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The role of ruminant *trans* fat as a potential nutraceutical in the prevention of cardiovascular disease

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ABSTRACT

Dietary *trans* fat has received increasing attention over the last few years. It is now appreciated that *trans* fatty acids (TFA) produced by ruminants (rTFA) via 'a natural biohydrogenation reaction' may have disparate health effects from those produced as a by-product of industrial processing present in partially hydrogenated vegetable oils. In this review, we discuss the most recent findings from human and animal intervention studies in order to evaluate the health implications and potential mechanisms of two major rTFA (*t*11-vaccenic acid and *c*9,*t*11-conjugated linoleic acid) in their purified form as well as in the format of dairy fat with regard to the development of cardiovascular diseases, with the aim to assess the potential of developing rTFA as novel components for functional foods.

1. Background

1.1. Trans fatty acids

The consumption of *trans* fatty acid (TFA) being causally associated with the development of cardiovascular disease (CVD) has been supported by epidemiological data, leading to a worldwide campaign of eliminating *trans* fat content in the food supply (Micha & Mozaffarian, 2009). The widely accepted definition of TFA is a class of fatty acids that contain one or more double bonds in the *trans* configuration. Most dietary TFA do not have a 'natural' origin, but have been introduced into commercial solid edible fats (e.g. margarine, shortening) produced via partial hydrogenation of vegetable oils aimed to elongate product shelf life and replace animal fat in cooking. Nutritional recommendations to eliminate dietary TFA have been established based on numerous epidemiological studies supporting a positive association between increased risk of coronary heart disease (CHD) and TFA consumption from foods containing partially hydrogenated vegetable oil (PHVO, e.g. margarine, cakes and cookies) (Oomen et al., 2001; Willett et al., 1993). For example, in 2002 the Panel on Macronutrients of the U.S. National Academies of Science, Institute of Medicine, recommended that *trans* fat consumption be as low as possible in a nutritionally adequate diet. Subsequently, in 2003 the World Health Organization recommended that *trans* fat intake be limited to less than 1% of total daily energy intake. In December 2005, Canada became the first country to mandate total *trans* fat content per serving must be listed for all prepackaged foods and was required to follow food labeling regulations. As a result in Canada, food containing more than 0.2 g per serving cannot be claimed as "*trans* fat-free". Other countries such as the US, Brazil and New Zealand have also adopted similar mandatory labeling requirements in 2006, whereas in most European countries the '*trans* fat free' campaign remains voluntary. Further evaluation of CHD risk indices has also revealed that TFA intake from PHVO might be associated with: higher blood concentrations of inflammatory markers (e.g. C-reactive protein); impaired endothelial function; reduced insulin sensitivity/secretion; impaired fetal development (anthropometric measures) and infant essential fatty acid status (Innis, 2006; Mozaffarian & Clarke, 2009). As a result of the collaborative effort in eliminating dietary TFA, recent consensuses showed a global decline of total TFA consumption from an average of 10 g/day worldwide a decade ago, to <4 g/day in Western countries, and <1 g/day in east Asian countries, during the past few years (Craig-Schmidt, 2006; Hulshof et al., 1999).

1.2. Ruminant trans fat

The complexities of the TFA issue have arisen particularly due to increasing recognition that TFA also occur naturally in various ruminant-

Abbreviations: CLA, conjugated linoleic acid; CHD, coronary heart disease; CVD, cardiovascular disease; EA, elaidic acid; FFA, free fatty acid; HDL, high density lipoprotein; IL, interleukin; iTFA, industrial *trans* fatty acid; LDL, low density lipoprotein; LDL-r, low density lipoprotein receptor; PHVO, partially hydrogenated vegetable oil; PPAR, peroxisome proliferator activated receptor; PUFA, polyunsaturated fatty acid; rTFA, ruminant *trans* fatty acid; SFA, saturated fatty acid; TFA, *trans* fatty acid; TG, triglyceride; TNF, tumor necrosis factor; TZD, thiazolidinedione; VA, vaccenic acid; VLDL, very low density lipoprotein

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based foods such as dairy, beef and lamb. Ruminant TFA (rTFA) contributes to approximately 10–20% of total TFA consumption in Europe and North America (Elias & Innis, 2002; Wolff & Precht, 2002). In Denmark where ruminant products are heavily consumed, rTFA may account for as high as 60–80% of total TFA intake (Jakobsen et al., 2006). Ruminant *trans* fats are produced by rumen bacteria during the biohydrogenation of polyunsaturated fatty acids (PUFA), primarily linoleic acid and α-linolenic acid (Lock & Bauman, 2004). There may be as many as 15 different positional isomers of *t*-16:1, *t*-18:1 and *t*-18:2 in ruminant-derived foods among which, the two predominant are *c*9,*t*11-18:2 (conjugated linoleic acid or CLA) and *t*-11 18:1 (vaccenic acid or VA) (Palmquist, Lock, Shingfield, & Bauman, 2005). *C*9,*t*11-CLA is present mostly as a transitory intermediate in the rumen whilst VA, the less well-studied rTFA, is the major biohydrogenation product that accumulates in the rumen and later in the tissue. Whole body pools of *c*9,*t*11-CLA have been shown to be primarily contributed by the endogenous synthesis from VA in liver and adipose tissue (Palmquist et al., 2005). In humans, the conversion of VA to *c*9,*t*11-CLA has been estimated to range from 11 to 30% (Turpeinen et al., 2002). In addition to dairy fat, PHVO act as additional dietary sources of VA, contributing to its total daily consumption varying from 0.3 to 2.0 g per day (Jakobsen et al., 2006; Wolff & Precht, 2002; Wolff et al., 2000). Similarly, the intake of *c*9,*t*11-CLA from non-supplement sources varies considerably from as low as 0.1 g to as high as 1.5 g per day (15-fold difference). Notably, the total *c*9, *t*11-CLA pool *in-vivo* may in fact be underestimated because an increased dietary intake of VA also adds cumulatively to whole body *c*9,*t*11-CLA pools.

Traditionally, rTFA has been considered to constitute a rather small part of the fat in dairy products (2–5% of total fatty acids), beef and lamb (3–9%) (Precht, 1995). However, dairy fat composition can be substantially altered by geographical and/or seasonal change, genetic engineering techniques, and variations in bovine feeding practice (Boeckaert et al., 2008; Rego et al., 2009; Taniguchi et al., 2004). It is now appreciated that ruminant TFA content can be increased up to 12% of total fat in dairy products (with different fatty acid profiles and additional reductions in saturated fat) (Mendis, Cruz-Hernandez, & Ratnayake, 2008; Palmquist et al., 2005). This increase in the abundance of rTFA in dairy-derived products has confounded the premise for minimizing total dietary TFA and sparked a critical need to better understand the bioactivity of rTFA isomers (e.g. VA), particularly in the context of CVD development.

There have been considerable epidemiological studies in the literature stating a positive association between TFA intake and CHD risk. Unfortunately however, very few have attempted to differentiate between industrial TFA (iTFA) and rTFA, possibly due to a lack of detailed TFA profiles in earlier food databases. Nevertheless, a number of epidemiological studies that were able to distinguish individual TFA isomers implied a positive association with CHD risk to exist only between TFA isomers generated by industrial means, but not between rTFA isomers formed during biohydrogenation reactions (e.g. *t*-16:1) (Lopez-Garcia et al., 2005; Mozaffarian et al., 2004). The Codex Alimentarius standard introduced a new definition of TFA to exclude all isomers with conjugated *trans* double bonds. This adjustment was thus perceived as official recognition of the discretionary health benefit of *c*9,*t*11-CLA, a natural TFA with such a characteristic structure. USA, Canada and Denmark are among the first countries to adopt similar conceptual changes in official dietary recommendations and food labeling regulations. Unfortunately, such a modification is not inclusive of all natural TFA isomers, nor is it inclusive of *t*11-VA, the most predominant natural TFA and also a major precursor to *c9, t11*-CLA *in-vivo*. This may possibly be due to the presence of VA in industrial *trans* fats, and more importantly, the lack of sufficient evidence demonstrating that VA elicits similar health benefit as *c9, t11*-CLA, potent enough to distinguish itself from other industrially produced *trans* fats. Interestingly however, the modification did exclude other CLA isomers (e.g. *t*,*t*-CLA) present in industrially produced *trans* fat despite a lack of solid scientific evidence.

1.3. Cardiovascular disease risk

Cardiovascular disease (CVD) has a complex etiology with contribution from both lipid metabolism and inflammatory pathways that cumulatively affect vascular function. Hypercholesterolemia, defined as elevated concentrations of circulating low-density lipoprotein (LDL) cholesterol, is traditionally regarded as the major modifiable CVD risk factor. Notably, small dense LDL particles tend to be considered more atherogenic due to their higher susceptibility to oxidation, thus accelerating the accumulation of macrophages (Krauss, 1995). Atherosclerotic plaque formation is also known to be exacerbated by elevated cholesterol-rich remnant lipoproteins derived from the intestine [i.e. chylomicron remnants (CM-r)] (Proctor, Vine, & Mamo, 2002). In a number of large-scale cohort studies, the literature also documents that in addition to hypercholesterolemia, fasting and non-fasting plasma TG concentrations act as independent predictors of CHD incidence, and are positively correlated with end-stage myocardial infarction, ischemic heart disease and related death (Bansal et al., 2007; Freiberg, Tybjaerg-Hansen, Jensen, & Nordestgaard, 2008; Langsted, Freiberg, & Nordestgaard, 2008).

In this review, we consider well-controlled intervention studies in both the human population and experimental models of CVD to help generate an understanding of the current progress on rTFA research. In order to assess the feasibility of nutraceutical application(s) of rTFA in the context of CVD prevention, we specifically focus our discussion on the effect of individual rTFA (i.e. *c*9,*t*11-CLA and VA) as well as dairy products naturally enriched with rTFA isomers on major CVD risk factors and occurrence of related health complications.

2. *C*9, *t*11-CLA and CVD

Over the past few decades, the health benefits of CLA to reduce CVD risk and/or immune function have been reported predominantly in animal models. Among the few reported double-blind randomized clinical trials that have assessed the effect of CLA on risk markers of CVD, only a limited number (summarized in Table 1) have used a purified preparation of *c*9,*t*11-CLA so as to eliminate the confounding effect of *t*10,*c*12-CLA isomer given its divergent bioactivity (Mensink, 2005). Interestingly, none of the clinical studies have reported improvements in blood lipids or body composition after *c*9,*t*11-CLA intervention. One study concluded that *c*9,*t*11-CLA alone had neutral effect on biochemical parameters associated with atherosclerosis and metabolic syndrome, whereas a CLA mixture (with various combinations of *c*9,*t*11- and *t*10, *c*12-CLA isomers) tend to be detrimental to CVD risk markers (Tholstrup et al., 2008). It is also worthwhile to note that most of these trials targeted the healthy population rather than patients diagnosed with cardiovascular complications *per se*, therefore the efficacy of CLA intervention remains largely limited.

Feeding CLA to animal models, particularly the *c*9,*t*11 isomer, has been reported to improve the blood lipid profile at an average dose of 1.0% w/w (equivalent to 3–5% of energy) (Table 1). Plasma triglyceride (TG), total cholesterol and LDL-cholesterol (especially small dense LDL) concentrations were reduced in hypercholesterolemic hamsters fed an average of 0.5–1% *c*9,*t*11-CLA for 12 weeks (LeDoux et al., 2007; Wilson, Nicolosi, Saati, Kotyla, & Kritchevsky, 2006). Data from our own research group has shown similar changes in these parameters in JCR:LA-*cp* rats, an established model of the metabolic syndrome that spontaneously develops CVD complications (Jacome-Sosa et al., 2010; Proctor, Kelly, Stanhope, Havel, & Russell, 2007). In addition to improved circulating cholesterol profile, plasma free fatty acid (FFA) concentration, hepatic de novo lipogenesis, liver TG deposition and postprandial lipaemia were also reduced in obese JCR:LA-*cp* rats fed 1.0% *c*9,*t*11-CLA for 16 weeks (Jacome-Sosa et al., 2010). There have been