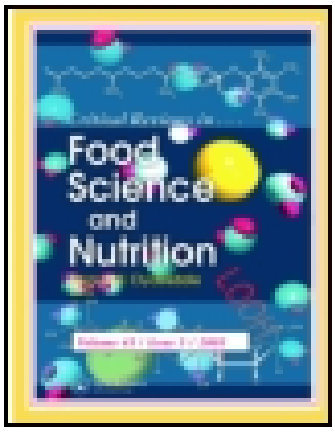

Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

Evaluation of the Impact of Ruminant trans Fatty Acids on Human Health: Important Aspects to Consider

Katrin Kuhnt[a], Christian Degen[a] & Gerhard Jahreis[a]
[a] Friedrich Schiller University, Institute of Nutrition, Dept. of Nutritional Physiology, Dornburger Str. 24, Jena, Germany, ,
Accepted author version posted online: 06 Mar 2015.

Evaluation of the impact of ruminant *trans* fatty acids on human health: important aspects to consider

Katrin Kuhnt*, Christian Degen, Gerhard Jahreis

Friedrich Schiller University, Institute of Nutrition, Dept. of Nutritional Physiology, Dornburger Str. 24, Jena, Germany

E-mail addresses:

* To whom correspondence should be addressed, katrin.kuhnt@uni-jena.de

Christian Degen: christian.degen@uni-jena.de

Gerhard Jahreis: b6jage@uni-jena.de

Abstract

The definition and evaluation of *trans* fatty acids (TFA) with regard to foodstuffs and health hazard are not consistent. Based on the current situation, the term should be restricted only to TFA with isolated double bonds in *trans*-configuration. Conjugated linoleic acids (CLA) should be separately assessed. Ideally, the origin of the consumed fat should be declared, i.e., ruminant TFA (R-TFA) and industrial TFA (non-ruminant; I-TFA).

In ruminant fat, more than 50% of R-TFA consists of vaccenic acid (C18:1 *t*11). In addition, natural CLA, i.e., *c*9,*t*11 CLA is also present. Both are elevated in products from organic farming. In contrast to elaidic acid (*t*9) and *t*10 which occur mainly in partially hydrogenated industrial fat, *t*11 is partially metabolized into *c*9,*t*11 CLA via Δ9-desaturation. This is the major metabolic criterion used to differentiate between *t*11 and other *trans* C18:1. *t*11 indicates health beneficial effects in several studies. Moreover, CLA in milk fat is associated with the prevention of allergy and asthma. An analysis of the few studies relating to R-TFA alone makes clear that no convincing adverse physiological effect can be attributed to R-TFA. Only extremely high R-TFA intakes cause negative change in blood lipids.

In conclusion, in most European countries, the intake of R-TFA is assessed as being low to moderate. Restriction of R-TFA would unjustifiably represent a disadvantage for organic farming of milk.

Keywords:

elaidic acid; vaccenic acid; conjugated linoleic acids; milk; lipoproteins; industrial TFA; organic farming; Tissues.

Review contents

1. Introduction

It is currently a matter of debate as to what extent ruminant *trans* fatty acids (R-TFA) raise the risk for cardiovascular diseases in comparison with non-ruminant industrially derived TFA (I-TFA). Epidemiological studies suggest that I-TFA generally have a negative effect on serum cholesterol and lipoprotein metabolism thereby increasing the risk for coronary heart disease. This is because of the high I-TFA intake resulting from ingestion of partially hydrogenated vegetable oils (PHVO) found in processed foods. However, regarding the relationship between ingested quantities of TFA and the associated risk of cardiovascular disease (CVD), R-TFA have to be evaluated separately to I-TFA. Moreover, the use of the term R-TFA which may include fatty acids with conjugated *trans* double bonds is inconsistent. Therefore, in this review we

discuss a number of aspects relevant to this research field and incorporate current studies as well as our latest as yet unpublished data.

2. Differences in *trans* isomer distribution in relation to TFA origin

In order to evaluate the difference between natural and industrial TFA with regard to their relevance for human health, the differing amounts of individual *trans* isomers must be considered in relation to the source of the fat. I-TFA originate mainly from catalytic hydrogenation of vegetable and fish oils (fat hardening) into solid or semi-solid PHVO. In contrast, R-TFA in milk, dairy products, and in the meat of ruminants are a result of bacterial biohydrogenation in the rumen. Therefore, I-TFA and R-TFA show clear differences in terms of isomer distribution and TFA fraction within the fat.

In addition, ruminant fat contains a maximum of 8% R-TFA, which is considerably less than the I-TFA content in PHVO still with up to 50%.

Trans hexadecenoic acids (trans C16:1)

In ruminant fat, small amounts of *trans* C16:1 isomers were found depending on the feed (0.3 - 0.8% of fat; Luna et al. 2009). In milk fat, the pattern of *trans* C16:1 was very similar to *trans* C18:1, but with a predominance of C16:1 *trans*9 (also published as *trans* palmitoleic acid) at about 30% of total *trans* C16:1 (Destaillats et al. 2000; unpublished observation).

Trans octadecenoic acids (trans C18:1)

In industrially produced food, mainly monounsaturated *trans* isomers of C18 but also some

polyunsaturated *trans* isomers of C18:2 and C18:3 are present. TFA found in food are identical in chemical structure but differ in amount (Kuhnt et al. 2011). Elaidic acid (C18:1 *trans*9; *t*9) and C18:1 *trans*10 (*t*10) dominate in food products containing PHVO. Milk fat contains many fatty acids with isolated and conjugated *trans* double bonds (Kramer et al. 2008). Here, vaccenic acid (C18:1 *trans*11; *t*11) prevail comprising about 40 - 80% of the total *trans* C18:1 isomers (Precht et al. 2001; Destaillats et al. 2007). The fatty acid profile of milk fat depends amongst others things to a great extent on the feed.

Conjugated linoleic acids (CLA)

Fatty acids with conjugated *trans* double bonds, e.g. CLA, are scarcely found in industrial PHVO. However, when CLA are present, these are in the form of *trans*,*trans* CLA and *t*10,*c*12 CLA (Table 1; unpublished observation). Industrially manufactured CLA products from vegetable oils usually contain two main isomers: *c*9,*t*11 and *t*10,*c*12 (Table 1). In contrast, in ruminant derived fats, the *c*9,*t*11 isomer is largely formed and accounts for 70 - 80% of the total CLA in dairy and meat products. The percentage of *t*10,*c*12 CLA in ruminant fat is very low, i.e. less than 5% of total CLA (<0.1% of fat; Table 1). In general, CLA isomers have shown a variety of biologically beneficial effects. CLA reduce body fat, CVD and cancer, and modulates immune and inflammatory responses as well as improves bone mass (rev. by Dilzer and Park 2012).

Conjugated trienes

In addition to CLA, other conjugated fatty acids with two and three conjugated double bonds have gained importance within the last decade due to their *in vitro* and *in vivo* anti-tumorigenic

activity (Degen et al. 2011a, Hennessy et al. 2011). Certain seed oils, especially common in Asian foods are rich in conjugated linolenic acids (CLnA) such as pomegranate (*c*9,*t*11,*c*13 CLnA), snake gourd, and bitter gourd (*c*9,*t*11,*t*13 CLnA; Table 1). Isomers of C18:3 with two conjugated double bonds also occur in ruminant fat but only in marginal amounts (e.g., *c*9,*t*11,*c*15; *t*9,*t*11,*c*15 C18:3; Table 1). Interestingly, CLnA can be converted to CLA (rev. by Hennessy et al. 2011, Degen et al. 2011a).

3. Definitions and regulations of TFA contents in foodstuffs

Regulations for including R-TFA and CLA with *trans*-double bonds into the TFA group and in foods differ from country to country. In some cases there exist no policies in this respect (Table 2).

In general, TFA can be defined according to the chemical and structural properties, physiological effects as well as origin. Irrespective of their genesis, the European Food Safety Authority (EFSA 2004) embraces CLA under the term TFA. However, the Food and Drug Administration (FDA 2003) limits the term TFA to its chemical structure, i.e. the FDA only refers to fatty acids with *trans* double bonds as TFA which excludes CLA. Due to the isomer-specific effects of some CLA (esp. *c*9,*t*11 vs. *t*10,*c*12) and the heterogeneous data available from human studies (Terpstra 2004, Wahle et al. 2004, Pariza et al. 2001), it seems plausible to exclude CLA from the group of TFA. Moreover, the definition of TFA based only on negative physiological effects is not possible, because some isolated *trans* isomers are not associated with negative effects, at least there is no evidence for this to date.

TFA can also be classified according to their genesis. At present, the focus is on the exceptional position of R-TFA resulting from biohydrogenation. Here, *t*11 thus receives special attention due to its higher concentration in R-TFA and because it acts as a precursor for *c*9,*t*11 CLA in human metabolism (Kuhnt et al. 2006). In addition, the high CLA content (*c*9,*t*11 CLA) in ruminant fat sets R-TFA (if CLA are included in the definition) apart from I-TFA. Regarding the origin of CLA, one could differentiate between naturally occurring (e.g., *c*9,*t*11 CLA; R-CLA) and industrially produced CLA (I-CLA).

The "Codex Committee on Food Labeling" and "Codex Committee on Nutrition and Foods for Special Dietary Uses" agreed in 2004 to label only fatty acids with isolated *trans* double bonds as TFA, i.e. to exclude CLA (Nishida and Uauy 2009). According to the WHO, efforts to reduce TFA content in foodstuffs are preferentially limited to PHVO-derived I-TFA and do not extend to R-TFA. However, the reduction of R-TFA intake was not part of this discussion (Nishida and Uauy 2009; Table 2). In general, the alleviation can be attributed to a low total R-TFA and CLA intake in relation to I-TFA intake.

4. Development and TFA intake data

According to an assessment by the WHO, TFA intake should be kept low in order to lower the risk of CVD (Nishida and Uauy 2009). It is recommended that TFA intake not exceed 1% of the energy intake (en%). Comparison with prior data, the TFA intake has currently decreased as a result of the implementation of labeling requirements, especially in the USA and Canada. The mean TFA intake of the US population was 2.5 en% (6.1 g/day, 1999 - 2002) from major food sources as cakes, cookies, pies, and pastries (Kris-Etherton et al. 2012). Other data confirmed

this (I-TFA 4.6 g/d, FDA 2003 to 1.3 g/d, Doell et al. 2012). In Europe, due to introduction of regulations and to efforts undertaken by the industry to reduce TFA content in foodstuff, TFA intake has also decreased, although the initial values were not really high. Unfortunately, availability of current data on TFA intake in Europe is limited. The Federal Institute of Risk Assessment Germany (BfR) estimated the TFA intake in Germany between 2005 and 2007 data as being below 1 en% (Table 3).

Whilst data from 1976 reveal the mean TFA intake in Western Europe as being 6 g/d, this value decreased to 2.6 g/d in 1996 according to results of the TRANSFAIR study (Stender et al. 2006). The average estimated TFA intake in the early 1990s was calculated as 1.9 g/d for women and 2.3 g/d for men (Fritsche and Steinhart 1997). However, several products with an alarming 35 - 50% TFA content in their fat are still available on the market (Stender et al. 2008, Kuhnt et al. 2011), probably representing the situation in other European countries. Therefore, individuals with certain dietary habits may still consume high amounts of I-TFA if certain brands or types of food products are frequently chosen.

Current calculations of the BfR based on the data of the National Nutrition Survey (NVS II) yield for Germany's 14 to 80 year-old subjects, gives an average total TFA intake as 1.94 g/d (Table 4). This corresponds to 0.77 en%. Men show a higher TFA intake (2.3 g/d; 0.80 en%) than women (1.6 g/d; 0.74 en%; Gabriel 2009).

With a declining I-TFA intake, the amount of R-TFA remains nearly constant, therefore the relative portion of R-TFA has risen to 86% of the total TFA intake (Jakobsen et al. 2006). Except for the USA, Canada and Iceland, data from other countries reviewed demonstrate that the largest proportion of TFA originates from ruminant fat (Table 3). Estimating the R-TFA intake is

made difficult by the fact that several different factors have to be taken into consideration (fat content of the foodstuff, feed, species of animal, processing of food, mixed fats, etc.). The daily intake of milk and dairy products established as part of the National Nutrition Survey II (2005 - 2007) revealed that men consumed on average of 265 ± 291 g/d and women 244 ± 209 g/d of milk and dairy products (Hilbig 2009). Our own calculations based on the data of the National Nutrition Survey II, the Max Rubner Institute and the Federal Ministry for Agriculture and Consumer Protection (BMELV) show a milk fat consumption of 20 – 45 g/d for the German population. The actual calculation of the R-TFA percentage in Germany amounts to about 50% of the consumed fat based on the appraisals of the BfR (Gabriel 2009; Table 3). This is due to the fact that butter and dairy products make up about half of the TFA intake. However, this proportion is believed to be higher, because cakes, biscuits and pastries may contain mixed fats with a ruminant fat portion. In addition, since meat products (beef, mutton) also contain R-TFA, the R-TFA proportion of total ingested TFA consumed in Germany amounts to about 60 - 80%. In addition, the ingested portion of R-TFA would be rather constant in the population. For example, in consumers with high total TFA intake, the determination of *t*11 portion showed that dairy products did not account for the higher TFA intake (Gabriel 2009).

5. Current TFA contents and *trans* C18:1 distribution in food products on the German market

Since industry has in part modified their fat hardening methods (food reformulation, genetic modification of FA composition, interesterification; Menaa et al. 2013), the TFA proportion in foodstuffs has been decreasing. Recent analyses of food products in the German market support a

general TFA decline (Kuhnt et al. 2011). In comparison to the past analyses, deep-fried potato products, e.g. chips and fries showed lower TFA contents of 0.5% in fat. In addition, commercially available baking and spread margarines showed decreasing TFA contents compared to prior analyses. In contrast, technologically utilized wholesale baking margarines showed considerably higher TFA contents. In particular the use of these industrial fats is responsible for the high TFA contents of 4.5% in the fat of the analysed bakery products such as Danish pastry, puff pastry, and doughnuts. Moreover, the use of PHVO in foods is not declared in over-the-counter bakery products (e.g., in Germany). The additional high fat content of these products (20 - 35%) is responsible for the high absolute TFA intake per consumed portion (Kuhnt et al. 2011). Therefore, amongst others bakery products and sweets represent a potential target for minimizing the TFA intake. Such strategies have been planned and initiated (oral communication; e.g., guidelines for minimizing TFA in foods; BMELV 2012). A trend to reduction of such *trans* fats in bakery products can be seen for example in Spanish bakery products in which the TFA content was below 1% according to a study in 2012 (Ansorena et al. 2013).

In the above mentioned analyses, the *trans* C18:1isomers were separately estimated (Kuhnt et al. 2011). The results show that *t*9 und *t*10 contents predominate in foodstuffs containing PHVO (e.g., margarine). Due to these *trans* C18:1 distribution patterns and with the help of the *t*9 to *t*11 ratio (so-called *t*9/*t*11 index), the origin of the processed fat in the end product can be identified. Whilst a *t*9/*t*11 index of <1.0 is typical for ruminant fat due to the predominance of *t*11 over *t*9, a quotient of >1.0 suggests the use of industrially hydrogenated fat. Butter fat from various producers with a mean *t*9/*t*11 index of 0.3 served as reference. The *t*9/*t*11 index of plant-derived

bakery products and frying fats can be as high as 4.4 - 5.2 (Kuhnt et al. 2011). In these cases, one can assume that the high TFA content of these bakery products results mainly from PHVO. Based on the *t*9/*t*11 index, a high portion of TFA from butter used in the traditional production of puff pastry can be excluded. Moreover, calculating the TFA intake according to the BfR (Gabriel 2009) and based on the *t*9/*t*11 index of about 2.0 suggests that the bulk of ingested TFA originates from I-TFA.

6. Metabolism and the pathophysiological effect of TFA

Differences in the metabolism

TFA such as *cis* fatty acids can be metabolized by oxidation, elongation, and desaturation processes. The TFAs are stored in adipocytes and incorporated into membrane lipids. However, both cell culture and animal studies show that the various TFA are metabolized and converted via differing processes, and, also act in a biochemically different manner (Degen et al. 2011a; Chardigny et al. 2007, Kadegowda et al. 2010). For example, *trans* isomers with a double bond in position 9 and 10 are not substrates for Δ9-desaturase. A significant increase of *c*9,*t*11 CLA in plasma and cellular blood fractions during *t*11 supplementation could be demonstrated in human studies. Therefore, *t*11 is a suitable precursor for synthesis of *c*9,*t*11 CLA (Turpeinen et al. 2002, Kuhnt et al. 2006). However, it results in a lower amount of *t*11 being available for incorporation into cell membranes. In contrast, *t*12 is not Δ9 desaturated (Kuhnt et al. 2006).

A survey of various data relating to the relevance of R-TFA to health, refers to the exceptional position of *t*11 in light of its conversion into *c*9,*t*11 CLA (Field et al. 2009). Consequently, *t*11 is rated as health-promoting. In particular, the anti-carcinogenic and partly anti-atherogenic effects

of *c*9,*t*11 CLA produced from *t*11 ascertained in animal models lead to this proposition (Lock et al. 2004, Corl et al. 2003, Parodi 2003). Moreover, in human T cells, *t*11 showed a cytokine reducing effect (interleukin-2 and tumor necrosis factor-α) which was independent of *c*9,*t*11 CLA since no bioconversion occurred. Thus, the data provide evidence that *t*11 acts mechanistically in a manner similar to *c*9,*t*11 CLA (Jaudszus et al. 2012).

(Patho)Physiological effects

Numerous prospective cohort studies verify the positive correlation between I-TFA intake and a heightened cardiovascular risk (Willett et al. 1993, Ascherio et al. 1996, Hu et al. 1997, Oomen et al. 2001). In a controlled intervention study, Mensink and Katan (1990) describe for the first time the increase of the total cholesterol (TC) and LDL-C concentrations with a simultaneous decrease of HDL-C levels in serum due to a TFA-rich diet. The TFA originated from partially hydrogenated *"high-oleic"* sunflower oil. Numerous clinical follow-up studies involving supplementing with partially hydrogenated vegetable and fish oils (*"high-oleic" sunflower oil*: Zock and Katan 1992, *vegetable oil:* Judd et al. 1994, *fish oil:* Almendingen et al. 1995, *sunflower oil:* Aro et al. 1997, s*oybean oil:* Sundram et al. 1997, *various vegetable oils*: Lichtenstein 1998, *shortenings and margarine:* Judd et al. 2002, *soybean oil:* Sundram et al. 2007 verify these results.

Epidemiological observations suggest an association between a daily I-TFA intake of 2 en% (approx. 5 g) and an increase in risk of more than 20% for ischaemic heart diseases (Katan 2006), mainly related to the negative influence of TFA on serum lipoproteins. A meta-analysis of prospective and retrospective studies by Mozaffarian et al. (2006) confirms this correlation.

However, the effect of TFA is not limited to an increase in the TC concentration. TFA also have an influence on lipoprotein(a), inflammatory phenomena, endothelial function, coagulation, insulin sensitivity, fatty acid patterns in membranes, and, in turn, on transporters and receptors as well as on the formation of eicosanoids (Mozaffarian et al. 2006). The increased I-TFA intake is also associated with an elevation of various systemic inflammatory markers (interleukin-6, tumor necrosis factor-α and C-reactive protein; Almendingen et al. 1995, Lichtenstein 1998, Baer et al. 2004, Mozaffarian et al. 2009, Lopez-Garcia et al. 2005).

7. Epidemiological and clinical studies concerning R-TFA or individual TFA

The WHO confirms the profound adverse effects of PHVO-derived I-TFA with regard to various cardiovascular risk factors which lead to an increase in the risk of coronary heart diseases (Nishida and Uauy 2009). According to several authors, currently there is no convincing evidence to substantiate an association between current R-TFA intake and increased risk of coronary heart diseases (Nishida and Uauy 2009; Pfeuffer and Schrezenmeir 2006).

Epidemiological studies

In Denmark, consumption of milk and dairy products is relatively high. Data show that around 86% of the ingested TFA in Denmark originates from ruminant fat. The median R-TFA intake is 1.7 g/d. About 90% of the Danish population ingests between 0.8 and 3.1 g/d R-TFA (0.4 - 1.1 en%; Jakobsen et al. 2006). In this context, the Danish population represents an appropriate model for investigating the effect of high R-TFA intake. In fact, a Danish longitudinal study that

lasted for 18 years concludes that a high R-TFA intake is not associated with a higher risk of coronary heart diseases (Jakobsen et al. 2008). Stender et al. (2008) concluded that a daily intake of up to 4 g R-TFA presented no health hazard. No other epidemiological studies have found a convincing association between *t*11 intake *via* animal fat, or R-TFA in general, and coronary heart diseases (Clifton et al. 2004, Hodgson et al. 1996, Sun et al. 2007, Mozaffarian et al. 2010). Even well-known large scale epidemiological studies (Nurses Health, ATBC, Framingham) could not ascertain any negative effects of R-TFA on the risk of coronary heart diseases (Table 5; Willett et al. 1993, Hu et al. 1997, Pietinen et al. 1997, Gillman et al. 1997, Oomen et al. 2001, Bolton-Smith et al. 1996). The ATBC study even claimed a beneficial influence of R-TFA and emphasized a clearly negative effect for intake of TFA with a preponderance of I-TFA.

Two case-control studies for instance demonstrated that elevated levels of *trans* isomers of C18:2 *c*9,*c*12 (linoleic acid) in erythrocytes and plasma phospholipids were associated with a higher risk of primary cardiac arrest and fatal ischemic heart failure or sudden cardiac death, respectively (Lemaitre et al. 2002; 2006). Levels of *trans* C18:1 were associated with lower risk. In contrast, the Physicians´ Health Study reported a lower risk of heart failure with higher concentrations of plasma phospholipid *trans* C18:2 in male physicians. In this study, plasma *trans* C16:1 and *trans* C18:1 were both not associated with a risk of heart failure (Tokede et al. 2013).

A recent study analysed the potential role of C16:1 *t*9 with respect to metabolic risk (Mozaffarian et al. 2010). The circulating C16:1 *t*9 is associated with lower insulin resistance, atherogenic dyslipidemia, and incident diabetes. The authors described the metabolic benefit consuming dairy products. In contrast, an increased risk of non-Hodgkin lymphoma was associated with the

intake of total TFA, but also with higher fat dairy products (Charbonneau et al. 2013). In general, the latter studies mainly reflect the situation between 1982 and 1999 (blood samples and food records; except Charbonneau et al. 2013) and not recent data.

By means of a quantitative review, Brouwer et al. (2010) concluded that all fatty acids with *trans*-double bonds (also including CLA) increase the LDL-C/HDL-C ratio in plasma. This statement was the source of a lively discussion among experts. There appears to be no justification for the retrospective aspect of the data, regardless of the purpose of each individual study. In addition, due to a number of methodical insufficiencies and the partial lack of comparability of the results of the review, the mean values of the calculated LDL-C/HDL-C differences in studies using I-TFA are four times as high compared to studies involving the sole administration of natural R-TFA (Table 6; calculation based on data by Brouwer et al. 2010).

A recent review and meta-analysis from limited numbers of observational studies showed that I-TFA may be positively associated to CHD, whereas R-TFA is not. The null association of R-TFA with CHD risk may be due to lower intake levels (Bendsen et al. 2011). Overall, it is important to consider into the TFA problematic the different analytical methods (Albuquerque et al. 2011), quantifying, analysed tissues, date of collected data, and the relatively low amounts of several *trans* isomers.

Intervention studies

Only a few intervention studies examining the effect of R-TFA with and without R-CLA are found in the literature. Hence, in order to evaluate the exclusive influence of R-TFAs, results

from both animal and human studies need to be considered.

Animal studies

One animal study involving „White New Zealand" rabbits whose diets were supplemented with butter enriched with *t*10 compared to *t*11 raised TC and LDL-C concentrations in plasma and increased lipid deposition in the aorta (Roy et al. 2007). In a study using LDL receptor-deficient mice, the effect of supplementing TFA derived from commercially hydrogenated vegetable shortening (1.5 en% *t*9), conventional butter (0.3 en% *t*11) and *t*11-enriched butter (1.5 en% *t*11) with and without added cholesterol was compared with respect to the development of atherosclerotic plaques. In this animal model, *t*9-containing fat stimulated the development of atherosclerotic lesions. However, in a direct comparison, *t*11-rich butter fat prevented the increase of atherosclerotic plaques, both with and without addition of cholesterol to the feed (Bassett et al. 2010). In rats, treatment with *t*10 enriched milk fat tended to increase triacylglycerides (TAG) concentration, whereas treatment with *t*11 and *c*9,*t*11 CLA containing milk fat was inclined to reduce it (Anadon et al. 2010). In addition to the problems relating to transfer findings from animal studies to humans, there is also the difficulty of comparing the action of single TFA, since many TFA act differently in a manner similar to the known isomer-specific effects of different CLA isomers (e.g., *c*9,*t*11 vs. *t*10,*c*12).

Studies in humans

For human studies, only those studies were selected (see Table 7) in which either *t*11 or R-TFA was supplemented with or without R-CLA, and also those in which I-TFA were directly compared to R-TFA. The mentioned studies were also used by Brouwer et al. (2010) for their

calculations of effects of I-TFA and R-TFA on HDL- and LCL-C levels in humans. However, our study (Kuhnt et al. (2006) was not included because the lipoprotein profile had at that time not been published.

Desroches et al. (2005) investigated the influence of CLA-enriched butter (4.2 g/100 g) compared to a control butter (0.4 g CLA/100 g). In contrast to the control group, the ratios of LDL-C/HDL-C and C/HDL-C were heightened. In the human study by Tricon et al. (2006), a daily amount of 1.5 g CLA and 4.7 g *t*11 (6.3 g Σ*trans* C18:1) was administered *via* dairy products. In a six weeks test phase, no effects on inflammatory biomarkers, insulin, glucose, TC, and TAG concentrations in serum were found. Tholstrup et al. (2006), used butter supplemented with *t*11 and *c*9,*t*11 CLA in a study involving healthy men over a period of five weeks. After the intervention, plasma TC and HDL-C concentrations were lower than those of the conventional control butter. The authors attribute this effect to the higher proportion of saturated fatty acids (SFA) in the control butter (Table 7). Also, after R-TFA intake via butter the authors concluded that dietary R-TFA equivalent to 1 en% has no significant effect on LDL-C and TAG but may be associated with a reduction in plasma HDL-C, particularly in overweight women (Lacroix et al. 2012).

Further studies supplementing sheep cheese naturally rich in *t*11- and R-CLA also showed no changes in risk of CVD risk (Pintus et al. 2012, Sofi et al. 2010) as well as a significant reduction of inflammatory markers (Sofi et al. 2010).

In an intervention study by our research group (Kuhnt et al. 2006) we compared the intake of 6.0 g of a mixture consisting of *t*11 and *t*12 from a synthetic supplement with the intake of a TFA-free placebo mix in healthy men and women. The placebo mix (1:1 palm kernel oil and rapeseed

oil) represents a TFA- and CLA-free fatty acid profile of an average European mixed diet. What distinguishes this study from other studies is that in both study groups the diet was free of ruminant fat (no R-CLA and R-TFA in the individual diet). Only *t*11, as representative for R-TFA was supplemented in the TFA mixture. A significant result of the study was that the high amounts of *t*11 and *t*12 (∑6 g/d over 6 weeks) had no influence on TC, HDL-C, LDL-C, and TAG concentrations (Table 8). Compared to the start of the study, the LDL-C/HDL-C ratio remained unchanged (-0.02) after TFA intervention. Furthermore, no differences between test and placebo groups were detected (Table 8). Moreover, no changes were found in biomarkers of the immune system and inflammation (interleukins, TNFα, C-reactive protein, secretory phospholipase A_2, intercellular adhesion molecule 1, leptin, adiponectin) (Kuhnt et al. 2007; Table 7).

At present, there are reports of only two studies that directly compare I-TFA with R-TFA in healthy subjects (Chardigny et al. 2008, Motard-Bélanger et al. 2008). In the double-blind randomized cross-over study by Chardigny et al. (2008), food products with high amounts of I-TFA and R-TFA with 5 en% respectively were ingested by male and female subjects. In males, results revealed no significant change in the lipoprotein profile for both diets. Yet, in female subjects, HDL-C, LDL-C and TC as well as TAG were lower on the I-TFA diet. However, intake of high levels of R-TFA resulted in increased concentrations of both LDL-C and TC in comparison to I-TFA. Regarding TC/HDL-C, no significant difference between the diets was determined. In the study by Motard-Bélanger et al. (2008) four diets comprising different concentrations of R-TFA and I-TFA were compared, administered exclusively to male participants. The diets contained a moderate R-TFA (1.5 en%), high R-TFA (3.7 en%) and high I-TFA (3.7 en%), as well as control diet (0.8 en%). In comparison with moderate R-TFA intake, I-

TFA increased TC, LDL-C and TC/HDL-C. In the group consuming high R-TFA, TC and LDL-C were raised compared to moderate R-TFA supplementation and to the control group. Moderate R-TFA intake showed no effect compared to control diet (Table 7).

Evaluation of the studies and outcomes

The currently available data for human studies regarding the effect of R-TFA, including R-CLA (*c*9,*t*11 CLA) is ambiguous due to a number of following reasons. Fat supplementation is often very high (e.g., 115 g butter daily; 135 g fat/d) and the short or no adaption/wash-out periods lead to a *per se* rise in TC and other biomarkers. In general, with regard to the cross-over intervention using lipids, the wash-out period is often too short in relation to the duration of the intervention. Further, the amounts of the respective supplemented fatty acids (e.g., *c*9,*t*11 CLA) what the subjects consumed by individual diet in addition to supplementation are not restricted during the study and/or not included in the calculations. Moreover, different methods used for statistical analysis lead to digressions and diverging conclusions. Finally, the often different baseline values of subjects from diverse groups are disregarded, and, in some cases, not published. This is particularly relevant as the inclusion of the baseline level as covariate would probably modify the outcome of the study.

Another important factor concerns gender since data in many of the studies relates to male subjects. Data from others and our own results make evident that gender-specific differences concerning the effect of different supplementations need to be considered (Kuhnt et al. 2006, Chardigny et al. 2008). Chardigny et al. (2008) found that men showed no effect to either TFA source. In women, a HDL-C decrease was measured after I-TFA supplementation, while R-TFA

intake caused a rise in LDL-C. In comparison to the mean initial values, no effect in the mean HCL-C and LDL-C of both genders could be detected after R-TFA supplementation (Table 6). Results from Motard-Bélanger et al. (2008) refer exclusively to studies with male subjects. Data from an analysis of females would probably deviate. Finally, in some instances, the study populations were too small to obtain conclusive data regarding gender-specific effects (e.g., Kuhnt et al. 2006). Furthermore, the fatty acid supplementation is not adjusted for energy intake. Thus, women often had a higher intake of the supplemented fatty acids compared to men.

Divergent study results can be traced back to the fact that SFA intake additionally influences the lipoprotein profile (Dubois et al. 2007). In several studies, the proportions of total SFA and individual SFA such as C14:0 and C16:0 are not taken into account. In the study by Chardigny et al. (2008), the total SFA intake is identical in both TFA groups, but C14:0 is higher in the R-TFA group (1.2% vs. 8.0%), whilst in the I-TFA group, the C16:0 proportion is higher (32% vs. 23%). Further, in the study by Tholstrup et al. (2006), the higher SFA in the control butter appears to hinder the comparability to *t*11-rich butter (C14:0 and C16:0 twice as high in control group). In some studies, modified butter fats were used and compared to normal butter as a control. However, butter can contain differing amounts of polyunsaturated fatty acids (PUFA) depending on the feed. For instance, butter made from milk from dairy cows fed on sunflower seeds (e.g. Tholstrup et al. 2006), has a higher proportion of PUFA (e.g., linoleic acid) and a lower concentration of SFA. Indeed, the choice of the control diet is decisive with respect to the main statement of these studies. In general, intervention with individual TFA isomers has proved to be difficult because in food products TFA comprises a mixture of various isomers. Although R-TFA and I-TFA contain chemically identical *trans* isomers of C18:1, distribution of these isomers is

significantly different. An equivalent proportion of individual fatty acids can frequently not be supplemented in the different study groups. Hence, the complexity of the interrelations and parameters weakens the main statement of the studies.

The observed adverse effects on the blood lipids seem to be in part due to the fat intake *per se*. The adverse effect rises proportionally to the fat supply. Notably, the proportion of SFA also plays a role here. When comparing the effects of TFA from vegetable and ruminant fats by means of intervention studies, the proportion of SFA as a 'confounder' represents a problem. Butter consumption alters the lipoprotein status negatively, except for the rise of HDL-C (Pfeuffer and Schrezenmeir 2000). In identical food products, SFA intake correlates with the TFA supply. Indications for an inverse association between the risk of coronary heart diseases and R-TFA intake are enhanced if SFA are accounted for as confounders (Jakobsen et al. 2008). In this way, SFA often impair the qualitative and especially the quantitative evaluation of the physiological effect of TFA.

Conclusion and relevance of the quantity of R-TFA

In the mentioned above studies the effects of consuming between three to tenfold amounts of R-TFA (and additional R-CLA) are compared to levels consumed by the average population. A detailed analysis of these studies clearly shows that in comparison to average R-TFA and R-CLA intake (considered as control), the effects on the TC and lipoprotein concentrations in serum are small or negligible. Other markers of CVD remained largely unaffected.

For example, an increased R-TFA intake of between 1.5 - 2.3 en% (5.5 - 6.3 g *trans* C18:1) with R-CLA proportion (0.6 - 1.5 g/d; Motard-Bélanger et al. 2008; Tricon et al. 2006, Tholstrup et al.

2006) compared to a low or normal R-TFA intake (0.4 - 0.8 en%) is not alarming in respect to CVD risk. It has to be noted that the R-TFA amount of 1.5 en% defined by Motard-Bélanger as moderate, with no effects compared to control, is related to average population intake in fact rated as being high. For example, the daily R-TFA intake of 1.5 en% corresponds to a daily consumption of 200 g cheese (33% fat), 500 ml milk (3.25% fat), 175 g yoghurt, and about 32 g butter (Motard-Bélanger et al. 2008).

To evaluate the relevance of R-TFA amounts used in these studies following calculation was made. Based on the BfR estimate for mean total TFA intake (0.77 en%; Table 4) and hypothesize a high R-TFA proportion of about 80%, the R-TFA proportion consumed in Germany is approximately 0.6 en% R-TFA (Figure 1). Thus, this estimated mean R-TFA intake in Germany is nearly equal to the amount used in the control group (0.8 en%) by Motard-Bélanger et al. (2008). Accordingly, if placed at the 95th percentile of R-TFA intake (based on mean total TFA intake 1.3 en%), the figure would be about 1.1 en% R-TFA in Germany. This amount lies between the amounts according to control and moderate R-TFA supplemented by Motard-Bélanger et al. 2008 (Figure 1). At such a high R-TFA intake, which is not common in practice, no difference to the control diet in this study was found (Motard-Bélanger et al. (2008). The amount of R-TFA that induced negative effects on blood lipids in this study are considered as extremely high (11 - 12 g/d; 3.7 en%) and not realistic, not even for an extraordinarily high consumption of products containing ruminant fat.

8. R-TFA and R-CLA content of milk and milk products, especially organic milk

Particularly, milk and dairy products of grazing cows and from mountainous regions is relatively

high in R-TFA compared to conventional produced milk (Kraft et al. 2003). In such milk, especially *t*11 is disproportionately high. Furthermore, grass-based milk has high R-CLA (especially *c*9,*t*11 and *t*11,*c*13) and omega-3 PUFA contents (Kraft et al. 2003, Degen et al. 2011b, Butler et al. 2011). Investigations on alpine milk and Bulgarian highland milk compared to conventionally produced milk have confirmed these findings to a large extent (own observation; Table 9).

Organic farming is often associated with grass feeding, but not exclusively. Other feeding strategies influence the profile and amount of R-TFA and R-CLA in milk. These are mainly diets providing lipid precursors for *t*11 formation (e.g., linoleic acid and α-linolenic acid) and modify microbial activity in the rumen (Chilliard et al. 2007). For example, the inclusion of plant oils (e.g., sunflower oil) and extruded seeds increase R-TFA and R-CLA in milk similar to pasture (Chilliard et al. 2007, Shingfield et al. 2010).

Since the R-TFA and R-CLA contents of organic and ecological produced milk (also beef) can be twofold to tenfold higher than that of conventional milk (Jahreis et al. 1997, Daley et al. 2010; Butler et al. 2011). There are also race- and species-specific differences since sheep and goat milk products are especially rich in R-TFA and R-CLA (Jahreis et al. 1999).

Our own analyses of 115 dairy products in 2005 and 220 milk and cheese samples in 2012 available on the German market yielded a mean R-TFA content of about 2 - 3% and a CLA proportion of about 0.7 - 1.3% in the fat. Higher values exist in biological compared to conventional dairy products. Therefore, the ingested amounts of R-TFA and R-CLA varied depending on the milk production system. For example, based on the average intake of 30 g/d dairy fat, through solely consumption of conventional dairy products the R-TFA and R-CLA

intake would amount to about 0.5 g/d and 0.1 g/d, respectively (Σ 0.6 g/d). In contrast, for consumption of organic milk and mountain milk products, R-TFA and R-CLA intake is considerably higher at approximately 1.5 g R-TFA and 1.0 g R-CLA/d (Σ 2.5 g/d), respectively. In addition, the intake of omega-3 PUFA intake is higher by biologically produced dairy and beef products (Daley et al. 2010, Butler et al. 2011, Molkentin 2009). But, of course, the ingested amount of these fatty acids depends on the fat content of each food product (milk vs. cheese).

This points out that the consumption of naturally and organically produced milk raises the intake of R-TFA and R-CLA. If there would be a general regulation and declaration of TFA including R-TFA, this would discriminate and impair organic and ecological milk production and would cause an uncertainty among consumers.

9. Dairy fat intake changed R-CLA, R-TFA and *t*9/*t*11 index in human body lipids

An increased intake of milk products by breastfeeding mothers is reflected in the TFA profile of breast milk. Mueller et al. (2010) were even able to trace a difference to organically produced milk products. The authors conclude that the higher the intake of milk products and especially of organically produced milk products, the higher the *t*11 proportion in the TFA of breast milk. We can emphasize here that the *t*9/*t*11 index is a suitable indicator for the portion of I-TFA related to R-TFA in the diet. Thus, the *t*9/*t*11 index of breast milk decreased with increased milk fat intake and the intake of organically produced milk products. High dairy fat intake of mothers increased *c*9,*t*11 CLA in blood lipids of their breastfed babies, but not of *t*11 (Enke et al. 2011). In case of high maternal dairy fat intake, *t*9/*t*11 indices were decreased in both maternal and fetal blood lipids (Enke et al. 2011).

In general, results of the KOALA Birth Cohort Study (Netherlands) found protective effects of ruminant fatty acids (e.g. *t*11 and *c*9,*t*11 CLA) against the development of atopic manifestations. The higher concentrations of these ruminant fatty acids including omega-3 PUFA in breast milk were associated with a lower risk of parent-reported eczema, atopic dermatitis, and sensitisation at 1 year of age (Thijs et al. 2011).

In intervention studies carried out with R-TFA and R-CLA-enriched dairy products, a significant rise of *t*11 and *c*9,*t*11 CLA in plasma reflect the higher dietary intake of these fatty acids (Pintus et al. 2012). In erythrocytes of Kenyan Maasai (Knoll et al. 2011) and Bulgarian shepherds, an increased incorporation of *t*11 and *c*9,*t*11 CLA has also been observed (Table 10). This is due to the high consumption of home-made products made from cow and ewe milk. The low *t*9/*t*11 index of these milk products correlates with a low *t*9/*t*11 index in the erythrocytes (unpublished observations). In erythrocytes of a study group without dietary ruminant fatty acids over 8 weeks, the levels of *t*11 and CLA were considerably lower. These data show that the *t*9/*t*11 index in erythrocytes clearly reflects the respective portion of dairy fat intake in the diet (Table 10).

According to current analyses of the *trans* isomers in human tissue samples in the German population (myocardium, subcutaneous fat), *t*9 is especially higher than *t*11 (Table 11; Baehr et al. 2011). This could support firstly a higher dietary amount of I-TFA represented by *t*9 as confirmed by BfR estimates (unpublished) and, secondly, the additional conversion of *t*11 into *c*9,*t*11 CLA.

Fortunately, in many countries the previously high I-TFA intake has been reversed due both to governmental regulations and consumer awareness, and has led to an average decrease of I-TFA in several foods reflected in decreased portions of TFA in plasma (Baylin 2013, Schwenke et al.

2013) and in adipose tissue (Clifton et al. 2004).

10. Milk and dairy products as complex foodstuffs

Besides R-TFA, R-CLA, and SFA, milk also contains short- and branch-chained as well as odd-numbered and omega-3 PUFA, phytanic acid and a multitude of bioactive components such as calcium, lysozyme, whey proteins (immunoglobulin A, M and G), sphingomyelin and bifidogenic glycomacropeptides. Most of these are associated with protective properties for public health. Thus, it is imperative not to reduce the nutritional relevance of milk to its fatty acids.

Results from several recent reviews and meta-analyses show no evidence for an association between dairy product intake and a heightened risk for coronary heart diseases (German et al. 2009, Elwood et al. 2010). Mente et al. (2009) were also able to demonstrate that there is no significant correlation between milk consumption and coronary heart diseases. Elwood et al. (2010) point to a lowered risk of ischaemic heart diseases through high milk consumption in contrast to low milk consumption. The authors conclude that no convincing evidence exists regarding health concerns through consumption of milk products. Thus, milk, as a staple food, contributes to the prevention of diet-related diseases, if embedded in a varied and well-balanced diet. The following maxim applies to the consumer: intake of a daily portion of milk contributes considerably to a healthy diet.

11. Conclusion

R-TFA have to be evaluated separately from I-TFA with respect to health hazard. Although, both sources mainly contain the same *trans* isomers, concentrations of the individual isomers differ. In addition, to evaluate impact for public health it has to be considered that the proportion of ingested amounts of R-TFA and I-TFA generally differ, also between populations and countries. It is important to clearly exclude R-TFA from mandatory restriction because no evident disadvantages have been reported in the literature with respect to consumption of an average amount of dairy products. Restriction and declaration of R-TFA would unjustifiably represent a disadvantage for organic milk and the ecological production of milk. Importantly, preventive benefits of milk consumption against several diseases such as allergy, and asthma that relate to ruminant fatty acids have been described.

Abbreviations

CLA: conjugated linoleic acids

CLnA: conjugated linolenic acids

CVD: cardiovascular disease

I-TFA: industrially derived *trans* fatty acids (non-ruminant)

PHVO: partially hydrogenated vegetable oils

PUFA: polyunsaturated fatty acids

R-CLA: ruminant-derived CLA

R-TFA: ruminant-derived *trans* fatty acids;

SFA: saturated fatty acids

*t*11: vaccenic acid (C18:1 *trans*11)

*t*9: elaidic acid (C18:1 *trans*9)

TAG: triacylglycerides

TC: total cholesterol

Author details

Department of Nutritional Physiology, Institute of Nutrition, Friedrich Schiller University, Jena, Germany.

Authors´ contribution

GJ and KK initiated the review. KK was responsible for the literature review and organization of the review, composition of the unpublished results and tables, statistical analysis, created the manuscript and worked through the submission process. CD conducted a portion of the literature review, provided unpublished data, and served as editor for the manuscript. GJ created a portion of the manuscript, provided unpublished data, and served as editor for the manuscript. All authors read and approved the manuscript.

Acknowledgement

The authors would like to thank Dr. Ulrich Schäfer for the proofreading and Nasim Krögel for the language editing of the manuscript.

Competing interests

The authors declare that they have no competing interests.

12. References

Albuquerque TG, Costa HS, Castilho MC, Sanches-Silva A: Trends in the analytical methods for the determination of trans fatty acids content in foods. *Trends in Food Science & Technology* 2011, **22**:543-560.

Almendingen K, Jordal O, Kierulf P, Sandstad B, Perdersen JI: Effects of partially hydrogenated fish oil, partially hydrogenated soybean oil, and butter on serum lipoproteins and Lp[a] in men. *J Lipid Res* 1995, **36**:1370-1384.

Anadon A, Martinez-Larranaga MR, Martinez MA, Ares I, Ramos E, Gomez-Cortes P, Juarez M, De la Fuente MA: Acute oral safety study of dairy fat rich in trans-10 C18:1 versus vaccenic plus conjugated linoleic acid in rats. *Food Chem Toxicol* 2010, **48**:591-598.

Ansorena D, Echarte A, Ollé R, Astiasarán I: 2012: no *trans* fatty acids in Spanish bakery products. *Food Chem* 2013, **138**:422-429.

Aro A, Jauhiainen M, Partanen R, Salminen I, Mutanen M: Stearic acid, *trans* fatty acids, and dairy fat: Effects on serum and lipoprotein lipids, apolipoproteins, lipoprotein(a), and lipid transfer proteins in healthy subjects. *Am Clin J Nutr* 1997, **65**:1419-1426.

Ascherio A, Rimm EB, Giovannucci EL, Spiegelinan D, Stampfer MJ, Willet WC: Dietary fat and risk of coronary heart disease in men, cohort follow up study in the United States. *Br Med J* 1996, **313**:84-90.

Baehr M, Jahreis G, Kuhnt K: *Trans*-Fettsäuren in Lebensmitteln auf dem deutschen Markt und in Humangeweben. *Ernahr-Umsch* 2011, **58:478-485.**

Baer DJ, Judd JT, Clevidence BA, Tracy RP: Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: A randomized crossover study. *Am J Clin*

Nutr 2004, **79**:969-973.

Bassett CM, Edel AL, Patenaude AF, McCullough RS, Blackwood DP, Chouinard PY, Paquin P, Lamarche B, Pierce GN: Dietary vaccenic acid has antiatherogenic effects in LDLr-/- mice. *J Nutr* 2010, **140**:18-24.

Baylin A: Secular trends in trans fatty acids: decreased trans fatty acids in the food supply are reflected in decreased trans fatty acids in plasma. *Am J Clin Nutr* 2013, **97**:665-666.

Bendsen NT, Christensen R, Bartels EM, Astrup A: Consumption of industrial and ruminant *trans* fatty acids and risk of coronary heart disease: a systematic review and meta-analysis of cohort studies. *Eur J Clin Nutr* 2011, **65**:773-783.

BMELV 2012. [http://www.bmelv.de/SharedDocs/Downloads/Ernaehrung/Rueckstaende/Trans-Fettsaeuren/TFA_Inhalt.pdf?__blob=publicationFile]

Bolton-Smith C, Woodward M, Fenton S, Brown CA: Does dietary trans fatty acid intake relate to the prevalence of coronary heart disease in Scotland? *Eur Heart J* 1996, **17**:837-845.

Brouwer IA, Wanders AJ, Katan MB: Effect of animal and industrial trans fatty acids on HDL and LDL cholesterol levels in humans- a quantitative review. *PLoS One* 2010, **5**(3):e9434. doi:10.1371/journal.pone.0009434.

Butler G, Nielsen JH, Larsen MK, Rehberger B, Stergiadis S, Canever A, Leifert C: The effects of dairy management and processing on quality characteristics of milk and dairy products. NJAS-*Wageningen Journal of Life Sciences* 2011, **58**:97-102.

Charbonneau B, O'Connor HM, Wang AH, Liebow M, Thompson CA, Fredericksen ZS, Macon WR, Slager SL, Call TG, Habermann TM, Cerhan JR: *Trans* fatty acid intake is associated with increased risk and n3 fatty acid intake with reduced risk of Non-Hodgkin Lymphoma. *J*

Nutr 2013, **143***:*672-681.

Chardigny JM, Clouet P, Combe N, Quignard-Boulangde A , Schmitt B, Lagarde M, Leger CL: Metabolism of trans and conjugated fatty acids. *Eur J Lipid Sci Technol* 2007, **109**:930-934.

Chardigny JM, Destaillats F, Malpuech-Brugère C, Moulin J, Bauman DE, Lock AL, Barbano DM, Mensink RP, Bezelgues JB, Chaumont P, Combe N, Cristiani I, Joffre F, German JB, Dionisi F, Boirie Y, Sébédio JL: Do *trans* fatty acids from industrially produced sources and from natural sources have the same effect on cardiovascular disease risk factors in healthy subjects? Results of the *trans* fatty ccids collaboration (TRANSFACT) study. *Am J Clin Nutr* 2008, **87**:558-566.

Chilliard Y, Glasser F, Ferlay A, Bernard L, Rouel J, Doreau M: Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. *Eur J Lipid Sci Technol* 2007, 109:828-855.

Clifton PM, Keogh JB, Noakes M: *Trans* fatty acids in adipose tissue and the food supply are associated with myocardial infarction. *J Nutr* 2004, **134**:874-879.

Corl BA, Barbano DM, Bauman DE, Ip C: *cis*-9, *trans*-11 CLA derived endogenously from *trans*-11 18:1 reduces cancer risk in rats. *J Nutr* 2003, **133**:2893-2900.

Daley CA, Abbott A, Doyle PS, Nader GA, Larson S: A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutr J* 2010, **9**:10. doi: 10.1186/1475-2891-9-10.

Degen C, Ecker J, Piegholdt S, Liebisch G, Schmitz G, Jahreis G: Metabolic and growth inhibitory effects of conjugated fatty acids in the cell line HT-29 with special regard to the conversion of *t*11,*t*13-CLA. *Biochim Biophys* Acta 2011a, **1811**:1070-1080.

Degen C, Lochner A, Keller S, Kuhnt K, Dänicke S, Jahreis G: Influence of in vitro

supplementation with lipids from conventional and Alpine milk on fatty acid distribution and cell growth of HT-29 cells. *Lipids Health Dis* 2011b, **10**:131.

Desroches S, Chouinard PY, Galibois I, Corneau L, Delisle J, Lamarche B, Couture P, Bergeron N: Lack of effect of dietary conjugated linoleic acids naturally incorporated into butter on the lipid profile and body composition of overweight and obese men. *Am J Clin Nutr* 2005, **82**:309-319.

Destaillats F, Golay PA, Joffre F, de Wispelaere M, Hug B, Giuffrida F, Fauconnot L, Dionisi F: Comparison of available analytical methods to measure trans-octadecenoic acid isomeric profile and content by gas-liquid chromatography in milk fat. *J Chromatogr A* 2007, **1145**:222-228.

Destaillats F, Wolff RL, Precht D, Molkentin J: Study of individual trans- and cis-16:1 isomers in cow, goat, and ewe cheese fats by gas-liquid chromatography with emphasis on the trans-delta3 isomer. *Lipids* 2000, **35**:1027-1032.

Dilzer A, Park Y: Implication of conjugated linoleic acid (CLA) in human health. *Crit Rev Food Sci Nutr* 2012, **52**:488-513.

Doell D, Folmer D, Lee H, Honigfort M, Carberry S: Updated estimate of *trans* fat intake by the US population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 2012, 29:861-874.

Elwood PC, Pickering JE, Givens DI, Gallacher JE: The consumption of milk and dairy foods and the incidence of vascular disease and diabetes: An overview of the evidence. *Lipids* 2010, **10**:925-939.

Enke U, Jaudszus A, Schleussner E, Seyfarth L, Jahreis G, Kuhnt K: Fatty acid distribution of

cord and maternal blood in human pregnancy: special focus on individual trans fatty acids and conjugated linoleic acids. *Lipids Health Dis* 2011, **10**:247.

EU Food Law: Iceland to introduce trans fat legislation. 2010. [http://www.eurofoodlaw.com/policy/iceland-to-introduce-trans-fat-legislation--1.htm]

European Food Safety Authority (EFSA): The Opinion of the Scientific Panel on dietetic Products, Nutrition and Allergies on a request from the Commission related to the presence of *trans*-fatty acids in foods and the effect on human health of the consumption of *trans* fatty acids. *EFSA J* 2004, **81**:1-49.

Federal Ministry of Health Austria: Stöger unterschreibt *Trans*fettsäuren-Verordnung. [http://www.ots.at/presseaussendung/OTS_20090820_OTS0082/gesundes-essen-stoeger-unterschreibt-transfettsaeuren-verordnung].

Field CJ, Blewett HH, Proctor S, Vine D: Human health benefits of vaccenic acid. *Appl Physiol Nutr Metab* 2009, **34**:979-991.

Food and Drug Administration (FDA): Food labeling: *Trans* fatty acids in nutrition labeling, nutrient content claims, and health claims (21 CFR Part 101). *Fed Regis* 2003, **68**:41434-41506.

Food Standards Australia New Zealand: *Trans* fatty acids in the New Zealand and Australia food supply: Review Report. Food Standards Australia New Zealand, Canberra/Wellington, 2007. [http://www.foodstandards.gov.au/newsroom/publications]

Fritsche J, Steinhart H: Contents of *trans* fatty acids (TFA) in German foods and estimation of daily intake. *Fett-Lipid* 1997, **99**:314-318.

Gabriel S: Ermittlung der Aufnahme von *trans*-Fettsäuren durch Lebensmittel in Deutschland.

Diploma thesis. The Federal Institute of Risk Assessment and Friedrich Schiller University Jena, Institute of Nutrition; 2009.

German JB, Gibson RA, Krauss RM, Nestel P, Lamarche B, van Staveren WA, Steijns JM, de Groot L, Lock AL, Destaillats F: A reappraisal of the impact of dairy foods and milk fat on cardiovascular disease risk. *Eur J Nutr* 2009, **48**:191-203.

Gillman MW, Cupples LA, Gagnon D, Millen BE, Ellison RC, Castelli WP: Margarine intake and subsequent coronary heart disease in men. *Epidemiology* 1997, **8**:144-149.

Gómez-Cortés P, Tyburczy C, Brenna JT, Juárez M, de la Fuente MA: Characterization of cis-9 trans-11 trans-15 C18:3 in milk fat by GC and covalent adduct chemical ionization tandem MS. *J Lipid Res* 2009, **50**:2412-2420.

Health Canada (2007): *Trans* Fats. [http://www.hc-sc.gc.ca/hl-vs/alt_formats/pacrb-dgapcr/pdf/iyh-vsv/food-aliment/trans-eng.pdf]

Hennessy AA, Ross RP, Devery R, Stanton C: The health promoting properties of the conjugated isomers of alpha-linolenic acid. *Lipids* 2011, **46**:105-119.

Hilbig A: Wie isst Deutschland? Auswertungen der Nationalen Verzehrsstudie II zum Lebensmittelverzehr. *Ernahr-Umsch* 2009, **56**:16-23.

Hodgson JM, Wahlqvist ML, Boxallc JA, Balazsd ND: Platelet *trans* fatty acids in relation to angiographically assessed coronary artery disease. *Atherosclerosis* 1996, **120**:147-154.

Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Rosner BA, Hennekens CH, Willett WC: Dietary fat intake and the risk of coronary heart disease in women. *N Engl J Med* 1997, **337**:1491-1499.

Hulshof K, van Erp-Baart MA, Anttolainen M, Becker W, Church SM, Couet C, Hermann-Kunz

E, Kesteloot H, Leth T, Martins I, Moreiras O, Moschandreas J, Pizzoferrato L, Rimestad AH, Thorgeirsdottir H, van Amelsvoort JMM, Aro A, Kafatos AG, Lanzmann-Petithory D, van Poppel G: Intake of fatty acids in Western Europe with emphasis on *trans* fatty acids: The TRANSFAIR study. *Eur J Clin Nutr* 1999, **53**:143-157.

Jahreis G, Fritsche J, Moeckel P, Schoene F, Moeller U, Steinhart H: The potential anticarcinogenic conjugated linoleic acid, cis-9,trans-11 C18:2, in milk of different species: Cow, goat, ewe, sow, mare, woman. *Nutr Res* 1999, **19**:1541-1549.

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

Jakobsen MU, Bysted A, Andersen NL, Heitmann BL, Hartkopp HB, Leth T, Overvad K, Dyerberg J: Intake of ruminant *trans* fatty acids in the Danish population aged 1-80 years. *Eur J Clin Nutr* 2006, **60**:312-318.

Jakobsen MU, Overvad K, Dyerberg J, Heitmann BL: Intake of ruminant trans fatty acids and risk of coronary heart disease. *Int J Epidemiol* 2008, **37**:173-182.

Jaudszus A, Jahreis G, Schloermann W, Fischer J, Kramer R, Degen C, Rohrer C, Roth A, Gabriel H, Barz D, Gruen M: Vaccenic acid-mediated reduction in cytokine production is independent of c9,t11-CLA in human peripheral blood mononuclear cells. *Biochim Biophys Acta* 2012, **1821**:1316-1322.

Judd JT, Baer DJ, Clevidence BA, Kris-Etherton P, Muesing RA, Iwane M: Dietary *cis* and *trans* nonounsaturated and saturated FA and plasma lipids and lipoproteins in men. *Lipids* 2002, **37**:123-131.

Judd JT: Dietary *trans* fatty acids: Effects on plasma lipids and lipoproteins of healthy men and

women. *Am J Clin Nutr* 1994, **59**:861-868.

Kadegowda AKG, Connor EE, Teter BB, Sampugna J, Delmonte P, Piperova LS, Erdman, RA**:** Dietary trans fatty acid isomers differ in their effects on mammary lipid metabolism as well as lipogenic gene expression in lactating mice. *J Nutr* 2010, **140**:919-924.

Katan MB: Regulation of *trans* fats: The gap, the polder, and McDonald's French fries. *Atheroscler Suppl* 2006, 7:63-66.

Knoll N, Kuhnt K, Kyallo FM, Kiage-Mokua BN, Jahreis G: High content of long-chain n-3 polyunsaturated fatty acids in red blood cells of Kenyan Maasai despite low dietary intake. *Lipids Health Dis* 2011, **10**:141.

Kraft J, Collomb M, Moeckel P, Sieber R, Jahreis G: Differences in CLA isomer distribution of cow's milk lipids. *Lipids* 2003, **38**:657-664.

Kramer JK, Hernandez M, Cruz-Hernandez C, Kraft J, Dugan ME: Combining results of two GC separations partly achieves determination of all cis and trans 16:1, 18:1, 18:2 and 18:3 except CLA isomers of milk fat as demonstrated using Ag-ion SPE fractionation. *Lipids* 2008, **43**:259-273.

Kris-Etherton PM, Lefevre M, Mensink RP, Petersen B, Fleming J, Flickinger BD: Trans fatty acid intakes and food sources in the US population: NHANES 1999-2002. *Lipids* 2012, **47**:931-940.

Kuhnt K, Baehr M, Rohrer C, Jahreis G: *Trans* **fatty acid isomers and the** *trans*-**9**/*trans*-**11 index in fat containing foods.** *Eur J Lipid Sci Technol* **2011, 113:1281-1292.**

Kuhnt K, Kraft J, Moeckel P, Jahreis G: *Trans*-11-18:1 is effectively Δ9-desaturated compared with *trans*-12-18:1 in humans. *Br J Nutr* 2006, **95**:752-761.

Kuhnt K, Kraft J, Vogelsang H, Eder K, Kratzsch J, Jahreis G: Dietary supplementation with *trans*-11- and *trans*-12-18:1 increases *cis*-9,*trans*-11-conjugated linoleic acid in human immune cells, but without effects on biomarkers of immune function and inflammation. *Brit J Nutr* 2007, **95**:1196-1205.

L'Abbé MR, Stender S, Skeaff M, Ghafoorunissa, Tavella M: Approaches to removing *trans* fats from the food supply in industrialized and developing countries. *Eur J Clin Nutr* 2009; **63**:50-67.

Lacroix E, Charest A, Cyr A, Baril-Gravel L, Lebeuf Y, Paquin P, Chouinard PY, Couture P, Lamarche B: Randomized controlled study of the effect of a butter naturally enriched in *trans* fatty acids on blood lipids in healthy women. *Am J Clin Nutr* 2012, **95**:318-325.

Lemaitre RN, King IB, Mozaffarian D, Sotoodehnia N, Rea TD, Kuller LH, Tracy RP, Siscovick DS: Plasma phospholipid trans fatty acids, fatal ischemic heart disease, and sudden cardiac death in older adults: the cardiovascular health study. *Circulation* 2006, **114**:209-215.

Lemaitre RN, King IB, Raghunathan TE, Pearce RM, Weinmann S, Knopp RH, Copass MK, Cobb LA, Siscovick DS: Cell membrane trans-fatty acids and the risk of primary cardiac arrest. *Circulation* 2002, **105**:697-701.

Leth T, Jensen HG, Mikkelsen AE, Bysted A: The effect of the regulation on *trans* fatty acid content in Danish food. *Atheroscler Suppl* 2006, **7**:53-56.

Lichtenstein AH: *Trans* fatty acids and blood lipid levels, Lp(a), parameters of cholesterol metabolism, and hemostatic factors. *J Nutr Biochem* 1998, **9**:244-248.

Lock AL, Corl BA, Barbano DM, Bauman DE, Ip C: The anticarcinogenic effect of *trans*-11 18:1 is dependent on its conversion to *cis*-9,*trans*-11 CLA by Δ9-desaturase in rats. *J Nutr*

2004, **134**:2698-2704.

Lopez-Garcia E, Schulze MB, Meigs JB, Manson JAE, Rifai N, Stampfer MJ, Willett WC, Hu FB: Consumption of *trans* fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction. *J Nutr* 2005, **135**:562-566.

Luna P, Rodriguez-Pino V, de la Fuente MA: Occurrence of C16:1 isomers in milk fats from ewes fed with different dietary lipid supplements. *Food Chem* 2009, **117**:248-253.

Menaa F, Menaa A, Tréton J, Menaa B: Technological approaches to minimize industrial trans fatty acids in foods. *J Food Sci* 2013, **78**:R377-386.

Mensink RP, Katan MB: Effect of dietary *trans* fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *New Engl J Med* 1990, **323**:439-445.

Mente A, de Koning L, Shannon HS, Anand SS: A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. *Arch Intern Med* 2009, **169**:659-669.

Molkentin J: Authentication of organic milk using delta13C and the alpha-linolenic acid content of milk fat. *J Agric Food Chem* 2009, **57**:785-790.

Motard-Bélanger A, Charest A, Grenier G, Paquin P, Chouinard Y, Lemieux S, Couture P, Lamarche B: Study of the effect of *trans* fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease. *Am J Clin Nutr* 2008, **87**:593-599.

Mozaffarian D, Aro A, Willett WC: Health effects of *trans*-fatty acids: experimental and observational evidence. *Eur J Clin Nutr* 2009, **63**:5-21.

Mozaffarian D, Cao H, King IB, Lemaitre RN, Song X, Siscovick DS, Hotamisligil GS: Trans-palmitoleic acid, metabolic risk factors, and new-onset diabetes in U.S. adults: a cohort study. *Ann Intern Med* 2010, **153**:790-799.

Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC: Medical progress - *Trans* fatty acids and cardiovascular disease. *N Engl J Med* 2006, **354**:1601-1613.

Mueller A, Thijs C, Rist L, Simões-Wüst AP, Huber M, Steinhart H: *Trans* fatty acids in human milk are an indicator of different maternal dietary sources containing *trans* fatty acids. *Lipids* 2010, **45**:245-251.

New York City Department of Health and Mental Hygiene (2007): Healthy Heart-Avoid *Trans* Fat. [http://www.nyc.gov/html/doh/html/cardio/cardio-transfat.shtml 12.10.2009]

Nishida C, Uauy R: WHO Scientific Update on health consequences of trans fatty acids: introduction. *Eur J Clin Nutr* 2009, **63**:1-4.

Oomen CM, Ocké MC, Feskens EJM, van Erp-Baart MJ, Kok FJ, Kromhout D: Association between *trans* fatty acid intake and 10-year risk of coronary heart disease in the Zutphen Elderly Study: a prospective population-based study. *Lancet* 2001, **357**:746-751.

Özgul-Yücel S: Determination of conjugated linolenic acid content of selected oil seeds grown in Turkey. *J AOAC* 2005, **82**:893-897.

Pariza MW, Park Y, Cook ME: The biologically active isomers of conjugated linoleic acid. *Prog Lipid Res* 2001, **40**:283-298.

Parodi PW: Anti-cancer agents in milk fat. *Austr J Dairy Techn* 2003, **58**:114-118.

Pfeuffer M, Schrezenmeir J: Bioactive substances in milk with properties decreasing risk of cardiovascular diseases. *Brit J Nutr* 2000, **84**:155-159.

Pfeuffer M, Schrezenmeir J: Impact of *trans* fatty acids of ruminant origin compared with those from partially hydrogenated vegetable oils on CHD risk. *Int Dairy Journal* 2006, **16**:1383-1388.

Pietinen P, Ascherio A, Korhonen P, Hartman AM, Willett WC, Albanes D, Virtamo J: Intake of fatty acids and risk of coronary heart disease in a cohort of finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. *Am J Epidemiol* 1997, **145**:876-887.

Pintus S, Murru E, Carta G, Cordeddu L, Batetta B, Accossu S, Pistis D, Uda S, Ghiani EM, Mele M, Secchiari P, Almerighi G, Pintus P, Banni S: Sheep cheese naturally enriched in α-linolenic, conjugated linoleic and vaccenic acids improves the lipid profile and reduces anandamide in the plasma of hypercholesterolaemic subjects. *Br J Nutr* 2013, **109**:1453-1462.

Precht D, Molkentin J, Destaillats F, Wolff RL: Comparative studies on individual isomeric 18:1 acids in cow, goat, and ewe milk fats by low-temperature high-resolution capillary gas–liquid chromatograph. *Lipids* 2001, **36**:827-32.

Richter EK, Shawish KA, Scheeder MRL, Colombani PC: *Trans* fatty acid content of selected Swiss foods: The TransSwissPilot Study. *J Food Comp Anal* 2009. doi:10.1016/j.jfca.2009.01.007.

Roy A, Chardigny JM, Bauchart D, Ferlay A, Lorenz S, Durand D, Gruffat D, Faulconnier Y, Sébédio JL, Chilliard Y: Butters rich either in trans-10-C18:1 or in trans-11-C18:1 plus cis-9, trans-11 CLA differentially affect plasma lipids and aortic fatty streak in experimental atherosclerosis in rabbits. *Animal* 2007, **1**:467-476.

Schwenke DC, Foreyt JP, Miller ER 3rd, Reeves RS, Vitolins MZ: Oxidative Stress Subgroup of

the Look AHEAD Research Group: Plasma concentrations of trans fatty acids in persons with type 2 diabetes between September 2002 and April 2004. *Am J Clin Nutr* 2013, **97**:862-871.

Scrimgeour CM and Harwood JL: Fatty acid and lipid structure. In *The lipid handbook.* 3rd edition. Edited by Gunstone FD, Harwood JL, Dijkstra AJ. CRC Press, Boca Raton; 2007:6-7.

Shingfield KJ, Bernard L, Leroux C, Chilliard Y: Role of *trans* fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. *Animal* 2010, 4:1140-1166.

Sklar LA, Hudson BS, Simoni RD: Parinaric acid from Parinarium glaberrimum. *Methods Enzymol* 1981, **72**:479-82.

Sofi F, Buccioni A, Cesari F, Gori AM, Minieri S, Mannini L, Casini A, Gensini GF, Abbate R, Antongiovanni M: Effects of a dairy product (pecorino cheese) naturally rich in cis-9, trans-11 conjugated linoleic acid on lipid, inflammatory and haemorheological variables: a dietary intervention study. *Nutr Metab Cardiovasc Dis* 2010, **20**:117-124.

Stender S, Astrup A, Dyerberg J: Ruminant and industrially produced *trans* fatty acids: Health aspects. *Food Nutr Res* 2008, **52**. doi: 10.3402/fnr.v52i0.1651.

Stender S, Dyerberg J, Bysted A, Leth T, Astrup A: A *trans* world journey. *Atheroscler Suppl* 2006, **7**:47-52.

Sun QMD, Campos H, Hankinson SE, Manson JE, Stampfer MJ, Rexrode KM, Willett WC, Hu FB: A prospective study of *trans* fatty acids in erythrocytes and risk of coronary heart disease. *Circulation* 2007, **115**:1858-1865.

Sundram K, Ismail A, Hayes KC, Jeyamalar R, Pathmanathan R: *Trans* (elaidic) fatty acids

adversely affect the lipoprotein profile relative to specific saturated fatty acids in humans. *J Nutr* 1997, **127**:514-520.

Sundram K, Karupaiah T, Hayes KC: Stearic acid-rich interesterified fat and *trans*-rich fat raise the LDL/HDL ratio and plasma glucose relative to palm olein in humans. *Nutr Metab* 2007, **4**:3. doi:10.1186/1743-7075-4-3.

Terpstra AH: Effect of conjugated linoleic acid on body composition and plasma lipids in humans: an overview of the literature. *Am J Clin Nutr* 2004, **79**:352-361.

Thijs C, Müller A, Rist L, Kummeling I, Snijders BE, Huber M, van Ree R, Simões-Wüst AP, Dagnelie PC, van den Brandt PA: Fatty acids in breast milk and development of atopic eczema and allergic sensitisation in infancy. *Allergy* 2011, **66**:58-67.

Tholstrup T, Raff M, Basu S, Nonboe P, Sejrsen K, Straarup E: Effects of butter high in ruminant *trans* on lipoproteins, fatty acid incorporation in lipid classes, C-reactive protein, oxidative stress, hemostatic variables and insulin in healthy, young men. *Am J Clin Nutr* 2006, **83**:237-243.

Tokede OA, Petrone AB, Hanson NQ, Tsai MY, Weir NA, Glynn RJ, Gaziano JM, Djoussé L: Plasma phospholipid trans fatty acids and risk of heart failure. *Am J Clin Nutr* 2013, **97**:698-705.

Tricon S, Burdge GC, Jones EL, Russell JJ, El-Khazen S, Moretti E, Hall WL, Gerry AB, Leake DS, Grimble RF, Williams CM, Calder PC, Yaqoob P: Effects of dairy products naturally enriched with *cis*-9,*trans*-11 conjugated linoleic acid on the blood lipid profile in healthy middle-aged men. *Am J Clin Nutr* 2006, **83**:744-753.

Turpeinen AM, Mutanen MAA, Salminen I, Basu S, Palmquist DL, Griinari JM: Bioconversion

of vaccenic acid to conjugated linoleic acid in humans. *Am J Clin Nutr* 2002, 76:504-510.

Valenzuela A, Nieto S, Peterson G, Tavella M: Comparative study in fried food about the stability of different vegetable oils. *Fat Oils Mag* 2004, **2**:22-28.

Wahle KWJ, Heys SD, Rotondo D: Conjugated linoleic acids: are they beneficial or detrimental to health? *Prog Lipid Res* 2004, **43**:553-587.

Willett WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA, Sampson LA, Hennekens CH: Intake of *trans* fatty acids and risk of coronary heart diseases among women. *Lancet* 1993, **34**:1581-1585.

Zock PL, Katan MB. Hydrogenation alternatives: Effects of *trans* fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in humans. *J Lipid Res* 1992, **33**:399-410.

Table 1 Overview of conjugated fatty acids isomers including trivial names, sources, and quantity of total fat.

Conjugated FA	Trivial name	Sources	% of fat	Reference
CLA (C18:2)				
*c*9,*t*11	Rumenic acid	Ruminant dairy products	0.3 - 4.3	Fritsche and Steinhart 1997
*t*10,*c*12	-	Ruminant dairy products	Tr	Kraft et al. 2003
*t*11,*c*13	-	Ruminant dairy products	Tr - 0.25	Shingfield et al. 2010
*t*7,*c*9	-	Ruminant dairy products	Tr - 0.2	Degen et al. 2011a
*t*9,*t*11	Ricinenic acid	Ruminant dairy products	Tr	
*t*11,*t*13	-	Ruminant dairy products	Tr	
Mix 50:50 *c*9,*t*11 + *t*10,*c*12	-	Alkaline isomerisation of linoleic acids	n/a	Pariza et al. 2001
Mix *t*,*t*/*c*,*t*&*t*,*c*/*c*,*c* CLA isomeres	-	Dietary CLA supplement (CLA-EXTREME™ year 2006)	∑55 (25/65/10)	Kuhnt unpublished observation
t,*t* CLA (13,15-7,9)	-	Crème-filled wafer	0.2	Kuhnt unpublished observation
CLnA (C18:3)				
*t*8,*t*10,*c*12	*α*-Calendic acid	Seed oil, pot marigold *(Calendula officinalis)*	18 - 62	Özgul-Yücel 2005 Hennessy et al. 2011
*t*8,*t*10,*t*12	*β*-Calendic acid	Seed oil, pot marigold *(Calendula officinalis)*	5 - 11	Scrimgeour and Harwood
*c*8,*t*10,*c*12	Jacaric acid	Seed oil, blue Jacaranda *(Jacaranda mimosifolia)*	36	Hennessy et al. 2011
*c*9,*t*11,*t*13	*α*-Eleostearic acid	Tung seed oil *(Aleurites fordii)*	68	
		Seed oil, bitter gourd (*Momordica charantia*)	50 - 56	
		Seed oil, snake gourd (*Trichosanthes cucumerina*)	30 - 50	
*t*9,*t*11,*t*13	*β*-Eleostearic acid	Seed oil *(Momordica c., Punica g., Aleurites f.)*	8 - 21	
*t*9,*t*11,*c*13	Catalpic acid	Seed oil, Chinese catalpa *(Catalpa ovata)*	15 - 42	
*c*9,*t*11,*c*13	Punicic acid	Seed oil, pomegranate *(Punica granatum)*	57 - 83	
		Rape seed oil (GMO)	2.5	
CALA (C18:3)				
*c*9,*t*11,*c*15	Rumelenic acid	*L. plantarum* AKU1009a; Intestinal bifidobacteria	67; 75	Hennessy et al. 2011

*t*9,*t*11,*c*15	-	*L. plantarum* AKU1009a	33	
*c*9,*t*11,*c*15	Rumelenic acid	Ruminant dairy products	0.03 - 0.5	Gómez-Cortés et al. 2009
*c*9,*t*11,*t*15	-	Ruminant dairy products	Tr - 0.21	
CSDA (C18:4)				
*c*9,*t*11,*t*13,*c*15	Parinaric acid	Seed oil *(Arabidopsis thaliana)*	n/a	Sklar et al. 1981

ALA, alpha-linolenic acid; C, conjugated; CLA, conjugated linoleic acids; CLnA, conjugated linolenic acids; GMO, genetically modified organisms; n/a, not applicable; SDA, stearidonic acid; Tr, traces.

Table 2 Regulations for reducing TFA content in foodstuff (voluntary/obliging).

Country	Year	Regulation	Reference	Regulation related to
The Netherlands	1995	Voluntary initiative of industry	Katan 2006	I-TFA
Argentina	2001	Co-operative agreement for the production of TFA-free sunflower oils	Valenzuela et al. 2004	I-TFA
Denmark	2004	Legally obliging limit	Leth et al. 2006	I-TFA
Canada	2005	Legally obliging declaration	Health Canada 2007	I-TFA + R-TFA[1]
USA	2006	Legally obliging declaration	FDA 2003	I-TFA + R-TFA[1]
New York City	2007	Legally obliging limit for restaurants	New York City Department of Health and Mental Hygiene 2009	I-TFA
Australia/ New Zealand	2007	Voluntary declaration; non-regulatory TFA-lowering is recommended (because of small intake)	L'Abbé et al. 2009	I-TFA + R-TFA[1]
Switzerland	2008	Legally obliging limit	Richter et al. 2009	I-TFA
Austria	2009	Legally obliging limit	Federal Ministry of Health 2009	I-TFA
Iceland	2010	Legally obliging limit similar to Denmark; at the planning stage	EU Food Law 2010	I-TFA

[1] without conjugated fatty acids or not clearly stated; no claim for completeness.

Table 3 Total TFA intake and R-TFA proportions in various countries.

Country	Total TFA [en%]	R-TFA [% of total TFA]	Reference
Germany	0.8	50	Gabriel 2009
Germany	0.8	79	Hulshof et al. 1999 (TRANSFAIR 1995-96)
Denmark	0.7	86	Jakobsen et al. 2006, L'Abbé et al. 2009
Iceland	2.1	~5	Hulshof et al. 1999 (TRANSFAIR 1995-96)
Australia	0.6	60	Food Standards Australia New Zealand 2007
Canada	2.2	19	Health Canada 2007
USA	2.6	21	FDA 2003

Table 4 Habitual total TFA intake in Germany (survey of n = 15371 people; Gabriel 2009).

Total TFA intake	**Mean**	**SD**	**Percentile** 25.	50.	75.	90.	95.	**Min**	**Max**
g/d	1.94	1.25	1.11	1.64	2.41	3.43	4.23	0.01	18.97
en%	0.77	0.33	0.54	0.71	0.94	1.17	1.34	0.02	5.54

Table 5 Epidemiological findings – correlation between TFA intake and risk of cardiovascular disease.

Prospective study	Country	Duration (years)	Total TFA intake (g/d)	Correlated with	
				Total TFA (I-TFA/R-TFA)	R-TFA (ruminant fat)
Willett et al. 1993 (Nurses Health)	USA n = 85,095 women	8	2.4 - 5.7 [1]	↑	↓n.s.
Hu et al. 1997 (Nurses Health)	USA n = 80,082 women	14	2.4 - 5.2	↑	↔
Pietinen et al. 1997 (ATBC)	FIN n = 21,930 men	5 - 8	1.8 - 6.2	↑	↓
Gillman et al. 1997 (Framingham)	USA n = 832 men	21	0 - 9 [2]	↑ (margarine)	↔ (butter)
Oomen et al. 2001 (Zutphen Elderly)	NL n = 667 men	10	5.2 - >15.6	↑	↑n.s.
Bolton-Smith et al. 1996	UK n = 10,359 men & women	n.a.	3.8 – 11.9	= (only men)	↓ (only men)

n.s., not significant; n.a., not applicable; [1] corresponds to 1.3 - 3.2 en%; [2] assessed: 15% TFA from margarine.

Table 6 Calculated mean change of LDL-C/HDL-C from human studies depending on origin of TFA.[1]

TFA origin	Number of studies	Mean difference LDL-C/HDL-C	Range
I-TFA	29	+0.34	+0.12 to +0.64
R-TFA (*trans* C18:1 and R-CLA)	6	+0.09	-0.10 to +0.23
CLA 50:50 (*c*9,*t*11:*t*10,*c*12)	12	+0.09	-0.37 to +0.25

[1] based on Brouwer et al. 2010.

Table 7 Comparison of data of human studies with R-TFA in combination with R-CLA; synthetic *t*11/*t*12 and vs. I-TFA.

Source	Study		Study information: Design	Supplement	Dose from diet & products [g/d] #	Intake: TFA en% excl. CLA	Total fat intake g/d [en%] baseline	Total fat intake g/d [en%] inter-vention	Results: C mmol/L start end	HDL-C mmol/L start end	LDL-C mmol/L start end	LDL-C/ HDL-C start end	C/ HDL-C start end	TAG mmol/L start end	Other parameters: Compared to control	Comment
ruminant CLA and TFA	**Desroches et al. 2005**	**control**	16 overweight men; 4 wk cross-over/ 7 wk wash-out butter	low CLA/ low R-TFA	CLA 0.2 *t*11 ~0.1 Σ*t*C18:1 ~0.4	~0.1	101 [34.5]	135 [41]	4.85 4.59	1.06 1.10	3.17 3.08	3.16 3.04	4.82 4.47	1.75 1.33	CRP, lipoprot., TAG↔ plasma ApoB↑ VLDL&LDL ApoB↔ LDL peak particle diameter↔	isoenergetic
		test		high CLA/ mod. R-TFA	CLA 2.6 *t*11 ~1.4 Σ*t*18:1 ~2.8	~0.9		133 [41]	4.76 4.74*	1.11 1.11	3.11 3.20	3.00 3.11*	4.55 4.54*	1.56 1.31		
	Tricon et al. 2006	**control**	32 healthy men; 6 wk cross-over/ 7 wk wash-out dairy products	low CLA/ low R-TFA	CLA 0.2 *t*11 0.3 Σ*t*18:1 0.8	~0.3	100 [35.9]	107 [38]	4.50 4.47	1.09 1.09	2.93 2.90	2.87 2.77	4.38 4.25	1.09 1.05	LDL density↔ LDL-C oxidation↔	
		test		high CLA/ mod. R-TFA	CLA 1.4 *t*11 4.7 Σ*t*18:1 6.3	~2.1		114 [38]	4.46 4.61	1.10 1.09	2.90 2.98	2.75 2.86*	4.21 4.41‡	1.02 1.19		
	Tholstrup et al. 2006	**control**	42 healthy men; 5 wk parallel control butter; enriched butte via sunflower seeds	low CLA/ low R-TFA	CLA 0.3 *t*11 0.5 Σ*t*18:1 1.0	~0.4	no data	min. 115 g butter [42]	4.05 4.87	1.40 1.54	2.72 3.44	1.94 2.24	2.89 3.16	0.79 0.89	CRP↔ hemostatic risk factors↔ insulin↔ glucose↔ oxid. stress↔	high SFA portion in control butter
		test		high CLA/ mod. R-TFA	CLA 1.5 *t*11 3.6 Σ*t*18:1 5.8	~2.3	no data	min. 115 g butter [45]	4.02 4.57*	1.32 1.39*	2.76 3.17	2.09 2.29	3.04 3.29	0.88 1.01		
synth. *t*11/*t*12	**Kuhnt et al. 2006, blood lipids unpublished**	**control**	healthy men & women (12/12) 6 wk	palm kernel/ rapeseed oil	CLA 0 *t*11 0	0	m: 85 [29] w: 58 [27]	m: 89 [31] w: 64 [30]	4.08 4.22	1.36 1.34	2.37 2.42	1.80 1.86	3.08 3.21	0.96 0.94	CRP↔ oxid.stress (8-oxodG↔, 15-kd-PGF$_{2\alpha}$↔; 8-	-endogenous synthesis of *c*9,*t*11 CLA -men &women -gender x diet

			parallel suppl. mixed in chocolate spread		Σ*t*18:1)										iso-$PGF_{2\alpha}$↑ Inflammatory markers↔	on lipoproteins -baseline as covariate
		test		synthetic *t*11/*t*12 mix	CLA 0.0 *t*11 3.0 Σ*t*18:1 5.0	m: 2.2 w: 2.7	m: 72 [27] w: 62 [26]	m: 77 [29] m: 66 [30]	4.44 4.41	1.40 1.37	2.77 2.68	2.06 2.04	3.26 3.33‡	0.97 0.92		
industrial TFA vs. ruminant TFA	**Chardigny et al. 2008**	**positive control**	healthy men/ women (19/21); 3 wk cross-over/ 1 wk wash-out butter, cheese, cookies	high I-TFA	m/w *t*9+*t*10 ~8.2/7.4 *t*11 ~1.3/1.2 Σ*t*18:1 ~12.9/11.7	~5.5	m: ~92 [37] w: ~74 [39]	m: ~84[36] w: ~78 [37]	4.73‡ ¥4.42	1.84 1.72	2.47 2.28	~1.34 ~1.33	~2.57 ~2.56	0.90 0.90	ApoA1↑, ApoB↑ CETP activity↔, Lp(a)↔	-only 1-wk wash out -men & women -gender x intervention on lipoproteins -‡only total baseline values available; -baseline as covariate
		test		high R-TFA	m/w *t*9+*t*10 ~1.8/1.6 *t*11 ~8.2/7.2 Σ*t*18:1 ~11.5/10.1	~4.8		m: ~86 [36] w: ~84 [40]	4.73 4.72*	1.84 1.77*	2.47 2.49*	~1.34 ~1.41	~2.57 ~2.67	0.90 1.01*		
	Motard-Bélanger et al. 2008	**control**	38 healthy men 3 wk cross-over/ 3-12 wk wash-out 1. control butter 2. shortening 3. mix: control/*t*11 4. *t*11-butter	control (low TFA)	*t*9+*t*10 ~0.5 *t*11 ~1.2 Σ*t*18:1 ~2.7	0.8	no data	~131 [37]	- 4.77	- 1.25	- 3.27	- 2.75	- 3.97	- 0.98	ApoA1↔, ApoB↔ CRP↔	-low *t*9 in shortening -low dietary CLA (0.2-0.6 g/d) -all values adjusted at baseline changed, except TAG, CRP - sign. to moderate R-TFA
		test		high I-TFA	*t*9+*t*10 ~4.2 *t*11 ~2.4 Σ*t*18:1 ~11.9	3.7		~134 [37]	- 4.88•	- 1.23	- 3.42•	- 2.94	- 4.16•	- 0.97•		
				mod. R-TFA	*t*9+*t*10 ~0.8 *t*11 ~2.3 Σ*t*18:1 ~5.2	1.5		~135 [37]	- 4.72	- 1.28	- 3.22	- 2.67	- 3.86	- 0.95		
				high R-TFA	*t*9+*t*10 ~1.9 *t*11 ~5.9 Σ*t*18:1 ~12.5	3.7		~136 [38]	- 4.92•	- 1.22•	- 3.47*•	- 3.02*•	- 4.23•	- 0.99•		

[#]CLA, mainly *c*9,*t*11-CLA; mod.: moderate; * significantly different to control: * P<0.05, [‡] P<0.1; ~calculated with published data (fat: 9 kcal/g); ↔ unchanged; ↑significantly increased in the test group; [¥] calculation: mg/dl into mmol/L (:38.67) or TAG: mg/dl into mmol/L (:88.57).

Table 8 Lipoprotein profile of subjects with supplementation of *t*11 and *t*12 mixture compared to placebo (unpublished results).

	TFA test group (6 g/d *t*11/*t*12 mixture; n=12)				**Placebo group** (no R-TFA, ruminant fat free; n=12)				***t*11/*t*12** vs. **placebo**
Serum [mmol/L]	**Start** day 0	**End** day 56	Change	P^1	**Start** day 0	**End** day 56	Change	P^1	P^2
Total C	4.44±0.78	4.41±0.79	-0.03	*.742*	4.08±0.55	4.22±0.43	+0.14	*.211*	***.457***
HDL-C	1.40±0.30	1.37±0.32	-0.03	*.232*	1.36±0.23	1.34±0.19	-0.02	*.515*	***.713***
LDL-C	2.77±0.71	2.68±0.69	-0.09	*.218*	2.37±0.64	2.42±0.55	+0.05	*.609*	***.538***
C/HDL-C	3.26±0.71	3.33±0.65	+0.07	*.387*	3.08±0.64	3.21±0.53	+0.13	*.072*	***.810***
LDL-C/ HDL-C	2.06±0.64	2.04±0.60	-0.02	*.839*	1.80±0.61	1.86±0.58	+0.06	*.372*	***.467***
TAG	0.97±0.41	0.92±0.33	-0.05	*.522*	0.96±0.36	0.94±0.34	-0.02	*.826*	***.799***

Values are means ± SD; study design and blood fatty acids published by Kuhnt et al. 2006;
[1]*P* value analysed by repeated measures; [2]*P* value analysed by univariate ANOVA with baseline as covariate (SPSS 19.0, IBM Corporation).

Table 9 R-TFA and R-CLA content in milk from cows and ewe under different feeding conditions (unpublished data).

Species, feeding Fatty acids as % of fat	**R-TFA**			**R-CLA**	
	Total *trans* C18:1	***t*11** [% of total *trans* C18:1]	***t*9/*t*11 index**	**Total CLA**	***c*9,*t*11 CLA** [% of total CLA]
Cow					
Conventional, concentrate [1]	1.5	0.3 [23%]	0.4	0.4	0.3 [78%]
Organic, grass [1]	3.8	2.0 [53%]	0.3	1.2	0.9 [73%]
Bulgarian highland, pasture[2]	5.2	3.0 [58%]	0.1	2.2	1.8 [82%]
Alpine milk, pasture 1200 m[3]	5.5	4.0 [67%]	0.08	2.6	2.3 [88%]
Alpine milk, pasture up to 2100 [2]	6.3	4.5 [67%]	0.07	3.1	2.7 [87%]
Ewe					
Organic, grass [1]	3.5	1.5 [43%]	0.2	1.3	1.0 [78%]
Bulgarian highland, pasture [2]	6.5	4.0 [62%]	0.09	2.9	2.3 [82%]

[1] Germany, [2] Bulgaria, [3] Switzerland.

Table 10 Fatty acid distribution in erythrocytes of populations with high and no dairy fat intake.

Study population	No dairy fat group[1] (8 wks)	Kenyan Maasai[2] (high dairy fat)	Bulgarian herdsmen[3] (high dairy fat)
	n=12	n=18	n=8
Dairy fat intake	~0	46 g/d	n.a.
FA [% of total FAME]			
Σ SFA	44.9±6.57	44.1±1.18	44.5±2.28
Σ MUFA	21.5±2.00	19.9±1.37	16.7±0.79
Σ PUFA	33.3±8.16	36.1±1.52	30.6±1.42
C14:0	0.34 ±0.10	0.42±0.11	0.55±0.27
C15:0	0.19±0.04[b]	0.32±0.07[a]	0.32±0.08[a]
C16:0	30.3±3.41[a]	28.2±1.66[b]	23.92±1.11
C17:0	0.38±0.08[b]	0.54±0.06[a]	0.51±0.08[a]
C18:0	12.1±3.10	11.8±1.53	15.34±1.08
C18:1 *c*9	18.0±1.68	17.1±1.39	12.69±0.63
C18:1 *t*9	0.16±0.05[a]	0.10±0.02[b]	0.10±0.02[b]
C18:1 *t*10	0.07± 0.03	0.03±0.01	0.10±0.02
C18:1 *t*11	0.08±0.02[c]	0.29[b]±0.08	0.48±0.09[a]
*t*9/*t*11 index	2.17[a]	0.39[b]	0.22[c]
Σ *trans* C18:1	0.58±0.13[c]	0.68±0.14 [b]	1.16±0.24
*t*9,*t*12 C18:2	0.07±0.01[a]	0.02±0.01[b]	0.01±0.01[b]
*c*9,*t*11 CLA	0.08±0.02[b]	0.41±0.12[a]	0.53±0.07[a]
Σ CLA	0.09±0.04[b]	0.54±0.14[a]	0.63±0.10[a]

Values are means ± SD; [1] Placebo group of the intervention study published by Kuhnt et al. 2006; [2] Knoll et al. 2011; [3] Samples from Bulgarian herdsmen from Rhodope Mountains (year 2007, unpublished data); [abc] post hoc Student-Newman-Keuls (SPSS 16); n.a., not analysed.

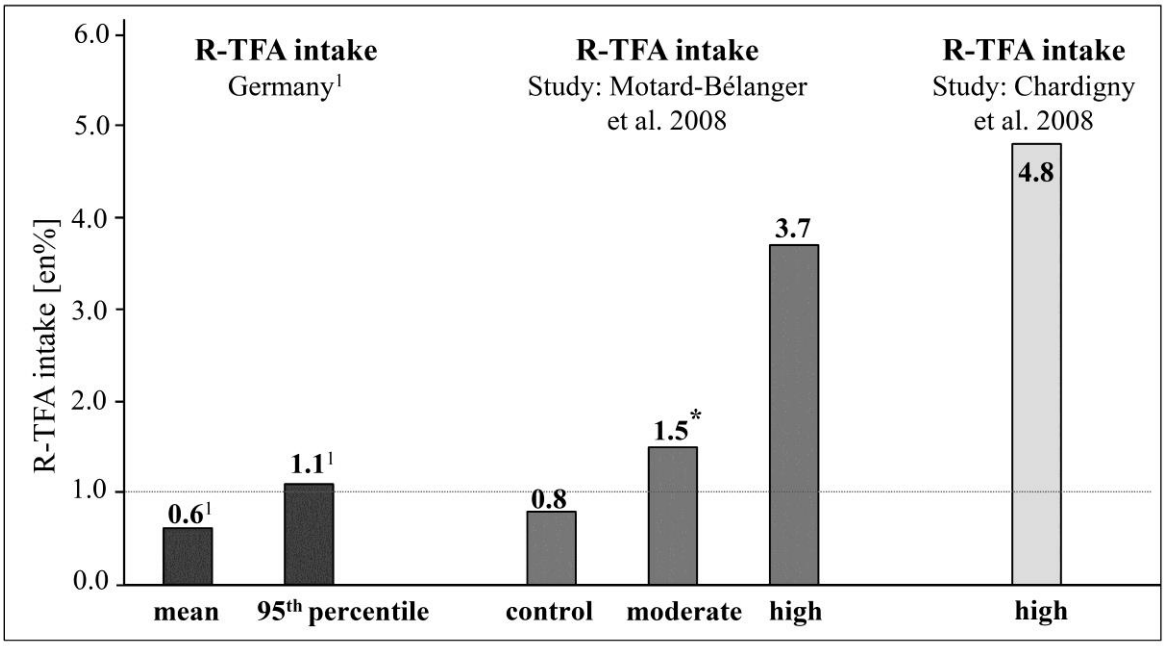

Figure 1

Figure 1 Comparison of an assumed high R-TFA intake in Germany and those amounts administered in the published studies (calculations without R-CLA).

[1] based on the BfR estimates of total TFA intake (Gabriel 2009), calculated with a theoretically high portion of R-TFA with 80% from total TFA.

* no effects on serum TC and lipoprotein profile compared to the control diet (Motard-Bélanger et al. 2008).