.03*	**0.29**	*0.11*	**0.21**	*0.06*	**0.22**	*0.06*
12,14 c/t	**0.47**	*0.01*	**0.37**	*0.03*	**0.34**	*0.08*	**0.33**	*0.04*
11t13c	**2.58**	*0.08*	**2.96**	*0.50*	**2.97**	*0.37*	**2.64**	*0.01*
11c13t	**0.17**	*0.03*	**0.31**	*0.04*	**0.42**	*0.16*	**0.48**	*0.33*
10t12c	**0.47**	*0.17*	**0.82**	*0.12*	**1.03**	*0.22*	**1.27**	*0.27*
9c11t	**86.79**	*0.19*	**84.77**	*1.07*	**84.43**	*1.53*	**84.90**	*1.82*
8t10c	**1.62**	*0.20*	**1.24**	*0.31*	**1.29**	*0.63*	**0.96**	*0.31*
7t9c	**3.71**	*0.14*	**4.24**	*0.33*	**4.36**	*0.74*	**3.72**	*0.61*
Sum t/t	**4.20**	*0.16*	**5.28**	*0.05*	**5.14**	*0.26*	**5.71**	*1.55*
Sum c/t	**95.80**	*0.15*	**94.72**	*0.05*	**94.86**	*0.26*	**94.29**	*1.55*

Table 21 Milling and cooking temperatures, heated milk cheese, CLA isomer composition [in % of total CLA isomers]

	Heated milk cheese					
	Milling 52°C Cooking 50°C		Milling 48°C Cooking 48°C		Milling 50°C Cooking 50°C	
CLA isomer	**mean [n=2]**	*SD*	**mean [n=2]**	*SD*	**mean [n=2]**	*SD*
12t14t	**0.83**	*0.05*	**0.94**	*0.33*	**0.82**	*0.03*
11t13t	**1.81**	*0.04*	**1.75**	*0.07*	**1.82**	*0.01*
10t12t	**0.33**	*0.03*	**0.30**	*0.04*	**0.36**	*0.05*
9t11t	**0.67**	*0.11*	**0.72**	*0.02*	**0.71**	*0.15*
8t10t	**0.37**	*0.06*	**0.38**	*0.00*	**0.36**	*0.11*
7t9t	**0.45**	*0.04*	**0.45**	*0.05*	**0.43**	*0.06*
6t8t	**0.20**	*0.00*	**0.22**	*0.03*	**0.19**	*0.01*
12,14 c/t	**0.32**	*0.01*	**0.32**	*0.05*	**0.32**	*0.01*
11t13c	**2.96**	*0.50*	**3.00**	*0.56*	**2.67**	*0.17*
11c13t	**0.19**	*0.00*	**0.23**	*0.12*	**0.56**	*0.45*
10t12c	**0.87**	*0.02*	**0.98**	*0.58*	**1.35**	*0.57*
9c11t	**85.14**	*1.41*	**84.30**	*1.82*	**85.75**	*1.07*
8t10c	**0.90**	*0.33*	**1.60**	*0.48*	**0.60**	*0.06*
7t9c	**4.98**	*0.39*	**4.82**	*0.39*	**4.06**	*0.22*
Sum t/t	**4.66**	*0.15*	**4.76**	*0.26*	**4.69**	*0.41*
Sum c/t	**95.34**	*0.15*	**95.24**	*0.26*	**95.31**	*0.41*

Table 22 Different strains of *Propionibacterium* spp., CLA isomer composition [in % of total CLA isomers]

	milk		normally used *Propionibacterium* sp.		high lipolytic *Propionibacterium* sp.		low lipolytic *Propionibacterium* sp.	
CLA isomer	**mean [n=2]**	*SD*	**mean [n=2]**	*SD*	**mean [n=2]**	*SD*	**mean [n=2]**	*SD*
12t14t	**0.95**	*0.01*	**0.85**	*0.01*	**0.91**	*0.01*	**0.97**	*0.05*
11t13t	**2.10**	*0.00*	**2.17**	*0.06*	**2.01**	*0.23*	**2.15**	*0.03*
10t12t	**0.25**	*0.00*	**0.35**	*0.20*	**0.20**	*0.01*	**0.21**	*0.02*
9t11t	**0.62**	*0.02*	**0.59**	*0.28*	**0.74**	*0.11*	**0.78**	*0.04*
8t10t	**0.14**	*0.05*	**0.13**	*0.01*	**0.21**	*0.10*	**0.14**	*0.05*
7t9t	**0.48**	*0.03*	**0.42**	*0.02*	**0.41**	*0.10*	**0.50**	*0.06*
6t8t	**0.14**	*0.00*	**0.12**	*0.01*	**0.12**	*0.06*	**0.14**	*0.03*
12,14 c/t	**0.43**	*0.00*	**0.84**	*0.63*	**0.31**	*0.13*	**0.49**	*0.01*
11t13c	**2.70**	*0.03*	**2.61**	*0.18*	**2.80**	*0.17*	**2.64**	*0.08*
11c13t	**0.14**	*0.04*	**0.32**	*0.13*	**0.44**	*0.47*	**0.08**	*0.02*
10t12c	**0.35**	*0.08*	**0.59**	*0.18*	**0.54**	*0.31*	**0.22**	*0.03*
9c11t	**86.52**	*0.39*	**85.61**	*0.57*	**86.57**	*0.75*	**87.04**	*0.28*
8t10c	**1.41**	*0.16*	**1.73**	*0.03*	**1.31**	*0.36*	**1.37**	*0.33*
7t9c	**3.76**	*0.01*	**3.67**	*0.64*	**3.43**	*0.02*	**3.28**	*0.05*
Sum t/t	**4.69**	*0.09*	**4.63**	*0.04*	**4.60**	*0.40*	**4.88**	*0.14*
Sum c/t	**95.31**	*0.09*	**95.37**	*0.04*	**95.40**	*0.41*	**95.12**	*0.14*

12.2.1.3 Fatty acid composition

Table 23 Cheese manufacturing, raw milk cheese, fatty acid profiles

	raw milk [n=3]		coagulated milk [n=3]		fresh curd [n=3]		salted curd [n=3]		cheese, 20 days [n=3]		final cheese [n=3]	
	mean	*SD*	**mean**	*SD*	**mean**	*SD*	**mean**	*SD*	**mean**	*SD*	**mean**	*SD*
C4:0	**4.42**	*0.19*	**4.27**	*0.05*	**4.32**	*0.15*	**4.21**	*0.09*	**4.25**	*0.02*	**4.24**	*0.04*
C6:0	**2.63**	*0.04*	**2.53**	*0.02*	**2.56**	*0.05*	**2.50**	*0.05*	**2.52**	*0.01*	**2.53**	*0.00*
C7:0	**0.04**	*0.00*	**0.04**	*0.01*	**0.04**	*0.01*	**0.04**	*0.00*	**0.04**	*0.01*	**0.04**	*0.00*
C8:0	**1.57**	*0.03*	**1.50**	*0.04*	**1.50**	*0.02*	**1.51**	*0.00*	**1.50**	*0.03*	**1.53**	*0.00*
C9:0	**0.05**	*0.01*	**0.05**	*0.01*	**0.04**	*0.01*	**0.05**	*0.01*	**0.05**	*0.01*	**0.06**	*0.01*
C10:0	**3.56**	*0.03*	**3.49**	*0.05*	**3.54**	*0.02*	**3.53**	*0.00*	**3.51**	*0.02*	**3.53**	*0.03*
C11:0	**0.34**	*0.01*	**0.32**	*0.00*	**0.34**	*0.00*	**0.33**	*0.00*	**0.33**	*0.02*	**0.32**	*0.01*
C12:0	**4.05**	*0.14*	**4.02**	*0.10*	**3.99**	*0.09*	**3.99**	*0.12*	**3.99**	*0.13*	**4.02**	*0.11*
C13:0	**0.13**	*0.02*	**0.12**	*0.02*	**0.12**	*0.01*	**0.12**	*0.00*	**0.12**	*0.00*	**0.12**	*0.00*
C14:0	**12.19**	*0.66*	**12.16**	*0.52*	**11.80**	*0.42*	**12.22**	*0.58*	**12.18**	*0.53*	**12.16**	*0.54*
C14:1	**0.89**	*0.02*	**0.90**	*0.01*	**0.90**	*0.01*	**0.88**	*0.04*	**0.89**	*0.01*	**0.90**	*0.01*
C15:0	**1.20**	*0.01*	**1.21**	*0.01*	**1.21**	*0.01*	**1.18**	*0.05*	**1.21**	*0.01*	**1.20**	*0.01*
C16:0	**28.18**	*0.94*	**28.25**	*0.79*	**27.75**	*0.60*	**28.38**	*0.78*	**28.29**	*0.69*	**28.23**	*0.74*
C16:1	**1.68**	*0.18*	**1.65**	*0.23*	**1.49**	*0.12*	**1.66**	*0.23*	**1.64**	*0.21*	**1.66**	*0.20*
C17:0	**0.77**	*0.03*	**0.73**	*0.01*	**0.72**	*0.02*	**0.74**	*0.03*	**0.73**	*0.01*	**0.76**	*0.01*
C18:0	**10.46**	*0.07*	**10.47**	*0.18*	**10.63**	*0.12*	**10.58**	*0.17*	**10.54**	*0.20*	**10.51**	*0.20*
9t-C18:1	**0.19**	*0.05*	**0.24**	*0.04*	**0.22**	*0.09*	**0.23**	*0.08*	**0.22**	*0.07*	**0.24**	*0.08*
11t-C18:1	**2.30**	*0.35*	**2.52**	*0.38*	**2.76**	*0.22*	**2.51**	*0.34*	**2.53**	*0.33*	**2.50**	*0.36*
9c-C18:1	**20.81**	*1.39*	**21.06**	*1.14*	**21.64**	*0.88*	**20.88**	*1.16*	**20.92**	*1.19*	**20.95**	*1.13*
9c12c-C18:2	**1.98**	*0.20*	**1.98**	*0.21*	**1.81**	*0.13*	**1.98**	*0.23*	**1.98**	*0.23*	**1.98**	*0.22*
C20:0	**0.16**	*0.02*	**0.16**	*0.01*	**0.15**	*0.02*	**0.17**	*0.03*	**0.17**	*0.03*	**0.16**	*0.02*
9c12c15c-C18:3	**0.88**	*0.06*	**0.86**	*0.08*	**0.91**	*0.05*	**0.86**	*0.06*	**0.88**	*0.03*	**0.87**	*0.06*
CLA	**1.06**	*0.09*	**1.05**	*0.15*	**1.14**	*0.10*	**1.03**	*0.15*	**1.04**	*0.16*	**1.06**	*0.13*
C22:0	**0.09**	*0.00*	**0.08**	*0.01*	**0.07**	*0.01*	**0.09**	*0.02*	**0.09**	*0.01*	**0.08**	*0.00*
8c11c14c-C20:3	**0.10**	*0.01*	**0.11**	*0.02*	**0.09**	*0.01*	**0.10**	*0.02*	**0.10**	*0.01*	**0.10**	*0.02*
5c8c11c14c-C20:4	**0.13**	*0.00*	**0.13**	*0.01*	**0.12**	*0.00*	**0.13**	*0.01*	**0.13**	*0.02*	**0.12**	*0.00*
5c8c11c14c17c-C20:5	**0.07**	*0.00*	**0.07**	*0.01*	**0.08**	*0.00*	**0.07**	*0.01*	**0.07**	*0.00*	**0.07**	*0.01*
C24:0	**0.05**	*0.00*	**0.05**	*0.00*	**0.05**	*0.00*	**0.05**	*0.01*	**0.05**	*0.00*	**0.04**	*0.00*

Table 24 Milling and cooking temperatures raw milk cheese, fatty acid profiles

	Raw milk cheese							
	raw milk		Milling 52°C Cooking 50°C		Milling 48°C Cooking 48°C		Milling 50°C Cooking 50°C	
	mean [n=3]	*SD*	**mean [n=3]**	*SD*	**mean [n=3]**	*SD*	**mean [n=3]**	*SD*
C4:0	**4.40**	*0.08*	**4.28**	*0.07*	**4.33**	*0.11*	**4.27**	*0.10*
C6:0	**2.61**	*0.04*	**2.55**	*0.04*	**2.58**	*0.03*	**2.56**	*0.06*
C7:0	**0.04**	*0.01*	**0.04**	*0.00*	**0.04**	*0.00*	**0.05**	*0.00*
C8:0	**1.56**	*0.02*	**1.53**	*0.00*	**1.54**	*0.02*	**1.51**	*0.04*
C9:0	**0.05**	*0.01*	**0.05**	*0.01*	**0.05**	*0.01*	**0.05**	*0.00*
C10:0	**3.53**	*0.05*	**3.50**	*0.07*	**3.51**	*0.02*	**3.48**	*0.04*
C11:0	**0.34**	*0.01*	**0.32**	*0.00*	**0.33**	*0.02*	**0.33**	*0.00*
C12:0	**4.02**	*0.11*	**4.00**	*0.08*	**4.01**	*0.11*	**3.99**	*0.08*
C13:0	**0.13**	*0.01*	**0.12**	*0.01*	**0.12**	*0.01*	**0.13**	*0.00*
C14:0	**12.04**	*0.46*	**12.03**	*0.44*	**12.08**	*0.47*	**12.06**	*0.49*
C14:1	**0.88**	*0.03*	**0.89**	*0.01*	**0.89**	*0.02*	**0.87**	*0.03*
C15:0	**1.19**	*0.03*	**1.20**	*0.01*	**1.21**	*0.00*	**1.19**	*0.02*
C16:0	**28.14**	*0.49*	**28.19**	*0.53*	**28.21**	*0.62*	**28.29**	*0.58*
C16:1	**1.81**	*0.27*	**1.78**	*0.26*	**1.77**	*0.26*	**1.78**	*0.27*
C17:0	**0.77**	*0.02*	**0.77**	*0.02*	**0.75**	*0.02*	**0.74**	*0.03*
C18:0	**10.52**	*0.21*	**10.56**	*0.17*	**10.53**	*0.14*	**10.58**	*0.13*
9t-C18:1	**0.22**	*0.11*	**0.26**	*0.06*	**0.22**	*0.03*	**0.21**	*0.02*
11t-C18:1	**2.39**	*0.17*	**2.52**	*0.26*	**2.49**	*0.29*	**2.50**	*0.31*
9c-C18:1	**20.91**	*0.86*	**20.96**	*0.80*	**20.95**	*0.79*	**21.02**	*0.83*
9c12c-C18:2	**1.96**	*0.13*	**1.96**	*0.16*	**1.94**	*0.16*	**1.95**	*0.16*
C20:0	**0.16**	*0.02*	**0.16**	*0.01*	**0.17**	*0.02*	**0.16**	*0.01*
9c12c15c-C18:3	**0.85**	*0.06*	**0.85**	*0.06*	**0.83**	*0.07*	**0.82**	*0.09*
CLA	**1.06**	*0.11*	**1.05**	*0.10*	**1.04**	*0.11*	**1.05**	*0.10*
C22:0	**0.08**	*0.01*	**0.08**	*0.00*	**0.08**	*0.01*	**0.08**	*0.01*
8c11c14c-C20:3	**0.10**	*0.01*	**0.10**	*0.02*	**0.10**	*0.02*	**0.10**	*0.02*
5c8c11c14c-C20:4	**0.13**	*0.01*	**0.13**	*0.00*	**0.13**	*0.01*	**0.13**	*0.01*
5c8c11c14c17c-C20:5	**0.07**	*0.01*	**0.08**	*0.00*	**0.07**	*0.01*	**0.07**	*0.01*
C24:0	**0.04**	*0.01*	**0.04**	*0.00*	**0.05**	*0.00*	**0.05**	*0.01*

Table 25 Milling and cooking temperatures part 2, heated milk cheese, fatty acid profiles

	Heated milk cheese					
	Milling 52°C Cooking 50°C		Milling 48°C Cooking 48°C		Milling 50°C Cooking 50°C	
	mean [n=3]	*SD*	**mean [n=3]**	*SD*	**mean [n=3]**	*SD*
C4:0	**4.27**	*0.11*	**4.29**	*0.15*	**4.26**	*0.10*
C6:0	**2.56**	*0.03*	**2.55**	*0.05*	**2.55**	*0.02*
C7:0	**0.03**	*0.00*	**0.04**	*0.00*	**0.04**	*0.01*
C8:0	**1.51**	*0.03*	**1.52**	*0.00*	**1.53**	*0.01*
C9:0	**0.04**	*0.00*	**0.04**	*0.01*	**0.04**	*0.01*
C10:0	**3.51**	*0.03*	**3.52**	*0.04*	**3.53**	*0.05*
C11:0	**0.34**	*0.01*	**0.34**	*0.01*	**0.34**	*0.01*
C12:0	**4.00**	*0.07*	**3.99**	*0.10*	**3.99**	*0.11*
C13:0	**0.12**	*0.00*	**0.12**	*0.02*	**0.12**	*0.01*
C14:0	**12.02**	*0.41*	**12.04**	*0.51*	**12.06**	*0.47*
C14:1	**0.89**	*0.02*	**0.89**	*0.02*	**0.88**	*0.04*
C15:0	**1.21**	*0.01*	**1.20**	*0.01*	**1.18**	*0.04*
C16:0	**28.15**	*0.53*	**28.18**	*0.65*	**28.15**	*0.59*
C16:1	**1.78**	*0.25*	**1.79**	*0.27*	**1.78**	*0.27*
C17:0	**0.76**	*0.03*	**0.77**	*0.05*	**0.76**	*0.04*
C18:0	**10.58**	*0.14*	**10.55**	*0.14*	**10.55**	*0.14*
9t-C18:1	**0.22**	*0.05*	**0.23**	*0.06*	**0.23**	*0.06*
11t-C18:1	**2.50**	*0.25*	**2.52**	*0.26*	**2.52**	*0.25*
9c-C18:1	**21.05**	*0.72*	**20.98**	*0.80*	**20.98**	*0.80*
9c12c-C18:2	**1.95**	*0.14*	**1.95**	*0.16*	**1.99**	*0.15*
C20:0	**0.17**	*0.01*	**0.17**	*0.02*	**0.17**	*0.02*
9c12c15c-C18:3	**0.85**	*0.06*	**0.84**	*0.06*	**0.86**	*0.05*
CLA	**1.06**	*0.10*	**1.06**	*0.09*	**1.04**	*0.11*
C22:0	**0.09**	*0.02*	**0.09**	*0.01*	**0.08**	*0.01*
8c11c14c-C20:3	**0.10**	*0.01*	**0.11**	*0.01*	**0.10**	*0.01*
5c8c11c14c-C20:4	**0.13**	*0.01*	**0.13**	*0.01*	**0.13**	*0.01*
5c8c11c14c17c-C20:5	**0.07**	*0.01*	**0.07**	*0.01*	**0.07**	*0.00*
C24:0	**0.05**	*0.00*	**0.05**	*0.01*	**0.05**	*0.00*

Table 26 Different strains of *Propionibacterium* spp., fatty acid profiles

	milk		normally used *Propionibacterium* sp.		high lipolytic *Propionibacterium* sp.		low lipolytic *Propionibacterium* sp.	
	mean [n=2]	*SD*	**mean [n=2]**	*SD*	**mean [n=2]**	*SD*	**mean [n=2]**	*SD*
C4:0	**4.20**	*0.03*	**4.26**	*0.15*	**4.33**	*0.19*	**4.29**	*0.04*
C6:0	**2.52**	*0.02*	**2.54**	*0.09*	**2.61**	*0.12*	**2.58**	*0.02*
C7:0	**0.04**	*0.01*	**0.04**	*0.01*	**0.05**	*0.01*	**0.04**	*0.01*
C8:0	**1.53**	*0.01*	**1.52**	*0.04*	**1.56**	*0.04*	**1.54**	*0.00*
C9:0	**0.04**	*0.00*	**0.06**	*0.00*	**0.05**	*0.00*	**0.04**	*0.00*
C10:0	**3.42**	*0.02*	**3.48**	*0.06*	**3.53**	*0.09*	**3.49**	*0.02*
C11:0	**0.32**	*0.01*	**0.33**	*0.03*	**0.36**	*0.01*	**0.34**	*0.01*
C12:0	**3.92**	*0.02*	**3.95**	*0.03*	**4.00**	*0.08*	**3.97**	*0.00*
C13:0	**0.12**	*0.00*	**0.13**	*0.00*	**0.13**	*0.00*	**0.12**	*0.00*
C14:0	**11.77**	*0.05*	**11.87**	*0.04*	**11.94**	*0.13*	**11.95**	*0.09*
C14:1	**0.92**	*0.00*	**0.92**	*0.01*	**0.91**	*0.00*	**0.92**	*0.01*
C15:0	**1.26**	*0.01*	**1.25**	*0.02*	**1.25**	*0.00*	**1.26**	*0.01*
C16:0	**27.75**	*0.09*	**27.77**	*0.14*	**27.67**	*0.02*	**27.68**	*0.03*
C16:1	**2.06**	*0.01*	**1.99**	*0.02*	**1.98**	*0.04*	**2.01**	*0.02*
C17:0	**0.78**	*0.01*	**0.78**	*0.00*	**0.77**	*0.00*	**0.78**	*0.00*
C18:0	**10.62**	*0.04*	**10.60**	*0.03*	**10.49**	*0.17*	**10.56**	*0.01*
9t-C18:1	**0.26**	*0.04*	**0.25**	*0.04*	**0.24**	*0.03*	**0.24**	*0.01*
11t-C18:1	**2.86**	*0.04*	**2.86**	*0.02*	**2.84**	*0.04*	**2.85**	*0.04*
9c-C18:1	**21.20**	*0.02*	**20.94**	*0.04*	**20.89**	*0.26*	**20.83**	*0.08*
9c12c-C18:2	**1.81**	*0.01*	**1.83**	*0.01*	**1.82**	*0.05*	**1.85**	*0.01*
C20:0	**0.13**	*0.01*	**0.14**	*0.02*	**0.15**	*0.01*	**0.15**	*0.02*
9c12c15c-C18:3	**0.86**	*0.02*	**0.86**	*0.00*	**0.88**	*0.05*	**0.88**	*0.03*
CLA	**1.20**	*0.02*	**1.20**	*0.03*	**1.19**	*0.02*	**1.21**	*0.01*
C22:0	**0.07**	*0.01*	**0.08**	*0.01*	**0.08**	*0.00*	**0.08**	*0.00*
8c11c14c-C20:3	**0.08**	*0.00*	**0.09**	*0.00*	**0.09**	*0.00*	**0.10**	*0.00*
5c8c11c14c-C20:4	**0.11**	*0.01*	**0.12**	*0.00*	**0.12**	*0.02*	**0.13**	*0.00*
5c8c11c14c17c-C20:5	**0.08**	*0.00*	**0.08**	*0.00*	**0.07**	*0.00*	**0.07**	*0.00*
C24:0	**0.05**	*0.00*	**0.06**	*0.01*	**0.04**	*0.00*	**0.04**	*0.00*

Table 27 Influence of cooking and grilling, fatty acid profiles

	Gratin			Gratin			Sauce Béchamel			Sauce Béchamel		
	cheese, fabricated in pilot-scale			cheese, industrially fabricated			cheese, fabricated in pilot-scale			cheese, industrially fabricated		
	raw, [n=1]	cooked, [n=3]		raw, [n=1]	cooked, [n=3]		raw, [n=1]	cooked, [n=3]		raw, [n=1]	cooked, [n=3]	
		mean	*SD*		**mean**	*SD*		**mean**	*SD*		**mean**	*SD*
C4:0	4.78	**4.27**	*0.06*	3.98	**4.45**	*0.05*	4.35	**4.39**	*0.02*	4.47	**4.48**	*0.04*
C6:0	2.76	**2.56**	*0.01*	2.35	**2.57**	*0.01*	2.57	**2.61**	*0.01*	2.62	**2.62**	*0.05*
C7:0	0.04	**0.04**	*0.00*	0.03	**0.04**	*0.00*	0.02	**0.03**	*0.01*	0.03	**0.03**	*0.00*
C8:0	1.56	**1.49**	*0.02*	1.37	**1.43**	*0.00*	1.46	**1.50**	*0.02*	1.45	**1.49**	*0.01*
C9:0	0.04	**0.04**	*0.01*	0.04	**0.04**	*0.00*	0.03	**0.04**	*0.01*	0.03	**0.05**	*0.01*
C10:0	3.36	**3.47**	*0.01*	3.16	**3.12**	*0.03*	3.36	**3.40**	*0.02*	3.24	**3.25**	*0.02*
C11:0	0.36	**0.33**	*0.00*	0.31	**0.34**	*0.00*	0.31	**0.32**	*0.00*	0.32	**0.34**	*0.01*
C12:0	3.78	**4.00**	*0.05*	3.66	**3.50**	*0.01*	3.80	**3.88**	*0.03*	3.60	**3.65**	*0.02*
C13:0	0.12	**0.12**	*0.01*	0.10	**0.11**	*0.01*	0.11	**0.11**	*0.01*	0.11	**0.11**	*0.00*
C14:0	12.20	**12.40**	*0.13*	11.41	**11.38**	*0.03*	12.15	**12.35**	*0.10*	11.74	**11.82**	*0.09*
C14:1	0.86	**0.86**	*0.01*	0.97	**0.98**	*0.01*	0.86	**0.88**	*0.01*	0.90	**0.94**	*0.00*
C15:0	1.23	**1.24**	*0.01*	1.33	**1.34**	*0.00*	1.31	**1.33**	*0.02*	1.35	**1.39**	*0.00*
C16:0	29.51	**29.77**	*0.13*	30.46	**30.37**	*0.05*	31.42	**31.40**	*0.10*	31.59	**31.54**	*0.12*
C16:1	1.48	**1.44**	*0.02*	1.93	**1.85**	*0.02*	1.65	**1.66**	*0.03*	1.80	**1.85**	*0.01*
C17:0	0.77	**0.74**	*0.03*	0.82	**0.79**	*0.03*	0.79	**0.81**	*0.02*	0.80	**0.83**	*0.03*
C18:0	10.34	**10.37**	*0.04*	10.99	**10.93**	*0.04*	9.80	**9.88**	*0.06*	10.01	**10.16**	*0.03*
9t-C18:1	0.22	**0.22**	*0.01*	0.17	**0.19**	*0.01*	0.18	**0.20**	*0.01*	0.17	**0.17**	*0.01*
11t-C18:1	2.04	**2.05**	*0.01*	2.40	**2.33**	*0.03*	1.62	**1.70**	*0.00*	1.74	**1.87**	*0.00*
9c-C18:1	20.42	**20.37**	*0.02*	21.12	**20.88**	*0.11*	19.66	**19.89**	*0.08*	19.82	**20.15**	*0.18*
9c12c-C18:2	2.04	**2.04**	*0.02*	1.25	**1.26**	*0.04*	1.59	**1.66**	*0.02*	1.28	**1.28**	*0.03*
C20:0	0.16	**0.16**	*0.00*	0.14	**0.14**	*0.01*	0.16	**0.16**	*0.02*	0.15	**0.15**	*0.00*
9c12c15c-C18:3	0.73	**0.77**	*0.01*	0.67	**0.66**	*0.04*	0.73	**0.73**	*0.02*	0.69	**0.71**	*0.02*
CLA	0.83	**0.83**	*0.02*	0.98	**0.95**	*0.02*	0.68	**0.69**	*0.01*	0.73	**0.78**	*0.01*
C22:0	0.08	**0.08**	*0.00*	0.08	**0.08**	*0.00*	0.08	**0.09**	*0.00*	0.08	**0.08**	*0.00*
8c11c14c-C20:3	0.09	**0.09**	*0.01*	0.08	**0.07**	*0.00*	0.07	**0.08**	*0.01*	0.06	**0.06**	*0.00*
5c8c11c14c-C20:4	0.13	**0.13**	*0.01*	0.10	**0.10**	*0.01*	0.11	**0.10**	*0.00*	0.10	**0.09**	*0.00*
5c8c11c14c17c-C20:5	0.06	**0.06**	*0.00*	0.06	**0.07**	*0.00*	0.06	**0.06**	*0.00*	0.06	**0.07**	*0.00*
C24:0	0.04	**0.05**	*0.01*	0.03	**0.04**	*0.01*	0.05	**0.05**	*0.00*	0.05	**0.04**	*0.00*

Table 28 Influence of cooking and grilling, fatty acid profiles

	Fondue savoyarde cheese, fabricated in pilot-scale			Fondue savoyarde cheese, industrially fabricated		
	raw, [n=1]	cooked, [n=3]		raw, [n=1]	cooked, [n=3]	
		mean	*SD*		**mean**	*SD*
C4:0	4.30	**4.34**	*0.07*	4.69	**4.53**	*0.03*
C6:0	2.59	**2.62**	*0.06*	2.73	**2.64**	*0.01*
C7:0	0.03	**0.04**	*0.00*	0.04	**0.04**	*0.00*
C8:0	1.55	**1.56**	*0.02*	1.52	**1.48**	*0.02*
C9:0	0.04	**0.04**	*0.00*	0.04	**0.05**	*0.00*
C10:0	3.63	**3.66**	*0.08*	3.28	**3.21**	*0.04*
C11:0	0.33	**0.33**	*0.01*	0.35	**0.35**	*0.01*
C12:0	4.18	**4.20**	*0.05*	3.60	**3.57**	*0.03*
C13:0	0.12	**0.12**	*0.01*	0.11	**0.11**	*0.01*
C14:0	12.69	**12.71**	*0.08*	11.44	**11.42**	*0.05*
C14:1	0.87	**0.86**	*0.01*	0.99	**0.99**	*0.01*
C15:0	1.24	**1.24**	*0.01*	1.35	**1.35**	*0.00*
C16:0	28.91	**28.86**	*0.09*	29.42	**29.56**	*0.03*
C16:1	1.37	**1.34**	*0.04*	1.82	**1.85**	*0.03*
C17:0	0.76	**0.72**	*0.03*	0.77	**0.80**	*0.02*
C18:0	10.38	**10.32**	*0.09*	11.02	**11.06**	*0.03*
9t-C18:1	0.22	**0.22**	*0.01*	0.20	**0.18**	*0.01*
11t-C18:1	2.14	**2.16**	*0.02*	2.51	**2.54**	*0.01*
9c-C18:1	20.43	**20.37**	*0.12*	20.94	**21.04**	*0.09*
9c12c-C18:2	2.01	**2.04**	*0.03*	1.05	**1.07**	*0.02*
C20:0	0.16	**0.16**	*0.01*	0.13	**0.12**	*0.00*
9c12c15c-C18:3	0.75	**0.78**	*0.01*	0.64	**0.67**	*0.02*
CLA	0.89	**0.90**	*0.02*	1.03	**1.04**	*0.02*
C22:0	0.09	**0.08**	*0.00*	0.09	**0.08**	*0.00*
8c11c14c-C20:3	0.09	**0.09**	*0.00*	0.07	**0.06**	*0.01*
5c8c11c14c-C20:4	0.11	**0.13**	*0.01*	0.09	**0.10**	*0.01*
5c8c11c14c17c-C20:5	0.07	**0.06**	*0.01*	0.07	**0.06**	*0.00*
C24:0	0.04	**0.05**	*0.00*	0.03	**0.03**	*0.00*

Table 29 Preparation of cheese spread, fatty acid profiles

	control		processing temperature 75°C heating time 0 min		processing temperature 75°C heating time 5 min		processing temperature 100°C heating time 0 min		processing temperature 100°C heating time 10 min	
	mean [n=2]	*SD*	**mean [n=2]**	*SD*	**mean [n=2]**	*SD*	**mean [n=2]**	SD	**mean [n=2]**	*SD*
C4:0	**4.26**	*0.11*	**4.27**	*0.05*	**4.25**	*0.03*	**4.29**	0.07	**4.26**	*0.02*
C6:0	**2.45**	*0.05*	**2.46**	*0.04*	**2.42**	*0.02*	**2.48**	0.04	**2.44**	*0.01*
C7:0	**0.02**	*0.00*	**0.03**	*0.00*	**0.03**	*0.00*	**0.02**	0.00	**0.03**	*0.00*
C8:0	**1.40**	*0.03*	**1.41**	*0.03*	**1.38**	*0.02*	**1.42**	0.04	**1.42**	*0.03*
C9:0	**0.03**	*0.00*	**0.03**	*0.01*	**0.03**	*0.00*	**0.03**	0.00	**0.03**	*0.00*
C10:0	**3.08**	*0.07*	**3.11**	*0.03*	**3.11**	*0.01*	**3.16**	0.07	**3.15**	*0.00*
C11:0	**0.32**	*0.01*	**0.32**	*0.02*	**0.32**	*0.01*	**0.33**	0.02	**0.32**	*0.00*
C12:0	**3.62**	*0.02*	**3.59**	*0.03*	**3.59**	*0.00*	**3.62**	0.09	**3.60**	*0.00*
C13:0	**0.11**	*0.00*	**0.11**	*0.00*	**0.11**	*0.00*	**0.11**	0.00	**0.11**	*0.00*
C14:0	**11.92**	*0.01*	**11.96**	*0.01*	**11.91**	*0.08*	**12.03**	0.24	**12.00**	*0.02*
C14:1	**0.91**	*0.02*	**0.94**	*0.00*	**0.91**	*0.02*	**0.92**	0.01	**0.92**	*0.01*
C15:0	**1.32**	*0.03*	**1.33**	*0.01*	**1.32**	*0.01*	**1.33**	0.01	**1.35**	*0.00*
C16:0	**30.27**	*0.08*	**30.36**	*0.00*	**30.40**	*0.04*	**30.37**	0.04	**30.37**	*0.02*
C16:1	**1.71**	*0.05*	**1.72**	*0.01*	**1.70**	*0.01*	**1.68**	0.03	**1.69**	*0.04*
C17:0	**0.85**	*0.04*	**0.84**	*0.06*	**0.85**	*0.04*	**0.86**	0.07	**0.88**	*0.04*
C18:0	**10.22**	*0.07*	**10.17**	*0.08*	**10.27**	*0.05*	**10.15**	0.19	**10.14**	*0.01*
9t-C18:1	**0.22**	*0.01*	**0.21**	*0.00*	**0.21**	*0.01*	**0.20**	0.00	**0.22**	*0.00*
11t-C18:1	**2.29**	*0.01*	**2.28**	*0.00*	**2.28**	*0.03*	**2.26**	0.06	**2.26**	*0.01*
9c-C18:1	**21.07**	*0.14*	**20.97**	*0.03*	**21.05**	*0.16*	**20.85**	0.19	**20.98**	*0.10*
9c12c-C18:2	**1.69**	*0.00*	**1.69**	*0.01*	**1.67**	*0.01*	**1.66**	0.03	**1.64**	*0.06*
C20:0	**0.15**	*0.00*	**0.15**	*0.01*	**0.15**	*0.01*	**0.15**	0.01	**0.14**	*0.01*
9c12c15c-C18:3	**0.72**	*0.02*	**0.70**	*0.02*	**0.69**	*0.02*	**0.71**	0.02	**0.71**	*0.01*
CLA	**0.99**	*0.01*	**0.98**	*0.01*	**0.98**	*0.01*	**1.01**	0.07	**0.98**	*0.02*
C22:0	**0.08**	*0.00*	**0.08**	*0.01*	**0.08**	*0.00*	**0.08**	0.01	**0.05**	*0.02*
8c11c14c-C20:3	**0.08**	*0.00*	**0.06**	*0.00*	**0.08**	*0.00*	**0.08**	0.00	**0.08**	*0.00*
5c8c11c14c-C20:4	**0.11**	*0.00*	**0.11**	*0.00*	**0.11**	*0.01*	**0.11**	0.00	**0.11**	*0.00*
5c8c11c14c17c-C20:5	**0.06**	*0.01*	**0.07**	*0.00*	**0.07**	*0.00*	**0.07**	0.00	**0.06**	*0.01*
C24:0	**0.05**	*0.00*	**0.05**	*0.01*	**0.04**	*0.00*	**0.04**	0.00	**0.05**	*0.00*

12.2.2 Desaturation and elongation of CLA and CLA metabolites

12.2.2.1 Composition of the experimental lipid free diet

Table 30 Composition of the experimental diet

	g / 950 g dry diet
casein	180
corn starch	460
sucrose	230
cellulose	20
mineral mixture*	50
vitamin mixture#	10

*mineral mixture (per 50g): $CaCO_3$ 12,0 g; K_2HPO_4 10,75 g; $CaHPO_4$ 10,75 g; $MgSO_4 \cdot 7H_20$ 5 g; NaCl 3 g; MgO 2 g; $FeSO_4 \cdot 7H_20$ 400 mg; $ZnSO_4 \cdot 7H_20$ 350 mg; $MnSO_4 \cdot H_20$ 100 mg; $CuSO_4 \cdot 5H_20$ 50 mg; $Na_2SiO_7 \cdot 3H_20$ 25mg; $AlK(SO_4)_2 \cdot 12H_20$ 10 mg; K_2CrO_4 7.5 mg; NaF 5 mg; $NiSO_4 \cdot 6H_20$ 5 mg; H_2BO_3 5 mg; $CoSO_4 \cdot 7H_20$ 2.5 mg; KIO_3 2 mg; $(NH_4)_6Mo_7O_{24} \cdot 4H_20$ 1 mg; LiCl 0.75 mg; Na_2SeO_3 0.75 mg; NH_4VO_3 0.5 mg; sucrose 5.5 g.

#vitamin mixture (per 10g): retinol acetate 5000 UI; cholecalciferol 1250 UI; DL-α-tocopherol acetate 100 UI; phyllokinone 1 mg; thiamin chlorhydrate 10 mg; riboflavin 10 mg; nicotinic acid 50 mg; Ca-pantothenate 25 mg; pyridoxin chlorhydrate 10 mg; D-biotin 0.2 mg; folic acid 2 mg; cyanocobalamin 25µg; choline chlorhydrate 1 g; DL-methionine 2 g; *p*-aminobenzoic acid 50 mg; inositol 100 mg; sucrose 5.5 g.

To 1000 g of the diet powder 500 ml of water were added. It was mixed until a homogenous pap was obtained.

12.2.2.2 Fatty acid composition of rat liver microsomes (fat free diet)

Table 31 Fatty acid composition of liver microsomes

	% of total fatty acids
C14:0	1.0
C14:1	0.2
C16:0	27.4
7c-C16:1	1.1
9c-C16:1	6.8
C18:0	17.2
9c-C18:1	16.4
11c-C18:1	6.3
9c12c-C18:2	3.3
6c9c12c-C18:3	0.2
11c-C20:1	0.2
5c8c11c-C20:3	4.6
8c11c14c-C20:3	0.8
5c8c11c14c-C20:4	9.4
7c10c13c16cC22:4	1.6
4c7c10c13c16c19cC22:6	3.5

Biological index of essential fatty acid deficiancy

5c8c11c-C20:3 / 5c8c11c14c-C20:4 = 4.6 / 9.4 = 0.49

12.2.2.3 Data of the Δ6-desaturation assay

Table 32 Results of the Δ6-desaturation assay

fatty acid	incubated quantity [nmol]	Substrate conversion in %	nmol	fatty acid	incubated quantity [nmol]	Substrate conversion in %	nmol
9c12c-C18:2	30	27.4	8.2	9c12c-C18:2	60	18.7	11.2
9c12c-C18:2	30	27.1	8.1	9c12c-C18:2	60	18.3	11.0
9c12c-C18:2	30	30.9	9.3	9c12c-C18:2	60	22.6	13.6
9c12c-C18:2	30	30.9	9.3	9c12c-C18:2	60	22.3	13.4
mean			**8.7**	**mean**			**12.3**
SD			*0.6*	*SD*			*1.4*
9c12c-C18:2	90	15.8	14.2	9c12c-C18:2	120	13.5	16.2
9c12c-C18:2	90	14.7	13.2	9c12c-C18:2	120	13.3	15.9
9c12c-C18:2	90	19.9	17.9	9c12c-C18:2	120	15.4	18.5
9c12c-C18:2	90	19.2	17.3	9c12c-C18:2	120	14.1	16.9
mean			**15.6**	**mean**			**16.9**
SD			*2.3*	*SD*			*1.1*

fatty acid	incubated quantity [nmol]	Substrate conversion in %	nmol	fatty acid	incubated quantity [nmol]	Substrate conversion in %	nmol
9c11t-C18:2	30	18.0	5.4	9c11t-C18:2	60	12.1	7.3
9c11t-C18:2	30	18.1	5.4	9c11t-C18:2	60	7.7	4.6
9c11t-C18:2	30	23.6	7.1	9c11t-C18:2	60	15.8	9.5
9c11t-C18:2	30	24.2	7.2	9c11t-C18:2	60	14.0	8.4
mean			**6.3**	**mean**			**7.4**
SD			*1.0*	*SD*			*2.1*
9c11t-C18:2	90	9.5	8.5	9c11t-C18:2	120	7.8	9.3
9c11t-C18:2	90	8.6	7.8	9c11t-C18:2	120	7.0	8.4
9c11t-C18:2	90	11.6	10.4	9c11t-C18:2	120	9.7	11.6
9c11t-C18:2	90	11.3	10.2	9c11t-C18:2	120	9.5	11.4
mean			**9.2**	**mean**			**10.2**
SD			*1.3*	*SD*			*1.6*

fatty acid	incubated quantity [nmol]	Substrate conversion in %	nmol	fatty acid	incubated quantity [nmol]	Substrate conversion in %	nmol
10t12c-C18:2	30	33.7	10.1	10t12c-C18:2	60	28.6	17.2
10t12c-C18:2	30	40.9	12.3	10t12c-C18:2	60	22.6	13.6
10t12c-C18:2	30	44.2	13.3	10t12c-C18:2	60	26.0	15.6
10t12c-C18:2	30	44.3	13.3	10t12c-C18:2	60	25.3	15.2
mean			**12.2**	**mean**			**15.4**
SD			*1.5*	*SD*			*1.5*
10t12c-C18:2	90	16.9	15.2	10t12c-C18:2	120	12.9	15.4
10t12c-C18:2	90	17.6	15.8	10t12c-C18:2	120	13.6	16.3
10t12c-C18:2	90	22.2	20.0	10t12c-C18:2	120	16.1	19.3
10t12c-C18:2	90	22.4	20.2	10t12c-C18:2	120	14.5	17.4
mean			**17.8**	**mean**			**17.1**
SD			*2.6*	*SD*			*1.7*

12.2.2.4 Data of the elongation assay

Table 33 Results of the elongation assay

fatty acid	incubated quantity [nmol]	Substrate conversion in %	nmol	fatty acid	incubated quantity [nmol]	Substrate conversion in %	nmol
6c9c12c-C18:3	10	62.2	6.2	6c9c12c-C18:3	20	35.9	7.2
6c9c12c-C18:3	10	65.1	6.5	6c9c12c-C18:3	20	41.5	8.3
6c9c12c-C18:3	10	60.3	6.0	6c9c12c-C18:3	20	45.3	9.1
6c9c12c-C18:3	10	65.7	6.6	6c9c12c-C18:3	20	34.8	7.0
mean			**6.3**	**mean**			**7.9**
SD			*0.3*	*SD*			*1.0*
6c9c12c-C18:3	40	20.8	8.3	6c9c12c-C18:3	60	8.4	5.1
6c9c12c-C18:3	40	10.9	4.3	6c9c12c-C18:3	60	7.2	4.3
6c9c12c-C18:3	40	16.7	6.7	6c9c12c-C18:3	60	9.0	5.4
6c9c12c-C18:3	40	16.8	6.7	6c9c12c-C18:3	60	12.0	7.2
mean			**6.5**	**mean**			**5.5**
SD			*1.6*	*SD*			*1.2*
6c9c12c-C18:3	80	7.7	6.1	6c9c12c-C18:3	100	4.9	4.9
6c9c12c-C18:3	80	6.8	5.4	6c9c12c-C18:3	100	4.1	4.1
6c9c12c-C18:3	80	5.7	4.6	6c9c12c-C18:3	100	4.6	4.6
6c9c12c-C18:3	80	3.0	2.4	6c9c12c-C18:3	100	3.2	3.2
mean			**4.6**	**mean**			**4.2**
SD			*1.6*	*SD*			*0.7*

fatty acid	incubated quantity [nmol]	Substrate conversion in %	nmol	fatty acid	incubated quantity [nmol]	Substrate conversion in %	nmol
6c9c11t-C18:3	10	76.2	7.6	6c9c11t-C18:3	20	66.8	13.4
6c9c11t-C18:3	10	82.0	8.2	6c9c11t-C18:3	20	54.4	10.9
6c9c11t-C18:3	10	80.8	8.1	6c9c11t-C18:3	20	62.4	12.5
6c9c11t-C18:3	10	77.8	7.8	6c9c11t-C18:3	20	55.7	11.1
mean			**7.9**	**mean**			**12.0**
SD			*0.3*	*SD*			*1.2*
6c9c11t-C18:3	40	26.4	10.5	6c9c11t-C18:3	60	16.2	9.7
6c9c11t-C18:3	40	21.8	8.7	6c9c11t-C18:3	60	12.8	7.7
6c9c11t-C18:3	40	28.5	11.4	6c9c11t-C18:3	60	16.5	9.9
6c9c11t-C18:3	40	25.3	10.1	6c9c11t-C18:3	60	15.2	9.1
mean			**10.2**	**mean**			**9.1**
SD			*1.1*	*SD*			*1.0*
6c9c11t-C18:3	80	8.9	7.1	6c9c11t-C18:3	100	6.7	6.7
6c9c11t-C18:3	80	9.6	7.7	6c9c11t-C18:3	100	5.2	5.2
6c9c11t-C18:3	80	9.6	7.7	6c9c11t-C18:3	100	6.4	6.4
6c9c11t-C18:3	80	7.1	5.7	6c9c11t-C18:3	100	5.7	5.7
mean			**7.0**	**mean**			**6.0**
SD			*0.9*	*SD*			*0.7*

12.2.2.5 Data of the Δ5-desaturation assay

Table 34 Results of the Δ5-desaturation assay

fatty acid	incubated quantity [nmol]	Substrate conversion in %	nmol	fatty acid	incubated quantity [nmol]	Substrate conversion in %	nmol
8c11c14c-C20:3	10	78.7	7.9	8c11c14c-C20:3	20	65.6	13.1
8c11c14c-C20:3	10	76.2	7.6	8c11c14c-C20:3	20	63.4	12.7
8c11c14c-C20:3	10	77.9	7.8	8c11c14c-C20:3	20	65.0	13.0
8c11c14c-C20:3	10			8c11c14c-C20:3	20	62.2	12.4
mean			**7.8**	**mean**			**12.8**
SD			*0.1*	*SD*			*0.3*
8c11c14c-C20:3	40	36.2	14.5	8c11c14c-C20:3	60	32.5	19.5
8c11c14c-C20:3	40	44.5	17.8	8c11c14c-C20:3	60	29.7	17.8
8c11c14c-C20:3	40	47.0	18.8	8c11c14c-C20:3	60	34.7	20.8
8c11c14c-C20:3	40	43.3	17.3	8c11c14c-C20:3	60	32.8	19.7
mean			**17.1**	**mean**			**19.4**
SD			*1.9*	*SD*			*1.2*
8c11c14c-C20:3	80	26.4	21.2	8c11c14c-C20:3	100	18.7	18.7
8c11c14c-C20:3	80	24.2	19.3	8c11c14c-C20:3	100	19.5	19.5
8c11c14c-C20:3	80	24.7	19.7	8c11c14c-C20:3	100	18.5	18.5
8c11c14c-C20:3	80	27.2	21.7	8c11c14c-C20:3	100	21.8	21.8
mean			**20.5**	**mean**			**19.6**
SD			*1.1*	*SD*			*1.5*

fatty acid	incubated quantity [nmol]	Substrate conversion in %	nmol	fatty acid	incubated quantity [nmol]	Substrate conversion in %	nmol
8c11c13t-C20:3	10	9.1	0.9	8c11c13t-C20:3	20	6.5	1.3
8c11c13t-C20:3	10	6.9	0.7	8c11c13t-C20:3	20	6.8	1.4
8c11c13t-C20:3	10	7.5	0.7	8c11c13t-C20:3	20	5.6	1.1
8c11c13t-C20:3	10	7.3	0.7	8c11c13t-C20:3	20		
mean			**0.8**	**mean**			**1.3**
SD			*0.1*	*SD*			*0.1*
8c11c13t-C20:3	40	5.3	2.1	8c11c13t-C20:3	60	5.7	3.4
8c11c13t-C20:3	40	4.3	1.7	8c11c13t-C20:3	60	4.7	2.8
8c11c13t-C20:3	40	5.6	2.2	8c11c13t-C20:3	60	5.7	3.4
8c11c13t-C20:3	40	6.3	2.5	8c11c13t-C20:3	60	6.0	3.6
mean			**2.1**	**mean**			**3.3**
SD			*0.3*	*SD*			*0.3*
8c11c13t-C20:3	80	5.6	4.5	8c11c13t-C20:3	100	4.53	4.5
8c11c13t-C20:3	80	5.6	4.5	8c11c13t-C20:3	100	4.73	4.7
8c11c13t-C20:3	80	5.5	4.4	8c11c13t-C20:3	100		
8c11c13t-C20:3	80	5.8	4.7	8c11c13t-C20:3	100	4.56	4.6
mean			**4.5**	**mean**			**4.6**
SD			*0.1*	*SD*			*0.1*

12.2.3 Influence of dietary CLA on PGI_2-and TXB_2 synthesis *in-vivo*

12.2.3.1 Composition of the experimental diet

Table 35 Composition of the experimental diet

	g / 940 g dry diet
casein	180
corn starch	460
sucrose	220
cellulose	20
mineral mixture*	50
vitamin mixture#	10

*mineral mixture (per 50g): $CaCO_3$ 12,0 g; K_2HPO_4 10,75 g; $CaHPO_4$ 10,75 g; $MgSO_4 \bullet 7H_20$ 5 g; NaCl 3 g; MgO 2 g; $FeSO_4 \bullet 7H_20$ 400 mg; $ZnSO_4 \bullet 7H_20$ 350 mg; $MnSO_4 \bullet H_20$ 100 mg; $CuSO_4 \bullet 5H_20$ 50 mg; $Na_2SiO_7 \bullet 3H_20$ 25mg; $AlK(SO_4)_2 \bullet 12H_20$ 10 mg; K_2CrO_4 7.5 mg; NaF 5 mg; $NiSO_4 \bullet 6H_20$ 5 mg; H_2BO_3 5 mg; $CoSO_4 \bullet 7H_20$ 2.5 mg; KIO_3 2 mg; $(NH_4)_6Mo_7O_{24} \bullet 4H_20$ 1 mg; LiCl 0.75 mg; Na_2SeO_3 0.75 mg; NH_4VO_3 0.5 mg; sucrose 5.5 g.

#vitamin mixture (per 10g): retinol acetate 5000 UI; cholecalciferol 1250 UI; DL-a-tocopherol acetate 100 UI; phyllokinone 1 mg; thiamin chlorhydrate 10 mg; riboflavin 10 mg; nicotinic acid 50 mg; Ca-pantothenate 25 mg; pyridoxin chlorhydrate 10 mg; D-biotin 0.2 mg; folic acid 2 mg; cyanocobalamin 25µg; choline chlorhydrate 1 g; DL-methionine 2 g; *p*-aminobenzoic acid 50 mg; inositol 100 mg; sucrose 5.5 g.

To 940 g of the diet powder 500 ml of water and 60 g of lipid according to the dietary group were added. The mixture was homogenized a pap was obtained.

The experimental lipids were prepared once for the whole study. Sunflower oil high in oleic acid and linseed oil (98:2; w / w) as basal lipid were mixed with 9c11t- or 10t12c-C18:2-triacylglycerols, respectively (basal lipid / 9c11t- or 10t12c-C18:2; 83.3:16.7; w / w). As control basal lipid was used. The fatty acid profile of the experimental lipids is given in table 36.

Table 36 Fatty acid composition of experimental lipids, given in diet

	% fatty acid of total fatty acids		
	group control	group 9c11t	group 10t12c
C14:0	0.05	0.04	0.04
5c-C14:1	0.02	0.01	0.01
C16:0	3.97	3.29	3.30
7c-C16:1	0.10	0.08	0.08
C18:0	2.12	3.25	2.79
trans-C18:1	0.17	0.21	0.25
9c-C18:1	77.64	64.40	64.15
9c12c-C18:2	13.55	11.19	11.19
C20:0	0.31	0.26	0.26
11c-20:1n-9	0.24	0.19	0.01
9c12c15c18:3	0.96	0.97	0.98
9c11t-C18:2		**15.22**	**0.52**
9t11t-C18:2		0.11	
10t12c-C18:2		**0.07**	**15.49**
10t12t-C18:2			0.19
C22:0	0.89	0.74	0.74

12.2.3.2 Data of TXB_2 in serum

Table 37 Determination of the TXB_2 content in serum (circulating TXB_2)

Diet group: Young-control	
	TXB_2 in ng / ml serum
sample 1	428
sample 2	619
sample 3	215
sample 4	333
sample 5	404
sample 6	350
mean	**391**
SD	*134*

Diet group: Young-9c11t	
	TXB_2 in ng / ml serum
sample 1	204
sample 2	297
sample 3	259
sample 4	161
sample 5	480
sample 6	254
mean	**276**
SD	*111*

Diet group: Young-10t12c	
	TXB_2 in ng / ml serum
sample 1	249.4
sample 2	196.7
sample 3	225.0
sample 4	141.3
sample 5	126.4
sample 6	133.9
mean	**178.8**
SD	*52.2*

Diet group: Adult-control	
	TXB_2 in ng / ml serum
sample 1	248
sample 2	609
sample 3	368
sample 4	428
sample 5	522
sample 6	434
mean	**435**
SD	*125*

Diet group: Adult-9c11t	
	TXB_2 in ng / ml serum
sample 1	490
sample 2	444
sample 3	655
sample 4	345
sample 5	415
sample 6	454
mean	**467**
SD	*104*

Diet group: Adult-10t12c	
	TXB_2 in ng / ml serum
sample 1	294
sample 2	328
sample 3	292
sample 4	314
sample 5	288
sample 6	492
mean	**335**
SD	*79*

12.2.3.3 Data of 6-keto-PGF$_{1\alpha}$ in plasma

Table 38 Determination of the 6-keto-PGF$_{1\alpha}$ content in plasma (circulating 6-keto-PGF$_{1\alpha}$)

Diet group: Young-control	
	6-keto-PGF$_{1\alpha}$ in pg / ml plasma
Sample 1	941
Sample 2	933
Sample 3	433
Sample 4	569
Sample 5	485
mean	**672**
SD	*247*

Diet group: Young-9c11t	
	6-keto-PGF$_{1\alpha}$ in pg / ml plasma
Sample 1	698
Sample 2	987
Sample 3	444
Sample 4	492
Sample 5	413
Sample 6	555
mean	**598**
SD	*215*

Diet group: Young-10t12c	
	6-keto-PGF$_{1\alpha}$ in pg / ml plasma
Sample 1	434
Sample 2	700
Sample 3	662
Sample 4	375
Sample 5	372
Sample 6	363
mean	**484**
SD	*155*

Diet group: Adult-control	
	6-keto-PGF$_{1\alpha}$ in pg / ml plasma
Sample 1	508
Sample 2	341
Sample 3	564
Sample 4	601
mean	**504**
SD	*115*

Diet group: Adult-9c11t	
	6-keto-PGF$_{1\alpha}$ in pg / ml plasma
Sample 1	573
Sample 2	592
Sample 3	330
Sample 4	275
Sample 5	437
mean	**441**
SD	*142*

Diet group: Adult-10t12c	
	6-keto-PGF$_{1\alpha}$ in pg / ml plasma
Sample 1	618
Sample 2	375
Sample 3	352
Sample 4	508
Sample 5	447
Sample 6	284
mean	**431**
SD	*120*

12.2.3.4 Data of 6-keto-PGF$_{1\alpha}$ in medium from incubated aorta

Table 39 Determination of the 6-keto-PGF$_{1\alpha}$ in medium of incubated aorta

Diet group: Young-control			
	6-keto-PGF$_{1\alpha}$ in ng / mg incubated aorta		
	incubation time		
	30 min	60 min	90min
Sample 1	7.1	10.4	26.6
Sample 2	9.0	25.5	46.5
Sample 3	33.7	74.5	9.4
Sample 4	34.0	43.2	38.9
Sample 5	12.9	36.2	39.2
Sample 6	20.5	35.2	34.6
mean	19.5	37.5	32.5
SD	12.0	21.4	13.1

Diet group: Adult-control			
	6-keto-PGF$_{1\alpha}$ in ng / mg incubated aorta		
	incubation time		
	30 min	60 min	90min
Sample 1	20.6	31.8	30.6
Sample 2	8.1	39.1	39.0
Sample 3	31.9	26.2	42.2
Sample 4	29.2	14.0	34.0
Sample 5	32.7	29.3	63.3
Sample 6	21.3	59.3	51.6
mean	24.0	33.3	43.5
SD	9.3	15.2	12.2

Diet group: Young-9c11t	6-keto-$PGF_{1\alpha}$ in ng / mg incubated aorta		
	incubation time		
	30 min	60 min	90min
Sample 1	2.4	10.0	12.9
Sample 2	8.2	15.7	19.3
Sample 3	9.4	29.4	20.0
Sample 4	32.5	19.9	19.4
Sample 5	8.6	18.7	15.8
Sample 6	10.6	22.6	32.1
mean	12.0	19.4	19.9
SD	10.5	6.5	6.6

Diet group: Adult-9c11t	6-keto-$PGF_{1\alpha}$ in ng / mg incubated aorta		
	incubation time		
	30 min	60 min	90min
Sample 1	4.6	16.5	26.3
Sample 2	3.2	3.5	29.2
Sample 3	13.6	47.9	41.9
Sample 4	6.7	46.1	91.3
Sample 5	32.5	65.2	46.3
Sample 6	41.8	53.8	46.4
mean	17.1	38.8	46.9
SD	16.2	23.7	23.4

Diet group: Young-10t12c	6-keto-$PGF_{1\alpha}$ in ng / mg incubated aorta		
	incubation time		
	30 min	60 min	90min
Sample 1	3.2	9.8	9.0
Sample 2	9.0	17.4	16.2
Sample 3	16.1	27.9	38.4
Sample 4	12.4	20.1	21.2
Sample 5	18.4	39.0	26.5
Sample 6	17.2	40.8	28.7
mean	12.7	25.8	23.4
SD	5.8	12.3	10.3

Diet group: Adult-10t12c	6-keto-$PGF_{1\alpha}$ in ng / mg incubated aorta		
	incubation time		
	30 min	60 min	90min
Sample 1	6.3	26.7	23.1
Sample 2	11.9	40.9	42.9
Sample 3	37.5	34.9	68.4
Sample 4	10.2	27.5	61.9
Sample 5	37.7	39.5	46.0
Sample 6	16.9	64.0	70.6
mean	20.1	38.9	52.1
SD	14.0	13.6	18.3

12.2.3.5 Fatty acid profiles of aorta, platelets and plasma lipid classes

Table 40 Fatty acid methyl ester composition in aorta

	control; young rats n=6		CLA 9,11; young rats n=6		CLA 10,12; young rats n=6		control; aged rats n=4		CLA 9,11; aged rats n=5		CLA 10,12; aged rats n=6	
	Mean	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD
C14:0	**0,11**	0,04	**0,29**	0,10	**0,14**	0,05	**0,12**	0,03	**0,22**	0,35	**0,12**	0,08
C14:1	**0,11**	0,13	**0,17**	0,06	**0,08**	0,02	**0,04**	0,01	**0,09**	0,14	**0,07**	0,05
C16:0	**11,23**	4,54	**19,19**	2,45	**11,67**	3,39	**9,33**	1,57	**11,85**	5,76	**13,99**	2,37
7c-C16:1	**0,35**	0,13	**0,82**	0,26	**0,39**	0,16	**0,19**	0,03	**0,31**	0,29	**0,23**	0,08
9c-C16:1	**1,01**	0,61	**1,92**	0,65	**0,62**	0,27	**0,42**	0,24	**0,83**	1,08	**0,79**	0,33
C17:0	**0,35**	0,12	**0,37**	0,13	**0,41**	0,12	**0,46**	0,09	**0,29**	0,10	**0,37**	0,07
C18:0	**18,35**	3,87	**16,17**	2,45	**20,98**	3,53	**23,71**	2,71	**21,47**	3,66	**18,61**	2,74
trans-18:1t	**0,25**	0,09	**0,21**	0,05	**0,30**	0,04	**0,24**	0,06	**0,22**	0,22	**0,25**	0,03
9c-C18:1	**23,21**	10,94	**23,54**	7,23	**17,86**	4,68	**13,03**	5,24	**13,17**	5,20	**19,11**	6,10
11c-18:1	**5,09**	0,48	**4,79**	0,51	**5,01**	0,22	**5,11**	0,29	**5,12**	0,23	**4,87**	0,16
9c12c-C18:2	**3,43**	1,54	**3,09**	0,57	**3,94**	0,46	**3,87**	1,59	**3,87**	0,98	**6,48**	1,99
C20:0	**0,35**	0,11	**0,18**	0,04	**0,21**	0,06	**0,23**	0,04	**0,22**	0,18	**0,14**	0,05
6c9c12c-C18:3	**0,04**	0,01	**0,04**	0,01	**0,03**	0,01	**0,03**	0,01	**0,04**	0,03	**0,03**	0,01
11c-20:1	**0,55**	0,09	**0,38**	0,06	**0,54**	0,23	**0,41**	0,04	**0,34**	0,02	**0,34**	0,04
13c-20:1	**0,18**	0,04	**0,12**	0,01	**0,18**	0,04	**0,15**	0,02	**0,13**	0,01	**0,14**	0,02
9c12c15c-C18:3	**0,10**	0,06	**0,10**	0,04	**0,09**	0,03	**0,13**	0,08	**0,14**	0,11	**0,25**	0,14
9c11t-C18:2			**0,86**	0,66					**0,41**	0,26		
10t12c-C18:2					**0,69**	0,28					**0,92**	0,39
C20:2	**0,13**	0,03	**0,09**	0,02	**0,20**	0,02	**0,26**	0,01	**0,26**	0,08	**0,31**	0,05
C22:0	**2,32**	0,83	**1,85**	0,60	**2,78**	0,24	**0,75**	0,28	**0,61**	0,09	**0,47**	0,10
5c11c14c-C20:3	**0,29**	0,10	**0,23**	0,06	**0,64**	0,12	**0,21**	0,03	**0,22**	0,05	**0,27**	0,06
8c11c14c-C20:3	**0,92**	0,31	**0,78**	0,24	**1,00**	0,11	**0,89**	0,14	**1,11**	0,23	**0,77**	0,16
13c-C22:1	**0,12**	0,05	**0,08**	0,02	**0,13**	0,02	**0,12**	0,02	**0,12**	0,02	**0,08**	0,02
5c8c11c14c-C20:4	**20,35**	7,26	**16,53**	4,69	**19,97**	2,63	**26,47**	3,49	**26,64**	6,08	**21,13**	4,06
5c8c11c14c17c-C20:5	**1,67**	0,69	**1,29**	0,38	**1,88**	0,12	**0,84**	0,30	**0,61**	0,14	**0,46**	0,11
7c10c13c16c-C22:4	**3,55**	1,53	**2,60**	0,76	**3,82**	0,50	**6,43**	1,07	**5,06**	1,29	**4,02**	0,98
4c7c10c13c16c-C22:5	**2,12**	0,91	**1,42**	0,48	**2,27**	0,43	**1,80**	0,26	**1,85**	0,47	**1,67**	0,45
7c10c13c16c19c-C22:5	**0,68**	0,29	**0,57**	0,19	**0,79**	0,13	**0,96**	0,23	**0,89**	0,27	**0,75**	0,13
4c7c10c13c16c19c-C22:6	**3,13**	1,23	**2,32**	0,84	**3,39**	0,54	**3,81**	0,25	**3,92**	1,16	**3,36**	0,58

Table 41 Fatty acid methyl ester composition in blood platelets

	control; young rats n=5		CLA 9,11; young rats n=6		CLA 10,12; young rats n=5		control; aged rats n=5		CLA 9,11; aged rats n=5		CLA 10,12; aged rats n=6	
	Mean	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD
C14:0	**0,12**	0,16	**0,15**	0,15	**0,52**	0,28	**0,58**	0,05	**0,41**	0,24	**0,58**	0,09
C14:1	**0,24**	0,27	**0,13**	0,04	**0,17**	0,04	**0,31**	0,02	**0,24**	0,05	**0,27**	0,02
C16:0	**18,86**	4,86	**21,72**	7,21	**27,53**	2,06	**29,53**	0,55	**28,14**	1,66	**28,26**	0,61
7c-C16:1	**0,30**	0,11	**0,43**	0,32	**0,49**	0,07	**0,51**	0,04	**0,50**	0,10	**0,44**	0,05
9c-C16:1	**0,30**	0,11	**0,49**	0,38	**0,40**	0,08	**0,57**	0,06	**0,62**	0,14	**0,37**	0,06
C17:0	**0,57**	0,33	**0,27**	0,15	**0,22**	0,04	**0,29**	0,03	**0,30**	0,12	**0,28**	0,04
C18:0	**17,92**	0,77	**17,24**	1,85	**16,21**	0,50	**15,09**	0,41	**16,00**	0,87	**16,82**	0,56
trans-18:1t	**0,20**	0,02	**0,17**	0,10	**0,23**	0,01	**0,09**	0,01	**0,11**	0,00	**0,19**	0,01
9c-C18:1	**8,99**	0,54	**7,70**	0,67	**7,86**	0,53	**7,39**	0,31	**6,57**	0,31	**6,23**	0,33
11c-18:1	**4,18**	0,17	**4,42**	0,19	**3,23**	0,04	**4,36**	0,11	**4,05**	0,25	**3,29**	0,10
9c12c-C18:2	**3,86**	0,23	**4,03**	0,26	**5,21**	0,33	**4,72**	0,35	**4,78**	0,55	**5,16**	0,21
C20:0	**0,41**	0,06	**0,40**	0,11	**0,39**	0,02	**0,35**	0,01	**0,34**	0,04	**0,36**	0,02
6c9c12c-C18:3	**0,09**	0,05	**0,06**	0,04	**0,08**	0,06	**0,04**	0,00	**0,04**	0,01	**0,04**	0,02
11c-20:1	**1,02**	0,07	**0,72**	0,38	**0,81**	0,09	**0,77**	0,05	**0,64**	0,06	**0,58**	0,05
13c-20:1	**0,17**	0,04	**0,31**	0,31	**0,16**	0,02	**0,19**	0,02	**0,18**	0,03	**0,16**	0,02
9c12c15c-C18:3	**0,08**	0,04	**0,07**	0,05	**0,08**	0,04	**0,07**	0,01	**0,08**	0,02	**0,09**	0,01
9c11t-C18:2			**0,54**	0,08					**0,43**	0,05		
10t12c-C18:2					**0,73**	0,04					**0,51**	0,05
C20:2	**0,45**	0,07	**0,40**	0,08	**0,39**	0,06	**0,41**	0,06	**0,39**	0,04	**0,46**	0,03
5c8c11c20:3	**0,73**	0,19	**0,71**	0,51	**0,38**	0,05	**0,37**	0,01	**0,38**	0,04	**0,35**	0,09
C22:0	**0,65**	0,15	**0,89**	0,44	**0,74**	0,08	**0,28**	0,03	**0,31**	0,03	**0,22**	0,10
5c11c14c-C20:3	**0,37**	0,08	**0,42**	0,12	**1,06**	0,12	**0,24**	0,02	**0,25**	0,05	**0,43**	0,06
8c11c14c-C20:3	**0,91**	0,14	**1,14**	0,28	**0,73**	0,03	**0,64**	0,09	**0,75**	0,08	**0,51**	0,04
13c-C22:1	**0,47**	0,02	**0,48**	0,11	**0,38**	0,07	**0,36**	0,02	**0,35**	0,03	**0,33**	0,04
5c8c11c14c-C20:4	**32,02**	3,15	**30,84**	3,82	**26,67**	1,49	**27,18**	0,46	**28,73**	0,86	**28,82**	1,02
C24:0	**0,63**	0,34	**0,63**	0,38	**0,44**	0,07	**0,23**	0,02	**0,23**	0,02	**0,11**	0,04
5c8c11c14c17c-C20:5	**0,29**	0,20	**0,40**	0,49	**0,15**	0,01	**0,17**	0,03	**0,23**	0,03	**0,13**	0,06
7c10c13c16c-C22:4	**4,49**	0,53	**3,92**	0,40	**2,79**	0,34	**4,24**	0,28	**3,99**	0,60	**3,69**	0,43
4c7c10c13c16c-C22:5	**0,72**	0,21	**0,44**	0,09	**1,11**	0,11	**0,29**	0,06	**0,23**	0,05	**0,38**	0,07
4c7c10c13c16c19c-C22:6	**0,93**	0,26	**0,87**	0,23	**0,85**	0,05	**0,73**	0,10	**0,72**	0,09	**0,92**	0,08

Table 42 Fatty acid methyl ester composition in the CE fraction of blood plasma

	control; young rats n=5		CLA 9,11; young rats n=5		CLA 10,12; young rats n=6		control; aged rats n=5		CLA 9,11; aged rats n=6		CLA 10,12; aged rats n=6	
	Mean	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD
C14:0	**1,09**	1,28	**0,38**	0,05	**0,62**	0,56	**0,57**	0,08	**0,84**	0,38	**0,19**	0,03
C14:1	**0,21**	0,12	**0,18**	0,01	**0,16**	0,02	**0,49**	0,20	**0,25**	0,05	**0,15**	0,01
C16:0	**8,81**	2,14	**7,97**	0,47	**7,86**	0,51	**7,37**	0,77	**7,36**	0,62	**6,13**	0,38
7c-C16:1	**0,93**	0,02	**0,95**	0,13	**0,46**	0,06	**0,77**	0,23	**0,57**	0,06	**0,29**	0,05
9c-C16:1	**2,42**	0,14	**3,00**	0,42	**1,19**	0,22	**2,06**	0,26	**1,97**	0,24	**0,86**	0,24
C17:0	**0,18**	0,08	**0,08**	0,02	**0,28**	0,13	**0,28**	0,10	**0,23**	0,17	**0,06**	0,00
C18:0	**1,13**	0,46	**0,63**	0,08	**1,32**	0,43	**0,75**	0,14	**0,82**	0,13	**0,63**	0,05
trans-18:1t	**0,16**	0,19	**0,06**	0,02	**0,23**	0,20	**0,28**	0,09	**0,12**	0,05	**0,06**	0,02
9c-C18:1	**13,34**	1,87	**10,91**	1,70	**10,69**	1,69	**8,08**	0,71	**8,56**	1,55	**6,15**	1,07
11c-18:1	**1,51**	0,14	**1,18**	0,14	**1,32**	0,18	**1,12**	0,06	**1,22**	0,18	**0,88**	0,11
9c12c-C18:2	**8,24**	0,69	**8,42**	0,94	**9,14**	0,62	**8,33**	0,76	**9,25**	1,37	**9,10**	0,72
C20:0	**0,05**	0,05	**0,04**	0,03	**0,04**	0,02	**0,03**	0,01	**0,04**	0,02	**0,01**	0,01
6c9c12c-C18:3	**0,61**	0,14	**0,63**	0,10	**0,25**	0,05	**0,55**	0,11	**0,53**	0,09	**0,35**	0,11
11c-20:1	**0,03**	0,03	**0,03**	0,02	**0,03**	0,03	**0,14**	0,05	**0,01**	0,01	**0,01**	0,00
13c-20:1	**0,03**	0,02	**0,02**	0,01	**0,03**	0,03	**0,07**	0,01	**0,05**	0,02	**0,01**	0,01
9c12c15c-C18:3	**0,08**	0,02	**0,09**	0,03	**0,09**	0,03	**0,17**	0,13	**0,17**	0,06	**0,13**	0,02
9c11t-C18:2			**0,44**	0,08					**0,41**	0,12		
10t12c-C18:2					**0,19**	0,04					**0,10**	0,01
C22:0	**0,70**	0,19	**1,12**	0,06	**0,70**	0,17	**0,27**	0,05	**0,30**	0,06	**0,19**	0,05
5c11c14c-C20:3	**0,30**	0,05	**0,33**	0,05	**0,86**	0,18	**0,24**	0,05	**0,21**	0,05	**0,37**	0,06
8c11c14c-C20:3	**0,26**	0,06	**0,33**	0,03	**0,24**	0,04	**0,22**	0,03	**0,22**	0,04	**0,15**	0,02
13c-C22:1	**0,09**	0,00	**0,06**	0,01	**0,14**	0,08	**0,06**	0,03	**0,05**	0,01	**0,06**	0,01
5c8c11c14c-C20:4	**58,32**	4,97	**61,23**	2,40	**60,23**	3,34	**66,23**	2,45	**65,00**	3,60	**70,98**	2,16
5c8c11c14c17c-C20:5	**0,16**	0,05	**0,28**	0,06	**0,10**	0,01	**0,27**	0,04	**0,28**	0,10	**0,19**	0,05
4c7c10c13c16c-C22:5	**0,25**	0,09	**0,20**	0,04	**1,10**	0,21	**0,12**	0,03	**0,09**	0,03	**0,29**	0,04
4c7c10c13c16c19c-C22:6	**1,09**	0,30	**1,46**	0,04	**2,73**	0,16	**1,51**	0,17	**1,48**	0,16	**2,68**	0,29

Table 43 Fatty acid methyl ester composition in the TAG fraction of blood plasma

	control; young rats n=6		CLA 9,11; young rats n=5		CLA 10,12; young rats n=6		control; aged rats n=5		CLA 9,11; aged rats n=6		CLA 10,12; aged rats n=6	
	Mean	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD
C14:0	**1,28**	0,37	**1,16**	0,29	**0,76**	0,13	**0,46**	0,04	**1,55**	0,39	**0,93**	0,26
C14:1	**0,42**	0,12	**0,27**	0,02	**0,16**	0,02	**0,24**	0,06	**0,59**	0,16	**0,49**	0,20
C16:0	**30,72**	1,36	**26,19**	1,23	**27,04**	1,19	**22,05**	0,83	**29,91**	2,64	**24,68**	0,74
7c-C16:1	**0,94**	0,25	**0,65**	0,08	**0,45**	0,05	**0,50**	0,05	**1,01**	0,19	**0,64**	0,21
9c-C16:1	**2,96**	0,71	**3,37**	0,27	**1,65**	0,21	**2,33**	0,43	**2,36**	0,30	**1,36**	0,22
C17:0	**0,33**	0,09	**0,16**	0,01	**0,17**	0,03	**0,15**	0,03	**0,70**	0,48	**0,37**	0,17
C18:0	**3,68**	0,68	**2,21**	0,21	**2,99**	0,22	**1,77**	0,43	**3,66**	0,50	**2,58**	0,23
trans-18:1t	**0,39**	0,09	**0,20**	0,02	**0,31**	0,04	**0,10**	0,01	**0,58**	0,14	**0,31**	0,12
9c-C18:1	**36,24**	2,88	**39,50**	1,22	**37,97**	2,64	**32,21**	2,30	**26,79**	2,21	**25,86**	3,46
11c-18:1	**3,46**	0,17	**3,07**	0,10	**2,70**	0,25	**3,28**	0,19	**3,62**	0,20	**2,39**	0,28
9c12c-C18:2	**8,95**	0,96	**11,33**	1,08	**13,16**	1,45	**22,36**	1,24	**14,76**	1,28	**24,49**	2,32
C20:0	**0,10**	0,01	**0,08**	0,02	**0,07**	0,03	**0,07**	0,04	**0,14**	0,06	**0,12**	0,04
6c9c12c-C18:3	**0,30**	0,10	**0,31**	0,08	**0,20**	0,04	**0,48**	0,08	**0,34**	0,10	**0,42**	0,16
11c-20:1	**0,20**	0,01	**0,16**	0,03	**0,20**	0,02	**0,14**	0,01	**0,38**	0,46	**0,13**	0,02
13c-20:1	**0,50**	0,16	**0,60**	0,18	**0,37**	0,20	**0,67**	0,44	**0,86**	0,11	**0,57**	0,12
9c12c15c-C18:3	**0,42**	0,06	**0,52**	0,06	**0,57**	0,10	**1,35**	0,14	**1,19**	0,36	**1,60**	0,18
9c11t-C18:2			**2,63**	0,05					**1,62**	0,25		
10t12c-C18:2					**1,13**	0,11					**0,75**	0,10
C20:2	**0,07**	0,02	**0,06**	0,00	**0,73**	0,33	**0,10**	0,01	**0,14**	0,15	**0,27**	0,04
5c8c11c20:3	**0,01**	0,01	**0,08**	0,01	**0,04**	0,00	**0,01**	0,00	**0,19**	0,38	**0,03**	0,01
C22:0	**1,21**	0,18	**1,28**	0,21	**0,82**	0,20	**0,68**	0,06	**0,57**	0,13	**0,34**	0,05
5c11c14c-C20:3	**0,06**	0,02	**0,06**	0,01	**0,17**	0,04	**0,08**	0,01	**0,11**	0,11	**0,10**	0,02
8c11c14c-C20:3	**0,11**	0,02	**0,10**	0,01	**0,08**	0,01	**0,10**	0,02	**0,13**	0,13	**0,08**	0,01
13c-C22:1	**0,17**	0,03	**0,11**	0,05	**0,10**	0,04	**0,03**	0,00	**0,37**	0,33	**0,11**	0,02
5c8c11c14c-C20:4	**4,92**	1,16	**3,61**	0,50	**4,17**	0,68	**6,96**	1,35	**4,72**	1,06	**7,32**	1,39
5c8c11c14c17c-C20:5	**0,30**	0,08	**0,32**	0,07	**0,22**	0,09	**0,64**	0,12	**0,82**	0,99	**0,43**	0,13
7c10c13c16c-C22:4	**0,56**	0,29	**0,33**	0,08	**0,94**	0,26	**0,54**	0,07	**0,32**	0,05	**0,46**	0,11
4c7c10c13c16c-C22:5	**0,68**	0,20	**0,42**	0,06	**1,45**	0,40	**0,48**	0,07	**0,31**	0,14	**0,53**	0,14
7c10c13c16c19c-C22:5	**0,12**	0,05	**0,27**	0,10	**0,39**	0,10	**0,35**	0,04	**0,79**	1,43	**0,48**	0,10
4c7c10c13c16c19c-C22:6	**0,88**	0,26	**0,92**	0,20	**1,00**	0,32	**1,87**	0,27	**1,45**	0,53	**2,12**	0,70

Table 44 Fatty acid methyl ester composition in the PL fraction of blood plasma

	control; young rats n=4		CLA 9,11; young rats n=5		CLA 10,12; young rats n=6		control; aged rats n=5		CLA 9,11; aged rats n=6		CLA 10,12; aged rats n=6	
	Mean	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD	**Mean**	SD
C14:0	**1,00**	0,20	**0,30**	0,04	**0,21**	0,01	**0,23**	0,03	**0,18**	0,02	**0,55**	0,21
C14:1	**1,95**	1,62	**0,16**	0,02	**0,13**	0,02	**0,23**	0,03	**0,17**	0,03	**1,00**	1,02
C16:0	**30,10**	0,83	**24,74**	2,19	**24,39**	0,82	**27,32**	1,85	**22,83**	2,34	**28,35**	2,16
7c-C16:1	**0,55**	0,06	**0,21**	0,03	**0,15**	0,02	**0,20**	0,01	**0,13**	0,01	**0,28**	0,05
9c-C16:1	**0,78**	0,07	**0,66**	0,06	**0,27**	0,02	**0,45**	0,05	**0,23**	0,05	**0,53**	0,07
C17:0	**2,84**	1,28	**0,28**	0,03	**0,23**	0,05	**0,56**	0,02	**0,43**	0,03	**1,00**	0,57
C18:0	**33,79**	1,56	**27,87**	2,26	**29,57**	3,21	**28,34**	0,63	**32,14**	2,59	**32,51**	2,52
trans-18:1t	**0,54**	0,21	**0,17**	0,01	**0,18**	0,03	**0,19**	0,02	**0,22**	0,07	**0,29**	0,08
9c-C18:1	**7,19**	0,47	**6,45**	3,51	**6,75**	0,53	**6,59**	0,19	**4,83**	0,69	**5,39**	0,40
11c-18:1	**2,79**	0,21	**3,48**	2,62	**1,73**	0,16	**3,63**	0,35	**2,03**	0,21	**2,73**	0,40
9c12c-C18:2	**4,03**	0,26	**5,54**	1,84	**6,50**	0,62	**8,21**	0,64	**8,13**	0,50	**6,80**	0,51
C20:0	**0,08**	0,04	**1,34**	2,87	**0,09**	0,07	**0,10**	0,01	**0,07**	0,01	**0,09**	0,03
6c9c12c-C18:3	**0,08**	0,05	**0,05**	0,01	**0,02**	0,00	**0,04**	0,01	**0,03**	0,02	**0,05**	0,02
11c-20:1	**0,23**	0,12	**0,10**	0,04	**0,13**	0,01	**0,19**	0,02	**0,10**	0,01	**0,13**	0,03
13c-20:1	**0,38**	0,06	**0,23**	0,07	**0,21**	0,09	**0,56**	0,06	**0,37**	0,03	**0,44**	0,07
9c12c15c-C18:3	**0,36**	0,04	**0,08**	0,14	**0,02**	0,00	**0,04**	0,01	**0,04**	0,01	**0,12**	0,04
9c11t-C18:2			**0,30**	0,06							**0,28**	0,06
10t12c-C18:2					**0,58**	0,08			**0,45**	0,06		
C20:2	**0,05**	0,02	**0,08**	0,01	**0,10**	0,01	**0,16**	0,02	**0,12**	0,01	**0,14**	0,08
5c8c11c20:3	**0,03**	0,01	**0,01**	0,00	**0,00**	0,00	**0,00**	0,00	**0,01**	0,01	**0,01**	0,01
C22:0	**0,25**	0,02	**0,59**	0,04	**0,38**	0,06	**0,24**	0,02	**0,14**	0,02	**0,20**	0,05
5c11c14c-C20:3	**0,13**	0,02	**0,27**	0,05	**0,68**	0,12	**0,23**	0,04	**0,30**	0,06	**0,13**	0,03
8c11c14c-C20:3	**0,18**	0,03	**0,36**	0,02	**0,29**	0,07	**0,33**	0,04	**0,22**	0,02	**0,21**	0,05
13c-C22:1	**0,05**	0,03	**0,03**	0,00	**0,03**	0,00	**0,03**	0,01	**0,03**	0,01	**0,03**	0,02
5c8c11c14c-C20:4	**10,35**	1,56	**21,44**	3,18	**15,68**	1,19	**17,74**	1,52	**19,51**	2,45	**15,33**	1,62
C24:0	**0,05**	0,01	**0,02**	0,01	**0,08**	0,02	**0,02**	0,01	**0,02**	0,01	**0,02**	0,01
5c8c11c14c17c-C20:5	**0,09**	0,03	**0,12**	0,03	**0,05**	0,01	**0,10**	0,01	**0,07**	0,03	**0,09**	0,01
7c10c13c16c-C22:4	**0,20**	0,07	**0,27**	0,04	**0,71**	0,18	**0,35**	0,04	**0,50**	0,08	**0,33**	0,04
4c7c10c13c16c-C22:5	**0,42**	0,09	**0,71**	0,20	**4,20**	1,10	**0,45**	0,11	**0,91**	0,24	**0,21**	0,08
7c10c13c16c19c-C22:5	**0,17**	0,11	**0,34**	0,10	**0,58**	0,15	**0,29**	0,05	**0,57**	0,09	**0,22**	0,04
4c7c10c13c16c19c-C22:6	**1,33**	0,26	**3,79**	1,01	**6,09**	1,30	**3,18**	0,57	**5,21**	1,37	**2,55**	0,55

12.3 Hazardous chemicals and apparatus equipment

12.3.1 Hazardous chemicals

Reagent	Symbol	Risk Phrases	Security Precautions
Acetic acid	C	R10-35	S23.2-26-45
Acetone	F	R11	S9-16-23.2-33
Acetonitrile	F, T	R11-23 / 24 / 25	S16-27-45
2-Amino-2-methylpropanol	Xi	R36 / 38	S26-37 / 39
Ammoniumchloride	Xn	R22-36	S22
ATP		R36 / 37 / 38	S26-36
Barium carbonate	Xn	R22	S24 / 25
Benzophenone			S24 / 25
Boron trifluoride in methanol (14%)	F, T	R11-23 / 25-34	S16-26-36 / 37 / 39-45
Butanol	Xn	R10-20	S16
tert-Butyldiphenylchlorosilane	C	R34	S25-36 / 37 / 39-45
Butyllithium in hexane (2.5M)	F, C	R14 / 15-17-34-48 / 20	S6.1-26-36 / 37 / 39-45
C23:0 methyl ester			
Calcium carbonate	Xi	R36	S26-39
Calcium hydride	F	R15	S7 / 8-24 / 25-43.6
Chloroform	Xn	R47-20 / 22-38-40-48	S35-36 / 37
CLA 9c11t- and 10t12c			
CLA 9c11t- and 10t12c-C18:2, TAG			
Coenzyme A, sodium salt			S24 / 25
Copper(II)sulfate, pentahydrate	Xn	R22-36 / 38	S22
Dichloromethane	Xn	R40	S23.2-24 / 25-36 / 37
Diethyl ether	F+	R12-19-22-66-67	S2-9-16-29-33
di-Homo-γ-linolenic acid			
3,4-Dihydropyran	F, Xi	R11-19-36 / 38	S9-16-29-43.3
Dimethylaminopyridine	T	R23 / 24 / 25-36 / 37 / 38	S26-28-36 / 37 / 39-45
EDTA	Xi	R36 / 37 / 38	S26-37 / 39
Ethanol	F	R11	S7-16
Florisil, (activated magnesium silicate)	Xi	R37	S22
Folin reagent (Folin & Ciocalteu's Phenol Reagent 2.0 N)	C	R34-29 / 21 / 21	S26-23-36 / 37 / 39
Glucose			
Gluthatione			S24 / 25
HEPES			
Heptane-1,7-diol			S24 / 25
Hexane	F, Xn	R11-48 / 20	S9-16-24 / 25-29-51
HMDS	F, Xn	R11-22-36 / 37 / 38	S9-16-26-29-37 / 39

Reagent	Symbol	Risk Phrases	Security Precautions
HMPA	T	R45-46	S45-53
Imidazole	Xi	R22-34	S25-36 / 37 / 39-45
Indomethacin	T+	R28-42 / 43-63	S45-36 / 37 / 39
Iodine	Xn	R20 / 21	S23-25
Linoleic acid			
γ-Linolenic acid			
Magnesium chloride, hexahydrate	Xi		S24 / 25
Magnesium turnings	F	R11-15	S7 / 8-43.6
Malonyl CoA		R36 / 37 / 38	S22-26-36
Methanol	F, T	11-23 / 25	S7-16-24-45
Molecular sieves 4Å			S24 / 25
NADPH		R23 / 24 / 25-36 / 37 / 38	S45
Nicotinamide	Xi		S24 / 25
PDC	O, T	R36 / 37 / 38-45-8	S17-45-53
Pentane	F	R11	S2-9-16-29-33-61-62
Petroleum ether	F, Xn	R11-52 / 53-65	S9-16-23.2-24-33-62
Potassium chloride	Xi	R36	S26-39
Potassium cyanide	T+	R26 / 27 / 28-32	S1 / 2-7-28-29-45
Potassium dihydrogenphosphate			
Potassium fluoride	T	R23 / 24 / 25	S26-45
Potassium-sodium tartrate			
PPTs	Xi	R36 / 37 / 38	S26-36
Propane-1,3-diol			S24 / 25
Silica gel			
Sodium	F, C	R14 / 15-34	S5.3-8-43.7-45
Sodium carbonate	Xi	R36	S22-26
Sodium chloride			
Sodium dihydrogenphosphate			
di-Sodium hydrogen phosphate, dihydrate			
Sodium hydrogen carbonate			
Sodium hydrogen sulfate	C	R34-37	S26-36 / 37 / 39-45
Sodium methoxide	F, C	R11-14-34	S8-16-26-43-45
Sodium sulfate			
Sodium thiosulfate			
Sodiumhydroxide	C	R35	S26-37 / 39-45
Sucrose			
Sulfuric acid	C	R35	S26-30-45
Tetra-n-butylammonium fluoride	Xi	R36 / 37 / 38	S26-37 / 39
THF	F, Xi	R11-19-36 / 37	S16-29-33
Toluene	F, Xn	R11-20	S16-25-29-33

Reagent	Symbol	Risk Phrases	Security Precautions
Triethylamine	F, C	R11-20/21/22-35	S3-16-26-29-36/37/39-45
Trifuoroacetic acid	C	R20-35	S9-26-27-28-45
Triphenylphosphin	Xn, N	R22-43-48/20/22-50/53	S26-36/37/39-61
TRIS	Xi	R36/38	

12.3.2 Radiolabeled chemicals

[^{14}C] Barium carbonate

[1-^{14}C] CLA 9c11t- and [1-^{14}C] 10t12c

[1-^{14}C] di-Homo-γ-linolenic acid

[1-^{14}C] Linoleic acid

[1-^{14}C] γ-Linolenic acid

General handling Precautions for 14C labeled reagents

1. Designate area for handling 14C and clearly label all containers.
2. Prohibit eating, drinking, smoking and mouth pipetting in room where 14C is handled.
3. Use transfer pipets, spill trays and absorbent coverings to confine contamination.
4. Handle potentially volatile compounds in ventilated enclosures.
5. If enhanced containment is necessary, handle volatile compounds in closed systems vented through suitable traps.
6. Sample exhausted effluent and room air by drawing a known volume through a membrane filter followed by an impinger containing dilute NaOH.
7. Wear disposable lab coats, wrist guards and gloves for secondary protection.
8. Select gloves appropriate for chemicals handled.
9. Maintain contamination and exposure control by regularly monitoring and promptly decontaminating gloves and surfaces.
10. Use pancake or end-window Geiger-Müller detectors or liquid scintillation counter to detect 14C.
11. Submit periodic urine and breath samples (as appropriate) for bioassay to determine uptake by personnel.
12. Isolate waste in sealed, clearly labeled containers and dispose according to approved guidelines.
13. Establish air concentrations, surface contamination and bioassay action levels below regulatory limits. Investigate and correct any conditions that may cause these levels to be exceeded.
14. On completing an operation, secure all 14C; remove and dispose of protective clothing and coverings; monitor and decontaminate self and surfaces; wash hands and monitor them again.

12.3.3 Instrumental equipment

Instrument	Name	Supplier
Centrifuge	Centrifuge 5804	Eppendorf
Centrifuge	GT422	Jouan
Centrifuge	J2-21M / E Centrifuge	Beckman
FTIR	FTS 60A	Bio-Rad
GC	HP 5890	Hewlett Packard
	HP 6890	Hewlett Packard
	Carlo Erba HRGC5300	Carlo Erba
	Packard 438A	Hewlett Packard
GC-Column	CPSil 88, 100m × 0.25mm I.D., 0.25µm	Chrompack
	BPX-70, 30m × 0.25mm I.D., 0.25µm	SGE
	BPX-70, 50m × 0.25mm I.D., 0.25µm	SGE
	DB5, 8m × 0.25mm I.D., 0.25µm	J&W Scientific
	DB5, 30m × 0.25mm I.D., 0.25µm	J&W Scientific
	HP1, 30m × 0.25mm I.D., 0.25µm	Hewlett Packard
	HP5, 30m × 0.25mm I.D., 0.25µm	Hewlett Packard
	Stabilwax, 60m × 0.53mm I.D., 0.5µm	Restek
HPLC	Gilson model 303	Gilson
	Hitachi 655A-12LC	Merck
	Shimadzu LC-10AS	Shimadzu
	Varian 9010	Varian
	Waters 600	Waters
HPLC-column	C18 nucleosil, 250mm × 10mm I.D., 5µm	Interchim
	ChromSpher 5 Lipids, 250mm × 4.6mm I.D., 5µm	Chrompack
	Zorbax SBC18, 250mm × 4.6mm I.D., 5µm	Chrompack
	Zorbax SBC18, 250mm × 21.2mm I.D., 5µm	
HPLC-detector	photodiode array detector JASCO-MD 1510	Varian
	photodiode array detector Waters PDA 996	Waters
	radioctive detector Berthold LB 2040	Berthold
	radioctive detector Berthold LB 503	Berthold
	radioctive detector Flo-One β Series A	Radiomatic Instruments
Microtiter-plate reader	MRX_{TC}	Dynatech Laboratories
MS detector	HP 5970	Hewlett Packard
	HP 5973	Hewlett Packard
NMR	FT-NMR	Bruker
Photometer	Uvikon 923 UV-VIS	Kontron
Ultracentrifuge	Centrikon T2060	Kontron
Ultraturrax	T25	Janke&Kunkel

13 BIBLIOGRAPHY

Abdel-Baky, S.; Giese, R.W.: Facile anhydride synthesis using trichlorotrifluoroacetone hydrate. J. Org. Chem. **51**, 3390-3391 (1986).

Ackman, R.G.: Laboratory preparation of conjugated linoleic acid. J. Am. Oil Chem. Soc. **75**, 1227 (1998).

Adlof, R.O.: Preparation of methyl *cis*-9, *trans*-11- and *trans*-9, *trans*-11-octadecadienoate-17,17,18,18-d_4, two of the isomers of conjugated linoleic acid. Chem. Phys. Lipids **88**, 107-112 (1997).

Adlof, R.O.: Preparation of unlabled and isotope-labeled conjugated linoleic and related fatty acid isomers. In: Yurawecz, M.P.; Mossoba, M.M.; Kramer, J.K.G.; Pariza, M.W.; Nelson, G.J. (Eds.), Advances in conjugated linoleic acid research, Vol. 1. AOCS Press, Champaign (USA). 21-38 (1999).

Azain, M.J.; Hausman, D.B.; Sisk, M.B.; Flatt, W.P.; Jewell, D.E.: Dietary conjugated linoleic acid reduces rat adipose tissue cell size rather than cell number. J. Nutr. **130**, 1548–1554 (2000).

Banni, S.; Day, B.W.; Evans, R.W.; Corongiu, F.P.; Lombardi, B.: Liquid chromatographic-mass spectrometric analysis of conjugated diene fatty acids in a partially hydrogenated fat. J. Am. Oil Chem. Soc. **71**, 1321-1325 (1994).

Banni, S.; Carta, G.; Contini, M.S.; Angioni, E.; Deiana, M.; Dessi, M.A.; Melis, M.P.; Corongiu, F.P.: Characterization of conjugated diene fatty acids in milk, dairy products, and lamb tissues. J. Nutr. Biochem. **7**, 150-155 (1996).

Banni, S.; Martin, J.C.: Conjugated linoleic acid and metabolites. In: Sébédio, J.L.; Christie, W.W. (Eds.), Trans fatty acids in Human Nutrition. The Oily Press, Dundee (Great Britain). 261-302 (1998).

Banni, S.; Angioni, E.; Casu, V.; Melis, M.P.; Carta, G.; Corongiu, F.P.; Thompson, H.; Ip, C.: Decrease in linoleic acid metabolites as a potential mechanism in cancer risk reduction by conjugated linoleic acid. Carcinogenesis **20**, 1019-1024 (1999a).

Banni, S.; Angioni, E.; Carta, G.; Casu, V.; Deiana, M.; Dessi, M.A.; Lucchi, L.; Melis, M.P.; Rosa, A.; Vargiolu, S.; Corongiu, F.P.: Influence of dietary conjugated linoleic acid on lipid metabolism in relation to its anticarcinogenic activity. In: Yurawecz, M.P.; Mossoba, M.M.; Kramer, J.K.G.; Pariza, M.W.; Nelson, G.J. (Eds.), Advances in conjugated linoleic acid research, Vol. 1. AOCS Press, Champaign (USA). 307-318 (1999b).

Banni, S.; Carta, G.; Angioni, E.; Murru, E.; Scanu, P.; Melis, M.P.; Bauman, D.E.; Fischer, S.M.; Ip, C.: Distribution of conjugated linoleic acid and metabolites in different lipid fractions in the rat liver. J. Lipid Res. **42**, 1056-1061 (2001).

Basu, S.; Smedman, A.; Vessby, B.: Conjugated linoleic acid induces lipid peroxidation in humans. FEBS Lett. **468**, 33-36 (2000a).

Basu, S.; Risérus, U.; Turpeinen, A.; Vesby, B.: Conjugated linoleic acid induces lipid peroxidation in men with abdominal obesity. Clin. Sci. **99**, 511-516 (2000b).

Baumgard, L.H.; Sangster, J.K.; Bauman, D.E.: Milk fat synthesis in dairy cows is progressively reduced by increasing supplemental amounts of trans-10, cis-12 conjugated linoleic acid (CLA). J. Nutr. **131**, 1764-1769 (2001).

Belitz, H.D.; Grosch, W.; Schieberle, P.: Lehrbuch der Lebensmittelchemie. 5. Aufl., Springer Verlag, Berlin (2001).

Belury, M.A.: Conjugated dienoic linoleate: a polyunsaturated fatty acid with unique chemoprotective properties. Nutr. Rev. **53**, 83-89 (1995).

Belury, M.A.; Nickel, K.P.; Bird, C.E.; Wu, Y.: Dietary conjugated linoleic acid modulation of phorbol ester skin tumor promotion. Nutr. Cancer **26**, 149-157 (1996).

Belury, M.A.; Kempa-Steczko, A.: Conjugated linoleic acid modulates hepatic lipid composition in mice. Lipids **32**, 199-204 (1997).

Belury, M.A.; Moya-Camarena, S.Y.; Liu, K.L.; Vanden Heuvel, J.P.: Dietary conjugated linoleic acid induces peroxisome-specific enzyme accumulation and ornithine decarboxylase activity in mouse liver. J. Nutr. Biochem. **8**, 579-584 (1997).

Benito, P.; Nelson, G.J.; Kelley, D.S.; Bartolini, G.; Schmidt, P.C.; Simon, V.: The effect of conjugated linoleic acid on plasma lipoproteins and tissue fatty acid composition in humans. Lipids **36**, 229-236 (2001a).

Benito, P.; Nelson, G.J.; Kelley, D.S.; Bartolini, G.; Schmidt, P.C.; Simon, V.: The effect of conjugated linoleic acid on platelet function, platelet fatty acid composition, and blood coagulation in humans. Lipids **36**, 221-227 (2001b).

Berdeaux, O.; Vatèle, J.M.; Eynard, T.; Nour, M.; Poullain, D.; Noël, J.P.; Sébédio, J.L.: Synthesis of (9Z,12E)-(1-14C) linoleic acid and (5Z,8Z,11Z,14E)-(1-14C) arachidonic acid. Chem. Phys. Lipids **78**, 71-80 (1995).

Berdeaux, O.; Christie, W.W.; Gunstone, F.D.; Sébédio, J.L.: Large-scale synthesis of methyl *cis*-9,*trans*-11-octadecadienoate from methyl ricinoleate. J. Am. Oil Chem. Soc. **74**, 1011-1015 (1997).

Berdeaux, O.; Voinot, L.; Angioni, E.; Juanéda, P.; Sébédio, J.L.: A Simple Method of Preparation of Methyl *Trans*-10,*Cis*-12- and *Cis*-9,*Trans*-11-Octadecadienoates from Methyl Linoleate. J. Am. Oil Chem. Soc. **75**, 1749-1755 (1998a).

Berdeaux, O.; Blond, J.P.; Bretillon, L.; Chardigny, J.M.; Mairot, T.; Vatèle, J.M.; Poullain, D.; Sébédio, J.L.: *In-vitro* desaturation and elongation of mono-*trans* isomers of linoleic acid by rat liver microsomes. Mol. Cell. Biochem. **185**, 17-25 (1998b).

Berlage, U.; Schmidt, J.; Peters, U.; Welzel, P.: A formal total synthesis of (±)-strigol. Tetrahedron Lett. **27**, 3091-3094 (1987).

Blankson, H.; Stakkestad, J.A.; Fagertun, H.; Thom, E.; Wadstein, J.; Gudmundsen, O.: Conjugated linoleic acid reduces body fat mass in overweight and obese humans. J. Nutr. **130**, 2943-2948 (2000).

Brenner, R.R.: The desaturation step in the animal biosynthesis of polyunsaturated fatty acids. Lipids **6**, 567-575 (1971).

Brenner, R.R.: Nutritional and hormonal factors influencing desaturation of essential fatty acids in animals. Prog. Lipid Res. **20**, 41-47 (1981).

Bretillon, L.; Chardigny, J.M.; Grégoire, S.; Berdeaux, O.; Sébédio, J.L.: Effects of conjugated linoleic acid isomers on the hepatic microsomal desaturation activities *in vitro*. Lipids, 965-969 (1999).

Britton, M.; Fong, C.; Wickens, D.; Yudkin, J.: Diet as a source of phospholipid esterfied 9,11-octadecdienoic acid in humans. Clin. Sci. **83**, 97-101 (1992).

Bulgarella, J.A.; Patton, D.; Bull, A.W.: Modulation of prostaglandin H synthase activity by conjugated linoleic acid (CLA) and specific CLA isomers. Lipids **36**, 407-412 (2001).

Carreau, J.P.; Dubacq, J.P.: Adaptation of a macro-scale method to the micro-scale for fatty acid methyl transesterification of biological lipid extracts. J. Chromatogr. **151**, 384-390 (1978).

Channing, M.; Simpson, N.J.: Radiosynthesis of 1-[11C]polyhomoallylic fatty acids. Labeled. Compds. Radiopharm. **6**, 541-546 (1993).

Chardigny, J.M.; Blond, J.P.; Bretillon, L.; Mager, E.; Poullain, D.; Martine, L.; Vatèle, J.M.; Noël, J.P.; Sébédio, J.L.: Conversion of 18:3 Δ 9*cis*, 12*cis*, 15*trans* in rat liver microsomes. Lipids **32**, 731-735 (1997).

Chen, Z.Y.; Chan, P.T.; Kwan, K.Y.; Zhang, A.: Reassessment of the antioxidant activity of conjugated linoleic acids. J. Am. Oil Chem. Soc. **73**, 749-753 (1997).

Chen, J.F.; Tai, C.Y.; Chen, Y.C.; Chen, B.H.: Effects of conjugated linoleic acid on the degradation and oxidation stability of model lipids during heating and illumination. Food Chem. **72**, 199-206 (2001).

Chin, S.F.; Liu, W.; Storkson, J.M.; Ha, Y.L.; Pariza, M.W.: Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J. Food Comp. Anal. **5**, 185-197 (1992).

Chipault, J.R.; Hawkins, J.M.: The determination of conjugated *cis-trans* and *trans-trans* methyl octadecadienoates by infrared spectrometry. J. Am. Oil Chem. Soc. **36**, 535-539 (1959).

Choi, Y.J.; Kim, Y.C.; Han, Y.B.; Park, Y.; Pariza, M.W.; Ntambi, J.M.: The *trans*-10,*cis*-12 isomer of conjugated linoleic acid downregulates stearoyl-CoA desaturase 1 gene expression in 3T3-L1 adipocytes. J. Nutr. **130**, 1920–1924 (2000).

Choi, Y.; Park, Y.; Pariza, M.W.; Ntambi, J.M.: Regulation of stearoyl-CoA desaturase activity by the *trans*-10,*cis*-12 isomer of conjugated linoleic acid in Hep2G cells. Biochem. Biophys. Res Comm. **284**, 689-693 (2001).

Chouinard, P.Y.; Corneau, L.; Saebo, A.; Bauman, D.E.: Milk Yield and composition during abomasal infusion of conjugated linoleic acids in dairy cows. J. Dairy Sci. **82**, 2737-2745 (1999).

Christie, W.W.: Isomers in commercial samples of conjugated linoleic acid. J. Am. Oil Chem. Soc. **74**, 1231 (1997).

Christie, W.W.; Sébédio, J.L.; Juaneda, P.: A practical guide to the analysis of conjugated linoleic acid. INFORM **12**, 147-152 (2001).

Cook, H.W.: Fatty acid desaturation and elongation eucaryotes. In: Vance, D.; Vance, J. (Eds.), Biochemistry of Lipid, Lipoproteins and Memebranes. Elsevier Science, Amsterdam (Netherlands) 141-169 (1991).

Cook, M.E.; Pariza, M.: The role of conjugated linoleic acid (CLA) in health. Int. Dairy J. **8**, 459-462 (1998).

Cook, M.E.; Drake, B.; Jerome, D.; Pariza, M.W.: The interaction of 9c,11t / 9t,11c and 10t,12c conjugated linoleic acid on the fat deposition in mice. Fed. Am. Soc. Exp. Biol. J. **13**, A1023 (1999).

Danieli, B.; Lesma, G.; Palmisano, G.; Tollari, S.: A specific and efficient synthesis of (±)-Dihydrocorynantheol. J. Chem. Soc. Perkin Trans. I, 1237-1240 (1984).

de Deckere, E.A.M.; van Amelsvoort, J.M.M.; McNeill, G.P.; Jones, P.: Effects of conjugated linoleic acid (CLA) isomers on lipid levels and peroxisome proliferation in the hamster. Br. J. Nutr. **82**, 309-317 (1999).

Dhiman, T.R.; Anand, G.R.; Satter, L.D.; Pariza, M.W.: Conjugated linoleic acid content of milk from cows fed different diets. J. Dairy Sci. **79 (Suppl.1)**, 35 (1996).

Dhiman, T.R.; Helmink, E.D.; McMahon, D.J.; Fife, R.L.; Pariza, M.W.: Conjugated linoleic acid content of milk and cheese from cows fed extruded oilseeds. J. Dairy Sci. **82**, 412-419 (1999).

Dobson, G.; Christie, W.W.: Structural analysis of fatty acids by mass spectrometry of picolinyl esters and dimethyloxazoline derivatives. Trends in Analytical Chemistry **15**, (1996).

Dobson, G.: Identification of conjugated fatty acids by gas chromatography-mass spectrometry of 4-methyl-1,2,4-triazoline-3,5-dione adducts. J. Am. Oil Chem. Soc. **74**, (1997).

Donovan, D.C.; Schingoethe, D.J.; Baer, R.J.; Ryali, J.; Hippen, A.R.; Franklin, S.T.: Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows. J. Dairy Sci. **83**, 2620-2628 (2000).

Durgam, V.R.; Fernandes, G.: The growth inhibitory effect of conjugated linoleic acid on MCF-7 cells is related to estrogen response system. Cancer Lett. **116**, 121-130 (1997).

Evans, M.; Geigerman, C.; Cook, J.; Curtis, L.; Kuebler, B.; McIntosh, M.: Conjugated linoleic acid suppresses triglyceride accumulation and induces apoptosis in 3T3-L1 preadipocytes. Lipids **35**, 899–910 (2000).

Eynard, T.; Vatèle, J.M.; Poullain, D.; Noël, J.P.; Chardigny, J.M.; Sébédio, J.L.: Synthesis of (9Z,12Z,15E)- and (9E,12Z,15Z)-octadecatrienoic acids and their (1-14C)- radiolabeled analogs. Chem. Phys. Lipids **74**, 175-184 (1994).

Fay, L.; Richli, U.: Location of double bonds in polyunsaturatde fatty acids by gas chromatography-mass spectrometry after 4,4-dimethyloxazoline derivatization. J. Chromatogr. **541**, 89-98 (1991).

Freeman, F.; Kim, D.S.H.L.: Preparation of 1,4-diketones and their reactions with bis(trialkyltin) or bis(triphenyltin) sulfide-boron trichoride. J. Org. Chem. **57**, 1722-1727 (1992).

Fritsche, J.; Mossoba, M.M.; Yurawecz, M.P.; Roach, J.A.G.; Sehat, N.; Ku, Y.; Steinhart, H.: Conjugated linoleic acid (CLA) isomers in human adipose tissue. Z. Lebensm. Unters. Forsch. A **205**, 415-418 (1997).

Fritsche, J.; Steinhart, H.: Analysis, occurence, and physiological properties of *trans* fatty acids (TFA) with particular emphasis on conjugated linoleic acid isomers (CLA) - a review. Fett/Lipid **100**, 190-210 (1998a).

Fritsche, J.; Steinhart, H.: Amounts of conjugated linoleic acid (CLA) in german foods and evaluation of daily intake. Z Lebensm. Unters. Forsch. A **206**, 77-82 (1998b).

Garcia-Lopez, S.; Echeverra, E.; Tsui, I.; Balch, B.: Changes in the content of conjugated linoleic acid (CLA) in processed cheese during processing. Food Res. Int., 61-64 (1994).

Garegg, P.J.; Samuelsson, B.: Novel reagent for converting a hydroxy-group into a aldo-group in carbohydrates with inversion of configuration. Part 2. J. Chem. Soc. Perkin Trans. I, 2866-2869 (1980).

Gavino, V.C.; Gavino, G.; Leblanc, M.J.; Tuchweber, B.: An isomeric mixture of conjugated linoleic acids but not pure *cis*-9,*trans*-11-octadecadienoic acid affects body weight gain and plasma lipids in hamsters. J. Nutr. **130**, 27–29 (2000).

Gnädig, S.: Konjugierte Linolsäureisomere in Lebensmitteln, humanem Fettgewebe und humanem Blutplasma. Diplomarbeit, Fachbereich Chemie, Inst. für Biochemie und Lebensmittelchemie, Abt. Lebensmittelchemie, Universität Hamburg, Hamburg (1996).

Griinari, J.M.; Cori, B.A.; Lacy, S.H.; Chouinard, P.Y.; Nurmela, K.V.V.; Bauman, D.E.: Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Delta(9)-desaturase. J. Nutr. **130**, 2285–2291 (2000).

Gudmundsen, O.; Wadstein, J.; Thom, E.: Human studies with CLA in Norway. 1st International Conference on Conjugated Linoleic Acid (CLA), Ålesund (Norway) (2001).

Ha, Y.L.; Grimm, N.K.; Pariza, M.W.: Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. Carcinogenis **8**, 1881-1887 (1987).

Ha, Y.L.; Grimm, N.K.; Pariza, M.W.: Newly recognized anticarcinogenic fatty acids: identification and quantification in natural and processed cheeses. J. Agric. Food Chem. **37**, 75-81 (1989).

Hamilton, R.J.: CLA is focus for SCI seminar. INFORM **12**, 629-633 (2001).

Harfoot, C.G.; Hazelwood, G.P.: Lipid metabolism in the rumen. In: Hobson, P. (Eds.), The rumen microbial ecosystem. Elsevier Sciences Publishers, London (Great Britain). 285-322 (1988).

Hayek, M.G.; Han, S.N.; Wu, D.Y.; Watkins, B.A.; Meydani, M.; Dorsey, J.L.; Smith, D.E.; Meydani, S.N.: Dietary conjugated linoleic acid influences the immune response of young and old C57BL/6NCrlBR mice. J. Nutr. **129**, 32-38 (1999).

Herbel, B.K.; Mcguire, M.K.; Mcguire, M.A.; Shultz, T.D.: Safflower oil consumption does not increase plasma conjugated linoleic acid concentrations in humans. Am. J. Clin. Nutr. **67**, 332-337 (1998).

Herscovici, J.; Egron, M.J.; Antonakis, K.: New oxidative systems for alcohols: molecular sieves with chromium-(VI) reagents. J. Chem. Soc. Perkins Trans. I, 1967-1973 (1982).

Houseknecht, K.L.; Vandenheuvel, J.P.; Moyacamarena, S.Y.; Portocarrero, C.P.; Peck, L.W.; Nickel, K.P.; Belury, M.A.: Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty fa/fa rat. Biochem. Biophys. Res. Comm. **244**, 678-682 (1998).

Howton, D.R.; Davis, R.H.; Nevenzel, J.C.: Unsaturated fatty acids. III. Preparation of 1-C14-linoleic acid. J. Am. Chem. Soc. **76**, 4970-4974 (1954).

Ip, C.; Chin, S.F.; Scimeca, J.A.; Pariza, M.W.: Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. Cancer Res. **51**, 6118-6124 (1991).

Ip, C.; Scimeca, J.A.; Thompson, H.J.: Conjugated linoleic acid: A powerful anticarcinogen from animal fat sources. Cancer **74**, 1050-1054 (1994a).

Ip, C.; Singh, M.; Thompson, H.J.; Scimeca, J.A.: Conjugated Linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. Cancer Res. **54**, 1212-1215 (1994b).

Ip, C.; Briggs, S.P.; Haegele, A.D.; Thompson, H.J.; Storkson, J.; Scimeca, J.A.: The efficacy of conjugated linoleic acid in mammary cancer prevention is independent of the level or type of fat in the diet. Carcinogenesis **17**, 1045-1050 (1996).

Ip, C.; Banni, S.; Angioni, E.; Carta, G.; McGinley, J.; Thompson, H.J.; Barbano, D.; Bauman, D.: Conjugated linoleic acid-enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. J. Nutr. **129**, 2135-2142 (1999a).

Ip, M.M.; Masso-Welch, P.A.; Shoemaker, S.F.; Shea-Eaton, W.K.; Ip, C.: Conjugated linoleic acid inhibits proliferation and induces apoptosis of normal rat mammary epithelial cells in primary culture. Exp. Cell Res. **250**, 22-34 (1999b).

Ip, C.; Ip, M.M.; Loftus, T.; Shoemaker, S.; Shea-Eaton, W.: Induction of apoptosis by conjugated linoleic acid in cultured mammary tumor cells and premalignant lesions of the rat mammary gland. Cancer Epidemiol. Biomarkers Prevent. **9**, 689–696 (2000).

Jahreis, G.; Fritsche, J.; Steinhart, H.: Monthly variation of milk composition with special regard to fatty acids depending on season and farm management systems - convential versus ecological. Fett/Lipid **98**, 356-359 (1996).

Jahreis, G.; Fritsche, J.; Steinhart, H.: Conjugated linoleic acid in milk fat - high variation depending on production system. Nutr. Res. **17**, 1479-1484 (1997).

Jahreis, G.; Fritsche, J.; Kraft, J.: Species-dependent, seasonal, and dietary variation of conjugated linoleic acid in milk. In: Yurawecz, M.P.; Mossoba, M.M.; Kramer, J.K.G.; Pariza, M.W.; Nelson, G.J. (Eds.), Advances in Conjugated Linoleic Acid Research, Vol. 1. AOCS Press, Champaign, IL. 215-225 (1999).

Jiang, J.; Bjoerck, L.; Fondén, R.; Emanuelson, M.: Occurence of conjugated *cis*-9,*trans*-11-octadecadienoic acid in bovine milk: effects of feed and dietary regimen. J. Dairy Sci. **79**, 438-445 (1996).

Jiang, J.; Bjorck, L.; Fonden, R.: Conjugated linoleic acid in Swedish dairy products with special reference to the manufacture of hard cheese. Int. Dairy J. **7**, 863-867 (1997).

Jiang, J.; Bjorck, L.; Fonden, R.: Production of conjugated linoleic acid by dairy starter cultures. J. Appl. Microbiol. **85**, 95-102 (1998).

Jiang, J.; Wolk, A.; Vessby, B.: Relation between the intake of milk fat and the occurence of conjugated linoleic acid in human adipose tissue. Am. J. Clin. Nutr **70**, 21-27 (1999).

Jones, D.F.; Weiss, W.P.; Palmquist, D.L.: Short communication: Influence of dietary tallow and fish oil on milk fat composition. J. Dairy Sci. **83**, 2024–2026 (2000).

Juaneda, P.; Cordier, O.; Gregoire, S.; Sebedio, J.L.: Conjugated linoleic acid (CLA) isomers in heat-treated vegetable oils. OCL **8**, 94-97 (2001).

Jung, M.Y.; Ha, Y.L.: Conjugated linoleic acid isomers in partially hydrogenated soybean oil obtained during nonselective and selective hydrogenation processes. J. Agric. Food Chem. **47**, 704-708 (1999).

Kavanaugh, C.J.; Liu, K.L.; Belury, M.A.: Effect of dietary conjugated linoleic acid on phorbol ester-induced PGE_2 production and hyperplasia in mouse epidermis. Nutr. Cancer **33**, 132-138 (1999).

Kelley, D.S.; Taylor, P.C.; Rudolph, I.L.; Benito, P.; Nelson, G.J.; Mackey, B.E.; Erickson, K.L.: Dietary conjugated linoleic acid did not alter immune status in young healthy woman. Lipids **35**, 1065-1071 (2000).

Kelly, M.L.; Berry, J.R.; Dwyer, D.A.; Griinari, J.M.; Chouinard, P.Y.; Vanamburgh, M.E.; Bauman, D.E.: Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. J. Nutr. **128**, 881-885 (1998).

Kemp, P.; White, R.W.; Lander, D.J.: The hydrogenation of unsaturated fatty acids by five bacterial isolates from the sheep rumen, including new species. J. General Microbiol. **90**, 100-114 (1975).

Kepler, C.R.; Tove, S.B.: Biohydrogenation of unsaturated fatty acids. J. Biol. Chem. **242**, 5686-5692 (1967).

Koolman, J.; Röhm, K.H.: Taschenatlas der Biochemie. Georg-Thieme-Verlag, 2.Aufl., Stuttgart, Germany (1997).

Krämer, J.: Lebensmittelmikrobiologie. Eugen Ulmer Verlag, 2. Aufl., Stuttgart, Germany (1996).

Kramer, J.K.G.; Fellner, V.; Dugan, M.E.R.; Sauer, F.D.; Mossoba, M.M.; Yurawecz, M.P.: Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. Lipids **32**, 1219-1228 (1997).

Kramer, J.K.G.; Sehat, N.; Dugan, M.E.R.; Mossoba, M.M.; Yurawecz, M.P.; Roach, J.A.G.; Eulitz, K.; Aalhus, J.L.; Schaefer, A.L.; Ku, Y.: Distributions of conjugated linoleic acid (CLA) isomers in tissue lipid classes of pigs fed a commercial CLA mixture determined by gas chromatography and silver ion-high-performance liquid chromatography. Lipids **33**, 549-558 (1998).

Kritchevsky, D.; Tepper, S.A.; Wright, S.; Tso, P.; Czarnecki, S.K.: Influence of conjugated linoleic acid (CLA) on establishment and progression of atherosclerosis in rabbits. J. Am. Coll. Nutr. **19**, 472S–477S (2000).

Labelle, M.; Falgueyret, J.P.; Riendeau, D.; Rokach, J.: Synthesis of two analogues of arachidonic acid and their reactions with 12-lipoxygenase. Tetrahedron **46**, 6301-6310 (1990).

Lavillonnière, F.; Martin, J.C.; Bougnoux, P.; Sébédio, J.L.: Analysis of conjugated linoleic acid isomers and content in French cheeses. J. Am. Oil Chem. Soc. **75**, 343-352 (1998).

Lee, K.N.; Kritchevsky, D.; Pariza, M.W.: Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis **108**, 19-25 (1994).

Lee, K.N.; Pariza, M.W.; Ntambi, J.M.: Conjugated linoleic acid decreases hepatic stearoyl-CoA desaturase mRNA expression. Biochem. Biophys. Res. Commun. **248**, 817-821 (1998).

Lehmann, L.: Identifizierung und stereoselektive Synthesen ungesättigter Signalstoffe. Doktorarbeit, Fachbereich Chemie, Inst. für Organische Chemie, Universität Hamburg, Hamburg (2001).

Li, Y.; Watkins, B.A.: Conjugated linoleic acids alter bone fatty acid composition and reduce *ex vivo* prostaglandin E-2 biosynthesis in rats fed n-6 or n-3 fatty acids. Lipids **33**, 417-425 (1998).

Lie Ken Jie, M.S.F.; Khysar Pasha, M.; Shahin Alam, M.: Synthesis and nuclear magnetic resonance properties of all geometrical isomers of conjugated linoleic acids. Lipids **32**, 1041-1044 (1997).

Lin, H.; Boylston, T.D.; Chang, M.J.; Luedecke, L.O.; Shultz, T.D.: Survey of the conjugated linoleic acid contents of dairy products. J. Dairy Sci. **78**, 2358-2365 (1995).

Lin, H.; Boylston, T.D.; Luedecke, L.O.; Shultz, T.D.: Factors affecting the conjugated linoleic acid content of Cheddar cheese. J. Agric. Food Chem. **46**, 801-807 (1998).

Lin, T.Y.; Lin, C.W.; Lee, C.H.: Conjugated linoleic acid concentration as affected by lactic cultures and added linoleic acid. Food Chem. **67**, 1-5 (1999a).

Lin, H.; Boylston, T.D.; Luedecke, L.O.; Shultz, T.D.: Conjugated linoleic acid content of cheddar-type cheeses as affected by processing. J. Food Sci. **64**, 874–878 (1999b).

Lin, T.Y.: Conjugated linoleic acid concentration as affected by lactic cultures and additives. Food Chem. **69**, 27–31 (2000).

Liu, K.L.; Belury, M.A.: Conjugated linoleic acid reduces arachidonic acid content and PGE(2) synthesis in murine keratinocytes. Cancer Lett. **127**, 15-22 (1998).

Loi, C.: Incorporation et conversion des acides gras polyinsaturés *trans* (n-3). Conséquences sur le métabolisme lipidique des plaquettes et des cellules endothéliales. U.F.R. des Sciences de la Vie et de la Santé, University of Burgundy, Dijon, France (1999).

Loor, J.J.; Herbein, J.H.: Exogenous conjugated linoleic acid isomers reduce bovine milk fat concentration and yield by inhibiting *de novo* fatty acid synthesis. J. Nutr. **128**, 2411–2419 (1998).

Loreau, O.; Maret, A.; Chardigny, J.M.; Sébédio, J.L.; Noel, J.P.: Sequential substitution of 1,2-dichloro-ethene: a convenient stereoselective route to (9Z,11E)-, (10E,12Z)- and (10Z,12Z)-[1-14C] conjugated linoleic acid isomers. Chem. Phys. Lipids **110**, 57-67 (2001).

Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J.: Proteins measurement with the folin reagent. J. Biol. Chem. 265-275 (1951).

Luthria, D.L.; Sprecher, H.W.: 2-Alkenyl-4,4-dimethyloxazolines as derivatives for the structural elucidation of isomeric unsaturated fatty acids. Lipids **28**, 561-564 (1993).

Mahfouz, M.M.; Kummerow, F.: Hydrogenated fat high in *trans* monoenes with an adequate level of linoleic acid has no effect on prostaglandin synthesis in rats. J. Nutr. **129**, 15-24 (1999).

Marcel, Y.L.; Christiansen, K.; Holman, R.T.: The preferred metabolic pathway from linoleic acid to arachidonic acid *in-vitro*. Biochim. Biophys. Acta **164**, 25-34 (1968).

Maryanoff, B.E.; Reitz, A.: Übersichtsartikel : The Wittig olefination Reaction and modifications involving phosphoryl-stabilized carbanions. stereochemistry, mechanism, and selected synthetic aspects. Chem. Rev. **89**, 863-927 (1989).

Medina, E.A.; Horn, W.F.; Keim, N.L.; Havel, P.J.; Benito, P.; Kelley, D.S.; Nelson, G.J.; Erickson, K.L.: Conjugated linoleic acid supplementation in humans: Effects on circulating leptin concentrations and appetite. Lipids **35**, 783-788 (2000).

Miller, C.C.; Park, Y.; M.W., P.; Cook, M.E.: Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection. Biochem. Biophys. Res. Commun. **198**, 1107-1112 (1994).

Mohede, I.; Albers, R.; van der Wielen, R.; Brink, L.; Dorovska-Taran, V.: Immunomodulation: CLA stimulates antigen specific antibody production in humans. 1st International Conference on Conjugated Linoleic Acid (CLA), Ålesund (Norway) (2001).

Mohrhauer, H.; Holman, R.: The effect of dose level of essential fatty acids upon fatty acid composition of the rat liver. J. Lipid Res. **4**, 151-159 (1963).

Moody, C.J.; Sie, E.R.H.B.; Kulagowski, J.J.: The use of diazophosphonates in the synthesis of cyclic ethers. Tetrahedron **48**, 3991-4004 (1992).

Mossoba, M.M.; Kramer, J.K.G.; Yurawecz, M.P.; Sehat, N.; Roach, J.A.G.; Eulitz, K.; Fritsche, J.; Dugan, M.E.R.; Ku, Y.: Impact of novel methodologies on the analysis of conjugated linoleic acid (CLA). Implications of CLA feeding studies. Fett / Lipid **101**, 235–243 (1999).

Moya-Camarena, S.Y.; Belury, M.A.: Species differences in the metabolism and regulation of gene expression by conjugated linoleic acid. Nutr. Rev. **57**, 336-340 (1999).

Moya-Camarena, S.Y.; Vanden Heuvel, J.P.; Belury, M.A.: Conjugated linoleic acid activates peroxisome proliferator-activated receptor α and β subtypes but does not induce hepatic peroxisome proliferation in Sprague-dawley rats. Biochim. Biophys. Acta **1436**, 331-342 (1999a).

Moya-Camarena, S.Y.; Vanden Heuvel, J.P.; Blanchard, S.G.; Leesnitzer, L.A.; Belury, M.A.: Conjugated linoleic acid is a potent naturally occuring ligand and activator of PPARα. J. Lipid Res. **40**, 1426-1433 (1999b).

Munday, J.S.; Thompson, K.G.; James, K.A.C.: Dietary conjugated linoleic acids promote fatty streak formation in the C57BL / 6 mouse atherosclerosis model. Br. J. Nutr. **81**, 251-255 (1999).

Nicolosi, R.J.; Rogers, E.J.; Kritchevsky, D.; Scimeca, J.A.; Huth, P.J.: Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. Artery **22**, 266-277 (1997).

O'Shea, M.; Lawless, F.; Stanton, C.; Devery, R.: Conjugated linoleic acid in bovine milk fat: a food-based approach to cancer chemoprevention. Trends in Food Science and Technology **9**, 192-196 (1998).

O'Shea, M.; Stanton, C.; Devery, R.: Antioxidant enzyme defense responses of human MCF-7 and SW480 cancer cells to conjugated linoleic acid. Anticancer Res. **19**, 1953–1959 (1999).

Ostrowska, E.; Muralitharan, M.; Cross, R.F.; Bauman, D.E.; Dunshea, F.R.: Dietary conjugated linoleic acids increase lean tissue and decrease fat deposition in growing pigs. J. Nutr. **129**, 2037–2042 (1999).

Pariza, M.W.; Hargraves, W.A.: A beef-derived mutagenesis modulator inhibits initiation of mouse epidermal tumors by 7,12-dimethylbenz[a]anthracene. Carcinogenesis **6**, 591-593 (1985).

Pariza, M.W.; Park, Y.; Cook, M.E.: Mechanisms of action of conjugated linoleic acid: evidence and speculation. Proc. Soc. Exp. Biol. Med. **223**, 8-13 (2000).

Park, Y.; Albright, K.J.; Liu, W.; Storkson, J.M.; Cook, M.E.; Pariza, M.W.: Effect of conjugated linoleic acid on body composition in mice. Lipids **32**, 853-858 (1997).

Park, Y.; McGuire, M.K.; Behr, R.; McGuire, M.A.; Evans, M.A.; Shultz, T.D.: High-fat dairy product consumption increases 9c,11t-18:2 (rumenic acid) and total lipid concentrations of human milk. Lipids **34**, 543-549 (1999a).

Park, Y.; Storkson, J.M.; Albright, K.J.; Liu, W.; Pariza, M.W.: Evidence that the *trans*-10,*cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. Lipids **34**, 235-241 (1999b).

Park, Y.; Storkson, J.M.; Ntambi, J.M.; Cook, M.E.; Sih, C.J.; Pariza, M.W.: Inhibition of hepatic stearoyl-CoA desaturase activity by *trans*-10,*cis*-12 conjugated linoleic acid and its derivatives. Biochim. Biophys. Acta **1486**, 285–292 (2000).

Park, Y.; Albright, K.J.; Cai, Z.Y.; Pariza, M.W.: Comparison of methylation procedures for conjugated linoleic acid and artifact formation by commercial (trimethylsilyl)diazomethane. J. Agric. Food Chem. **49**, 1158-1164 (2001).

Precht, D.; Molkentin, J.; Vahlendick, M.: Influence of the heating temperature on the fat composition of milk fat with the emphasis on *cis-/trans*-isomerization. Nahrung / Food **43**, 25-33 (1999).

Reaney, M.J.T.; Liu, Y.D.; Westcott, N.D.: Commercial production of conjugated linoleic acid. In: Yurawecz, M.P.; Mossoba, M.M.; Kramer, J.K.G.; Pariza, M.W.; Nelson, G.J. (Eds.), Advances in conjugated linoleic acid research, Vol. 1. AOCS Press, Champaign (USA). 39-54 (1999).

Rickert, R.; Steinhart, H.; Fritsche, J.; Sehat, N.; Yurawecz, M.P.; Mossoba, M.M.; Roach, J.A.G.; Eulitz, K.; Ku, Y.; Kramer, J.K.G.: Enhanced Resolution of conjugated linoleic acid isomers by tandem-column silver-ion high performance liquid chromatography. J. High Res. Chromatogr. **22**, 144-148 (1999).

Rickert, R.; Steinhart, H.: Bedeutung, Analytik sowie Vorkommen von konjugierten Linolsäureisomeren (CLA) in Lebensmitteln. Ernährungs-Umschau **48**, 4-7 (2001).

Riserus, U.; Vessby, B.: Diverging effects of CLA isomers on insulin resistance and lipid metabolism in obese men with metabolic syndrome. 1st International Conference on Conjugated Linoleic Acid (CLA), Ålesund (Norway) (2001).

Ritzenthaler, K.L.; McGuire, M.K.; Falen, R.; Shultz, T.D.; Dasgupta, N.; McGuire, M.A.: Estimation of conjugated linoleic acid intake by written dietary assessment methodologies underestimates actual intake evaluated by food duplicate methodology. J. Nutr. **131**, 1548-1554 (2001).

Ryder, J.W.; Portocarrero, C.P.; Song, X.M.; Cui, L.; Yu, M.; Combatsiaris, T.; Galuska, D.; Bauman, D.E.; Barbano, D.M.; Charron, M.J.; Zierath, J.R.; Houseknecht, K.L.: Isomer-specific antidiabetic properties of conjugated linoleic acid - Improved glucose tolerance, skeletal muscle insulin action, and UCP-2 gene expression. Diabetes **50**, 1149-1157 (2001).

Sébédio, J.L.; Juaneda, P.; Dobson, G.; Ramilison, I.; Martin, J.C.; Chardigny, J.M.; Christie, W.W.: Metabolites of conjugated isomers of linoleic acid (CLA) in the rat. Biochim. Biophys. Acta **1345**, 5-10 (1997).

Sébédio, J.L.; Gnädig, S.; Chardigny, J.M.: Recent advances in conjugated linoleic acid. Curr. Opin. Clin. Nutr. Metab. Care **2**, 499-506 (1999).

Sébédio, J.L.; Angioni, E.; Chardigny, J.M.; Gregoire, S.; Juaneda, P.; Berdeaux, O.: The effect of conjugated linoleic acid isomers on fatty acid profiles of liver and adipose tissues and their conversion to isomers of 16:2 and 18:3 conjugated fatty acids in rats. Lipids **36**, 575-582 (2001).

Sehat, N.; Kramer, J.K.G.; Mossoba, M.M.; Yurawecz, M.P.; Roach, J.A.G.; Eulitz, K.; Morehouse, K.M.; Ku, Y.: Identification of conjugated linoleic acid isomers in cheese by gas chromatography, silver ion high performance liquid chromatography and mass spectral reconstructed ion profiles. Comparison of chromatographic elution sequences. Lipids **33**, 963-971 (1998).

Sehat, N.; Rickert, R.; Mossoba, M.M.; Kramer, J.K.G.; Yurawecz, M.P.; Roach, J.A.G.; Adlof, R.O.; Morehouse, K.M.; Fritsche, J.; Eulitz, K.D.; Steinhart, H.; Ku, Y.: Improved separation of conjugated fatty acid methyl esters by silver ion-high-performance liquid chromatography. Lipids **34**, 407-413 (1999).

Shantha, N.C.; Decker, E.A.; Ustunol, Z.: Conjugated linoleic acid concentration in processed cheese. J. Am. Oil Chem. Soc. **69**, 425-428 (1992).

Shantha, N.C.; Decker, E.A.; Hennig, B.: Comparison of methylation methods for the quantitation of conjugated linoleic acid isomers. J. AOAC Int. **76**, 644-649 (1993).

Shantha, N.C.; Crum, A.D.; Decker, E.A.: Evaluation of conjugated linoleic acid concentrations in cooked beef. J. Agric. Food Chem. **42**, 1757-1760 (1994).

Shantha, D.C.; Decker, E.A.: Conjugated linoleic acid concentrations in cooked beef containing antioxidants and hydrogen donors. J. Food Lipids **2**, 57-64 (1995).

Shantha, N.C.; Ram, L.N.; O'Leary, J.; Hicks, C.L.; Decker, E.A.: Conjugated linoleic acid concentrations in dairy products as affected by processing and storage. J. Food Res. **60**, 695-720 (1995).

Sisk, M.B.; Hausman, D.B.; Martin, R.J.; Azain, M.J.: Dietary conjugated linoleic acid reduces adiposity in lean but not obese Zucker rats. J. Nutr. **131**, 1668-1674 (2001).

Sonnet, P.E.: Direct conversion of an alcohol tetrahydropyranyl ether to a bromide, chloride, methyl ether, nitrile or trifluoroacetate. Synth. Comm. **6**, 21 (1976).

Sprecher, H.: Biosynthetic pathways of polyunsaturated fatty acids. Adv. Exp. Med. Biol. **83**, 35-50 (1977).

Sprecher, H.; Lutheria, D.L.; Mohammed, B.S.; Baykousheva, S.P.: Reevaluation of the pathways for the biosynthesis of polyunsaturated fatty acids. J. Lipid Res. **36**, 2471-2477 (1995).

Stangl, G.I.; Müller, H.; Kirchgessner, M.: Conjugated linoleic acid effects on circulating hormones, metabolites and lipoproteins, and its proportion in fasting serum and erythrocyte membranes of swine. Eur. J. Nutr. **38**, 271-277 (1999).

Stangl, G.I.: High dietary levels of conjugated linoleic acid mixture alter hepatic glycerophospholipid class profile and cholesterol-carrying serum lipoprotrins of rats. J. Nutr. Biochem. **11**, 184-191 (2000).

Stanton, C.; Lawless, F.; Kjellmer, G.; Harrington, D.; Devery, R.; Connolly, J.F.; Murphy, J.: Dietary influences on bovine milk cis-9,trans-11-conjugated linoleic acid content. J. Food Sci. **62**, 1083-1086 (1997).

Sugano, M.; Tsujita, A.; Yamasaki, K.; Ikeda, I.; Kritchevsky, D.: Lymphatic recovery, tissue distribution, and metabolic effects of conjugated linoleic acid in rats. J. Nutr. Biochem. **8**, 38-43 (1997).

Sugano, M.; Tsujita, A.; Yamasaki, M.; Noguchi, M.; Yamada, K.: Conjugated linoleic acid modulates tissue levels of chemical mediators and immunoglobulins in rats. Lipids **33**, 521-527 (1998).

Tanaka, T.; Hattori, T.; Kouchi, M.; Hirano, K.; Satouchi, K.: Methylene-interrupted double bond in polyunsaturated fatty acid is an essential structure for metabolism by the fatty acid chain elongation system of rat liver. Biochim. Biophys. Acta **1393**, 299-306 (1998).

Truitt, A.; McNeill, G.; Vanderhoek, J.Y.: Antiplatelet effects of conjugated linoleic acid isomers. Biochim. Biophys. Acta **1438**, 239-246 (1999).

Tsuboyama-Kasaoka, N.; Takahashi, M.; Tanemura, K.; Kim, H.J.; Tsuyoshi, T.; Okuyama, H.; Kasai, M.; Ikemoto, S.; Ezaki, O.: Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. Diabetes **49**, 1534-1542 (2000).

Turek, J.J.; Li, Y.; Schoenlein, I.A.; Allen, K.G.D.; Watkins, B.A.: Modulation of macrophage cytokine production by conjugated linoleic acids is influenced by the dietary n-6:n-3 fatty acid ratio. J. Nutr. Biochem. **9**, 258-266 (1998).

Ulberth, F.; Gabering, R.G.; Schrammel, F.: Flame-Ionization detector response to methyl, ethyl, propyl, and butyl esters of fatty esters. J. Am. Oil Chem. Soc. **76**, 263-266 (1999).

Vatèle, J.M.; Dong, H.D.; Chardigny, J.M.; Sébédio, J.L.; Grandgirard, A.: Synthesis of methyl (5Z,8Z,11Z,14Z,17E)-eicosapentaenoate and methyl 4Z,7Z,10Z,13Z,16Z,19E)-docosahexaenoate. Chem. Phys. Lipids **74**, 185-193 (1994).

Vessby, B.; Riserus, U.; Smedman, A.; Basu, S.: Metabolic effects of conjugated linoleic acid (CLA) in humans. 1st International Conference on Conjugated Linoleic Acid (CLA), Ålesund (Norway) (2001).

Welsch, C.W.: Enhancement of mammary tumorigenesisi by dietary fat: review of potential mechanisms. Am. J. Clin. Nutr. **45**, 192-202 (1987).

Werner, S.A.; Luedecke, L.O.; Shultz, T.D.: Determination of conjugated linoleic acid content and isomer distribution in three cheddar-type cheeses: effects of cheese culture, processing and aging. J. Agric. Food Chem. **40**, 1817-1821 (1992).

West, D.B.; Delany, J.P.; Camet, P.M.; Blohm, F.; Truett, A.A.; Scimeca, J.: Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse. Am. J. Physiol. **275**, R667-R672 (1998).

Whigham, L.D.; Cook, M.E.; Atkinson, R.L.: Conjugated linoleic acid: Implications for human health. Pharmacol. Res. **42**, 503-510 (2000).

Whigham, L.D.; Cook, E.B.; Stahl, J.L.; Saban, R.; Bjorling, D.E.; Pariza, M.W.; Cook, M.E.: CLA reduces antigen-induced histamine and PGE(2) release from sensitized guinea pig tracheae. Am J. Physiol. Reg. Integr. Comp. Physiol. **280**, R908-R912 (2001).

Yang, L.; Leung, L.K.; Huang, Y.; Chen, Z.Y.: Oxidative stability of conjugated linoleic acid isomers. J. Agric. Food Chem. **48**, 3072-3076 (2000).

Yurawecz, M.P.; Kramer, J.K.G.; Dugan, M.E.R.; Sehat, N.; Mossoba, M.M.; Yin, J.J.; Ku, Y.: Incorporation of conjugated fatty acid into biological matrices. In: Yurawecz, M.P.; Mossoba, M.M.; Kramer, J.K.G.; Pariza, M.W.; Nelson, G.J. (Eds.), Advances in Conjugated Linoleic Acid Research, Vol. 1. AOCS Press, Champaign, IL. 238-252 (1999).

Zambell, K.L.; Keim, N.L.; Van Loan, M.D.; Gale, B.; Benito, P.; Kelley, D.S.; Nelson, G.J.: Conjugated linoleic acid supplementation in humans: Effects on body composition and energy expenditure. Lipids **35**, 777–782 (2000).

Zhang, J.Y.; Yu, Q.T.; Liu, B.N.; Huang, Z.H.: Chemical modification in mass spetrometry IV - 2-alkenyl-4,4-dimethyloxazolines as derivatives for the double bond location of long-chain olefinic acids. Biomed. Environ. Mass Spectrom. **15**, 33-44 (1988).

Zhang, A.; Chen, Z.Y.: Oxidative stability of conjugated linoleic acids relative to other polyunsaturated fatty acids. J. Am. Oil Chem. Soc. **74**, 1611-1613 (1997).

14 CURRICULUM VITAE

Silke GNÄDIG

Morgensternstr. 11a, 12207 Berlin

born, May 1st, 1972 in Berlin, single

School education

09 / 79-07 / 85	Elementary school in Berlin
09 / 85-06 / 91	High school, Goethe-Oberschule, Berlin-Lichterfelde
06 / 91	Abitur (High School Diploma),

University education

10 / 91-09 / 93	Foundation studies of food chemistry, Technical University, Berlin
12 / 92	Klaus-Koch-Fellowship for excellent studies
09 / 93	Pre-exam of food chemistry
10 / 93-09 / 95	Main studies of food chemistry, Technical University, Berlin
01 / 96	First exam of food chemistry
04 / 96-12 / 96	Diplomarbeit (Master of Science), Institute of Food Chemistry, University of Hamburg, Title: «Conjugated linoleic acid in food, human blood plasma and human adipose tissue»
11 / 96-04 / 97	Trainee at the Institute of Food Chemistry, University of Hamburg, in fulfillment of the practical year as food chemist
11 / 97-04 / 98	Trainee at the Hygiene-Institute of Hamburg, in fulfillment of the practical year as food chemist
07 / 98	Second exam of food chemistry
since 02 / 99	External Ph.D. student of the faculty of chemistry, University of Hamburg, Marie-Curie-Fellowship of the European Union.

Professional experience

03/92	Gesellschaft für Lebensmittelforschung, Berlin (Food analysis and research)
09/94	Federal institute of health consumer protection and veterinary medicine (BgVV), Berlin
05/97	Institute of Food Chemistry, University of Hamburg (Optimization and Validation of methods for the analysis of cadmium and PCB)
06/97-07/97	National Institute of Agricultural Research, aroma research unit, Dijon, FRANCE
09/98-01/99	Research Scientist, Institute of Food Chemistry, University of Hamburg, (Coordination of scientific research proposal on conjugated linoleic acid)
02/99-07/01	Research Scientist, National Institute of Agricultural Research, lipid research unit, Dijon, FRANCE

List of Publications

Original Papers and Reviews

Sébédio, J.L.; Gnädig, S.; Chardigny, J.M.: Recent advances in conjugated linoleic acid. Curr. Opin. Clin. Nutr. Metab. Care **2**, p. 499-506 (1999)

Gnädig, S.; Rickert, R.; Sébédio, J.L.; Steinhart, H.: Conjugated linoleic acid (CLA): Physiological effects and production. Eur. J. Lipid Sci. Technol. **103**, p. 56-61 (2001)

Gnädig, S.; Berdeaux, O.; Loreau, O.; Noël, J.P.; Sébédio, J.L.: Synthesis of (6Z,9Z,11E)-octadecatrienoic and (8Z,11Z,13E)-eicosatrienoic acids and their [1-^{14}C]-radiolabeled analogs. Chem. Phys. Lipids **112**, p. 121-135 (2001)

Berdeaux, O.; Gnädig, S.;.Chardigny, J.M.; Loreau, O.; Noël, J.P.; Sébédio, J.L.: In-vitro desaturation and elongation of rumenic acid by rat liver microsomes. Submitted to Lipids

Gnädig, S.; Chamba, J.F.; Perreard, E.; Chappaz. S.; Rickert, R.; Steinhart, H.; Sébédio, J.L.: Influence of Processing on Conjugated Linoleic Acid (CLA) Content and CLA-Isomer Composition in French Emmental Cheese. In preparation

Oral communications

Gnädig, S.; Chardigny, J.M.; Berdeaux, O.; Grégoire, S.; Sébédio, J.L.: *In-vitro* and *in-vivo* metabolism of single CLA-isomers into long chain polyunsaturated fatty acids. 1st International Conference on Conjugated Linoleic Acid (CLA), Ålesund (Norway), June 10-13, 2001

Berdeaux, O.; Gnädig, S.; Loreau, O.; Sébédio, J.L: *In-vitro* desaturation and elongation of rumenic acid by rat liver microsomes. 24th World Congress and Exhibition of the International Society of Fat Research (ISF), Berlin (Germany), September 16-20, 2001

Gnädig, S.; Sébédio, J.L.; Chardigny, J.M.; Grégoire, S.; Genty, M.; Almanza, S.*: In-vivo* effects of CLA on eicosanoid production. 93rd AOCS Annual Meeting & Expo, Montréal (Canada), May 5-8, 2002

Poster presentations

Berdeaux, O.; Gnädig, S.; Loreau, O.; Noël, J.P.; Sébédio, J.L.: Syntheses of C18:3 and C20:3 desaturation and elongation metabolites of 9c,11t-18:2. 3rd Meeting of the European Section of AOCS, Helsinki (Finland), June, 2000

Gnädig, S.; Chamba, J.F.; Perreard, E.; Chappaz. S.; Rickert, R.; Steinhart, H.; Berdeaux, O.: Influence of Cheese Processing on Conjugated Linoleic Acid (CLA) Content and CLA-Isomer Composition. 17th International Congress of Nutrition, Vienna (Austria), August 27-31, 2001