Effect of *Trans* Fatty Acids on Milk Fat and Their Impact on Human Health

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Abstract

Advances in our understanding of lipid metabolism in the ruminant and the effect of specific fatty acids on both ruminant metabolism and human health has increased significantly in recent years. Unique biohydrogenation intermediates formed in the rumen have been shown to be potent inhibitors of milk fat synthesis, and the biology behind this and its potential application and benefit to both animal welfare and animal productivity are active areas of research. In spite of the negative image of dietary *trans* fatty acids on human health, the two major *trans* fatty acids found in ruminant meat and milk, vaccenic acid and CLA, have been shown to be effective in preventing cancer and atherogenesis in biomedical studies with animal models. The enrichment of these fatty acids in milk by natural means offers potential to not only benefit human health but also improve the public perception of milk and dairy products.

Introduction

There has been an increased interest over the last decade in understanding the metabolism of dietary lipids and their role in health and well-being. This interest has been complimented by analytical advances which have improved our ability to quantify specific fatty acids and the intermediates of fatty acid metabolism. This interest has also been fueled by the recognition that certain fatty acids are key regulators of gene expression and metabolic processes. The role of specific fatty acids in eicosanoid synthesis and reproductive processes, as well as the ability of specific conjugated linoleic acids to regulate fat synthesis are two examples featured in these proceedings.

Interest in fatty acids has also come from the recognition that certain dietary fatty acids can impact human health. In particular, the association between the intake of *trans* fatty acids and the risk of coronary heart disease (CHD) has been a recent medical focus (Institute of Medicine, 2002). Consequently the FDA has announced that the of the content of *trans* fat in foods and dietary supplements will be mandatory on nutritional labels beginning in 2006 (US FDA, 2003). In the following section we will provide background information on *trans* fatty acids and their presence in ruminant tissues and ruminant-derived food products. The *trans* fatty acid content relates to rumen biohydrogenation, therefore additional sections will discuss their origin in ruminants and their recently discovered role in regulating milk fat synthesis. Finally we will examine the human consumption of *trans* fatty acids and recent results investigating their possible effects on human health.

Background

Trans Fatty Acids

Unsaturated fatty acids are typically present in feeds of plant origin, with linoleic acid (18:2) and linolenic acid (18:3) being the most common ones in seeds and forages, respectively. In nature, the double bonds in these

unsaturated fatty acids typically have a *cis* orientation. This geometric configuration has the hydrogen atoms of the two carbons connected by a double bond located on the same side of the fatty acid. As shown by the example of linoleic acid (*cis*-9, *cis*-12 18:2), this results in a bend in its structure (Figure 1). In contrast, *trans* fatty acids contain at least one double bond in which the double bond has a *trans* geometric configuration where the hydrogen atoms are located on opposite sides of the carbons adjoined by a double bond. This results in a structure where the fatty acid carbon chain is straight as demonstrated by vaccenic acid (*trans*-11 18:1; VA; Figure 1).

The most common *trans* fatty acids in human (and animal) diets are eighteen carbon fatty acids with a single double bond (18:1 *trans*) and for this paper we will refer to these as TFA. In the US diet, about 80-90% of the TFA originate from partially hydrogenated vegetable oils (PHVO) that are used in cooking and preparation of processed foods (US FDA, 2003). The remainder of the dietary intake of TFA comes from food products derived from ruminants. The *trans* double bond can exist at various positions in the octadecenoic fatty acid. In milk fat and meat from ruminants, VA is the most common TFA, accounting for 60-80% of the total (Emken, 1995; Craig-Schmidt, 1998). In contrast, PHVO contains large amounts of many *trans* 18:1 isomers. These differences in isomer distribution of the TFA may be of special significance in relation to human health effects as will be discussed subsequently.

Conjugated Linoleic Acids

Conjugated linoleic acids (CLA) are also unsaturated fatty acids that contain two double bonds. These double bond pairs can be *trans/trans*, *trans/cis*, *cis/trans* or *cis/cis* (Sehat et al., 1998). Thus, many of the CLA are also a specialized form of *trans* fatty acids. However, CLA differ from naturally occurring fatty acids in that the double bonds are located on adjacent carbons. For example, in *cis*-9, *trans*-11 CLA the double bonds are located between carbons 9-10 and 11-12 (Figure 1). This contrasts with typical PUFA where there is an interceding methylene group (CH_2) between the double bonds as illustrated for linoleic acid (Figure 1; double bonds located at carbons 9-10 and 12-13 separated by the carbon 11 methylene group).

The predominant source of CLA in human diets is ruminant-derived food products. Dairy products provide about 70% of the intake and beef products account for another 25% (Ritzenthaler et al., 2001). Many different isomers of CLA are found in ruminant fat, but *cis*-9, *trans*-11 CLA is the major form and represents about 75-90% of the total. The second most common form is *trans*-7, *cis*-9 CLA and it represents about 10% of the total. The remainder of the CLA is composed of other *trans/trans*, *trans/cis*, *cis/trans* or *cis/cis* forms, with each individual isomer typically representing a small portion (<1%) of the total (Bauman et al., 2003).

The Cow Dimension

Rumen Biohydrogenation

The presence of different isomers of TFA and CLA is a consequence of the biohydrogenation of dietary polyunsaturated fatty acids (PUFA) by rumen bacteria (Harfoot and Hazlewood, 1997). The major pathways for the biohydrogenation of linoleic acid and linolenic acid are shown in Figure 2. Complete biohydrogenation results in the production of stearic acid (18:0), but VA is an intermediate in the pathways for both of these unsaturated fatty acids. As a consequence of some of this intermediate escaping complete biohydrogenation in the rumen, VA represents about 60 – 70% of the TFA in milk and meat from ruminants (Emken, 1995). Other TFA isomers in milk fat are also of rumen origin being formed by less prominent pathways for PUFA biohydrogenation. In addition, recent studies have shown that *trans* 18:1 isomers are also formed in the rumen from oleic acid (*cis*-9 18:1; Mosley et al., 2002).

As mentioned earlier the most common CLA isomer is *cis*-9, *trans*-11 CLA, and it is only formed as the initial intermediate in the biohydrogenation of linoleic acid (Figure 2). Based on this it was assumed that escape from the rumen was the origin of this CLA isomer in milk fat. However, detailed studies across a wide range of diets demonstrated this was incorrect (Griinari et al., 2000; Corl et al., 2001; Lock and Garnsworthy, 2002; Piperova et al., 2002; Shinfield et al., 2003; Kay et al., 2004). Rather the predominant source of the *cis*-9, *trans*-11 CLA in milk fat is endogenous synthesis involving the enzyme Δ^9-desaturase with VA as the substrate (Figure 2). As a result, the ratio of VA:*cis*-9, *trans*-11 CLA in milk fat is typically about 3:1. The *trans*-7, *cis*-9 CLA in milk fat is also almost exclusively of endogenous origin being synthesized by Δ^9-desaturase with rumen-derived *trans*-7 18:1 as the

precursor (Corl et al., 2002; Piperova et al., 2002). However, other CLA isomers are of rumen origin and formed as intermediates in the bacterial biohydrogenation of polyunsaturated fatty acids by less prominent pathways.

Milk Fat Synthesis

It has been know for over a century that certain types of diets cause a marked reduction in milk fat secretion. This is commonly referred to as milk fat depression (MFD) and typically occurs with diets that are low in effective fiber, for example low forage diets or diets where the forage has been chopped or pelleted, or when dietary supplements of plant or fish oils are fed (Davis and Brown, 1970). This has been an active area of research over the last 50 years and many theories have been considered and found to be inadequate to explain MFD (see reviews Erdman, 1996; Bauman et al., 2001; 2003). However, recent research has established that diet-induced MFD is related to rumen biohydrogenation and requires two conditions: 1) a change in rumen environment resulting in a shift in the rumen bacteria population which is often associated with a reduction in rumen pH and 2) consumption of a diet containing PUFA.

Diet-induced MFD results in an increase in the milk fat content of TFA, specifically an increase in *trans*-10 18:1 (Griinari et al., 1998). Based on this and other recent developments, Bauman and Griinari (2001; 2003) proposed the "biohydrogenation theory" to explain the cause and mechanism of MFD. They hypothesized that under certain dietary conditions the pathways of rumen biohydrogenation are altered to produce unique fatty acid intermediates that are potent inhibitors of milk fat synthesis. Consistent with this concept, Baumgard et al. (2000) were the first to demonstrate that *trans*-10, *cis*-12 CLA was a potent inhibitor of milk fat synthesis. Subsequent abomasal infusion studies established the dose response relationship between *trans*-10, *cis*-12 CLA and the reduction in milk fat with as little as 3 g/d causing over a 20% reduction in milk fat yield (Baumgard et al, 2001; Peterson et al., 2002). Thus, with certain diets the rumen environment is altered so that a portion of the biohydrogenation involves a pathway that produces this CLA isomer (Figure 3). In this case, the first step in the biohydrogenation of linoleic acid involves an isomerization of the *cis*-9 double bond resulting in the formation of *trans*-10, *cis*-12 CLA (Figure 3). Based on comparisons between the milk fat content of *trans*-10, *cis*-12 CLA in studies of diet-induced MFD and during dose response studies with the pure isomer, we have proposed that there must be additional unique biohydrogenation intermediates that can inhibit milk fat synthesis (Bauman and Griinari, 2001; Peterson et al., 2003a). The increases in milk fat content of *trans*-10 18:1 that occur during diet-induced MFD raise the possibility that this fatty acid might have an effect. This has not been directly examined, but consideration of results from studies with PHVO indicate that if *trans*-10 18:1 does effect milk fat synthesis, its potency must be several orders of magnitude less than *trans*-10, *cis*-12 CLA. Identifying additional biohydrogenation intermediates that effect milk fat synthesis is an exciting and active area of research.

The mechanism for MFD focuses on the mammary gland and involves coordinated reductions in mRNA abundance of genes for key enzymes associated with all major aspects of milk fat synthesis. These processes include de novo synthesis of fatty acids, uptake and transport of preformed fatty acids, desaturation, and packaging into triglycerides (Piperova et al., 2000; Ahnadi et al., 2002; Baumgard et al., 2002; Peterson et al., 2003a). Furthermore, reductions in the expression of lipogenic genes were related to the presence of *trans*-10, *cis*-12 CLA in milk fat for both dietary-induced MFD and the MFD occurring with abomasal infusion of pure *trans*-10, *cis*-12 CLA (Baumgard et al., 2002; Peterson et al., 2003a). These results provide support for a mechanism involving alterations in transcription activation of these genes, and our recent work suggests the sterol response element-binding protein (SREBP) family of transcription factors are probably involved in the intracellular signaling pathway (Peterson et al., 2003b).

Certain situations exist where a technology to reduce milk fat output in a controlled manner could be of benefit to both animal and producer. These include markets where producers are regulated by a quota system based on milk fat, as well as periods where animals cannot consume sufficient energy to meet requirements (ie. early lactation, episodes of heat stress, or when poor weather limits forage growth in pasture-based systems). Recent long-term studies (up to 20 wk) have shown that a controlled reduction in milk fat yield is possible with supplementation of rumen-protected CLA supplements (Perfield et al., 2002; Bernal-Santos et al., 2003). This reduction is specific for milk fat and has no adverse effects on animal well-being, and termination of supplementation results in return of milk fat yield to previous levels. Investigation of reduced milk fat yield as a potential management tool continues to be an active area of research (see review by Griinari and Bauman, 2003).

Implications for Human Health

Coronary Heart Disease

Current public health policy strongly recommends a reduction in the intake of TFA. This recommendation is based on the possible adverse effects of TFA related to their putative association with elevated plasma concentrations of cholesterol and LDL along with lower concentrations of HDL (Williams, 2000; Institute of Medicine, 2002; Danish Nutrition Council, 2003). These changes in blood lipids are associated with an increased risk of CHD and, as mentioned earlier, beginning January 1, 2006 there is a mandatory requirement for food labels to list content of *trans* fat just as currently occurs for saturated fat (US FDA, 2003). As will be subsequently discussed, many beneficial effects have been identified for CLA isomers and because of this the FDA has excluded these special *trans* fatty acids from their definition of "*trans* fat" for the purposes of nutritional labeling.

Unfortunately, little or no distinction has been made between the biological effects of different TFA isomers. Most of the available data examining variables associated with CHD relate to TFA from PHVO and specific studies with *trans*-9 18:1 (elaidic acid). These data have been broadly extrapolated to imply that high consumption of any and all *trans* fatty acid isomers is associated with increased CHD. However, double bond position in fatty acids is an important consideration in terms of biological effects. Ruminant-derived lipids typically contain 1 - 8% of their total fatty acids as TFA (Craig-Schmidt, 1998) with the predominant isomer being VA (Emken, 1995; Figure 4). In contrast, PHVO derived from the commercial hydrogenation of vegetable and marine oils can contain 40 - 60% TFA with a Gaussian distribution of the *trans* 18:1 isomers that centers on *trans*-9, *trans*-10, and *trans*-11 (Emken, 1995; Craig-Schmidt, 1998; Figure 4). Holmer (1998) reviewed a number of studies that investigated the metabolism of TFA with the majority of these comparing oleic to elaidic acid. However the properties of elaidic acid will not necessarily be representative of all *trans*-18:1 acids. For example, Hodgson et al. (1996) found that the intake of *trans*-9 and *trans*-10 18:1 were positively correlated with CHD, whereas the intake of VA was not.

A number of epidemiological studies have investigated the relationship between dietary intake of TFA and CHD, and these are cited as strong evidence for the need to reduce the intake of TFA (Institute of Medicine, 2002). However, as discussed above, differences among TFA isomers (i.e. food sources) may be an important consideration. Some epidemiological studies have data that allow a comparison of food sources. Our assessment of these indicates that the positive relationship between TFA and CHD risk is specifically related to the intake of TFA derived from vegetable fats (Willet et al., 1993; Ascherio et al., 1993; Bolton Smith et al., 1996; Gillman et al., 1997; Pietinen et al., 1997). In fact, in three of these studies (Willet et al., 1993; Bolton Smith et al., 1996; Pietinen et al., 1997) there was a negative association between the intake of TFA of animal origin and the risk of CHD. As the intake of TFA from vegetable fats progressively increases, the relative risk of CHD also increases with a risk of 1.78 at the highest quintile (Table 1). In contrast, CHD risk decreases with increasing intake of TFA from animal sources (Table 1). Furthermore, a German case-control study reported that subjects with angiographically documented CHD had less *cis*-9, *trans*-11 CLA in their adipose tissue (i.e. less intake of ruminant fats) than control subjects (Fritsche et al., 1998). Although some studies have reported no differences in the effects of elaidic and VA on blood lipoprotein concentrations (Meijer et al., 2001) the weight of evidence does not support these findings. In fact it has been proposed (Ackman, 1997) that evolution and long-term coexistence have adapted humans to cope with the type of TFA present in ruminant products.

Based on the above results, we conclude that TFA from ruminant fats differ in their relationship to the risk of CHD, and we suggest that this difference relates to the type of TFA, specifically the presence of VA and CLA. Studies have examined the effects of CLA on CHD and related variables in a number of animal models. These results indicate that dietary CLA can reduce plasma cholesterol and suppress cholesterol-induced atherosclerosis. Initial investigations used the rabbit as the model and demonstrated that feeding 0.5% CLA resulted in reductions in plasma concentrations of total and LDL-cholesterol (Lee et al., 1994). Using the hamster model, Nicolosi et al. (1997) demonstrated a reduction in total and non-HDL cholesterol levels when a commercial CLA preparation was fed at 0.025 to 0.5% CLA. However, Munday et al. (1999) found no beneficial effect of dietary CLA supplements on CHD-related variables in a mouse model. Nevertheless, recent work to assess the effects of feeding pure *cis*-9, *trans*-11 CLA has shown that it provides the same level of protection against cholesterol-induced atherogenesis in rabbits compared with synthetic mixtures of CLA isomers (Kritchevsky, 2003). It therefore seems logical that

dietary VA supplied by dairy products will also have beneficial effects on variables associated with increased risk for CHD as it could be used via endogenous synthesis to provide additional *cis*-9, *trans*-11 CLA.

Cancer

The predominance of human health related work has been concerned with the anticancer properties of CLA. Investigations with animal and cell models have established that CLA is anti-carcinogenic for many types of cancer. This work has typically been performed using commercially available synthetic CLA isomers. However, in collaboration with scientists at Roswell Park Cancer Institute (Buffalo, NY) we demonstrated that dietary consumption of a VA/CLA-enriched butter was effective in reducing the incidence of tumors in a rat-model of mammary carcinogenesis (Ip et al., 1999). These results were among the first to demonstrate that a naturally produced anti-carcinogen, consumed as a component of a natural food, is effective in preventing cancer. During this study we made the unexpected observation that the tissue content of *cis*-9, *trans*-11 CLA was greater when the CLA was supplied by butter than for a comparable amount of the same chemically prepared CLA isomer (Ip et al., 1999). We postulated this difference was related to endogenous synthesis of *cis*-9, *trans*-11 CLA from the VA present in the dietary supply of butter. An initial study supported this when we observed that feeding rats increasing amounts of pure VA resulted in a progressive increase in the tissue concentrations of *cis*-9, *trans*-11 CLA, and a corresponding reduction in the number of premalignant mammary lesions, an early marker for mammary cancer (Banni et al., 2001). Based on these results, we investigated this more extensively in the rat mammary cancer model by using naturally produced dairy products to formulate diets that varied in the content of VA and *cis*-9, *trans*-11 CLA. Fatty acid analysis showed that the conversion of dietary VA to CLA resulted in a dose-dependent increase in the accumulation of *cis*-9, *trans*-11 CLA in the mammary fat pad and this was accompanied by decreases in both tumor incidence and tumor number (Table 2; Corl et al., 2003). We are currently investigating the extent to which the observed cancer-preventing effect is specific to VA as compared to effects being due to the conversion of VA to *cis*-9, *trans*-11 CLA. Nevertheless, from these data it is clear that VA, the predominant TFA present in dairy products, is anticarcinogenic.

The endogenous synthesis of *cis*-9, *trans*-11 CLA from VA is dependent on the enzyme Δ^9-desaturase, and this is the major source of CLA in ruminant food products as discussed earlier. Of special importance, this enzyme is also present in human tissues and several studies have established that humans are capable of converting VA to *cis*-9, *trans*-11 CLA (Salminen et al., 1998; Adlof et al., 2000; Turpeinen et al., 2002). In fact, Turpeinen et al. (2002) reported that approximately 20 % of VA is converted to CLA in humans, thereby doubling CLA supply to tissues. Thus, this enzyme system is the key in differentiating VA from other *trans* 18:1 isomers. Although difficult to show a direct effect of an anticarcinogen in the human diet, it was recently observed that there was an inverse relationship between dietary and serum CLA and the risk of breast cancer in postmenopausal women (Aro et al., 2000).

Additional Health Effects

In the last decade CLA have been shown to have a number of additional beneficial health effects in biomedical studies with animal models. These include reducing the onset and severity of diabetes and obesity, immune modulation, and altering the rate of bone formation. Although research in these areas are limited compared with the effects of CLA on cancer, they still merit consideration and further research. Due to the limited scope of this paper the reader is directed to the following reviews for further information: Belury (2002), Parodi (2002) and Whigham et al. (2000).

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Figure 1. Chemical structures of linoleic acid (*cis*-9, *cis*-12 18:2; A), *cis*-9, *trans*-11 CLA (B) and *trans*-11 18:1 (C). Arrows indicate location of double bonds. Adapted from Bauman et al. (2000).

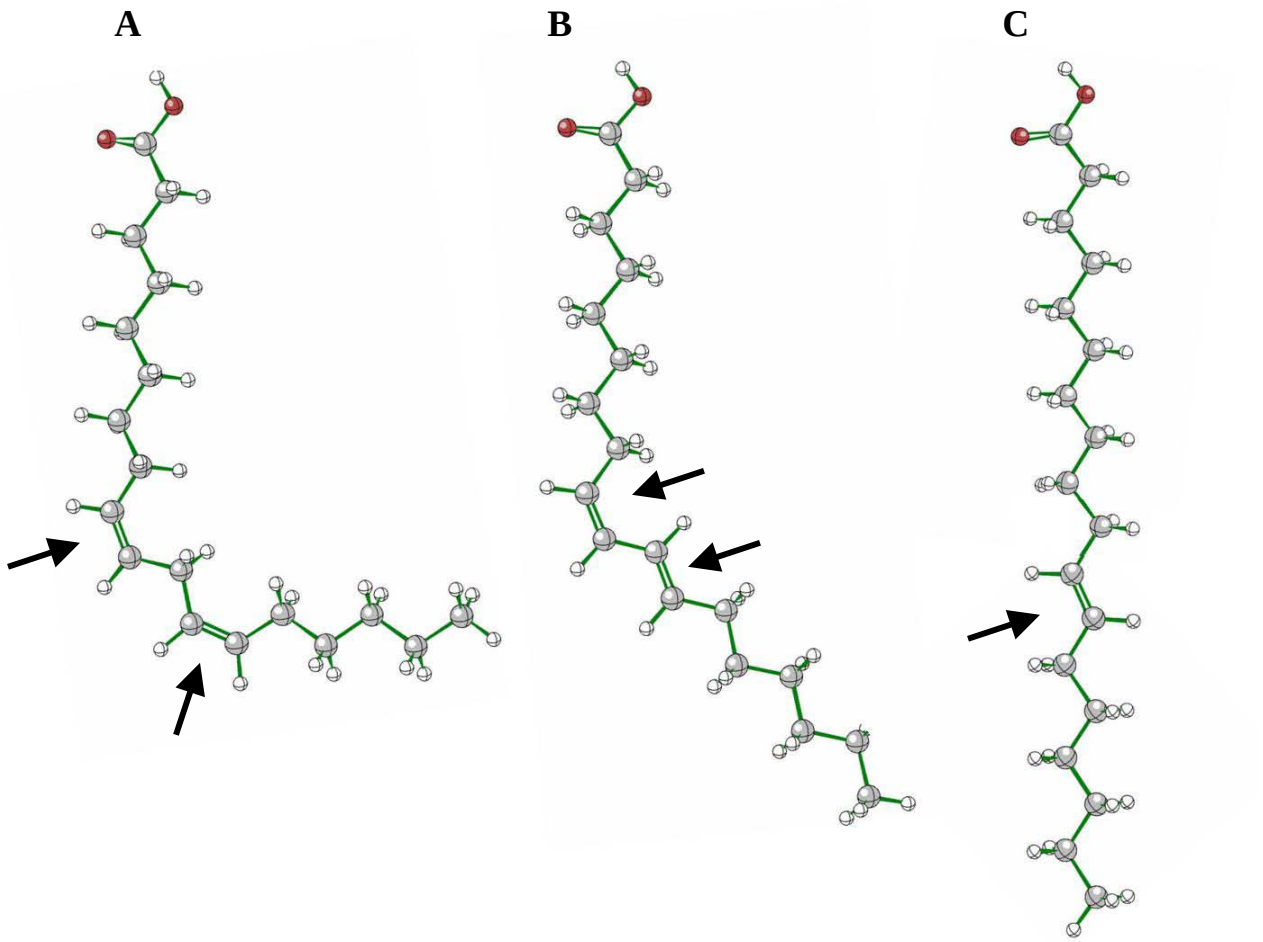

Figure 2. Pathways for rumen and endogenous synthesis of *cis*-9, *trans*-11 CLA in the dairy cow. Adapted from Bauman et al. (2003).

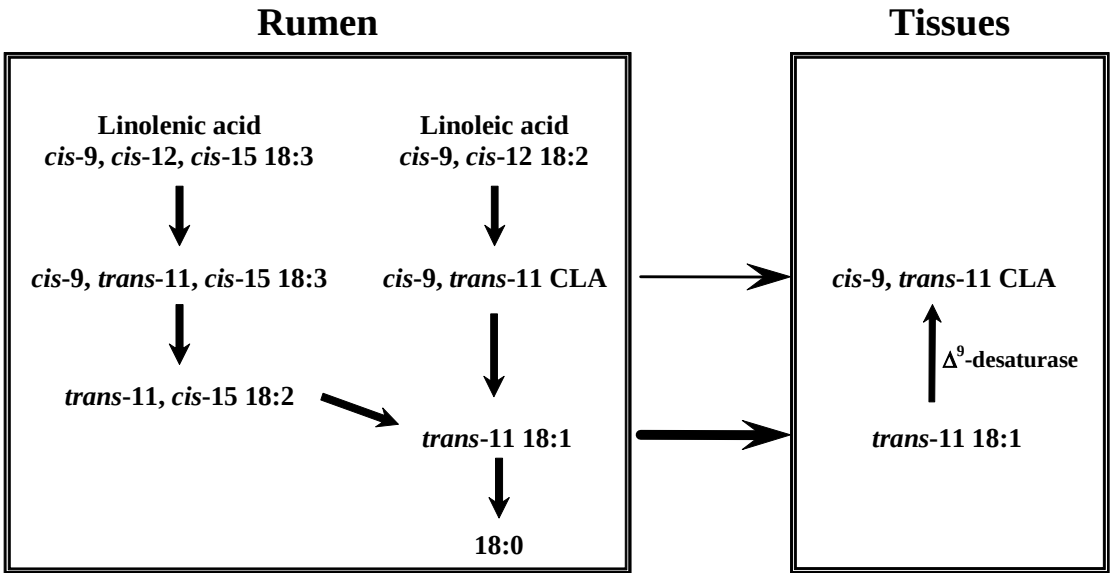

Figure 3. Alteration in the biohydrogenation pathway of linoleic acid. Adapted from Griinari and Bauman (1999).

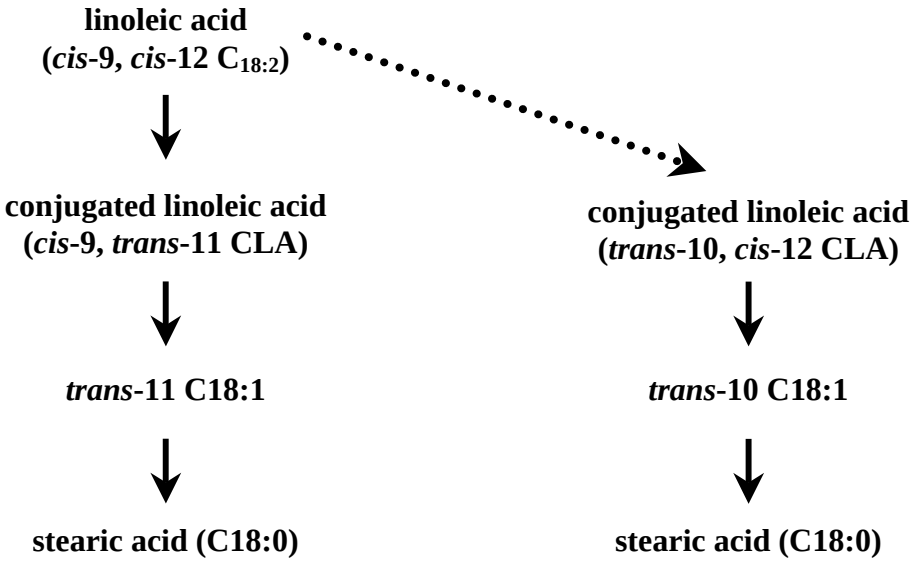

Figure 4. Typical distribution of *trans* fatty acids in ruminant lipids and partially hydrogenated vegetable oils. Adapted from Danish National Council (2003).

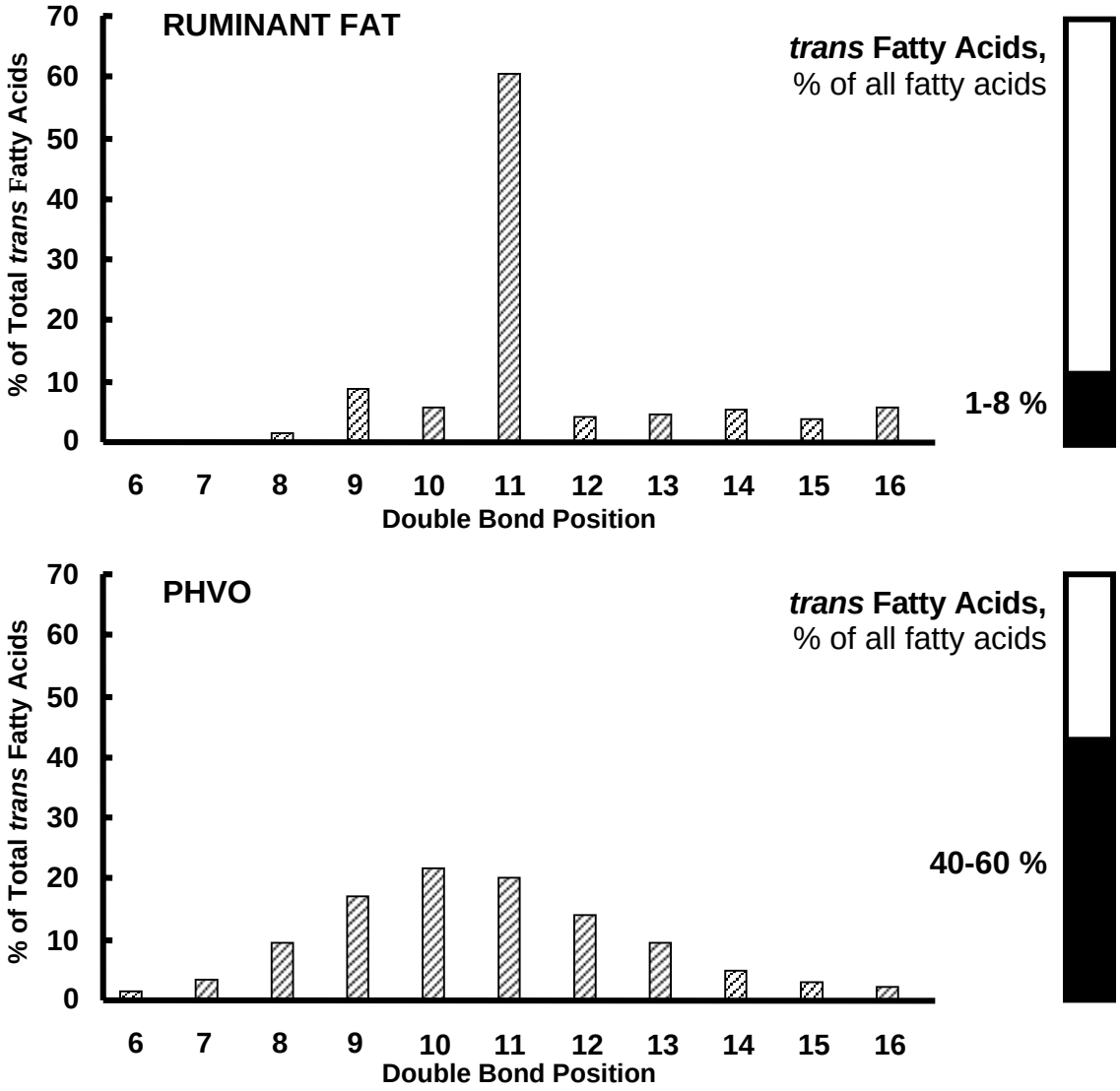

Table 1. Relative risk of coronary heart disease in women[1]. Adapted from Willet et al. (1993).

	Relative Risk in Quintile				
Trans Isomer Source	1	2	3	4	5
Vegetable fats	1.00	1.43	1.11	1.39	1.78
Animal fats	1.00	0.76	0.69	0.55	0.59

[1]Nurses health study: 69,181 women.

Table 2. Effect of altering the amount of CLA and VA in the diet on mammary fatty acid content and tumor development in the mammary cancer rodent model[1]. Adapted from Corl et al. (2003).

	Fatty Acid Percent of Diet[2]		Mammary Fat Pad Content (g/100 g fatty acids)		Mammary Tumors	
Diet	*trans* -11 18:1	*cis* - 9, *trans* - 11 CLA	*trans* -11 18:1	*cis* - 9, *trans* - 11 CLA	Incidence (%)	Total Number
A	0.13	0.05	0.36	0.48	93	91
B	0.13	0.18	0.37	0.57	93	85
C	0.13	0.24	0.37	0.59	83	74
D	0.13	0.37	0.38	0.66	77	65
E	0.73	0.18	2.15	2.21	70	63
F	1.00	0.24	2.75	2.85	47	49
G	1.60	0.37	4.17	4.14	40	36

[1]Rats were injected with a single dose of methylnitrosourea, a chemical carcinogen, and then fed dietary treatments for 24 weeks until killed for tissue analysis. Variations in dietary levels of VA and CLA were provided by combinations of a control butter and a VA/CLA enriched butter.
[2]Diets A, B, C and D have a similar content of VA, but differ in the percent of CLA. Diets E vs B, F vs C and G vs D represent comparisons of dietary supply of VA at comparable levels of CLA.