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Health benefits and safety evaluation of certain food components

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IDF news

Schedule of future IDF events*

2002		
23-28 Sept.	IDF World Dairy Summit and Congress	Paris (FR)
2003		
14-16 May	Ice Cream Symposium	Thessaloniki (GR)
24-27 Aug.	2nd World Symposium on Dairy Foods in Human Nutrition and Health	Melbourne (AU)
8-12 Sept.	IDF World Dairy Summit and Centenary	Bruges (BE)
2004		
Spring	IDF Symposium on Dairy Hygiene and Safety	Durban (ZA)
21-25 March	IDF Symposium on Cheese: Ripening, Characterization & Technology	Prague (CZ)
9-12 May	4th International Symposium on Recombined Milk & Milk Products	Cancun (ME)
Sept./Oct.	IDF World Dairy Summit	To be determined
2005		
To be determined	IDF World Dairy Summit	Vancouver (CA)
To be determined	IDF Mastitis Seminar	The Netherlands

* Further details of these events can be obtained from the IDF General Secretariat.

Health benefits and safety evaluation of certain food components

Foreword

The four articles in this issue of the Bulletin of IDF have been developed by experts involved in the work of the IDF Standing Committee on Nutrition and health. They deal with topical issues of current interest to the dairysector .

IDF is very grateful to the authors of the four papers for their efforts:

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1 Food Microorganisms - Health Benefits, Safety Evaluation and Strains with Documented History of Use in Foods

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ABSTRACT

This scientific summary reviews the latest research related to health benefits and safety evaluation of lactic acid bacteria (LAB) and introduces an inventory of food microorganism species with a history of safe use in foods. The inventory has been produced in collaboration between the International Dairy Federation (IDF) and European Food and Feed Cultures Association (EFFCA). It is not to be regarded as being complete since many locally used dairy or food starter strains with a long history of safe use have not yet been included.

The literature on recent research on the use of various LAB in clinical and nutritional treatment of severely compromised individuals has mainly disclosed a positive influence on the treatment and prevention of various diseases. Reports on adverse effects and even infections caused by LAB have appeared occasionally, but mostly in cases where the LAB appear accidentally and rarely in cases where LAB has been administered intentionally.

Increasing evidence is accumulating that certain LAB are able to interact with the gastrointestinal tract and its mucosal immune system.

No adverse effects of using LAB have been reported in the great number of controlled human trials, which is a strong indication that laboratory (dairy) strains of probiotic LAB are safe – even when administered to severely immune compromised individuals.

1 INTRODUCTION

The technology of producing fermented foods has been known since ancient times in most parts of the world.

Originally fermented foods were "spontaneously" fermented by autochthonous strains, but the use of inoculation material containing the fermenting microorganisms has been known for the past 100 years.

Systematic use of starter cultures has only been exploited since the middle of the 20th century. At the turn of the century the science of microbiology advanced rapidly. The role of microorganisms in disease and food production became realised. Microbial hygiene was understood and pasteurization of perishable foods was introduced to combat infectious diseases.

Along with the introduction of pasteurization, it became a necessity to inoculate products to initiate fer-

mentation, and the use of starter cultures became common practice.

In the beginning, starter cultures were isolates from earlier fermentations that were maintained and propagated at the site of production.

Owing to problems in maintaining the quality of such undefined, multistrain cultures, companies started to specialize, produce and maintain such cultures.

However, bacteriophage attack, mutations and seasonal variations in composition of inoculation material (milk, vegetables, grapes, etc.) often made it difficult to secure stability of such multistrain, wild type cultures. As a result development of starters based on single strains or defined multistrain starter cultures were initiated.

Today, more and more starter cultures are composed of single defined strains as compared to the undefined multistrain cultures. Such starter cultures, however, do not possess the same multiplicity of strains and many producers within the food industry still prefer the old, undefined multistrain starter cultures to secure a more cultured "buttery" flavour of their product.

It will be possible to compose starter cultures that contribute flavour notes that have not been known hitherto. There are principally two ways in which this can be achieved. Either by composing starter cultures from strains that are out of natural balance and which possibly may even contain strains that cannot be naturally propagated together, or by using gene modified strains.

In order to exploit the potential of developing cultures that consist of single strains, it is important that sufficient numbers of acceptable strains are available. These are strains that raise no question as to their usability in a food context, meaning that they should be safe.

A good criterion for general acceptability of a bacteria strain or culture as safe for use in foods is that the strain or culture has a history of safe use in foods.

In the USA, Title 21 of the Code of Federal Regulations (21 CFR) and the US FDA Office of Premarket Approval, lists microorganisms which are approved food additives or which enjoy Generally Recognized as Safe (GRAS) status. "GRAS" status is always considered only for a specified use. Thus, for instance, microbes themselves are not considered GRAS, but their traditional use in dairy foods is. Currently harmless lactic acid producing bacteria, including *Lactobacillus acidophilus* and other lactic acid bacteria (specifically *Streptococcus thermophilus, Str cremoris, Str lactis* and *Str lactis ssp diacetylactis, Lactobacillus bulgaricus, L. fermentum, L. lactis* and three Leuconostoc species (*Leuconostoc citovorum, Leuconostoc dextranicum* and *Leuconostoc mesenteroides*) can be used as additional ingredients in specified standardized foods such as cultured milk including e.g. buttermilk, sour cream, cottage cheese and yoghurt, provided that the mandatory cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are also used in yoghurt.

The FDA continuously assesses petitions for GRAS status for strains or cultures for specific food application. As an example FDA has approved GRAS petitions by Nestle US for *Bifidobacterium lactis* Bb12 and *Streptococcus thermophilus* TH4 for use in formulas and foods for infants and children.

2 CLINICAL STUDIES ON HEALTH BENEFITS INVOLVING LAB

Evaluating health benefits and possible hazards related to the use of LAB in foods is complicated.

Several research papers points to the importance of ingested dose of microorganisms in relation to health benefit as well as possible hazard [4,21]. Reliable biomarkers and methodologies are still to be developed [28].

In contrast to the few reported cases in which dairy LAB used clinically have been involved in infections, more and more scientific papers document a health improving effect of LAB even in severely weakened or immune compromised individuals.

Recently Naidu et al., 1999 [19] reviewed probiotics and recorded 143 human clinical trials with probiotics carried out between 1961 and 1998, comprising more than 7500 individuals. No illness or discomfort was reported.

Wolf et al., 1998 [29] administered *Lactobacillus reuteri* to HIV patients and no adverse effect was noted.

Aso et al. 1995 [3] demonstrated a significant positive effect of administering *Lactobacillus casei* in preventing recurrence of superficial bladder cancer in a double-blind placebo controlled trial comprising 138 patients. Diarrhoea and constipation occurred equally in patients receiving LAB and patients receiving placebo (4.6 and 4.4 % respectively) and resolved without treatment.

Pedone et al., 1999 [20] conducted a study on the effect of *Lactobacillus casei* preparation on the incidence of diarrhoea. The study comprised 127 children from day care centres given yoghurt, yoghurt with added *L. casei* and placebo, respectively. No significant effect was shown between treatments in incidences of diarrhoea, but the severity of the diarrhoea was significantly reduced in the *L. casei* treated group.

Guandalini et al., 2000 [9] carried out a European multicentre, double blind and placebo controlled study which comprised administration of *Lactobacillus rhamnosus GG* in a rehydration solution to children with acute diarrhoea. The investigation involved 127 children aged 1 month to 3 years from 11 day-care centres and represented 10 different European countries. The results showed that administration of the LAB was safe and resulted in a significant shorter duration of diarrhoea, less chance of protracted course of the illness and faster discharge from hospital.

Gionchetti et al., 2000 [7] carried out a double blind, placebo controlled trial on the effect of a LAB preparation on chronic pouchitis. Forty patients with chronic pouchitis were grouped equally. One group received a LAB preparation containing 300 billion live microbes comprising *L. casei, L. plantarum, L. acidophilus, L. bulgaricus, B. longum, B. breve, B. infantis* and *S. thermophilus*. The results of the trial showed that 17 of the 20 patients receiving the LAB preparation had no relapse during the 9-month follow up period as compared to 20 out of 20 elapsed in the placebo group. No side effects and no significant changes in the laboratory parameters examined were registered in either group of patients, demonstrating the safety in using a multispecies-multistrain LAB preparation for treating patients with severe intestinal conditions.

Recently Candy et al., 2001 [5] reported a case of a newborn male child hospitalized with severe bile stained vomiting and gross abdominal distension caused by anal

atresia. The child was subjected to perineal anoplasty and resection of the ileum and colon. Jejunostomy was performed and the child was left with 60 cm jejunum. The postoperative course was complicated by sepsis. At 7 months of age, jejunal-rectal anastomy was performed. At 11 months of age the child was transferred to oral feeding.

The jejunal effluent yielded a rich microbial flora that was evaluated as being a danger to the function of his *residual jejunum*. It was therefore decided to try probiotic therapy.

At the age of 12 months the child was subject to probiotic therapy by administering 1.5 x 10^9 viable *Lactobacillus casei* Shirota 3 times a day.

After 3 days of treatment the stool contained an abundant amount of lactobacilli, urine function normalized and the stool frequency decreased form 12 to 4 times per day.

Two years later the child was still under probiotic therapy. He consumes a variety of normal foods. He still passes 4 stools per day but is able to pass the night without defecation.

All the above examples strongly point to the safety of LAB, even when ingested by severely weakened persons suffering gastro intestinal disorders and sepsis.

In spite of the comprehensive documentation of positive health effects of LAB, the reported cases of adverse effects and sepsis remain an issue of concern to the scientific community as to the general safety of LAB.

Accordingly, there have been many in vitro studies dealing with the safety aspect of lactic acid bacteria in an indirect manner. In these investigations, biomarkers of safety have been investigated such as: ability to adhere to human intestinal epithelial cells, ability to translocate human cells in cell culture experiments and properties like lack of antibiotic resistance and lack of ability to transfer antibiotic resistance genes to other intestinal microbes or pathogens. Translocation is a double-edged sword as lack of LAB may facilitate translocation of harmful bacteria.

Most such studies point to lactic acid bacteria as being safe, although the possibility for some strains to be potentially pathogenic in persons with severe conditions such as underlying disease, surgery or extreme immunocompromised is usually not excluded.

In individuals weakened in one way or another and from whom LAB were isolated in connection with sepsis, it is very difficult to evaluate the actual hazard of these LAB's as they may only be growing opportunistically in tissue damaged by other causes. In most cases of recorded LAB infection, the infection undoubtedly originates from the individuals own intestinal flora, although other sources such as ingested LAB cannot be excluded.

However, there has been no clinical tradition for isolation and identification of LAB so there may be an underestimation of clinical cases of infections due to LAB.

Limits defining safety are difficult to determine. Markers like adhesion or colonization are usually regarded as a positive functional property, but may also be one of the mechanisms of systemic entrance of the organisms. Systemic translocation of LAB in sterile rodents has been demonstrated, but the animals did not suffer from the translocated LAB and cleared themselves during 4 weeks [11,32]. Furthermore it was demonstrated that rodents mono-associated with *Bifidobacterium longum* had a significantly improved resistance towards translocation by pathogens [31]. The mechanism is not known but may involve activation of the immune system, as a significant increase in secreted immunoglobulin A (SIgA) was demonstrated [27].

There is increasing evidence that a major part of the positive (probiotic) effects of lactic acid bacteria are to be attributed to an interaction with the bowel as an immune organ.

- It has been demonstrated that some LAB are able to modulate the immune system [15,18].
- Probiotic LAB are more effective towards virus infections than against microbial (bacteria) infections [9].
- Probiotic LAB are able to reduce atopic hypersensitivity [12,23].
- Most lymph nodes (Peyer's patches) are located in the small intestine, where ingested LAB greatly outnumbers the autochthonous flora [16,17,18].
- In the colon the autochthonous flora greatly outnumbers ingested LAB [17].

3 SAFETY EVALUATION OF LAB

This review focus on lactic acid bacteria generally ascribed a probiotic effect. This safety evaluation does not include enterococci but do include bifidobacteria.

Lactic acid bacteria (LAB) are consumed in enormous quantities, primarily through consumption of fermented foods. According to the latest statistics as published in bulletin No. 355 [30] from the International Dairy Federation (IDF), the average annual consumption of fermented milk products is 22 kg per capita in Europe. In total, this amounts to about 8.5 billion kg fermented milk per year.

With an average microbial content in these fermented products of 10^8 bacteria per gram (or ml), this amounts to a total of 8.5 x 10^{20} lactic acid bacteria.

Assuming one bacterial cell weighs 4 x 10^{-12} gram, this means that 3400 tonnes of pure lactic acid bacterial cells are consumed every year in Europe.

To the best of the authors' knowledge, there have been only a few reported cases of infection per se due to consumption of probiotic bacteria or fermented milk containing probiotic bacteria, unless in cases where other microorganisms have contaminated the product [13,21].

One paper provided strong evidence that *L. rhamnosus* has been involved in liver abscesses [22]. In this case the person concerned was elderly, weakened and had chewed capsules containing the probiotic shortly after dental treatment. As such the concentration of the probiotic was greatly above what is normally consumed.

Other cases in which LAB have been involved, include human bacteraemia, and infections have been reported on numerous occasions in the literature, although rarely without the existence of an underlying factor [1,2,6,8,14,22].

The overwhelming part of the literature, however, describes a positive or inert health effect of ingesting LAB (probiotics). The present states of the art of the effect of probiotics have recently been thoroughly reviewed [3,14,22,23,24,25,26]. The literature is in no way unambiguous in relation to the safety evaluation of LAB. Most scientific papers document a beneficial health effect of LAB even in severely weakened or immune compromised individuals.

4 CLINICAL CASES INVOLVING LAB

Clinical cases up to 1994 that involve lactic acid bacteria and bifidobacteria have been thoroughly reviewed by Aguirre and Collins, 1993 [1] and Gasser, 1994 [6].

The review by Aguirre and Collins comprises 68 clinical cases where *Lactobacillus* spp. have been isolated, 27 cases where *Leuconostoc* species have been isolated and 18 cases where *Pediococcus* spp. have been isolated from persons with clinical infections.

Out of the 68 clinical cases of infections in which *Lactobacillus* species have been involved, 35 cases were identified as infections in the heart region (endocarditis) and 10 cases were blood infections (bacteraemia). The remaining 23 cases comprised erysipeloid, throat infection, endometritis, sepsis after peritonitis, congenital pneumonia in newborns, chest infection, septic urinary infections, liver abscess, septic wounds and lung abscess.

In 7 cases no underlying diseases were diagnosed and in two cases it was not specified whether or not an underlying disease had been diagnosed.

Out of the 27 cases involving *Leuconostoc* species, 10 cases were identified as bacteraemia and the remaining 8 cases comprised meningitis (2), septic wounds (3), peritoneal infection, dental infection and one not specified.

Underlying diseases were diagnosed in all but two cases. In one case it was not specified whether or not an underlying disease was diagnosed.

In 18 cases where *Pediococcus* spp. was involved in clinical infections, 11 were identified as blood infections (bacteraemia). The disease for the remaining 7 cases was not specified. In all but one case, underlying disease was diagnosed. In one case, it was not specified whether or not an underlying disease was diagnosed.

The review from Gasser covers more or less the same scientific reports as reviewed by Aguirre and Collins and the conclusion is very much the same. However, Gasser includes bifidobacteria and reviewed 9 cases where *Bifidobacterium* species have been identified in relation to human infections. In all 9 cases bifidobacteria were isolated from the patients' blood, however, underlying diseases were diagnosed in all cases. In 5 of the cases, *B. eriksonii* was isolated. In one case, *B. adolescentis* was isolated and in 3 cases the identification was not carried out to species level.

Table 1 summarizes the results described in the reviews by Aguirre & Collins and Gasser.

The figures in the table represent the reported cases of human infections from the first reported case of endocarditis in 1938 until 1994.

In total, the table comprises 155 cases where LAB or bifidobacteria have been involved in human infections. 95 of the cases involved *Lactobacillus* species, 33 cases involved *Leuconostoc* species, 18 cases involved *Pediococcus* species and 9 cases involved *Bifidobacterium* species.

Endocarditis is clearly the most frequent infection in which *Lactobacillus* species have been involved with strains of species of *Lactobacillus rhamnosus/casei* most often being isolated.

Bifidobacterium species and *Pediococcus* species have not been isolated from cases of endocarditis and only 2 cases are reported where *Leuconocstoc* species were isolated.

In more non-specific blood infections (bacteraemia), however, *Leuconostoc, Pediococcus* and *Bifidobacterium* species have been isolated more frequently than *Lactobacillus* species.

Regarding other infections not identified as either endocarditis or bacteraemia, there seems to be no difference in the frequency in which LAB have been isolated.

Recently Golan et al. 2001 [8] reported two cases of bacteraemia caused by *Leuconostoc mesenteroides* in bone marrow transplanted individuals. The cases were serious and probably partly caused by the fact that the patients were initially treated unsuccessfully with vancomycin. This, however, does not disregard the fact that *Leuconostoc mesenteroides* was isolated from the blood of the patients, who responded positively upon treatment with dapomycin.

5 IDF/EFFCA INVENTORY OF MICROORGANISMS WITH A HISTORY OF USE IN FOODS

Safety of food is a global concern. Safety documentation of microorganisms used in food manufactured either through clinical tests or through a documented history of safe use in foods will be required by most authorities in the future. This trend has strengthened in recent years.

Recently the European community introduced "The Novel Food Directive" (Regulation (EC) No 258/97), which states that: "Foods and food ingredients which have not hitherto been used for human consumption to a significant degree within the community shall acquire permission from the member state concerned, in accordance with the requirements laid down in the Novel Food Directive".

Clinical cases in which lactic acid bacteria or bifidobacteria have been isolated

Clinical outcome	*Lacto-bacillus* spp.	*L. acido-philus*	*L. rham-nosus*	*L. casei*	*L. plan-tarum*	*Leuco-nostoc*	*Pedio-coccus* spp.	*Bifido-bacterium* spp.	Total
Endocarditis	7	3	19	12	11	2	-		54
Bactereamia	8	3	5	-	2	23	11	9	61
Other infections	19	2	3	-	1	8	7		40
Total	34	8	27	12	14	33	18	9	155

Of the infected blood samples tested (more than 100 000 samples) app. 0.1 % contained *lactobacillus* and 0.01 % contained *Leuconostoc.*

In 142 cases out of the 155 (92%) the patients had a severe underlying disease identified.

Adapted after: Aguirre and Collins, 1993 and Gasser 1994

As there may be cases where it is not obvious whether or not a LAB species, or strains of this species alone or contained in a starter culture, have been used for human consumption to a significant degree IDF and EFFCA has taken initiative to produce the attached inventory of starter species with a documented history of use in food manufacture and accordingly for consumption by humans [10].

The inventory has also been acknowledged by the European industrial platform for lactic acid bacteria (LABIP).

The inventory contains species that have a record for use in food production either as single strain cultures, single species cultures, or multiple species cultures as defined by IDF (IDF standard 149A: 1997). The species/strain may also be a well-defined specie/strain contained in a commercial starter culture composed of otherwise unknown species of LAB.

The history of use in foods of the species in the inventory is documented by either scientific literature references or by statements in good faith from the companies producing and/or selling strains belonging to the species or cultures that are composed of or contain strains belonging to the species.

The inventory is not to be regarded as comprehensive, because strains may exist that have been used for food manufacture without being recognised. Also species/strains produced by the food industry for "in company" use in products is not included.

It also has to be considered that the taxonomy of lactic acid bacteria and other food microorganisms have been subject to rather drastic changes due to the development of genetic methodologies in the (their) classification. Bergeys Manual of Systematic Bacteriology (9th edition, 1986) is thus in many respects outdated, as many of the microorganisms recorded have been renamed. Species have been merged and new species have been added.

The strains in the IDF/EFFCA inventory have been sold by one or more of the EFFCA member companies for food purposes since the year "sold since" indicated in the inventory.

Documentation for sold quantities is available but regarded confidential by the companies.

Beyond sold quantities the inventory mentions major applications for the species concerned and provide 121 representative literature references documenting the mentioned applications.

6 CONCLUSION

There are abundant and rapidly accumulating scientific reports on the beneficial health effects of administering LAB and/or *Bifidobacterium* within dairy foods.

The literature on lactic acid bacteria (LAB) reports some cases in which such organisms have been involved in clinical cases of infections in human subjects who mostly had serious or even fatal underlying diseases.

There is, however, only one obvious but occasional report that describes a case in which administered food strains of LAB have caused adverse effects – even when seriously immune compromised individuals are included.

There is accumulating evidence that LAB interact with the gastrointestinal tract as the major immune compartment by enhancing the gut barrier and modulating the immune system. This is in accordance with the fact that probiotic LAB and Bifidobacterium seems to yield better protection against viral infections than against bacterial infections. However, more mechanistic work on competitive exclusion of pathogens needs to be conducted.

Food strains of LAB and other food microorganisms appear to be safe in food use, while strains of unknown origin, isolated from patients suffering from bacteraemia, may represent a risk.

Even though the risk of using LAB's and *Bifidobacterium* in foods may not be zero, the benefits are abundant.

The species of food microorganisms contained in the IDF/EFFCA inventory are all strains with a history of safe use in foods and such they are to be regarded as safe. As the inventory is not complete, other strains of food microorganisms that are not on this list may also be regarded as safe. Considering the large amounts of LAB consumed by the general public, the many reports on positive health effects of selected strains and the very few reports on adverse effects of LAB, the benefits of LAB appear to far outweigh the potential and minute risk involved in consuming LAB.

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2 Inventory of Microorganisms with a Documented History of Use in Food

Same authors as Chapter 1
See page 4

ABSTRACT

In order to classify traditionally used microorganisms (starter cultures) as safe food ingredients, The IDF in collaboration with EFFCA (European Food and Fed Cultures Association) has compiled an inventory of microorganisms with a documented history of use in food. The inventory is supplied with explanatory text dealing with taxonomic changes that have taken place over time. The use of the microorganisms is documented by sales information and through literature references.

The inventory is by no means exhaustive, and is based on the knowledge of the participating experts, not that of the whole food or dairy industry. This inventory is intended for information only, not as a complete inventory of microorganisms for use in food, or to exclude any other existing products unknown to the participating experts.

The list mentions "major applications" for the included culture or strain. This information is taken from the literature and it does not imply that the strain or culture could not be applied in the production of other foodstuffs.

1 INTRODUCTION

During historic time microorganisms have constituted an integral part of fermented foods like: wine, beer, dairy products, bread and fermented vegetables. At the turn of this century the science of microbiology advanced very fast and the role of microbes in the fermentation of foods became understood. Commercial starter cultures for use in the fermented food industry were developed.

IDF and EFFCA have jointly produced this inventory of starter species for which a documented history for use in food manufacture to a significant degree*) exists, as there may be cases where it is not obvious whether or not a lactic acid bacteria species or strains of this species, alone or contained in a starter culture have been used for human consumption to a significant degree. The history of use in foods of the species in the inventory is documented by scientific literature references and statements in good faith from the companies producing and selling strains belonging to the species or cultures composed of or containing strains belonging to the species.

The inventory contains species that have a record for use in food production either as single strain cultures, single species cultures of multiple species cultures as defined by International Dairy Federation (IDF standard 149A:1997). The specie/strain may also be contained in a commercial starter culture composed of otherwise unknown species.

Strains may exist that have been used by food manufacture without being recognised.

The inventory is conservative in the sense that many commonly used artisanal starter cultures contain a variety of strains belonging to the different species for which no documented record exists. To this must be added that natural changes due to mutations may be expected in an undefined artisanal starter culture.

Bergeys Manual of Systematic Bacteriology (9th Edition, 1986) is now in many respects out of date, as many of the microorganisms recorded has been renamed due to the development of genetic methodologies in the classification. It also has to be considered that the taxonomy of lactic acid bacteria and other food microorganisms have been subject to changes. Strains have been merged and new strains have been added.

As such, the inventory is not comprehensive. New entities will be added and modifications made in accordance with latest scientific findings.

The inventory consists of lactic acid bacteria and some other bacterial species belonging to *Enterococcus*, *Streptococcus*, as well as yeasts and moulds. These strains used by the food industry have a long history of use in food without any adverse effects.

*) The term "Used in food to a significant degree" is in this list defined as: Species or cultures sold for human consumption in quantities exceeding an equivalent of 10 kg of freeze dried culture. This amount of culture used for inoculation in amounts of 0.01 % corresponds to a produced amount of app. 100 tonnes of fermented products containing approx. 10^8 microorganisms per gram of product.

2 MICROORGANISMS WITH A DOCUMENTED HISTORY OF USE IN FOODS

Table 1: EFFCA/IDF– inventory of microorganisms with documented history of use in human food

Used since	Group / Genera / Species	Major application(s)	Reference	Taxonomy
	Bacteria			
	Arthrobacter			
1997	Arthrobacter globiformis	Citrus fermentation to remove limonin and reduce bitterness.	22) 115)	17a) 17a)
<1996	Arthrobacter nicotianae	Cheese maturation.		
	Bacillus			
1996	Bacillus coagulans	Sold as probiotic culture for humans and animals. **)	23), 24)	17a)
	Bifidobacterium			
1991	Bifidobacterium adolescentis	Used in fermented milks. Probiotic properties. **)	1), 25), 23)	17a)
1980	Bifidobacterium animalis	Used in fermented milks. Probiotic properties. **)	23), 26)	17a)
1970	Bifidobacterium bifidum	Used in fermented milk as probiotic ingredient. **)	23),	17a)
1980	Bifidobacterium breve	Used as probiotics in fermented milks and infant formulas. **).	27), 28)	17a)
1980	Bifidobacterium infantis	Used as probiotics in fermented milks and infant formulas. **)	28), 23)	17a)
1980	Bifidobacterium lactis = (B. animalis)	Fermented milks with probiotic properties. Common in European Fermented milks. (Similar to B. animalis. Species named in 1997.)	26), 28)	17a)
1980	Bifidobacterium longum	Fermented milks with probiotic properties. **)	29), 30), 23)	17a)
1991	Bifidobacterium pseudolongum	Fermented milk and probiotic for animals.	31)	17a)
	Brevibacterium			
1992	Brevibacterium casei	Used for cheese production.	4)	17a), 4)
1960	Brevibacterium linens	Used for cheese production.	4)	17a), 4)
	Corynebacterium			
1997	Corynebacterium ammoniagenes	Cheese ripening.	116)	17a)
<1996	Corynebacterium flavescens	Used in cheese ripening cultures.	35)	17a)
	Enterobacter			
1982	Enterobacter aerogenes	Bread fermentation. **)	117)	17a)
	Enterococcus			
1982	Enterococcus durans	Cheese and sour dough fermentation and used as human probiotic.	9)	17a)
1980	Enterococcus faecium	Cheese and fermented milk with probiotic effect.	2), 36)	17a)
	Hafnia			
<1996	Hafnia alvei	Ground beef and ripening of meat.	37)	17a)
	Halomonas			
1980	Halomonas elongata	Ripening of ham.	20)	17a)
	Kocuria			
< 1989	Kocuria varians	In meat product or as surface ripening flora and biopreservation.	38), 118), 119)	17a)
	Lactobacillus			
1950	Lactobacillus acidophilus	Fermented milk and probiotics. **)	2), 3), 23)	17a)

1991	Lactobacillus alimentarius	Fermented sausages.	39)	17a)
1996	Lactobacillus amylovorus	Bread fermentation and production of glucoamylase.	40), 41)	17a)
1995	Lactobacillus bavaricus	Meat fermentation and biopreservation of meat.	42)	17a)
1980	Lactobacillus brevis	Bread fermentation. **)	43), 23)	17a)
1987	Lactobacillus buchneri	Malolactic fermentation in wine.	44),	17a)
1970	Lactobacillus casei	Dairy starter. Cheese ripening. **)	2), 45)	17a)
1988	Lactobacillus coryniformis	Fermentation of cheese and cassava.	21), 46),	17a)
1988	Lactobacillus crispatus	Probiotics.	47)	17a)
1993	Lactobacillus curvatus	Cheese ripening. **)	10), 23)	17a), 16)
1960	Lactobacillus delbruecki subsp. delbruecki	Fermented milks. **)	23)	17a)
1930	Lactobacillus delbrueckii subsp. bulgaricus	Yoghurt and other fermented milks. **)	2), 3), 23)	17a)
1949	Lactobacillus delbrueckii subsp. lactis	Fermented milk and cheese.	2)	17a)
1980	Lactobacillus farciminis	Fermentation of bread.		17a)
1980	Lactobacillus fermentum	Fermented milks. **)	2), 23	17a)
1980	Lactobacillus gasseri	Fermented milk and probiotics.	2), 12), 48)	17a)
1930	Lactobacillus helveticus	Starter for cheese. Cheese ripening.	2), 49)	17a)
1996	Lactobacillus heterohiochi (= L. fructivorans)	Bread fermentation. **)	68), 66)	17a)
<1995	Lactobacillus hilgardii	Malolactic fermentation of wine.	50)	17a)
1986	Lactobacillus jensenii	Fermentation of cerealies.	13)	17a), 8)
1962	Lactobacillus johnsonii	Biopreservation and probiotics.	7), 12), 51), 52)	17a), 11)
1950	Lactobacillus kefirgranum	Fermented milk (Kefir). Reduction of bitter taste in citrus juice.	53)	17a)
1950	Lactobacillus kefiri	Fermented milk (Kefir). Reduction of bitter taste in citrus juice.	3), 54), 55), 56)	17a)
1986	Lactobacillus lactis	Fermented milk and Emmental cheese starter. **)	23)	17a)
1970	Lactobacillus paracasei	Cheese fermentation. Probiotic cheese. Probiotics.	15), 57), 58)	17a), 5)
1987	Lactobacillus pentosus	Meat fermentation and biopreservation of meat.	59)	17a)
1965	Lactobacillus plantarum	Silage inoculum. Fermentation of vegetables. Malolactic fermentation. **)	23), 60),61)	17a)
1980	Lactobacillus reuteri	Probiotics. **)	2), 62), 23)	17a)
1980	Lactobacillus rhamnosus	Probiotic culture / Additional starter for Emmental cheese.	1), 63)	17a)
1993	Lactobacillus sakei subsp. carnosus (= L. curvatus)	Meat fermentation.	111)	17a)
1991	Lactobacillus sakei subsp. sakei	Fermentation cheese and meat products.	64), 43)	17a)
1996	Lactobacillus salivarius	Cheese fermentation and probiotics.	65)	17a)
1950	Lactobacillus sanfranciscensis (= L. sanfrancisco)	Fermentation of bread.	66)	17a)
1950	Lactobacillus sanfrancisco (= L. sanfranciscensis)	Fermentation of bread.	66)	17a)
1960	Lactobacillus xylosus (= L. lactis subsp. lactis)	Cheese culture for enhanced ripening.	114)	17a)
<1996	Lactobacillus zeae (= L. casei subsp. casei /L. rhamnosus)	Cheese production and probiotics.	6), 112), 113)	17a)
	Lactococcus			
1903	Lactococcus lactis subsp. cremoris	Common in dairy starter.	2)	17a)
1903	Lactococcus lactis subsp. lactis	Common in dairy starter.	2)	17a)
1903	Lactococcus lactis subsp. lactis biovar diacetilactis	Common in dairy starter.	2), 34)	17a)
	Leuconostoc			
1903	Leuconostoc lactis	Common in dairy starter.	2)	17a)
1903	Leuconostoc mesenteroides subsp. cremoris	Common in dairy starter.	2)	17a)
1903	Leuconostoc mesenteroides subsp. dextranicum	Common in dairy starter.	2)	17a)

1949	Leuconostoc mesenteroides subsp. mesenteroides	Common in dairy starter.	2)	17a)
	Micrococcus			
<1996	Micrococcus varians (= Kucuria varians)	Meat fermentation and biopreservation of meat.	67)	17a)
	Oenococcus			
1980	Oenococcus oeni (= Leuconostoc oeni)	Malolactic fermentation of wine.	68), 69)	17a)
	Pediococcus			
1970	Pediococcus acidilactici	Meat fermentation and biopreservation of meat. Cheese starter.	70)	17a)
1970	Pediococcus pentosaceus	Meat fermentation and biopreservation of meat.	2), 71), 72)	17a)
	Propionibacterium			
1949	Propionibacterium acidipropionici	Meat fermentation and biopreservation of meat.	70)	17a)
1982	Propionibacterium arabinosum	Cheese fermentation. Probiotic cheese. Probiotics.	1), 72), 73)	17a)
1949	Propionibacterium freudenreichii subsp. freudenreichii	Cheese fermentation (Emmental cheese starter). **)	73), 23)	17a)
1949	Propionibacterium freudenreichii subsp. shermanii	Cheese fermentation (Emmental cheese starter). **)	73), 23)	17a)
1999	Propionibacterium thoenii	Biopreservation of foods.	74)	17a)
	Staphylococcus			
1970	Staphylococcus carnosus	Meat fermentation.	19), 75)	17a)
1970	Staphylococcus carnosus subsp. carnosus	Meat fermentation and biopreservation of meat.	76)	17a)
1970	Staphylococcus carnosus subsp. utilis (= S. carnosus)	Meat fermentation.	119)	17a)
1997	Staphylococcus equorum	Biopreservation of cheese.	77)	17a)
1997	Staphylococcus sciuri	Cheese fermentation. Probiotic cheese. Probiotics.	78), 120)	17a)
<1996	Staphylococcus vitulinus (= S. pulveri)	Meat fermentation.	119)	17a)
1980	Staphylococcus xylosus	Meat and cheese fermentation cheese.	79), 80), 81)	17a)
	Streptococcus			
1980	Streptococcus salivarius	Fermented milk and cheese.	82), 83)	17a)
1930	Streptococcus thermophilus	Cheese starter and yoghurt.	2), 3), 83)	17a)
	Fungi **Mycelium fungi / Mould**			
	Fusarium			
1996	Fusarium solani	Used for cheese production (Vacherin cheese; Switzerland).	85)	17a), 17b)
	Penicillium			
1980	Penicillium album (= P. caseicolum, P.candidum, or P. camemberti)	White mould cheeses (camembert type).	86), 90)	17a), 17b)
1960	Penicillium camemberti (= P. caseicolum, P. candidum, or P. album)	White mould cheeses (camembert type).	87), 90)	17a), 17b)
1960	Penicillium candidum (= P. caseicolum, P. camembertii, or P. album)	White mould cheeses (camembert type).	88)	
1980	Penicillium chrysogenum	Meat fermentation and biopreservation of meat.	89)	17a), 17b)
1980	Penicillium nalgiovense (= P. jensenii)	Meat (sausage) fermentation.	90)	17a), 17b)
1950	Penicillium roqueforti	Blue mould cheeses.	90), 93), 92)	17a), 17b)

	Verticillium			
1986	Verticillium lecanii	White mould cheeses.	32)	17a), 17b)
	Trichothecium			
1986	Trichothecium domesticum	Surface flora of St. Nectaire cheese.	32), 33)	17a), 17b)
	Yeast			
	Candida			
<1996	Candida famata	Fermentation of blue vein cheese and biopreservation of citrus.	91)	17a), 17b)
1950	Candida kefyr (= C. pseudotropicalis)	Kefir fermentation and cheese maturation.	94), 95)	17a), 17b)
1993	Candida friedricchi	Kefir fermentation.	121)	17a), 17b
1986	Candida Holmii	Kefir Fermentation	121)	17a), 17b
1950	Candida krusei	Kefir fermentation. Sour dough fermentation.	95), 43), 96), 97)	17a), 17b)
1950	Candida pseudotropicalis (= C. kefyr)	Kefir fermentation.	94), 95)	17a), 17b)
1980	Candida utilis	Fortification of corn meal by fermentation.	99)	17a), 17b)
<1996	Candida valida	Used for cheese ripening.	98)	17a), 17b)
	Debaryomyces			
1980	Debaryomyces hansenii	Cheese maturation.	93)	17a), 17b)
	Geotrichum			
1970	Geotrichum candidum	Ripening of soft and semisoft cheeses or fermented milks	100), 101), 102)	17a), 17b)
	Hansenula			
1950	Hansenula mrakii (= Williopsis mrakii)	Kefir fermentation.	14), 103)	17a), 17b)
	Kluyveromyces			
1950	Kluyveromyces fragilis (= Kluyveromyces marxianus)	Fermentation of soy milk. Fortification of soft cheese. Flavour enhancer.	3), 104), 105)	17a), 17b)
1980	Kluyveromyces lactis	Cheese ripening.	95)	17a), 17b)
1980	Kluyveromyces marxianus (= Kluyveromyces fragilis)	Fermentation of soy milk. Fortification of soft cheese. Flavour enhancer.	3), 104), 105)	17a), 17b)
	Pichia			
<1996	Pichia fermentans	Isolated from fermented olives.	106)	17a), 17b)
	Saccharomyces			
1950	Saccharomyces bayanus	Kefir fermentation. Juice and wine fermentation.	107), 108)	17a), 17b)
1980	Saccharomyces cerevisiae	Among others used for beer and cheese production.	109)	17a), 17b)
1990	Saccharomyces cerevisiae subsp. boulardii	Used as probiotic culture.	110)	17a), 17b)
1950	Saccharomyces florentius	Kefir fermentation.	18)	17a), 17b)
1986	Saccharomyces unisporus	Kefir fermentation.	121)	17a), 17b)
	Williopsis			
1950	Williopsis mrakii (= Hansenula mrakii)	Kefir and cheese fermentation.	14), 103)	17a), 17b)

**) These cultures or strains are specifically mentioned in relation to GRAS status.

GRAS status is often connected with an approval by the US FDA. However, in the USA, Code of Federal Regulations (21 CFR) and the US FDA Office of Premarket Approval, lists microorganisms which are approved food additives or which enjoy Generally Recognized as Safe (GRAS) status. "GRAS" status is always considered only for a specified use. Thus, for instance, microbes themselves are not considered GRAS, but their traditional use in dairy foods is. Currently harmless lactic acid producing bacteria, including Lactobacillus acidophilus and other lactic acid bacteria (specifically *Streptococcus thermophilus, Str. cremoris, Str. lactis and Str. lactis ssp. diacetylactis, Lactobacillus bulgaricus, L. fermentum, L. lactis and three Leuconostoc species (Leuconostoc citovorum, Leuconostoc dextranicum* and *Leuconostoc mesenteroides*) can be used in as additional ingredients in specified standardized foods such as cultured milk (including e.g. yoghurt and buttermilk), sour cream, cottage cheese and yoghurt, provided that the mandatory cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are also used in yoghurt.

The FDA continuously grant GRAS status for strains or cultures for specific food application. As an example FDA has approved GRAS petitions by Nestle US for Bifidobacterium lactis Bb12 and Streptococcus thermophilus Th4 for use in formulas and foods for infants and children.

Reference web-site: http://vm.cfsan.fda.gov/~dms/opa-micr.html

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3 Trans Fatty Acids

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by their kind courtesy

ABSTRACT

The expression 'trans fatty acids' encompasses a large number of substances having highly heterogeneous roles. Several experimental and epidemiological studies suggest that trans fatty acids (mainly elaidic acid) would seem to have negative effects on plasma cholesterol levels and the incidence of cardiovascular disease. These can be found for instance in hydrogenated or deodorized vegetable fats. Conjugated linoleic acids (CLA) and possibly also vaccenic acid on the other hand seem to have beneficial effects on different body functions as well as a protective effect on some pathologies such as cancer, obesity, cardiovascular disease and diabetes. These occur naturally in fats of dairy origin. Some trans fatty acids may be active in very low concentrations, either positively (CLA in dairy fats) or negatively (TFA of alpha-linolenic acid in oils. At the present time, scientific knowledge of trans fatty acids is relatively fragmented.

1 INTRODUCTION

Two carbon atoms connected by a double bond cannot rotate about the double linkage as they can about a single bond. This offers opportunity for two different spatial arrangements of the substituent groups if the groups on each unsaturated carbon atom are different from each other. When both hydrogens of the ethylenic double bond of a fatty acid lie on the same side of the molecule the name is prefixed by *cis*-(Latin, on this side). When the hydrogens are positioned on opposite sides of the molecule the prefix trans- (Latin, across) is used. This phenomenon is referred to as geometrical isomerism. The *cis*-configuration produces a rigid 30° bend in the hydrocarbon chain of the fatty acid, whereas the *trans*-configuration resembles more the straight chain arrangement of saturated fatty acids (Figure 1). The bend in the hydrocarbon chain interferes with efficient crystalline packing and melting point decreases with the number of double bonds. Change in melting point with configuration is important in oil and fat technology, while configuration of an acid has important biological consequences for membrane systems.

In addition to spatial isomerism the double bond may occupy different positions along the hydrocarbon chain of the fatty acid molecule. The resulting isomers are referred to as positional isomers. Two systems exist side-by-side to denote the position a double bond occupies in a fatty acid. Firstly, in a system preferred by biochemists, double bonds are indicated by the symbol Δ and numbered from the carboxyl carbon end e.g. linoleic acid would be named *cis*-9, *cis*-12-octadecadienoic acid with double bonds at the Δ9,12-positions. Secondly, in a less precise system used by nutritionists, polyunsatu-

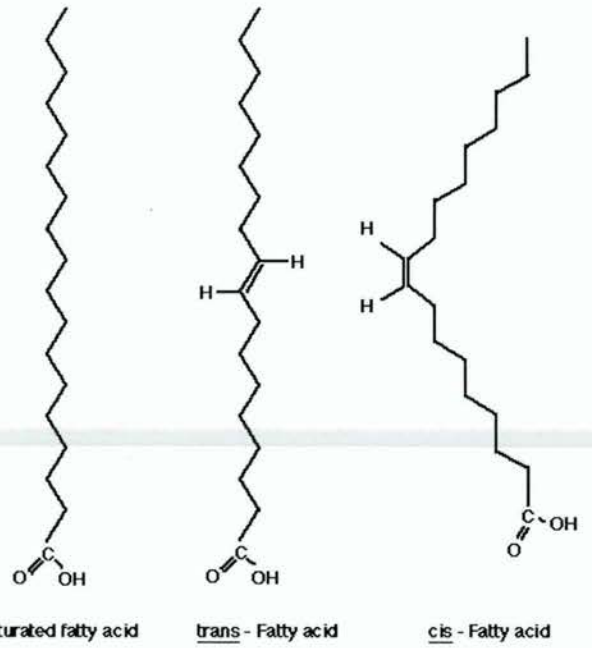


Figure 1: *Structure of 18 carbon atom saturated, trans- and cis- fatty acids*

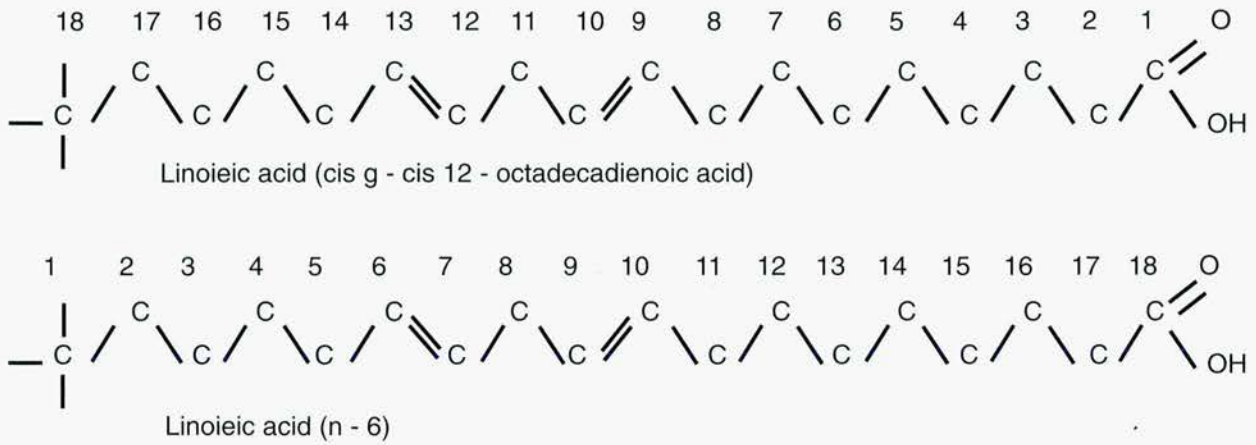


Figure 2: Two methods of naming linoleic acid

rated fatty acids are classified into three groups n-3 (omega-3), n-6 or n-9, the numbers representing the first double bond from the methyl-terminal end of the fatty acid (Figure 2).

Polyunsaturated fatty acids present in nature usually have methylene-interrupted double bonds. Hydrogenation mediated isomerism may produce double bonds with conjugated unsaturation.

2 TRANS FATTY ACIDS IN FOOD

In man and most mammals, food intake chiefly provides *cis* fatty acids, but also contains *trans* fatty acids in some cases [10,12]. These *trans* fatty acids are absorbed, incorporated into various tissues and are used in several metabolic pathways, which they may or may not disturb [9,37,38,50].

Schematically, the formation of *trans* isomers is closely connected with the hydrogenation of fatty acids (FA). The sources of *trans* FA total three in number: biohydrogenation by rumen flora of polyunsaturated *cis* fatty acids eaten by ruminants, partial catalytic hydrogenation of oils and vegetable fats, and the heating of vegetable oils especially during the deodorizing of oils at the refining stage [1,50,66].

Biohydrogenation and partial catalytic hydrogenation mainly lead to geometrical isomers and positional isomers of oleic acid (18:1); biohydrogenation also produces conjugated isomers of linoleic acid (18:2) (including the Conjugated Linoleic Acids – CLAs) of which some have one or two *trans* configuration bonds; deodorization and, more generally, heat treatments applied to oils, lead to the formation of geometrical isomers of essential fatty acids (linoleic acid, n-6 C18:2, and linolenic acid, n-3 C18:3) [11,13,23,68,71].

2.1 Milk and dairy fats

2.1.1 Cow milk

Milk fat is complex, being composed of triacylglycerols, themselves composed of several hundred different fatty acids from C2 to C28, even or uneven, saturated, mono-unsaturated or poly-unsaturated, *cis* or *trans*, linear or branched, ketonic or hydroxylated [15,59]. The lipid content of cow milk lies between 3.5 and 4.3%. With the introduction of high performance analytical methods, the data available on trans fatty acids is now becoming more extensive and relatively precise (Table 1) [50,67,73,74].

The *trans* configuration of milk fatty acids is found irrespective of the length of the carbon chain, but it is the monounsaturated fatty acid isomers with 18 carbon atoms (18:1) which account for the most part of *trans*-FAs in dairy products. The *trans* forms make up 1.5 to 6.5% of the total fatty acids in dairy fats [21,27,49,66]. A German study estimates that the *trans* isomers account for 3.7 to 4.3% of fatty acids in unprocessed milk and from 2 to 6% of fatty acids in derivative products such as butter, cheese, etc [21]. In France, WOLFF reports an average annual TFA content of 3.3% in total milk FAs with a maximum average of 4.3% in Spring [66]. These variations are attributable to several parameters that are often involved, such as seasonal variations, type of food, animal breed, geographical situation [35,48,52].

Table 1: Total *trans*-18:1 isomers in cow dairy fat [as a % (g/ 100g) of total fatty acids] [38].

Average	σ	Min.	Max.	n	Country	Year
-	-	4.30	7.60	18	Au	1983 (46)
6.28	-	-	-	5	DK	1983 (40)
3.33	0.99	1.75	5.20	31	A	1994 (26)
3.75	-	2.46	5.18	24	F	1994 (67)
3.62	1.22	1.29	6.75	1756	D	1995 (51)
5.91	0.92	-	-	22	IRL	1995 (51)
2.70	-	1.81	3.43	-	D	1996 (27)
3.13	0.24	-	-	7	D	1997 (21)
2.53	1.34	-	-	12	D	1997 (21)
3.83	1.34	1.91	6.34	100	D	1996 (53)

Table 2: Distribution of trans-18:1 positional isomers in cow dairy fats [as a % of total trans-18:1] [38].

Country:	Au	Au	DK	F	F	D
Products: nb samples	Milk 18	Butter 18	Butter 6	Butter 12	Butter 12	Butter 1756
trans bond position						
Δ4	min.	max.				1.6
Δ5						1.5
Δ6-8	1.8	4.2	2.1		7.2	9.6	4.7
Δ9	7.3	14.6	8.8				6.9
				67.9			
Δ10	4 .4	13.1	5.5				4.7
Δ11	28.4	55.1	60.5		58.2	5.4	43.2
Δ12	3.9	9.0	4.1	5.1	6.2	7.2	6.3
Δ13/14	8.9	18.5	9.6	15.0	15.4	17.2	14.2
Δ15	4,7	7.5	3.9	5.4	5.7	6.6	7.9
Δ16	5.7	9.4	5.5	6.7	7.3	9.0	9.8

Trans monounsaturated acids, isomers of oleic acid (Δ9c 18:1), make up 97-98% of the *trans* isomers occurring in dairy fats [49,50]. *Trans*-18:1 levels in dairy fats vary between 2.5 and 6.3% of fatty acids (Table 1). The most important isomer from a quantitative viewpoint is vaccenic acid, a C18 monounsaturated acid having a double *trans* bond at position 11 after carboxyl, generally designated Δ11*t*. 18:-1. This acid alone represents approximately one half of the *trans*-18 isomers of dairy fats. Elaidic acid, Δ9t-18:-1, accounts for 6.9 to 14.6% and the Δ13*t* and Δ14t isomers for 9.6 to 17.2% of *trans*-18:1 isomers. Other positional isomers occur in low proportions (Table 2).

In addition to these 18:1 acids, dairy fats contain traces of "minor" *trans* fatty acids: 14:1, 15:1, 16:1.

Following after are the *trans*-18:2 isomers of polyenoic acids. On average these represent between 0.4 and 1% of the total fatty acids in dairy fats (Table 3). Some authors report levels possibly varying between 0.56 and 1.58% [53]. According to these authors, dairy products apparently contain 62% more *trans*-18:2 than margarine, and 77% more than shortenings or heated oils.

Compared with other fats, dairy products are relatively rich in Conjugated Linoleic Acids –CLAs, with a content of 1.7 to 7.1 mg CLA/g of fat depending upon the origin of the milk fat [14]. The main isomer is the acid Δ9*c*,11*t*-18.2, called "rumenic" acid, which accounts for approximately 90% of CLAs in dairy fats, i.e. from 0.34 to 0.85% of total fatty acids on average (Table 3) with maximum levels possibly reaching 1.95% [14,27,44,62]. Rumenic acid forms 46.6% of the *trans*-18:2 isomers in milk. This information is of essential importance bearing in mind the biological properties of this "rumenic" acid [7,25,47].

2.1.2 Goat and ewe milks

Very few studies have been made into the occurrence of *trans* fatty acids in ovine and caprine dairy products [39]. WOLFF [69] reports results on the analysis of

Table 3: *Trans* isomers (other than 18:1) in cow dairy fats [as a % (g/ 100g) of total fatty acids] [38].

trans fatty acids	Average	Year	Country
14:1	0.24[1] 0.29[1] 0.32[1]	1996	D
	0.02[1] 0.03[2] 0.03[3]	1997	
15:1	0.03[2]	1983	DK
	0.18[2]	1983	DK
	0.08-0.13[2]	1994	F
16:1	0.13[2]	1995	D
	0.41[1] 0.52[1] 0.49[1]	1996	D
	0.19[1] 0.19[2] 0.09[3]	1997	D
	0.51[2]	1993	A
	0.59[2]	1994	A
18:2	0.52[1] 0.45[1] 0.74[1]	1996	D
	0.99[2]	1997	D
	0.62[1] 0.74[2] 0.72[3]	1997	D
18:2CLA	0.34[1] 0.61[1] 0.80[1]	1996	D
	0.85[2]	1997	

[1] *milk* ; [2] *butter* ; [3] *cheese.*

Table 4: Distribution (%) of trans-18:1 isomers in ewe and goat cheeses

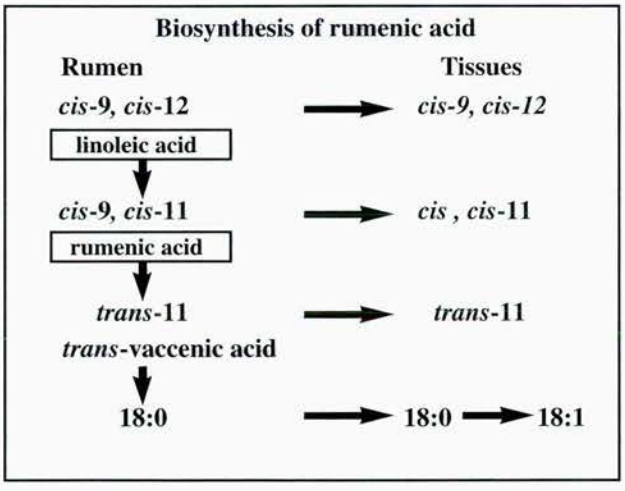
	D6-9	D10,11	D12	D13,14	D15	D16
Ewe	8.3	56.7	6.7	15.6	5.2	7.5
Goat	12.6	44.9	8.7	18.2	6.8	8.8

cheeses: ewe-milk cheese (n=8) shows an average *trans*-octadecenoic content of 4.53% of total fatty acids (with extreme values of 3.02% and 6.17%); goat cheeses only contain 2.68% (from 1.75 to 4.50%). As in bovine dairy products, vaccenic acid is predominant in goat and ewe milk (table 4) [7,45,69]. The distribution of *trans*-18:1 isomers in goat milk fat compares with the level observed in cow milk. Also, distributions of *trans*-16:1 isomers are comparable between the fats of cow, goat and ewe cheeses [16]; the Δ9*t*-16:1 isomer proves to be predominant in the series of *trans* hexadecenoic acids. The occurrence of the Δ3*t*-16:1 isomer in substantial proportions is apparently directly due to the pasture grazed by livestock.

2.1.3 Source of *trans* isomers of fatty acids in ruminant milk

Mammals do not seem to synthesize *trans* fatty acids. The presence of these isomers in ruminant milk is mainly due to the metabolism of rumen flora [8,36]. Some rumen bacteria, such as *Butyrivibrio fibrisolvens*, convert linoleic acid (18:2) and linolenic acid (18:3) into octadecenoic acids (18:1) at the time of a true spate of successive isomerization and biohydrogenation reactions; these 18:1 acids are then converted into stearic acid (18:0). The onset of the different isomers is not simultaneous and depends upon the specificity of microbial ecology [13]. The initial intermediaries in the conversion of linoleic acid are apparently the conjugated isomers of this acid (CLA) mainly rumenic acid Δ9*c*,11*t*-18.2 [25,28]; the acids of mono-trans form are apparently intermediaries for subsequent steps [28,49,61].

18:2 Æ CLA 18:2 Æ trans-18:1 Æ 18:0.



The α-linolenic and γ-linolenic acids are converted firstly into conjugated 18:3 isomers, and are then hydrogenated into octadecenoïc acids, which also include *trans* configurations, further biohydrogenation produces chiefly vaccenic acid 11*t*-18:1, a common intermediary for the biohydrogenation of α- and γ-linolenic acid and linoleic acid. Migrations of double bonds along the carbon chains and the action of bacterial enzymes specific to *cis/trans* isomerization in the rumen could account for the production of other minor conjugated fatty acid isomers together with other minor isomers of trans-monounsaturated fatty acids.

However, the CLA levels in the rumen are not sufficient to account for the quantities of conjugated isomers of linoleic acid occurring in milk. During lactation, a Δ9-desaturase of the mammary gland would seem to convert the vaccenic acid 11*t*-18:1 into rumenic acid 9*cis*, 11*t*-18:2. The differences in activity of this desaturase in relation to type of food apparently accounts for the variations in CLA levels in milk in relation to diet [18,19,20,24,31,32,33,48].

The conversion of octadecenoïc acids into stearic acid, the final step in these conversions, is apparently not catalysed by the microbial enzymes but by animal-specific enzymes.

Several studies demonstrate a seasonal variation in the *trans* fatty acid content of milk, with a minimum in winter and a maximum in spring. The source of food substrates partly accounts for these variations. The lipid content of young grass is high, up to 75% of lipids being polyunsaturated fatty acids (PUFAs) which tallies with the maximum *trans* isomer contents reported in spring and summer milk [35,73]. On the contrary, animal stalling times relate to food with a low PUFA content leading to low levels of *trans* fatty acids [35].

A study on French butter [66] showed *trans*-18:1 levels of 3.22% (winter average) to 4.28% (spring/summer average) of total fatty acids. These seasonal variations show practically the same pattern for all *trans*-18:1 isomers but with varying intensities [52]. Vaccenic acid appears to undergo the greatest variations in absolute value increasing from 0.93% to 2.87% of total fatty acids during the transition period from stalls to grazing land ; at the same time, elaidic acid increases from 0.21% to 0.26% and total *trans*-18:1 from 2.65% to 5.08%.

These conclusions have led several research teams to studying the influence of bovine food rations on the production of *trans* fatty acids in milk. It would seem that the lipid composition of diet has an important influence on the composition of dairy fat [60].

Seasonal variations reflect differences in pasture composition; the breed of cow may have a slight influence also, it can be expected that animals which stay longer on pastures have higher annual averages of *trans* fatty acids.

2.2 Vegetable fats

Hydrogenation provides a means of converting liquid vegetable oils, and in some cases marine oils, into semi-solid plastic fats for use in margarine and shortening production. Hydrogenation consists of the addition of hydrogen to the ethylenic double bonds by reaction of hydrogen gas in the presence of a metal catalyst, usually nickel, under controlled conditions. The hydrogenation reaction is accompanied by simultaneous isomerization,

both positional and geometrical, of the unsaturated bonds. Thus hydrogen can add to the *cis*-double bonds to produce a saturated acid. At the same time, fatty acids with the *cis*-configuration react with hydrogen at the catalyst surface to produce the *trans*-configuration. Additionally, both *cis*- and *trans*- bonds can migrate along the hydrocarbon chain to produce a mixture of positional isomers.

2.2.1 Margarines, shortenings and hydrogenated oils

Partial catalytic hydrogenation of vegetable oils is intended firstly to increase their melting point so that they can be hardened, and secondly to increase their oxidizing stability.

Unlike dairy products, the levels of *trans* fatty acids formed during the partial catalytic hydrogenation of oils vary immensely. Numerous parameters are involved: type and composition of the unsaturated fatty acids in the oil, type of catalyst, hydrogenation conditions (temperature, pressure, stirring), extent of hydrogenation reached [50,66]. *Trans* fatty acids may represent from 1-2% (household margarines) up to 40-50% (shortenings) of partially hydrogenated oil fats [1,2,66].

Partial catalytic hydrogenation generates 85 to 95% of *trans*-18:1 octadecenoic fatty acids, the remaining 5% being essentially 18:2 octadecadienoic acids and traces of 18:3 trienoic acids (up to 0.7%) and 16:1 hexadecenoic acids (Table 6) [11,44,53,71].

Table 6: *Trans* fatty acids in margarines (as a % of total fatty acids)

18:1	18:2	18:3	Country
10.7 – 30.1	1.9	n.s.	USA
9 – 22	n.s.	0.9 - 1	F
1.2 – 20.71	0.2 – 1.9	n.s.	D
7.3 – 24.4	n.s.	n.s.	EEC*

n.p. = not specified;
** the authors point out that most values are lower than those obtained for US margarines.*

In respect of the 18:1 isomers, the proportion of the different isomers is very different to that of milk. Vaccenic acid is not the majority component in this case since it only represents around 10 to 15% of total *trans*-18:1. The distribution of *trans*-18:1 isomers in margarines appears to be Gauss-like, centred on the Δ9 isomers (elaidic) and Δ10 which may represent up to around twenty per cent of total *trans*-18:1 [4,22,43].

The 18:2 acids Δ9*c*,12t and Δ9*t*,12c represent 85% of *trans*-18:2 isomers in margarines and hydrogenated oils (compared with 9.3% in dairy fats). Some authors report contents of conjugated isomers of linoleic acid ranging from 0.1 to 0.7 mg CLA/g of lipids in vegetable oils [14]; rumenic acid apparently accounts for less than 50% of total CLAs in oils. These are low contents compared with CLA levels measured in dairy fats. For these same authors, the proportions of CLAs in manufactured products reflect the initial levels of the raw materials. On the other hand, a vast survey made on German food products did not find any CLAs in margarines or in shortenings [53].

Small traces of hexadecenoic *trans*-16:1 are found in margarines (0.04% of total fatty acids); on the other hand, partially hydrogenated fish oils may contain up to 3.03% of these trans-16:1 [53].

In Europe, "household" margarines contain increasingly fewer *trans* fatty acids, due firstly to replacement of oil hydrogenation by interesterification, and secondly to the choice of raw materials [6]. Hence, recent data show that most margarines in France currently contain less than 1% trans fatty acids (compared with 30 to 40% found in American margarines).

On the other hand it would seem that TFA levels have not been reduced in industrial foods which use former technology vegetable fats.

2.2.2 Refined oils (deodorized)

The *trans* isomers of essential fatty acids (linoleic acid 18:2 and α-linolenic 18:3) have been detected in non-hydrogenated refined oils subjected to deodorization at temperatures in the order of 220-260°C [1], and in frying oils (the percentage of *trans* fatty acids increasing in waste frying oils) [11,56,68]. Since these isomers do not occur in virgin oils [1,65], the isomerization phenomenon in this case is solely of heat origin and only concerns geometrical isomerism. The extent of isomerization is dependent upon temperature and process application time [17,23,69].

α-linolenic acid is the most sensitive to this type of isomerization; its *trans* isomers may represent up to 3.5% of the total fatty acids in refined oils [56,70]. It is always the four same compounds which are found out of the seven that are theoretically possible; the relative proportions of these four isomers seem to be fairly constant and unrelated to the initial content of α-linolenic acid in the oil [65,69]. The18:3 isomers Δ9*c*,12*c*,15*t* or Δ9*t*,12*c*,15*c* alone represent 85-90% of the *trans*-18:3 that are formed [1,64,65,69].

The isomers of 18:2 linoleic acid are found in lesser proportion (up to 1% of total fatty acids in refined oils) and above all are 18:2 mono-trans isomers: Δ9*c*,12*t* or Δ9*t*,12*c* [53,56,65]. No isomerization apparently takes place with oleic acid, a mono-unsaturated fatty acid of the same series (18:1) [68].

2.3 Meat and meat products

Animal fats may contain *trans* fatty acids, especially isomers of 18:1 oleic acid. As a general rule, ruminants show the highest levels. Their *trans* fatty acids derive from the activity of the rumen while in monogastrics they are a reflection of diet [14,74].

The *trans* fatty acid content of meats ranges from 0.2% of total fatty acids for horse-meat to 10.6% for mutton [22]; beef suet may contain between 2 and 4.6% of *trans*-18:1 [50,66]. Some authors report high levels of conjugated linoleic acids in lamb with 5.6 mg CLA/g of fat, and beef with 2.9 to 4.3 mg CLA/g of fat [14]. The fats of non-ruminants show the lowest levels with 0.6, 0.9 and 0.3-0.6 mg CLA/ g of fat for pork, chicken and fish respectively. There does not appear to be any seasonal variation in the trans fatty acid content of muscles in ruminants. The occurrence of trans fatty acids in non-ruminants is related to diet; therefore the trans fatty acid profile in pigs fed with milk powder is identical to that of dairy fats.

3 TRANS FATTY ACIDS AND HEALTH

Several recent experimental and epidemiological studies emphasize the involvement of *trans* fatty acids on plasma lipoprotein levels and the possible repercussions of the presence of these isomers in food and on the incidence of cardiovascular disease [3,5,30,34,41,42,63,76]. In contrast conjugated linoleic acids (CLAs) apparently have a protective effect against some pathologies [57]. The evidence is reviewed hereunder.

3.1 Between CLA and CLA: The beneficial effects of CLA

Since the 80s-90s, the attention given to CLAs has increasingly grown on account of their biological properties [116]. However, between CLA and CLA it is not always easy to find one's bearings... It is indeed a complex family of compounds owing to the variety of the double bond positions and their isometry, and most studies have used synthetic mixtures chiefly containing two isomers: rumenic acid (9*c*, 11*t*-18:2) and the 12*c*, 10*t*-18:2 isomer, but also - depending upon commercially available sources – the 8, 10 and 11,13 isomers. These fatty acids are apparently active in very small concentrations.

3.1.1 Anticarcinogenic potential

Numerous experiments performed on different animal models have shown CLAs to have substantial anticarcinogenic potential [78,81,88,102]. *In vivo* and *in vitro* experiments performed on mice and rats have shown favourable effects on the induction and development of mammary and skin tumours but also on stomach and bowel cancers. The addition of a mixture of different isomers to the diet significantly slows down the development of chemically-induced tumours. Moreover, the number of primary tumours is reduced compared with a group fed with a diet non-supplement with CLAs. These results were backed by a recent study [87] which shows that butter enriched with CLAs provides protection against experimental mammary cancer. A recent publication [91] shows that dietary CLA (1.5% of intake) significantly reduces the synthesis of PGE2 in the epidermis of mice, suggesting that CLA takes part by mediating the production of this prostaglandin. Another mechanism of action has also been put forward, suggesting the inhibition of cell growth and cell differentiation in primary cultures through a reduction in DNA synthesis and stimulation of apoptosis [87]. More recently, it has been suggested that CLAs may have an effect on the development of metastases [86].

3.1.2 Antiatherogenic effects

Antiatherogenic effects have also been described in the rabbit and hamster. In rabbits, the addition of 0.5 g of CLAs per day to diet leads to a reduction in the accumulation of aortic atherosclerotic lesions The LDL/HDL ratio was also significantly reduced. In hamsters, histological examination of the aorta showed a reduction in aortic fatty streak area in the group supplemented with CLA [97,100,101].

3.1.3 Anabolizing effects

CLAs apparently also have anabolizing effects: experiments in mice and swine have shown that they reduce fat mass and increase the lean mass, in mice more so than in swine. An effect that is probably due to reduced depositing of lipids and increased lipolysis in the adipocytes [104,105], and which could be chiefly attributed to the 12cis, 10trans-18:2 isomer [77,82,100].

3.1.4 Effects on the immune system

Recent studies also suggest effects on the immunity system. A specific influence on different categories of antibodies, such as an increase in IgA, IgG and IgM with parallel IgE reduction has been described [111].

3.1.5 Antidiabetogenic properties

Dietetary CLA normalized tolerance to glucose and the hyperinsulinaemia of diabetic rats, wich suggests a potentially beneficial effect on non-insuli dependent diabetis.

In a diabetic fatty rat model dietary CLA normalized glucose tolerance, improved hyperinsulinemia and lowered circulating free fatty acids, which prevented or delayed the onset of hyperglycemia and diabetes [75,84].

3.1.6 Changes in fatty acid profile

CLAs can induce changes in the fatty acid profile of various tissues. A possible explanation is an interaction of CLAs with some desaturases. It has been shown, for example, that CLAs lead to a reduction in the activity and expression of RNA messengers of D9-desaturase (stearoyl-CoA desaturase) [79,98].

However, the active isomer or isomers and their action mechanisms remain ill-known [109]. The effects of natural isomers such as 7*trans*,9-*cis*-18:2 have not been the subject of any study. Similarly, no research has been made into the 11*cis*,13*trans* isomer occurring in appreciable quantities in some synthetic mixtures used in experiments, even though it would seem to incorporate itself specifically into some classes of phospholipids [94]. Very few studies have been conducted in Man, but there are many in progress on the food intake of trans vaccenic acid in particular which could contribute to increasing the bio-availability of CLA.

Research into CLAs is only just starting [80,85,99,103,106,107,113]. Other studies are needed to appreciate the true impact on different physiological functions and pathologies in Man and to discover which CLA (CLAs?) are active.

3.2 Between TFA and TFA: The disparate biological effects of *trans* fatty acid isomers

3.2.1 Health implications of *trans* fatty acids: Elaidic versus trans-vaccenic acid

In most [137,139,147,148], but not all [134,136] of the controlled clinical studies performed trans fatty acids increased plasma cholesterol levels, particularly LDL cholesterol levels. Epidemiological data suggest that increased intake of trans fatty acids is associated with higher cholesterol levels [144]. As high cholesterol levels are associated with an increased risk of developing CHD, trans fatty acids may (indirectly) promote CHD. In this respect trans fatty acids appear more atherogenic than saturated fatty acids with 12-16 C-atoms. While saturated fatty acids may increase both (CHD risk-increasing) LDL- and (CHD risk-decreasing) HDL cholesterol levels, trans fatty acids increase only LDL-cholesterol levels. The

Nurses' Health Study was the first to suggest that increased intake of trans fatty acids was associated with a higher risk of coronary heart disease [145]. Later the Health Professionals Follow-up Study and the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study came to the same conclusion [review by Ascherio et al., 119].

But there are more lines of evidence linking trans fatty acids with risk for CHD. According to clinical trials trans fatty acids increase plasma levels of LP(a) [124,138,139], another risk factor for CHD, though one study did not [121]. Lp(a) is an independent risk factor for CHD. In the Lp(a) particle the protein (a) is linked to an LDL particle in an disulfidic bond. Protein (a) has sequence homologies to the proenzyme plasminogen. The active enzyme plasmin is responsible for the dissolution of existing fibrin clots in the blood vessel. Therefore, one assumes that Lp(a) competes with plasminogen for binding sites at the fibrin molecule and thus inhibits plasmin(ogen) action. Such an impaired dissolution of fibrin clots would be a plausible explanation for the CHD risk associated with high Lp(a) levels.

Trans fatty acids change cell membrane composition [126,131] and thus possibly membrane properties, they modulate activities of some enzymes in lipid metabolism, like Δ-6 desaturase [117,120] and CETP [129], and possibly others in an untoward way. Via changes in Δ-6 desaturase activity trans fatty acids interfere with biosynthesis of long-chain polyunsaturated [128] and thus prostaglandin metabolism. Elaidic acid diet given to rats promoted carcinogenesis [125].

It is yet open whether and to what extent these changes observed in vitro are relevant to metabolism and health of humans. Whether these effects become apparent will most certainly depend on the amount of *trans* fatty acids consumed and on the individual sensitivity. The phenomenon of hypo- and hypersensitivity was shown with respect to both dietary fatty acids and cholesterol and is probably also valid with respect to trans fatty acids. A high consumption of *trans* fatty acids generated during hydrogenation of vegetable oil seems undesireable, particularly for subject carrying a high CHD risk.

The study of Willett et al. [145] found that only *trans* fatty acids from hydrogenated oils, but not of animal (ruminant) fat were associated with an increased CHDrisk. This finding drew wider attention to the difference between trans fatty acids originating from hydrogenated oils (the major one being elaidic acid, abbreviated c18:1Δ9*trans* or t9-c18:1) and ruminant fats (trans-vaccenic acid, abbreviated c18:1Δ11*trans* or t11-c18:1).

Differences between the metabolism of elaidic and *trans*-vaccenic acid, which might imply a lower or even non-existent CHD risk associated with *trans*-vaccenic acid:

- *Trans*-vaccenic acid stimulates the lipoprotein secretion of intestinal Caco-2 cells (both triglycerides and apolipoproteins B100 and B48) less than elaidic acid does [123]. This might have consequences for postprandial and eventually fasting plasma lipid levels. And as the authors argue, increased triglyceride levels may finally be followed by decreased HDL cholesterol levels.
- Vaccenic acid is more rapidly metabolized by ß-oxidation than elaidic acid [130].
- Elaidic acid, but not trans-vaccenic acid, inhibits the Δ5-desaturase [141]. This enzyme is essential for the conversion of linoleic acid and arachidonic acid in their respective biologically active metabolites. An inhibition might disturb the metabolism of these essential fatty acids.
- When labelled linoleic acid was incubated together with unlabelled elaidic or unlabelled trans-vaccenic acid in hepatocyte cell culture experiments more elaidic than trans-vaccenic acid was incorporated into cellular phospholipids, especially in phosphatidylethanolamine [146]. Whether there is a biological consequence of this finding is yet open.
- Trans-vaccenic, but not elaidic acid is an important precursor for CLA synthesis in the (human) body. Studies in humans [142], mice [143] and cow [124] give evidence of a "reverse" CLA synthesis from trans-vaccenic acid (*t*11-18:1). This reverse synthesis is probably catalyzed by a Δ9-desaturase in the liver and might be the explanation why the CLA concentration is even higher in the body fat of the cow than in the milk fat. This property of trans-vaccenic acid may be the most important when weighing against elaidic acid.

Once more attention was paid to the individual *trans* fatty acid isomers, there was growing interest in their effect on lipoprotein metabolism, too. Several studies tried to address this question, by exchanging butter for margarine or other hydrogenated oils in the diet of humans [118,122,127,132,133,135,147]. Zock and Katan [149] reviewed the studies published up to 1997 and concluded that replacement of butter by hard-stick margarine decreased total and LDL cholesterol, and did hardly increase the total/HDL-cholesterol ratio. This finding is basically confirmed by the later studies. The difficulty with all these studies is, that the exchange of the trans fatty acids is associated with changes of other fatty acids which affect cholesterol level. Butter diets were always richer in saturated fatty acids and lower in polyunsaturated fatty acids than the margarine/hydrogenated oil diet. This means that the question of the effect of individual *trans* fatty isomers on lipid levels can hardly be solved by using natural fats.

A recent report from the Zuthphen Elderly follow-up epidemiological study [140] confirmed that a high intake of *trans* fatty acids was positively correlated with an increased risk of CHD. The statistical analysis did not distinguish between ruminant and industrially manufactured *trans* fatty acids. But it reported two interesting details: (i) that the intake of industrially manufactured trans fatty acids was severalfold higher in 1985 than intake of ruminant trans fatty acids, and (ii) that during the 10 yr observation period there was a clear-cut reduction of industrially manufactured *trans* fatty acids but not of ruminant trans fatty acids. This means that risk reduction was presumably due to a decrease of intake of industrially manufactured *trans* fatty acids.

Other studies suggest that deodorization methods, which may lead to converting 40% of cis alpha-linolenic acid into *trans* could be major importance. Indeed, only very small quantities of these *trans* isomers are apparently able to alter the lipid profile by increasing LDL and reducing HDL.

In summary there is only evidence for an undesired effect of hydrogenated vegetable oil containing trans fatty acids. The *trans* fatty acid which seems to mediate the effects is probably elaidic acid or any other substance which is generated during the hydrogenation and is correlated with *trans* fatty acid concentration. Vaccenic acid or CLA do not seem to make these effects or might even exert beneficial effects.

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4 Milk Lipids in Diet and Health - Medium Chain Fatty Acids (MCFA)

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ABSTRACT

The majority of absorbed MCFA are transported in the portal vein. The proportion transported in the lymph increases with increasing chain length and with chronic administration. Simultaneous administration of MCT and LCT increase MCFA appearance in the lymph. Vice versa, MCFA in structured triglycerides improve absorption of (polyunsaturated) LCFA in sn-2 position. There are some reports of lower bile cholesterol and phospholipid concentrations and lower pancreatic enzyme output with MCFA or MCT administration. There is no obvious hyper- or hypocholesterolemic effect of MCFA. Fasting plasma triglyceride levels become elevated, while the postprandial triglyceride response and, in parallel, lymph phospholipid and apo A-IV output are decreased. Long-term feeding also decreases the postprandial response to a standard LCT meal. MCFA increase both resting metabolic rate and postprandial energy expenditure, though this stimulatory effect may disappear with longer administration. Adipose tissue deposition in experimental animals is decreased, and some experiments in human suggest improved weight loss in overweight subjects. MCFA improve insulin sensitivity, but blood pressure is not affected. This means that quite a few metabolic effects of MCFA may help to reduce the risk of developing features of the metabolic syndrome. It is yet open to what extent these effects can be achieved and maintained under a regular diet regimen. There are also some reports of a differing immunological response in the intestine compared with LCFA.

1 INTRODUCTION

Medium chain fatty acids (MCFA) comprise saturated fatty acids with 8-12 carbons. Commercially prepared medium chain triglycerides (MCT) are mostly composed of C8:0 and C10:0, with only minor amounts of fatty acids shorter than 8 or longer than 10 carbon atoms. They are available as oil or, somewhat more enriched with long-chain fatty acids (LCFA), as spread. Butterfat, coconut oil and other palm kernel oils are rich natural sources of MCFA. Dietary MCFA are mostly contained in triglycerides, either pure MCT or in combination with LCFA. In mixed triglycerides, they occupy preferentially the sn-1 and sn-3 position.

Due to its unique absorption and metabolism characteristics, medium chain triglyceride (MCT) preparations have been used therapeutically since the 1950s, mostly for parenteral nutrition and in the treatment of long-chain triglyceride (LCT) malabsorption, or as concentrated source of energy for preterm infants. It is the aim of this review to look at the potential role of MCFA/MCT on health and performance in an everyday setting and in modulating Western life style health risks like the metabolic syndrome, which includes coronary heart disease (CHD), hyperlipidemia, diabetes type 2 (non-insulin dependent diabetes mellitus, NIDDM), hypertension and obesity.

2 DIGESTION AND LYMPHATIC TRANSPORT

MCFA are faster released from triglycerides than LCFA, because lingual, gastric and pancreatic lipases do (i) preferentially hydrolyse the sn-1 and sn-3 ester bonds of the triglycerides, and (ii) have also a higher activity towards short- and medium chain fatty acids than towards LCFA. The products of MCT hydrolysis are absorbed faster than those of LCT hydrolysis, and as fast as glucose [references in 1]. Recovery of MCFA in the lymph is usually low. One explanation is that they are in part digested and absorbed in the stomach [2]. Furthermore, after triglyceride hydrolysis in the intestine, short- and medium-chain fatty acids are transported directly to the liver via the portal venous system, whereas LCFA are incorporated into chylomicrons and transported through the lymphatic system to the peripheral circulation. This strict position, however, needs correction. All fatty acids use both portal and lymphatic transport systems in varying propor-tions. The degree of recovery of MCFA in the lymph is influenced by a number of factors which deserve closer attention.

(a) Lymphatic recovery of MCFA increases with chain length. Recovery of C10:0 was severalfold higher than of C8:0 [3,4], despite a higher proportion of C8:0 in the dietary fat. Lymphatic recovery in rats of MCFA from intra-gastrically infused structured triglycerides increased

with chain length, from 7.3 to 26.3 and 81.7% for C8:0, C10:0 and C12:0, respectively [5]. Similar recoveries were found for C10:0 [6,7]. But when a fat-rich diet was consumed orally for 3 wk, more than 30% of C8:0 and more than 60% of C10:0 appeared in the lymph chylomicrons [4].

(b) The latter observation may be due to oral uptake of fats within a mixed meal rather than gastic infusion of emulsions. But it might as well be due to long-term feeding practices, because this seems to increase MCFA appearance in lymph chylomicrons. After six days on a MCFA-rich dietary regimen the total amount of MCFA in chylomicron triglycerides of human plasma had increased from 8 to 15% [3]. One explanation for adjustment with time may be that oral administration of MCT increases octanoyl-CoA synthetase activity in rat intestinal mucosa nearly 2-fold, whereas oral administration of LCT does not affect palmitoyl-CoA synthetase activity [8]. This probably promotes incorporation of MCFA into triglycerides, as after CoA activation, octanoate, like palmitate, is incorporated into phospholipids and triglycerides.

(c) The positional distribution of fatty acids in the dietary mixed MCFA/LCFA triglycerides is highly maintained in the chylomicron triglycerides, no matter whether mainly LCFA [5,9] or MCFA [4] occupy the sn-2 position. This is not too surprising, because the fatty acid in the sn-2 position of triglycerides is preferentially absorbed as 2-monoglyceride and serves as a template for reesterification in intestinal cells. However, the proportion of total MCFA in chylomicron triglycerides was not affected by the position of MCFA in the dietary triglycerides [4].

(d) The simultaneous administration of MCT and LCT increased MCFA appearance in the lymph [10]. In some studies MCFA appearance was improved when physical mixtures or randomized oils were given instead of structured triglycerides with relatively more of the LCFA in sn-2 position [11-13], but this was not so in other rat studies [4,6,7,14].
Vice versa, combination of MCFA with LCFA improved LCFA absorption. Labeled linoleic acid in the sn-2 position was severalfold better absorbed in an ex-vivo system when MCFA rather than oleic acid occupied position sn-1 and sn-3 [15]. Other rat studies showed that absorption of LCFA in sn-2 position of LCFA/MCFA mixed triglycerides was even better than from a native soybean oil [14] or rapeseed oil [6] with equal or higher amounts of the LCFA at the sn-2 position.

(e) In order to address the question whether the position of the LCFA within the triglyceride molecules affects absorption efficiency, physical mixtures of fats or randomized triglycerides were compared with structured, i.e. chemically or lipase-interesterified lipids of the same fatty acid pattern. The latter contained LCFA primarily in the sn-2 and MCFA in the sn-1 and sn-3 position. Indeed, LCFA were better absorbed from structured triglycerides as compared to randomized triglycerides [14] or physical fat mixtures [13]. Similar trends were observed both in normal-absorbing and malabsorbing rats [6]. Such structured triglycerides improved also absorption of tocopherol and retinol as compared to a physical mix [16]. The proportion of MCFA in the serum, liver and adipose tissue triacylglycerols was not affected by the MCFA distribution of the dietary fats [17].

Yet there are two studies to the contrary. Upon infusion of structured rather than random triglycerides the accumulated lymphatic transport of eicosapentanoic (EPA) and docosahexanoic acid (DHA) was significantly higher during the first 8h too, but this difference disappeared after 24h [7]. This means that the absorption of the random structure triglycerides was merely prolonged as compared to a specific structure. Likewise, 24h accumulated lymphatic recovery of EPA and DHA was not increased after administration of a specific oil as compared to a randomized oil, a physical mixture of tridecanoin and fish oil, or fish oil alone [11]. In both studies, ω3-fatty acids were the predominant source of LCFA, and the total amount of LCFA in the dietary oils was lower than in the previously described ones. Maybe this gives a clue for the differing study outcomes.

(f) Previous chronic feeding of MCT seems not only to improve the lymphatic transport of dietary MCFA, but also to impair that of LCFA. In rats prefed for 4 wk a diet rich in MCT, the intraduodenal infusion of a LCFA emulsion increased total lymph lipid output within 3 h only weakly, while there was a pronounced increase in menhaden oil- and sunflower oil-prefed animals [18]. The authors explained this effect with a lower biliary phospholipid output in MCT-prefed animals, but did not provide evidence. Biliary phospholipids are essential for efficient lymphatic lipid output [19]. Another rat study showed indeed that 48h infusion of LCT emulsions increases biliary cholesterol and phospholipid concentrations and increases the lithogenic index, while MCT and MCT/LCT emulsions do not. Bile flow was not changed by the type of fat infused [20]. In a long-term rat feeding study, LCT but not MCT increased intestinal bile acid concentration [21]. In humans, a single liquid meal containing LCT increased bile acid flow to the intestine, while MCT did not [22].
In pigs [23], MCT and LCT infusions affected the secretory pattern of the exocrine pancreas differently. There was a significantly lower secretory volume and decreased lipase, and colipase output with MCT, and plasma cholecystokinin concentration was decreased. This suggests an immediate different response to fats of differing chain length. Similar changes were observed with long-term coconut oil as compared with fish oil feeding [24]. Nevertheless, fat digestibility was not different between groups.

3 INTERMEDIARY METABOLISM

The main differences in the intermediary metabolism of LCFA and MCFA are: (i) MCFA are less bound to albumin, (ii) MCFA do not have to combine with the fatty-acid binding protein in the cell, (iii) MCFA are able to penetrate the inner mitochondrial membrane independent of esterification to carnitine and without involvement of the carnitine-acylcarnitine translocase. Thus they quickly enter the mitochondria and undergo rapid beta-oxidation, whereas most long-chain fatty acids are packaged into triglycerides in the hepatocytes. It is generally assumed that MCFA do not require carnitine to cross the mitochondrial membrane, at least not in liver, kidney and heart [25]. Yet carnitine may to some degree support MCFA utilization. Intravenous infusion of MCT/LCT instead of LCT emulsions in healthy male subjects induced both

significantly lower plasma levels of free carnitine and higher levels of ß-hydroxybutyrate and short-chain acylcarnitine [26]. Long-chain acylcarnitine was decreased with simultaneous octanoate as compared to oleate infusion [27]. Urinary acylcarnitine excretion was higher in infants fed a MCT-enriched formula as compared to a low-MCT formula [28], and in diabetics on a MCT diet [29]. It was assumed that carnitine may facilitate removal from the liver if there is an excess short-chain acyl moieties generated by ß-oxidation, possibly for utilization by other tissues. No changes of plasma long-chain or short-chain acylcarnitine levels were observed in NIDDM subjects [29,30]. Another parameter to judge from is urinary dicarboxylic acid excretion. This is a minor pathway for MCFA elimination, but is seen as a useful marker of the efficiency of utilization of MCFA as a source of energy. Excretion increased with increasing proportions of MCFA from 0 to 50% in infant formulas [31a], and following a 30 d MCT diet in NIDDM patients [30]. When term infants were given MCT-enriched formulas with or without supplementary L-carnitine for 56 d, they excreted significantly less medium-chain dicarboxylic acids on the carnitine-supplemented diet [28]. Excretion was significantly higher with longer-term administration.

Once in the mitochondrial matrix, ß-oxidation is the almost exclusive fate of all fatty acids, whatever the chain length. The oxidation of MCFA is poorly affected by the nutritional or hormonal status of the body and the oxidation rate of MCFA is greater and faster than of LCFA [references in 31]. This has been confirmed by stable isotope tracer studies in humans [32,33] and by studies on the cellular level. Octanoate is oxidized to a greater degree than oleate in adipocytes [34] and isolated hepatocytes [25,35], whereas oleate is preferentially esterified both in adipocytes [34] and hepatocytes [25]. Both oxidation of octanoate and oleate was higher in hepatocytes of rats adapted to a MCT rather than a LCT diet [25]. In humans, the oxidation of endogenous LCFA was increased with longer-term MCT feeding [33]. The stimulation of octanoate oxidation supports the idea that there is an effect of long-term MCFA consumption on non-carnitine-dependent fatty acid transport. While oxidation rates of LCFA are usually depressed when the diet is simultaneously high in carbohydrates, this is not the case for MCFA [27].

Rapid oxidation is accompanied by increased ketone body formation. Their ketogenic character is one of the main properties of MCT [31]. Feeding studies in animals [25,36-38] and humans [39,40] have demonstrated that MCFA as compared with LCFA increase plasma ketone body concentrations. But increased ß-hydroxybutyrate levels were only observed in normotriglyceridemic non-diabetic subjects, while there was no such increase in diabetics after 5 d on a predominantly MCT dietary regimen [27]. Likewise, there was no significant change of plasma levels in NIDDM subjects on a MCT or LCT diet [30]. Ketone body production was severalfold higher with octanoate rather than oleate incubation in isolated hepatocytes [25]. Again, as with oxidation, ketone body formation was higher in hepatocytes of rats adapted to a MCT rather than a LCT diet [25], both with oleate and octanoate as substrate. But MCT-induced hyperketonemia in intact animals was attenuated with longer-time feeding [41].

Numerous studies have observed increased lipogenesis, i.e. de novo fatty acid synthesis and triglyceride synthesis, with MCFA as compared with LCFA [review in 31]. Increased liver lipogenic enzyme activities have been shown in rats chronically fed MCT rather than LCT [37,42-44], both in genetically lean and obese animals [45]. When rats were weaned on a carbohydrate-rich diet, the key lipogenic enzymes in liver, i.e. fatty acid synthetase (FAS) and acetyl-CoA-carboxylase (ACC), increased within the next nine days. When diets rich in both carbohydrates and MCT or LCT were supplied, this carbohydrate-induced increase was inhibited by LCT, but only delayed by MCT [46]. There were, however, tissue-specific differences. In the adipose tissue, both LCT and MCT inhibited the carbohydrate-induced increase of ACC activity, while only LCT inhibited the FAS activity increase (slightly) [46]. Lipogenesis was increased in isolated hepatocytes of rats chronically fed MCT rather than LCT diets [25], both with oleate or octanoate as substrate. The enrichment of plasma triglycerides with MCFA and palmitic acid in humans gives indirect evidence [47,48]. This phenomenon of increased lipogenesis seems at first sight in conflict with the firmly established observation that MCFA are preferentially used for oxidation instead of storage. But animal or man adapted to a diet containing MCT may carry out rapid ß-oxidation and ketogenesis at the same time as increased lipogenesis, at least when lipids/energy are provided in excess of requirement [25].

In *in vitro* systems lipolysis of MCT was faster than of LCT [49,50]. Rate of lipolysis decreased with increasing chain length [50]. Thus it seems not surprising that overall lipoprotein lipase (LPL) activity in human plasma was higher following a 6 h parenteral infusion of MCT instead of LCT emulsion [51] and that there was a faster clearance of the MCT emulsion [52]. There is an excellent correspondence between lipid hydrolysis and rate of clearance [52]. Likewise, the rate of triglyceride uptake into tissue is proportional to tissue LPL activity. Yet, one must pay attention to the fact that the effect on LPL activities of MCFA as compared to LCFA differs among tissues. This is in analogy to the organ-specific regulation of lipogenesis [46]. While LPL acitivity was higher with MCT instead of LCT in the rat diaphragm [52], as was hepatic lipase [52,53], it was actually decreased in adipose tissue [45,53] and heart [53]. This disparity between tissues helps to reconcile the fact that MCT feeding at the same time decreases fat deposition and increases lipogenesis and plasma triglyceride levels. It seems that MCT are preferentially directed towards liver and muscles rather than to adipose tissue. Rapid clearance promotes rapid oxidation. But in the liver, rapid oxidation may be accompanied by increased lipogenesis, as mentioned before.

In FAO rat hepatoma cells, as well as in normal rat hepatocytes, octanoate did not upregulate the expression of the gene for liver fatty acid-binding protein (FABP), while LCFA (palmitic, oleic, linoleic, linolenic and arachidonic acid) did so in a time- and dose-dependent manner [54].

Obviously the vast majority, but not all MCFA, undergo intrahepatic bioconversion [28]. However, the progressive rise of MCFA in the lymph with longer-term MCT administration [3] and the associated attenuation of hyperketonemia [41] suggests that extrahepatic tissues play increasingly important roles in the behavior of MCFA with time. The shift in partitioning between liver and

extrahepatic tissues may explain why some effects exerted with short-term admistration of MCT, like increased thermogenesis, may disappear with prolonged administration.

4 EFFECT ON PLASMA CHOLESTEROL

Saturated fatty acids are hypercholesterolemic when directly compared with mono- or polyunsaturated fatty acids [review in 55]. It was not examined in this context whether short- and medium-chain act in the same way as long-chain saturated fatty acids. There are some reports of a hypocholesterolemic effect of MCFA in animals [42, further references in 1]. Of the more recent rat experiments only one observed a hypocholesterolemic effect of MCT as compared with lard and trimyristin diets [21], but others found no different effect of MCT and LCT diets in lean and obese Zucker rats [36,45] or after a 14d intragastric administration of a TPN (total parenteral nutrition) solution containing either MCT/LCT or LCT alone [38]. Yet intravenous administration of the MCT-solution increased plasma cholesterol levels in this study [38].

Of the more recent studies in humans, two showed no effect on total cholesterol as com-pared to mono- and polyunsaturated long-chain fatty acids [47,56] or a non-significant de-crease [57], while others observed a hyper-cholesterolemic effect as compared to an oleic acid-rich fat [58,59] or corn oil [60,61]. Compared with a basal polyunsaturated fatty acid (PUFA) diet, C8:0 and C10:0 diets were slightly hypercholesterolemic, though less than a C12:0 diet [62]. But as no PUFA control group was carried through the 4 wk experimental period in this study, this might merely have been a nonspecific time effect. Except for one study [60] all test diets provided 40-53% of energy as fat. MCFA were C8:0 and C10:0, with only traces of C12:0.

There is no straightforward explanation for this discrepancy in cholesterol response. As cholesterol absorption was not differently affected by MCFA or LCFA in the diet [21], endogenous metabolic regulations must be responsible for any change of plasma cholesterol levels. On the basis of the data of Ney et al. [38] one may speculate that substrate availability for cholesterol synthesis may have played a role, as only intravenous, but not intragastric infusion of a MCT-LCT emulsion increased plasma cholesterol levels. Only with intravenous infusion there were also higher acyl-CoA concentrations in liver, which serve as substrate for both cholesterol and fatty acid biosynthesis, as well as higher liver cholesterol concentrations [38]. As rat liver is more central to total body cholesterol synthesis than is human liver, any shift of partitioning of substrate may be of more relevance to this species. The human experiments of Hill et al. [47] and Swift et al. [57] lasted just 6 d and used liquid formula regimen which contained almost exclusively either MCFA or LCFA, while the other human studies lasted 2-4 wk and approximately 20 to 40% of fatty acids in the MCT diet were non-MCFA. With the short time a new steady state may have not been reached yet. Hayes [63] concluded that the switch from a LCT to a MCT diet was mostly associated with a reduced intake of polyunsaturated fatty acids, and that the hypocholesterolemic C18:2 was mostly decreased below a 5-6% critical threshold concentration in the MCT diet fats. Thus, the apparent hypercholestesterolemic effect of MCT may merely highlight the lost hypocholesterolemic effect of C18:2.

5 EFFECT ON POSTPRANDIAL TRIGLYCERIDE RESPONSE

Plasma triglyceride levels rise after a fat meal and return to baseline levels 6-12 hours later. This means that humans spend most of the day in a postprandial state. Zilversmit postulated as early as 1979 that post-prandial lipoproteins are atherogenic. Epidemiological [64] and case-contol studies [65] showed a close positive correlation between postprandial triglyceride changes and CHD risk, and our group has suggested a link to the metabolic syndrome [66,67]. A number of studies showed that the postprandial triglyceride response, usually expressed as area under the postprandial curve (AUC), is more pronounced with saturated fatty acids rather than PUFA [68]. However, a comparison between butter- and olive oil-meals showed no higher postprandial total triglyceride response following the butter meal, but a higher chylomicron triglyceride response [70]. In two other studies the response to butter fat was compareable to that of a PUFA-rich oil [71,72]. This may be due to the higher amount of MCFA in butter fat. Numerous studies found that postprandial triglyceride response is lower with MCFA than with LCFA intake [3,47,57,60,73-76]. This was paralleled by a diminished lymph phospholipid and apo A-IV output [73].

Borel et al. [74] determined postprandial triglyceride response following two consecutive LCT meals, as compared to an (exclusively) MCT meal followed by a LCT meal. There was virtually no postprandial change of plasma triglyceride levels after the MCT meal. But under the MCT-LCT experimental design the response to the following LCT meal was significantly higher than under the LCT-LCT regimen, and the AUC of both periods combined was close to that of the LCT-LCT regimen. The authors concluded that a fraction of the MCFA had been stored temporarily in the mucosa and secreted after the second meal, and that LCFA are absolutely required for chylomicron formation. It may however be questioned whether lack of LCFA is a sufficient explanation, as chylomicron triglycerides are generally enriched in endogenous (long-chain) fatty acids [77]. Lack of phospholipids for chylomicron synthesis may be of more relevance [19,20,73].

One explanation for this low to absent postprandial triglyceride increase with MCT may be the low inflow of triglycerides, due to gastric digestion and absorption of MCFA and the preferential transport of absorbed MCFA in the portal vein instead of the lymph. Another one is faster clearance, as chylomicrons obtained from butter- and fish oil-fed rats were also more rapidly cleared from liver perfusates than remnants derived from olive, corn and palm oil [77]. MCT feeding resulted in smaller chylomicrons than PUFA-rich diets. These chylomicrons were faster cleared from the circulation when injected into chow-fed recipient rats [78]. The pattern of postprandial lymph fatty acids after infusion of a standard lipid emulsion was different if rats were chronically fed MCT as compared to sunflower or menhaden oil or a low-fat diet, with relatively more oleic and less linoleic acid following the chronic MCT feeding [18]. The positional distribution of MCFA in the dietary triglycerides had no significant effect on lymph flow, triglyceride output, lipid composition, or particle size. Nevertheless, positional distribution of MCFA in lymph chylomicron triglycerides may be relevant to the catabolism of chylomicrons in blood

plasma, because chylomicrons containing triglycerides with MCFA in sn-2 position have a prolonged half-life in the circulation [4].

Borel et al. [74] observed an impaired absorption of ß-carotene and retinyl palmitate administered together with MCT. But again, they used exclusively MCT, while MCFA in combination with LCFA may act quite differently. Indeed, there was a significantly better absorption of gastrically administered labeled tocopherol and retinol, when animals were fed randomly interesterified fish oil/medium-chain structured triglycerides, i.e. when more of the long-chain fatty acids were in the sn-2 position, rather than a physical mixture of fish oil and MCT [16]. Furthermore, as demonstrated by Mendeloff as early as 1954, a retinyl palmitate peak may appear later after a second meal, independent of the fat content of this meal. Anyhow, such a delayed postprandial appearance is not necessarily limited to MCFA intake. Several researchers observed secondary postprandial triglyceride peaks and found that fat from a first meal contributes to the triglyceride response of a consecutive meal [review in 69].

It might be assumed that chronic feeding of MCFA attenuates postprandial triglyceride response less, as there is an increasing proportion of MCFA in the lymph with chronic feeding. But in rats prefed for 4 wk a diet rich in MCT, even the intraduodenal infusion of a LCFA emulsion did hardly increase total lymph lipid output, while there was a pronounced increase in low fat-fed and even more in menhaden oil- and sunflower oil-prefed animals [18]. Labeled oleic and arachidonic acids from the emulsion did appear in the lymph, but in MCT-prefed animals they were obviously only weakly diluted with endogenous fatty acids.

The degree of postprandial triglyceride response is positively associated with levels of several pro-coagulant CHD risk factors like factor VII [71,79]. Meals rich in MCT do usually not induce factor VII activation [76,79], though in one case the response to a butter-, an oleate- and a MCT-oleate-rich diet was similar [80]. Thus, MCFA may or may not exert a positive effect on these CHD risk factors, and the habitual background diet may affect the degree of response.

6 EFFECT ON PLASMA FASTING TRIGLYCERIDE LEVELS

Once MCFA are preferred over LCFA for oxidation, one might assume that this results in lower plasma triglyceride levels. While there is one rat study which observed no different effect of MCT vs. LCT on plasma triglycerides [36], other animal studies showed that longer-term MCFA consumption causes hypertriglyceridemia [45,53, more references in 31]. Ney et al. [38] found a hypertriglyceridemic effect of MCT both with intravenous and intragastric infusion of a TPN solution. Also, four of the above mentioned human studies [47,57,58,61] observed significantly higher fasting serum triglycerides with MCFA in-stead of LCFA consumption.

Basically, two metabolic processes could be responsible for this phenomenon, increased triglyceride synthesis and decreased triglyceride clearance from the plasma. It is universally recognized that lipogenesis is down-regulated when a low-fat diet is shifted to a high-fat one, but MCFA seem to operate less efficiently. As pointed out before, MCT diets, especially when fed in excess of caloric needs, might lead to increased de novo fatty acid synthesis and enhanced fatty acid elongation activity by the liver. This, in turn, would be expected to increase hepatic triglyceride production and very low-density lipoprotein (VLDL) secretion, and could account for the elevated fasting plasma triglyceride levels.

As mentioned before, LPL activity in human plasma is higher following MCT instead of LCT [51], and there is faster clearance of intravenously administered MCT emulsions [52]. This might protect from increased plasma triglyceride levels. But as LPL activity is actually less with MCT instead of LCT in adipose tissue [45,53,78], while hepatic lipase activity is increased [53], MCT are less readily available to adipose tissue, but are readily available to muscles and liver. In the liver, rapid oxidation may be accompanied by increased lipogenesis. In conclusion, reduced LPL activity in adipose tissue may indirectly contribute to fasting hypertriglyceridemia.

To some degree a calculatory aspect may contribute to an (apparent) hypertriglyceridemic effect of MCT. If infused or fed on an equal weight basis with LCT, MCT provide about 1.8 times more triacylglycerol molecules than LCT [52].

7 EFFECT ON WEIGHT CONTROL

In westernized countries, relative adiposity is a growing health problem affecting a sizeable proportion of the population. It is generally assumed that a high fat intake favors excess energy intake. The lack of efficiency of classical treatments for obesity has led to search for alternative strategies. It is widely specu-lated that exchange of LCFA for MCFA may favor weight control, as MCFA are preferentially oxidized rather than deposited in adipose tissue triglycerides. The arguments in favor of or against a role of MCFA in weight control have been extensivly reviewed by Bach et al. [31].

There is indeed scientific evidence suggesting that MCFA may facilitate weight regulation or even promote weight loss. Due to the lower molecular weight of a MCFA molecule the energy density on a per weight basis is lower for MCT than LCT. Some human experiments suggested that spontaneous behaviour is such that food and thus energy intake is less with MCFA than with LCFA consumption. When men were put on high-fat diets for 14 d, they consumed less energy when MCT was the predominant dietary fat [81]. MCT in the breakfast decreased also lunch size and thus lunch energy intake [40]. But there was no such decreased food intake in rat experiments [37,44].

Decreased weight gain was observed in rat studies [35,37,41,43,45,82,83]. Weight gain was avoided with MCT in contrast to LCT in a high-fat diet of humans [81]. Japanese overweight subjects (BMI ≥ 23 kg/m^2) on a weight maintenance diet with 10g MCT per day and fat providing 26% of total energy lost more weight within 8 wk than control subjects [56]. There was also a 13% greater weight loss with MCT instead of LCT in obese women on a hypocaloric diet for 4 or 12 wk, with fat providing 30% of dietary energy [84]. This effect was not significant, possibly due to the small number of participants, or because total fat intake was very low. There are reports of increased resting metabolic rate with MCT instead of LCT in rats [45,85] and humans [86]. When rats were starved for two weeks and then refed either a

low-fat diet or various fat-rich diets, coconut oil and safflower oil diets resulted in high energy expenditures, in the case of coconut oil very close to that of low-fat refed animals, quite opposite to lard [87]. An increased postprandial energy expenditure was also observed in human studies [39,86,88,93]. Seaton et al. [39] calculated that this increase was equivalent to 13% of the energy contained in the MCT meal. This diminished energy efficiency may be related to the inefficiency forcibly associated with the cycling of carbons between ß-oxidation and lipogenesis. White et al. [86] found that the increased postprandial energy expenditure with MCT rather than LCT diets was less after 14 rather than after 7 d, and that the difference was then no longer significant. This study was somewhat unusual in that a mixture of butter and coconut oil was used as source of MCFA, which provided twice as much C12:0 as C8:0-10:0. This may explain the moderate effect to start with, and the (apparent) attenuation with time. Nevertheless, metabolic adaptations, showing attenuated effects with longer-term MCFA feeding, have been observed in animals and humans. This raises the question whether MCFA hold a potential as weight-reducing agent in the long run. Besides, the efficacy of MCFA in reducing body weight is expected to diminish in proportion to its dilution in mixed regimens [31].

Rat feeding experiments showed that MCT feeding diminishes fat deposition [37,43,45,82,83,85,89]. In humans the area of subcutaneous fat was significantly more reduced with MCT rather than LCT [56]. As outlined before, MCFA were more efficiently oxidized than LCFA [25,32,33-35]. LPL activity and thus fatty acid uptake was lower in adipose tissue with MCT instead of LCT feeding [45, 53]. Furthermore, octanoate did not stimulate preadipocyte differentiation, while oleate did [34]. Adipocytes were smaller in animals on a MCT instead of a LCT feeding regimen [45,89]. A recent study showed that the ß-oxidation of LCFA is impaired in obese as compared to normal-weight subjects, but that this is not the case for the oxidation of MCFA [90]. Therefore, MCFA may also be of interest in the dietary management of existing obesity.

8 EFFECT ON DIABETES RISK

In addition to genetic predisposition, dietary factors have been linked to the pathogenesis of insulin resistance, especially a high intake of fat. A high-fat, low-carbohydrate intake reduces the ability of insulin to suppress endogenous glucose production and decreases glucose oxidation [91]. LCFA and MCFA are different in this respect: When LCT or a 50:50 mixture of MCT and LCT were infused concomitantly with glucose and insulin in healthy subjects, in amounts similar to those used in total parenteral nutrition, LCT infusion decreased glucose oxidation (expressed in percent of total glucose disappearance) significantly more than MCT/LCT [92]. A clamp study with parallel infusions of [1-^{14}C]oleate or [1-^{14}C] octanoate in healthy subjects showed that a high glucose supply decreases oleate, but not octanoate oxidation [27]. Oxidation rates of LCFA are usually depressed when the diet is simultaneously high in carbohydrates. This means that glucose controls entry of LCFA, but not of MCFA, into mitochondria.

It has often been reported that administration of MCT over the short- or long-term decreases plasma glucose levels, which is explained by enhanced insulin-mediated glucose disposal [39, more references in 25]. The stimulation of insulin secretion is in turn attributed to increased ketone body production [31]. But many studies found no change in fasting glucose levels with chronic MCT instead of LCT administration in rats [36,38,83] or humans [30,47,84]. Likewise, there was no significant change of fasting insulin levels in rats [38,45,83,89] or humans [30,47]. There was no different postprandial insulin response in humans [40,47,93], while postprandial glucose response was either not different [40], or significantly [30] or non-significantly [47] lower with MCT instead of LCT diets.

Clamping studies were performed in humans on either a MCFA or LCFA-rich diet to determine the impact of MCFA consumption on insulin sensitivity. One experiment was carried out in NIDDM and nondiabetic hypertriglyceridemic and normotriglyceridemic subjects after 5 d on a diet with LCT or 80% MCT plus 20% LCT (crossover design). Fat did provide 40% of energy [29]. Another euglycemic clamp study was done in healthy women who had been on a 800 kcal hypocaloric diet for 4 or 12 weeks, either LCT or (largely) MCT, with only 30% of energy as fat [84]. Both studies found an enhanced insulin sensitivity following MCFA, i.e. there was an increased glucose requirement to maintain euglycemia during clamp. But when another insulin/glucose euglycemic clamp experiment was performed in NIDDM subjects who had been on such an either LCT or largely MCT diet for 30 d (crossover design), MCT did not improve insulin-mediated glucose metabolism [30], despite the above mentioned attenuated postprandial glucose response. It might be that the dietary intake of the free-living subjects of Yost et al. [30] was probably not so closely controlled as the formula diets in the other two studies, or that the amount of MCFA (43% instead of 80% of the dietary fatty acids) was too low.

9 EFFECT ON HYPERTENSION

Hypertension is another feature of the metabolic syndrome, often associated with insulin resistance and obesity. However, MCT, as compared to safflower oil or a chow diet, did not affect the blood pressure [83].

10 EFFECT ON EXERCISE PERFORMANCE

Persons who have undergone endurance training have greater fat oxidation during exercise than untrained persons exercising at the same absolute intensity. The substrate for this increased fat oxidation are non-plasma-derived fatty acids, perhaps from intramuscular stores. But while oleate oxidation was higher in the trained group, there was no difference of octanoate oxidation between trained and untrained sedentary subjects [94]. Fatty acid oxidation during endurance exercise permits sustained physical performance and delays glycogen depletion and hypoglycemia. As parenteral lipid supplementation during exercise increases fat oxidation [95], the question was raised whether dietary MCFA might improve fat oxidation, too, and might provide an alternative carbon source for muscles during prolonged exer-

cise. MCFA might have several advantages over LCFA: They are more rapidly emptied from the stomach and more rapidly hydrolysed and absorbed. They are not esterified but instead transported quickly to the mitochondria for oxidation, independent of carnitine. And last but not least, MCFA are metabolized as fast as glucose. The next question is whether MCFA should be administered on its own or in combination with carbohydrates in order to improve endurance performance. Carbohydrate availability may directly regulate fat oxidation during exercise. But whereas glucose inhibited LCFA oxidation, it did not inhibit MCFA oxidation [94,96]. MCT coingested with carbohydrates were even more rapidly oxidized than MCT alone, i.e. maximal MCT oxidation rates were reached more quickly [97].

Few studies have investigated the effect of MCT ingestion on exercise performance. In two experiments of similar design [97,98], amounts of 85 g MCT were given as drinks, successively during continued cycling performance. Reference groups received carbohydrate drinks, carbohydrates plus MCT, and, in one of the studies, water as control. Van Zyl et al. [98] observed a better performance with glucose plus MCT than with glucose alone, while MCT alone showed an impaired performance. The study of Jeukendrup et al. [97] found no difference in performance with glucose, glucose plus MCT or water, while MCT alone had also a negative effect. The two studies differed in several aspects: duration of the performance test (60 or 15 min), time of the day (morning or early afternoon), the type of MCT used (C8:0 plus C10:0 or C8:0 only) and the carbohydrate used (short-chain glucose polymers or glucose). Nevertheless, Jeukendrup et al. [97] attributed the absence of an MCT effect mainly to gastrointestinal complaints reported in the experimental subjects. In summary, the majority of studies investigating the role of MCT on exercise found no sparing effect of muscle glycogen after consumption of MCT. At present, there is insufficient evidence to recommend that athletes either ingest fat in the form of MCT during exercise, or "fat-adapt" in the weeks prior to a major endurance event in order to improve athletic performance [99].

11 EFFECT ON IMMUNE RESPONSE

Another interesting aspect is the role of MCFA in the modulation of immune response, in the intestine and possibly beyond. There are different proinflammatory activities of MCFA and LCFA in the intestine. Both enhanced IL-1ß- and TNF-α-induced inflammatory responses in an intestinal epithelial cell line, but the effect of LCFA was stronger than of MCFA [100]. Absorption of oleic acid but not of octanoic acid produced a significant elevation of lymphocyte flux and increased proliferative response of lymphocytes in intestinal lymph [101]. LCT as compared to MCT feeding markedly enhanced mucosal damage in experimental enteritis in rats [102] and increased jejunal permeability in piglets, another model of intestinal injury [103].

12 PERSPECTIVES

This review lists potentially positive and negative effects of MCFA on health and physical performance. Very interesting is the weak postprandial triglyceride response and the decreased lipid deposition in adipose tissue. Quite a few claims raised about the health benefits of MCT are questionable or no yet answered conclusively. Furthermore, it needs to be ascertained whether all effects observed in rats hold also true for humans, particularly if peroxisome proliferator-activated receptors (PPARs) should be involved in gene regulation of relevant enzymes [104].

Questions which deserve more attention are first, how human metabolism adapts to long-term consumption of MCFA. There are reports that increased postprandial thermogenesis and insulin sensitivity are attenuated with longer administration, and that ketone body production is diminished. Secondly is the question of how MCFA behave in combination with long-chain fatty acids. A number of the "classical" studies did a complete exchange of LCT for MCT, and not much attention was paid to the fatty acid pattern of the LCT meal or diet. What would be the outcome if MCFA were given in reasonable and practical amounts for everyday diets, and if compared with saturated long-chain fatty acids? Thirdly, more information needs to be obtained concerning the impact of MCFA on the metabolism of LCFA. MCFA in structured triglycerides may improve the absorption of essential polyunsaturated LCFA. This is an interesting feature and highlights the potential of "tailor-made" triglycerides for special needs, lipids which combine the benefits of both short-chain and long-chain polyunsaturated fatty acids. It is improbable that consumption of natural MCFA-rich foods alone could mediate the positive effect of MCFA, at least not without concomitantly introducing the negative effects of longer-chain saturated fatty acids. Intake of pure MCT preparations is limited by the fact that high amounts of MCFA may cause gastrointestinal com-plaints and because monotony may arise if one would have to give up tasty fat-rich natural foods for rather bland MCT. For sure, MCT are not a "calorie-free" fat, and whatever the question asked, total energy supply and the percentage of fat in the diet will most probably affect the outcome of a study.

An interesting feature of MCT under aspects of functionality is their transport-enhancing effects across the intestinal mucosa for hydrophilic bioactive compounds and drugs. Among the fatty acids and acylglycerols tested, diacylglycerols (1,2-dicaproin), monoacylglycerols (monocaprin, monocaprylin) and sodium salts of MCFA effectively enhanced the transport across Caco-2 cell monolayers on a permeable support [105,106]. This effect was proportional to the applied dose at lower concentrations, but levelled off at higher concentrations.

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Health benefits and safety evaluation of certain food components

Food microorganisms - health benefits, safety evaluation and strains with documented history of use in foods

G. Mogensen, S. Salminen, J. O'Brien, A. Ouwehand, W. Holzapfel, C. Shortt, R. Fondén, G.D. Miller, D. Donohue, M. Playne, R. Crittenden & B. Bianchi Salvadori

This scientific summary reviews the latest research related to health benefits and safety evaluation of lactic acid bacteria (LAB) and introduces an inventory of food microorganism species with a history of safe use in foods.

6pp - English only

Index: acid; bacteria; culture; food; health; inventory; lactic; microorganism; safety; strain

Inventory of Microorganisms with adocumented history of use in food

J. Seifert & G. Mogensen

In order to classify traditionally used microorganisms (starter cultures) as safe food ingredients, the IDF in collaboration with EFFCA (European Food and Fed Cultures Association) has compiled an inventory of microorganisms with a documented history of use in food. The inventory is contained in full in this paper.

10pp - English only

Index: acid; bacteria; cheese; culture; fermentation; food; inventory; lactic; microorganism; probiotic; strain; yoghurt

Trans Fatty Acids

Y. Soustre, B. Laurent, J. Schrezenmeir, M. Pfeuffer, G. Miller & P. Parodi

The expression 'trans fatty acids' encompasses a large number of substances having highly heterogeneous roles. Several experimental and epidemiological studies suggest that trans fatty acids (mainly elaidic acid) would seem to have negative effects on plasma cholesterol levels and the incidence of cardiovascular disease, as described in this paper.

12pp - English only

Index: acid; bacteria; cardiovascular; cis; disease; fatty; food; health; isomer; meat; milk; oil; trans; vegetable

Milk lipids in diet and health – Medium Chain Fatty Acids (MCFA)

M. Pfeuffer & J. Schrezenmeir

Due to its unique absorption and metabolism characteristics, medium chain triglyceride (MCT) preparations have been used therapeutically since the 1950s, mostly for parenteral nutrition and in the treatment of long-chain triglyceride (LCT) malabsorption, or as concentrated source of energy for preterm infants. This paper looks at the potential role of MCFA/MCT on health and performance in an everyday setting and in modulating Western life style health risks.

11 pp - English only

Index: acid; chain; cholesterol; diabetes; fatty; health; hypertension; lipid; long; lymph; medium; metabolism; saturated; triglyceride

Total: 42 pp

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(d) volume number
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(d) Name of the publishers & city or town
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Example: 2 Walstra, P. The role of proteins in the stabilization of emulsions. In: G.O. Phillips, D.J. Wedlock & P.A. William (Editors), Gums & Stabilizers in the Food Industry - 4. IRL Press, Oxford (1988).

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"	*Usually double quotes and not single quotes*
? !	*Half-space before and after question marks, and exclamation marks*
±	*Half-space before and after*
micro<u>oo</u>rganisms	*Without a hyphen*
Infra-red	*With a hyphen*
et al.	*Not underlined nor italic*
e.g., i.e.,....	*Spelled out in English - for example, that is*
li<u>tre</u>	*Not liter unless the author is American*
ml, mg,...	*Space between number and ml, mg,...*
skimmilk	*One word if adjective, two words if substantive*
sulfuric, sulfite, sulfate	*Not sulphuric, sulphite, sulphate (as agreed by IUPAC)*
AOAC <u>International</u>	*Not AOACI*
<u>programme</u>	*Not program unless a) author is American or b) computer program*
milk and milk product	*rather than "milk and dairy product" - Normally some latitude can be allowed in non scientific texts*
-ize, -ization	*Not -ise, -isation with a few exceptions*
Decimal comma	*in Standards (only) in both languages (as agreed by ISO)*

No space between figure and % - i.e. 6%, etc.

Milkfat	*One word*
USA, UK, GB	*No stops*
Figure	*To be written out in full*
1000-9000	*No comma*
10 000, etc.	*No comma, but space*
hours	*ø h*
second	*ø s*
litre	*ø l*

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[2] Danish Dairy Board .....

IDF does not spell out international organizations

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