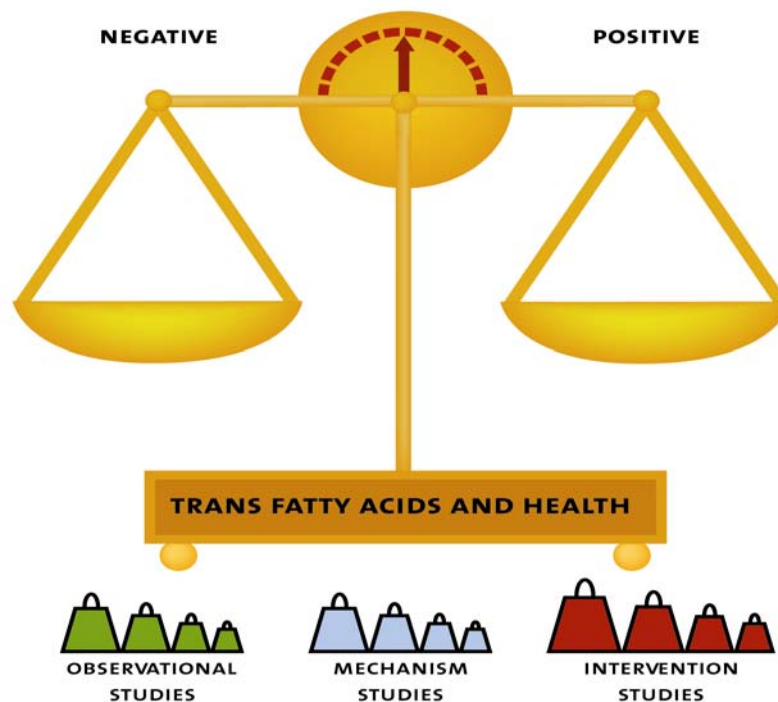


**First International Symposium**  
**on**  
**TRANS FATTY ACIDS AND HEALTH**

**Rungstedgaard**

Rungsted Kyst, Copenhagen, Denmark, 11-13 September 2005

**Programme**  
**and**  
**Abstract Book**



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## Programme and list of contents

### Sunday the 11<sup>th</sup> of September 2005

16:00-18:00 Registration

19:00-22:00 Get-together party

### Monday the 12<sup>th</sup> of September 2005, 09:00 – 17:30

#### 09:00 – 09:15 Opening of the symposium

*B Richelsen*, Chairman of the Danish Nutrition Council

*L Barfoed*, Minister of Family and Consumer Affairs

*S Stender*, Chairman of the Organising and Scientific Committee

#### Epidemiological data

#### Abstracts on page

Chairman: *J Dyerberg*

- 09:15 – 09:45 Worldwide consumption of trans fatty acids 4  
*MC Craig-Schmidt*
- 09:45 – 10:15 The role of *Trans*-Fatty Acids and Coronary Heart Disease –  
Epidemiological Data 5  
*WC Willett*
- 10:15 – 10:45 Trans fatty acids and sudden cardiac death 6  
*RN Lemaitre*
- 10:45 – 11.15 Coffee break and posters
- 11:15 – 11.35 Intake of ruminant trans fatty acids and risk of coronary heart disease 7  
*M Uhre Jakobsen*
- 11:35 – 11:55 Trans fatty acids and diabetes mellitus 8  
*J Salmerón*
- 12:00 – 13:30 Lunch and posters
- 13:30 – 14.00 Trans fatty acids, pregnancy and infancy 9  
*SM Innis*
- 14.00 – 14.30 Trans fatty acids and birth outcome: the MEFAB Study and ABCD cohorts 10  
*G Hornstra*

#### Risk markers – Intervention studies

Chairman: *S Stender*

- 14:30 – 14:50 Trans fatty acids and blood lipids 11  
*A Ascherio*
- 14:50 – 15:10 Trans fatty acids and systemic inflammation 12  
*D Mozaffarian*
- 15:10 – 15:30 Trans fatty acids and vascular function – heart rate 13  
*J Dyerberg*
- 15:30 – 15:50 Effects of dietary *trans* fatty acids on insulin sensitivity 14  
*U Risérus*
- 15:50 – 16:20 Coffee break and posters

**Mechanisms of action**

Chairman: *HS Hansen*

- 16:20 – 16:40** Membrane function and membrane lipids: role of fatty acids 15  
*AM Katz*

**Denmark and the world**

Chairman: *HS Hansen*

- 16:40 – 17:00** The trans fatty acid story in Denmark 16  
*A Astrup*

- 17:00 – 17:20** A trans world journey 17  
*S Stender*

**19:00 – 23:00** Dinner

**Tuesday the 13<sup>th</sup> of September 2005, 09:00-14:00**

**The future for trans fatty acids**

Chairman: *J Dyerberg*

- 09:00 – 09:20** The effect of the regulation on trans fatty acid content in Danish food 18  
*T Leth*

- 09:20 – 09:40** Labeling of *trans* fatty acid content in food, regulations and what limits, pros and cons - the FDA view 19  
*J Moss*

- 09:40 – 10:00** Labeling of trans fatty acid content in food, regulations and what limits, pros and cons - the Danish view 20  
*HG Jensen*

- 10:00 – 10:20** Is the quality and cost of food affected if industrially produced trans fatty acids are removed? 21  
*K Nielsen*

**10:20 – 10:50** Coffee break and posters

**The Scientific basis for TFA regulations**

Chairman: *A Astrup*

- 10:50 – 11:15** The scientific basis for TFA regulations – is it sufficient? 22  
*A Aro*

- 11:15 – 11:40** The scientific basis for TFA regulations – is it sufficient? 23  
*M Katan*

- 11:40 – 12:05** The scientific basis for TFA regulations – is it sufficient? 24  
*WC Willett*

- 12:05 – 12:10** Conclusion and farewell  
*S Stender*

**12:15 - 14:00** Lunch

## World-wide Consumption of *Trans*-Fatty Acids

Craig-Schmidt M.C., Professor, Ph.D., Auburn University, Auburn, AL 36849 USA

*Trans*-fatty acids originate in the diet from commercially hydrogenated oils, as well as from dairy and meat fats containing *trans*-fatty acids as the result of ruminant biohydrogenation. The amount of *trans*-fatty acids in the diet is of interest because of the possible adverse effects of these isomers with respect to cardiovascular disease and infant development.

Estimates of *trans*-fatty acids in the diet vary not only with the dietary habits and food supply of a population, but also with the method used to estimate consumption. The methods used to measure *trans*-fatty acid consumption in various countries throughout the world include 1) estimates based on "food disappearance" or market share data, 2) analysis of dietary consumption data of a representative population, 3) laboratory analysis of duplicate portions or composite diets, and 4) estimates based on the *trans*-fatty acid content of biological tissues, such as human milk, red blood cell membranes and adipose tissue. Each method has inherent advantages and disadvantages. In general, estimates of *trans*-fatty acid intake based on disappearance data tend to be greater than those which are based on individual consumption data. Estimates of intake are also confounded by an ever-changing food supply which results in food databases which are quickly outdated or incomplete.

Estimates of the *trans*-fatty acid content of the diet range from less than 1 g/person/ day in Asian/Pacific countries to 10 to 20 g/person/day in subpopulations of some Western countries.\* In North America, average daily intakes of *trans*-fatty acids have been estimated by food frequency questionnaire methods to be 3 to 4 g/person and by extrapolation of human milk data to be greater than 10 g/person. "Consensus" values by two expert panels are given as 6.4 g/person/day for women and 8.1 to 12.8 g/day for general per capita consumption in North America. Average values for daily *trans*-fatty acid intake in the United Kingdom range from 2 to 7 g/person. The diet in northern European countries has traditionally contained more *trans*-fatty acids than the diet in Mediterranean countries in which olive oil is commonly used. Mean daily intakes of *trans*-fatty acids in European countries range from minimal values in Italy, Portugal, Greece and Spain to greater values for Germany, Finland, Denmark, Sweden, France, United Kingdom, Belgium, Norway, the Netherlands, and Iceland (given in increasing order). Estimates for the typical Australian diet are in the same range as Western diets, with values ranging from 3 to 8 g/person/day. Traditional diets in Korea and Japan, however, contain only small quantities of *trans*-fatty acids, with estimates of 0.6 g/person/day reported for Korea and 0.1 to 0.3 g/person/day reported for Japan.

In some populations, such as Japan, the average *trans* content of the diet appears to be increasing, whereas in some European countries and in North America, recent decreases in the *trans* content of the diet have been observed, presumably due to modifications of commercially available fats or changes in consumer choices. The impact of changes in legislation restricting use of *trans*-fatty acids in food products and requiring *trans*-fatty acids content on food labels awaits future studies.

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\*Craig-Schmidt, M. C. Worldwide consumption of *trans*-fatty acids, *Trans Fatty Acids in Human Nutrition* (J. L. Sébédio and W. W. Christie, ed.), The Oily Press, Dundee, Scotland, 1998, pp. 50-113.

## The Role of *Trans*-Fatty Acids and Coronary Heart Disease: Epidemiologic Data

Willett W.C., M.D., Dr. P.H., Harvard University, Boston, MA

As expected, the change from *cis* to *trans* configuration of double bonds induced by partial hydrogenation profoundly alters not just the physical shape but also the biological function of unsaturated fatty acids. *Trans*-fatty acids have many adverse metabolic effects including elevation of LDL cholesterol, triglycerides and LP(a), reduction in HDL cholesterol, and adverse effects on endothelial function, inflammatory markers, and probably insulin resistance.

In general, countries with high intake of *trans* fatty acids tend to have higher rates of coronary heart disease. The relation between intake of *trans*-fatty acids and risk of coronary heart disease has been examined in multiple case-control and cohort studies using both biochemical measurements of *trans*-fatty acids and assessments of dietary intake. The early case-control studies tended to be too small to detect even substantial associations, and blood measurements after the occurrence of myocardial infarction may not represent *trans* fat intake before the event. In a multi-center case-control study in Europe that used adipose levels of *trans* fatty acids, a 40% increase in risk was seen in the highest quintile after excluding the Spanish centers, where nearly all persons had low levels. Several large prospective studies have now been conducted and positive associations have been seen consistently, although the association was not statistically significant in each study. In the Nurses' Health Study, which used repeated measures of intake to account for changes in food processing and preferences, we found an approximately 80% increase in risk for a 2% energy increment in *trans* fat intake. The magnitude of excess risk in epidemiological studies is substantially greater than expected by the effects of *trans* fats on blood LDL and HDL. However, recent evidence that *trans* fat adversely affects inflammatory markers and other risk factors may account for this apparent paradox. Because high intake of *trans* fat is almost universal in many populations, it can account for as much as 30-40% of coronary heart disease in these countries.

Elimination of partial hydrogenation of vegetable oils is one of the most cost-effective means to improve health because this avoids both the destruction of essential polyunsaturated fatty acids and the creation of *trans*-fatty acids.

## Trans-fatty acids and sudden cardiac death

Lemaitre RN<sup>1)</sup>, King IB<sup>2)</sup>, Mozaffarian D<sup>3)</sup>, Sootodehnia D<sup>1)</sup>, Siscovick DS<sup>1)</sup>

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- 3) Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, and the Departments of Nutrition and Epidemiology, Harvard School of Public Health, Boston, MA.

While trans fatty acids (TFA) are associated with risk of CHD and atherosclerosis risk factors, less is known about a possible relation to sudden cardiac death. In addition, the association of different classes of TFA, difficult to assess from the diet, has received limited attention.

Using bloods collected by paramedics at the time of cardiac arrest, we have reported the association of red blood cell TFA, a biomarker of intake, with out-of-hospital cardiac arrest (CA) as a first manifestation of heart disease. In a case-control study with 179 cases and 285 matched controls, higher levels of trans 18:2 were associated with higher risk of out-of-hospital CA (Odds ratio [OR] for interquintile range, adjusted for risk factors: 3.1, 95% CI: 1.7-5.4). In contrast, trans 18:1, the most abundant TFA in the diet, and trans 16:1 were not associated with risk.

Following these observations, we investigated the association of TFA with fatal ischemic heart disease (IHD) in a case-control study nested in the prospective Cardiovascular Health Study. "We identified 214 incident cases of fatal IHD (fatal myocardial infarction and coronary heart disease death) and 214 controls matched to the cases on age, gender, clinic, prior clinical cardiovascular disease and blood draw date. Higher levels of plasma phospholipid trans 18:2 in bloods collected on average 3 years earlier were associated with higher risk of fatal IHD (OR for interquintile range, adjusted for risk factors: 2.3, 95% CI : 1.4-3.8). In contrast, higher levels of trans 18:1 appeared associated with lower risk of fatal IHD (OR: 0.6, 95% CI : 0.4-0.9). Trans 16:1 was not associated with risk. Similar results were obtained in analyses limited to fatal IHD cases due to arrhythmia." ("unpublished data").

Higher blood levels of trans 18:2 but not trans 18:1, are associated with higher risk of out-of-hospital CA and fatal IHD. These studies suggest that current efforts at decreasing TFA in foods should take into consideration the trans 18:2 content as well.

## Intake of ruminant trans fatty acids and risk of coronary heart disease

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<sup>4)</sup> Department of Human Nutrition, The Royal Veterinary and Agricultural University, Denmark and Capio Diagnostik A/S, Denmark

**Objective:** We did a prospective cohort study in order to describe the association between the intake of ruminant *trans* fatty acids (TFA) and the risk of coronary heart disease (CHD), assessing associations for both the absolute and the energy-adjusted intakes of ruminant TFA.

**Methods:** The study included 3,686 middle-aged Danish women and men without previous CHD enrolled between 1974-93. Usual dietary intake was determined at baseline using a 7-day weighed food record. During 18 years follow-up, 374 participants with fatal or nonfatal CHD events were identified.

**Results:** In multivariate analysis, the absolute intake of ruminant TFA in grams (0.5 unit) was not associated with the risk of CHD among women. However, after additional adjustment for intake of saturated fat, the absolute intake of ruminant TFA was borderline significantly associated with a 16 % lower risk of CHD (hazard ratio (HR) = 0.84, 95% confidence interval (CI): 0.70, 1.01). There was no association between the absolute intake of ruminant TFA and the risk of CHD among men. In multivariate analysis, for a fixed total energy intake, the energy-adjusted intake of ruminant TFA in grams (0.5 unit) was not associated with the risk of CHD among neither women nor men. However, after additional adjustment for saturated fat, there was a trend for an inverse association among women (HR = 0.77, 95% CI: 0.55, 1.09) but not among men (HR = 1.06, 95% CI: 0.95, 1.18). In spline regression analyses, the associations for both the absolute and the energy-adjusted intakes of ruminant TFA appeared linear over the range of intakes among both women and men.

**Conclusion:** These results suggest that intake of ruminant TFA is not associated with a higher risk of CHD. Whether ruminant TFA intake is innocuous or even protective against CHD cannot be concluded from this study.

## ***Trans* fatty acids and diabetes mellitus**

Salmerón J, Dr., Unidad de Investigación Epidemiológica y en Servicios de Salud, Instituto Mexicano del Seguro Social, Cuernavaca Morelos, México.

Background: Metabolic studies suggest that insulin resistance may be increased by greater intake of saturated and *trans*-fatty acids and reduced by mono or polyunsaturated fatty acids. However, the long-term relations between specific types of dietary fat and risk of type 2 diabetes remain unclear.

Objective: Our objective was to examine the relations between dietary fat intakes and the risk of type 2 diabetes in women.

Methods: We prospectively followed 84204 women aged 34–59 y with no diabetes, cardiovascular disease, or cancer in 1980. Detailed dietary information was assessed at baseline and updated in 1984, 1986, and 1990 by using validated questionnaires. Relative risks of type 2 diabetes were obtained from pooled logistic models adjusted for non-dietary and dietary covariates.

Results: During 14 y of follow-up of the Nurses' Health Study, 2507 incident cases of type 2 diabetes were documented. Total fat intake, compared with equivalent energy intake from carbohydrates, was not associated with risk of type 2 diabetes; for a 5% increase in total energy from fat, the relative risk (RR) was 0.98 (95% CI: 0.94, 1.02).

Intakes of saturated or monounsaturated fatty acids were also not significantly associated with the risk of diabetes. However, for a 5% increase in energy from polyunsaturated fat, the RR was 0.63 (0.53, 0.76;  $P < 0.0001$ ) and for a 2% increase in energy from *trans* fatty acids the RR was 1.39 (1.15, 1.67;  $P = 0.0006$ ). We estimated that replacing 2% of energy from *trans* fatty acids isoenergetically with polyunsaturated fat would lead to a 40% lower risk (RR: 0.60; 95% CI: 0.48, 0.75).

Conclusions: These data suggest that total fat and saturated and monounsaturated fatty acid intakes are not associated with risk of type 2 diabetes in women, but that *trans* fatty acids increase and polyunsaturated fatty acids reduce risk. Substituting nonhydrogenated polyunsaturated fatty acids for *trans* fatty acids would likely reduce the risk of type 2 diabetes substantially.

Based on the previously reported data:

Dietary fat intake and risk of type 2 diabetes in women

Salmerón J, Hu FB, Manson JA, Stampfer MJ, Colditz GA, Rimm EB, Willett WC.

Am J Clin Nutr 2001;73:1019-26.

## **Trans Fatty Acids: Pregnancy and Infancy**

Innis SM, Ph.D. - Director, Nutrition Research Program, Child and Family Research Institute, Professor, Department of Paediatrics, University of British Columbia Vancouver, B.C. Canada V5Z 4H4

The all *cis* n-6 and n-3 polyunsaturated fatty acids (PUFA) are essential nutrients required for growth and development and support a wide range of physiological and biochemical pathways critical to optimal health. The essential fatty acids n-6 linoleic acid (LA, 18:2n-6) and n-3 alpha linolenic acid (ALA, 18:3n-3) are predominantly found in vegetable oils. LA and ALA are converted by desaturation and elongation to the n-6 arachidonic acid (ARA, 20:4n-6) and the n-3 eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Whereas ARA is essential for growth and eicosanoid pathways, DHA is particularly important in the central nervous system and reduced DHA in the brain and retina is associated with decreased visual and cognitive functioning. Industrial hydrogenation of dietary fats and oils results in loss of *cis* PUFA, particularly ALA, which could lead to inadequate n-3 fatty acid intakes, and creation of *trans* fatty acids.

However, concern has also been raised that high intakes of *trans* fatty acids could impair the conversion of LA to ARA and of ALA to EPA and DHA, thus potentially also adversely affecting fetal and infant growth and development. On the other, the placenta is believed to preferentially transport ARA and DHA from mother to fetus, and ARA and DHA are both present in human milk. We determined the intake of the all *cis* and *trans* fatty acids among pregnant and lactating women and young children, and quantified *cis* and *trans* fatty acids in matched maternal-infant cord plasma, human breast milk, breast feeding infants, and in preschool children. The mean daily intake of *trans* fatty acids among pregnant women in western Canada was  $1.4 \pm 0.06\%$  energy, and about 3 fold higher than the intake of ALA ( $1.6 \pm 0.1\text{g/day}$ ). The mean intake of DHA was 160mg/day, with only 16% of women consuming  $\geq 300\text{mg DHA/day}$ . Human milk (n=103) had 7.1g *trans* /100g fatty acids (range 2.2-18.8 g). *Trans* fatty acids in maternal plasma and human milk were significantly related ( $P < 0.001$ ) to the concentration in infant cord plasma or breast-fed infant plasma, respectively. *Trans* fatty acids in milk were inversely related to LA and ALA, but not ARA and DHA, but dietary *trans* fat as well as LA intakes were inversely related to DHA in young children, when controlling for n-3 intake. We found no evidence of any relation between cord plasma triglyceride or phospholipid *trans* fatty acids and infant birth weight or length, or length of gestation. 78% of dietary *trans* fatty acid intake among pregnant and lactating women was from processed foods, 11% from table spreads, and 11% from ruminant meat and milk. In this population, high intakes of *trans* fatty acids occur concurrently with low intakes of ALA and DHA, which were associated with lower developmental outcome measures. This suggests the need to combine strategies to increase n-3 intakes at the same time as lowering *trans* fatty acids.

## **Trans fatty acids and birth outcome: some first results of the MEFAB and ABCD cohorts**

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The long-chain polyunsaturated fatty acids (LCPUFA) arachidonic acid (AA) and docosahexaenoic acid (DHA) are generally acknowledged to play essential roles in fetal growth and brain development. Moreover, the DHA status at birth has been shown to be positively associated with various aspects of later brain function and child behavior. Therefore, the neonatal LCPUFA status should be adequate.

Industrial hydrogenation of edible oils causes the formation of *trans* isomers of unsaturated fatty acids, which are readily absorbed and metabolized by man. These fatty acids inhibit the synthesis of LCPUFA from their essential fatty acid precursors and may impair placental LCPUFA transfer. As a consequence, *trans* unsaturated fatty acids may lower the fetal LCPUFA status and, thereby, compromise fetal development.

We tested this hypothesis in newborns of the Maastricht Essential Fatty Acid Birth (MEFAB) cohort by relating the relative amounts (% of fatty acids) of the most common dietary *trans* fatty acid, elaidic acid (ELA, C18:1*t*), in their cord plasma, cord erythrocyte, and cord vessel wall phospholipids (PL) to birth weight (BW), birth length (BL), and head circumference (HC).

From simple linear regression analyses it appeared that birth length was negatively associated with ELA contents in the phospholipids of cord venous and arterial vessel walls, whereas head circumference was negatively associated with the plasma phospholipid ELA content ( $n = 425-556$ ,  $B = -2.57$  to  $-5.53$ ,  $p = 0.027-0.01$ ).

In most cord domains, AA and DHA levels are negatively related to ELA concentrations, and very often these LCPUFA appeared significantly related to various birth outcome measures themselves. Therefore, the ELA-BO relationships were corrected for AA and DHA concentrations. This attenuated most of these relationships, indicating an intermediating role of LCPUFA in the possible link between ELA and birth outcome. However, the ELA-HC relationship in plasma PL remained significant, and in erythrocyte PL relationships with ELA became apparent for birth weight. This suggests an LCPUFA-independent role of ELA as well.

Using multiple linear regression analyses, associations between neonatal ELA status and birth outcome variables were also corrected for a number of potential confounders. In most cases this strengthened these associations. After further correction for AA and DHA, the following ELA-BO relationships remained (or became) significant: plasma ELA and HC, ELA in cord artery walls and BL as well as HC, and ELA in umbilical venous walls and BL ( $n$  between 329 and 461,  $B$  between  $-2.14$  and  $-4.89$ ,  $p$  between 0.047 and 0.020). Man cannot synthesize *trans* fatty acids, and, consequently, fetal *trans* fatty acids must have been obtained from the mother. The significant relationship between maternal *trans* intake (duplicate portion analysis) and *trans* concentration in cord plasma phospholipids supports this view.

The potential association between maternal *trans* status and neonatal birth weight is presently under investigation in mothers of the Amsterdam Born Children and their Development (ABCD) cohort who donated blood at their first antenatal screening. The relative ELA concentrations in plasma phospholipids of these mothers were negatively associated with the birth weight of their term children ( $n = 3466$ ,  $B = -237$ ,  $p = 0.004$ ). Although adjustment for gestational length attenuated this relationship, it remained significant. After adjustments for additional confounders, the association lost significance. Maternal plasma PL concentrations of AA and DHA were negatively correlated with ELA levels. In addition, BW correlated with AA in a negative and with DHA in a positive way. Therefore, the ELA-BW associations were adjusted for these LCPUFA as well but, again, ELA was no longer significantly associated with birth weight.

Although the negative associations between neonatal or maternal elaidic acid concentrations and selected birth outcome variables are relatively weak, it seems nonetheless prudent to minimize *trans* intake during pregnancy and lactation until further studies give a more decisive answer about the potential risk of *trans* fatty acids for fetal and infant development.

## **Trans fatty acids and blood lipids**

Ascherio A, M.D., Dr. P.H., Harvard University, Boston, MA

Early metabolic studies focused on total cholesterol and thus did not detect the opposite effects that trans fatty acid intake has on low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol. Rigorous randomized trials to establish the effects of hydrogenated fat or trans fatty acid intake on individual lipoprotein classes started in 1990, when a report from the Netherlands suggested that a diet enriched in elaidic acid (a subfraction of 18:1 trans), compared to one enriched in oleic acid (18:1 cis), increased total and low-density lipoprotein LDL cholesterol concentrations and decreased high-density lipoprotein HDL cholesterol concentration, hence resulting in a less favorable total cholesterol/HDL cholesterol ratio. In contrast, enrichment of the diet with saturated fatty acids increased LDL cholesterol, but had no effect on HDL cholesterol, thus resulting in a less adverse change than elaidic acid.

Subsequently, numerous studies (mostly using a mixture of isomers obtained by partial hydrogenation of vegetable oils) have demonstrated a dose-dependent relationship between trans fatty acid intake and the LDL:HDL ratio, and found that the magnitude of this effect is about twice greater for trans fatty acids compared to saturated fatty acids. Based on these metabolic results, and on the relationship between this ratio and coronary heart disease (CHD), we estimated that an average reduction in trans fatty acid intake of 2 percent of calories (the average intake in the USA) would predict a 7% decline in CHD mortality. Since this is much less than the effect suggested by epidemiological studies, it is likely that trans fatty acids have adverse effects beyond those on plasma HDL and LDL. These may include effects on other lipid fractions, LDL particle size, and postprandial lipids. Recent investigations have attempted to establish the mechanisms by which trans fatty acid intake affects plasma lipids and to examine the effects of specific trans isomers.

## **Trans-fatty acids and systemic inflammation**

Mozaffarian D, M.D. M.P.H. F.A.C.C. - The Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, and the Departments of Nutrition and Epidemiology, Harvard School of Public Health, Boston, MA.  
Correspondence: Dr. Mozaffarian, e-mail: [dmozaffa@hsph.harvard.edu](mailto:dmozaffa@hsph.harvard.edu).

Consumption of trans-fatty acids (TFAs) predicts higher risk of coronary heart disease, sudden death, and possibly diabetes mellitus. These associations are greater than would be predicted by effects of TFAs on serum lipoproteins alone. Systemic inflammation may be involved in the pathogenesis of atherosclerosis, acute coronary syndromes, sudden death, insulin resistance, dyslipidemia, and heart failure. Evidence from both observational and experimental studies indicates that TFAs are proinflammatory. Limited evidence suggests that proinflammatory effects may be stronger for trans-isomers of linoleic acid (t18:2) and oleic acid (t18:1), rather than of palmitoleic acid (t16:1), but further study of potential isomer-specific effects is needed. The mechanisms underlying the proinflammatory effects of TFAs are not well-established, but may involve TFA incorporation into endothelial cell, monocyte, or macrophage cell membranes (affecting membrane signaling pathway relating to inflammation) or ligand-dependent effects on peroxisome proliferator-activated receptor (PPAR) or retinoid X receptor (RXR) pathways of adipocytes. Activation of inflammatory responses may represent an important mediating step between TFA consumption and risk of coronary heart disease, sudden death, and diabetes. Further study is indicated to investigate the proinflammatory effects of TFAs and the implications of such effects for cardiovascular health.

## Trans fatty acids and vascular function – heart rate

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The magnitude of increased risk for coronary heart disease, associated to the intake of Industrially Produced Trans Fatty Acids (IP-TFA) is, as estimated from prospective epidemiological studies, substantially greater than the risk increment accounted for by the effect of IP-TFA on blood lipids and – lipoproteins.

To add to the clarification of any hitherto unnoticed effect(s), we performed a controlled dietary intervention trial comparing the effect of IP-TFA and long chained n-3 PUFA on cardiac risk markers.

Eighty seven healthy males were randomly assigned to 8 weeks of daily intake of either 20 g of IP-TFA, 4 g n-3 PUFA, or control fat, incorporated in 33 g of fat used for baking of identical bakery products (bread and cakes) given as part of the daily food consume.

High-density lipoprotein cholesterol decreased in the TFA-group and triglycerides and mean arterial blood pressure decreased in the n-3 group, compared to the control group. Heart rate variability (HRV), arterial dilatory capacity, compliance, and distensibility were unchanged.

Post-hoc, we performed a subgroup analysis of the results from the subjects with normal HRV, leaving out the quartile of study participants with increased HRV. In this, 24-hour heart rate was increased by 3 beats per minute in the TFA group, in contrast to a decreased 24-h heart rate of the same magnitude in the n-3 group. A high heart rate is associated with an increased cardiovascular mortality and *vice-versa*.

A recent study, independent of the present study, supports indirectly the validity of the present post-hoc analysis by finding that supplementation with 1.5 g/day of n-3 PUFA decreases heart rate by 2.1 beats/min.

Our findings thus support the idea that IP-TFA and n-3 PUFA may affect risk for cardiovascular mortality via mechanisms that is not related to the traditional risk markers as plasma lipids and lipoproteins, but also calls for further studies along these lines.

## Effects of dietary *trans* fatty acids on insulin sensitivity

Risérus U. Dr., Oxford Centre for Diabetes, Endocrinology and Metabolism,  
Churchill Hospital, Oxford University, UK

The association between dietary *trans* fatty acids, serum lipid levels and cardiovascular risk has been described in a number of studies. The link between *trans* fatty acids and glucose metabolism is however more unclear. As *trans* fatty acids might interfere with cell membrane functions due to alterations of membrane fatty acid composition, there are reasons to believe that high intake of *trans* fatty acids might affect cellular glucose transport and consequently insulin sensitivity. Whether *trans* fatty acids impair glucose metabolism is uncertain since the available intervention studies show divergent and inconclusive results. The divergent results might be due to methodological differences, differences between individual *trans* fatty acids, and genetic and dietary differences in the background population under investigation. *Trans* conjugated linoleic acid isomers (has one double bond in *trans* configuration) has however shown to impair insulin sensitivity in insulin resistant subjects. The current evidence from controlled interventions studies has been reviewed and will be presented at the meeting. More controlled intervention studies are apparently needed before we can conclude that *trans* fatty acids in general impair insulin sensitivity in healthy or diabetic subjects.

## Membrane Function and Membrane Lipids: Role of Fatty Acids

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Membranes contain a mixture of lipids, most of which are amphipathic in that they contain both hydrophilic and hydrophobic moieties. Most of these lipids are built upon glycerol, a 3-carbon sugar that is esterified to a hydrophilic "head group" and one or two hydrophobic fatty acyl chain "tails". The head groups usually contain charged anionic phosphate compounds, hence the term phospholipid. Cholesterol, which is found in the plasma membrane, "stiffens" the bilayer by reducing membrane fluidity. Virtually all membrane lipids contain fatty acids with an even number of carbon atoms; these are mainly palmitic and stearic acids (saturated C<sub>16</sub> and C<sub>18</sub>), and oleic, linoleic, and linolenic acids (unsaturated C<sub>18</sub> fatty acids with 1, 2 and 3 double bonds respectively). Phospholipids containing saturated fatty acids form relatively ordered regions in membrane bilayers, whereas regions containing unsaturated fatty acids are more fluid. Natural unsaturated membrane fatty acids are *cis*-isomers, in which the fatty acyl chains adjacent to the double bond are on the same side of the molecule. *Trans*-fatty acids, in which the fatty acyl chains are on opposite (*trans*) sides of the hydrocarbon fatty acyl chain, modify membrane structure in part because this configuration expands the bilayer.

Most of the important activities of biological membranes are mediated by intrinsic membrane proteins that are imbedded in the bilayer. These proteins, which can make up more than half of the weight of a membrane, include receptors, enzymes, channels, carriers, pumps, and exchangers. The extracellular portions of plasma membrane proteins often contain covalently bound lipid (lipoproteins) or carbohydrate (glycoproteins).

The fluidity of the lipid bilayer allows membrane proteins to move in the plane of the membrane, much as icebergs float in the sea. The lipids that surround the hydrophobic surfaces of the membrane proteins, sometimes called the *boundary layer lipids* or *annulus*, play an important role in regulating the activity of these proteins; this is seen in the ability of a variety of fatty acids to cause specific functional modifications of the calcium pump of the sarcoplasmic reticulum. Similar effects allow drugs, most of which are amphipathic molecules, to modify cardiac function after they enter the bilayer and interact with the hydrophobic surfaces of membrane proteins. Changes in dietary fatty acid intake may influence the risk of arrhythmias and other manifestations of heart disease by changing the concentrations of these amphipathic molecules in the boundary layer lipids around membrane proteins where, like drugs, they can modify critical physiological functions.

## **The trans fatty acid story in Denmark**

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In a publication by Willett et al. in *The Lancet* 6<sup>th</sup> March 1993 it was suggested that industrially produced trans fatty acids (IP-TFA) in foods posed a hitherto unrecognised health threat. The analyses of results from The Nurse's Health Study found that a high intake of IP-TFA as compared with saturated fat was associated with increased risk of ischemic heart disease (IHD) in women. The finding was regarded to be important by The Danish Nutrition Council, and the subsequent actions of this body triggered a series of important events in Denmark, resulting eleven years later in legislation restricting the content of IP-TFA to a maximum of 2% of the fat in foods.

The first report from the Council in 1994 concluded that dietary IP-TFA promotes IHD at least as much as equivalent amounts of saturated fat, and probably even more. The Council recommended that IP-TFA should be eliminated from foods, and that the European Commission should consider including IP-TFA in the declarations on food products. The report and its conclusions were heavily criticised by the European margarine industry and by scientists from the other Nordic countries, but were backed by the Danish margarine industry.

The Danish Nutrition Council maintained its position that IP-TFA could be eliminated from foods without negative influence on taste, price or health, and that the suspected higher risk associated with ingestion of IP-TFA than with saturated fat required protection of the consumer. The Danish margarine producers agreed to reduce the IP-TFA content of their products.

In a follow-up report in 2001 the evidence of adverse effects of IP-TFA on IHD was strengthened by further studies, and these suggested that a high intake of IP-TFA also increases the risk of type 2 diabetes, cancer, and allergic diseases, and negatively influences foetal growth and early development. Although it was concluded that the intake of IP-TFA in Denmark had decreased substantially since 1994, the report also identified a subgroup in the Danish population that could have a very high intake of IP-TFA due to frequent intake of French fries, micro oven popcorn, chocolate bars, and fast food. The Danish Nutrition Council therefore recommended that addition of IP-TFA to foods should cease before 2005. In 2003 a third report by The Council further substantiated the evidence and the Council recommended that addition of IP-TFA to foodstuffs should be terminated as soon as possible.

As a consequence of this in 2002 the Danish government proposed regulation of IP-TFA to minimise the IHD risk. The regulation was submitted to the Member states of the EU. The views of the Member states differed, but Denmark was allowed to implement the regulation from January 1<sup>st</sup> 2004. Recent analyses of high-risk foods from a selection of European cities clearly demonstrates that IP-TFA have been almost eliminated from foods in Denmark, also in food menus from the international fast food chains which continue to sell foods with high levels of IP-TFA in other countries. The Danish experience clearly shows that IP-TFA can be eliminated from foods without adverse effects on price, taste or shelf-life.

## A Trans World Journey

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We have identified foods with high contents of industrially produced trans fatty acids (IP-TFA) sold in various countries around the world.

Prepacked foods were bought if “partially hydrogenated vegetable fat” or similar terms were high on the list of ingredients. Foods were obtained in 12 European countries, in North America, Russia, and in Tonga. In addition French fries and chicken nuggets were bought at two international fast food chains in each of the European countries.

The IP-TFA content was analysed by standardised methodology.

The content of IP-TFA in the fats of the 350 products varied from below 1 to above 40 %. In all countries foods with percentages above 15 were found. In 50 % of the foods more than 2 % of the fat was IP-TFA.

Some items, especially microwave oven popcorn, contained more than 10 gram per 100-gram product. French fries contained 0.1-7.5 gram, nuggets 0.1-8.0 gram, and biscuits from 3.3-10.4 gram IP-TFA per 100 gram product with large variation from country to country, also within the same fast food chain. About 20% of the prepacked foods i.e. cakes, wafers and biscuits contained more than 1 gram IP-TFA per 100-gram product. In most of the countries similar products were available without significant amounts of IP-TFA.

As the products are popular food items bought in major groceries and fast-food chains, our findings suggest that a considerable number of persons, even in countries with a low average intake of IP-TFA, ingest 5-10 gram IP-TFA on a daily basis. Without a regulation of allowable IP-TFA content in fat for food production, this level can change substantially (as observed for identical products in different countries) depending on commercial interests.

Intake of about 5 gram IP-TFA daily is associated with a 25% increased risk of ischemic heart disease (IHD). This is 4-5 times higher than the IHD risk associated with intake of the same amount of saturated fatty acids.

Foods with an extremely high IP-TFA content are especially available in Eastern Europe – these countries also experience a high and increasing IHD prevalence.

In Denmark a regulation was introduced in 2004 restricting the use of IP-TFA to a maximum of 2% of the fat in foods. This makes it nearly impossible to consume more than 1 gram IP-TFA on a daily basis. The regulation had no noticeable effect on availability, price and quality of foodstuffs previously containing high amounts of IP-TFA.

In conclusion: Our results suggest that millions of people are exposed to an unnecessary and avoidable health hazard. It seems sensible to introduce a regulation regarding the content of IP-TFA in food as soon as possible.

## The effect of the regulation on trans fatty acid content in Danish food

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Danish Institute for Food and Veterinary Research has monitored the *trans* fatty acid (TFA) content in Danish foods for the last 30 years, from the seventies with fatty acid analysis on packed columns until today's use of 100 m capillary columns, silver ion chromatography and GC-MS identification.

In the seventies margarines and shortenings contained large amounts of both short chain and long chain TFA, in average about 10 g/100 g margarine, and even margarines with more than 40% linoleic acid contained significant amounts of TFA, in average 5 g/100 g. As late as in 1995 most shortenings still contained long chain TFA from hardened fish oil in contrary to retail margarines. However, retail margarines still contained C18:1 *trans* in amounts about 5 g/100 g margarine and little had happened since the beginning of the nineties, except a reduction of TFA in margarines with 40% linoleic acid. In 1999 TFA were out of the retail margarines and looking over the period from 1992 to 1999 without increasing the amount of saturated fatty acids, if anything even with an increase in the monounsaturated fatty acids. However, there was still about 6 g TFA/100 g in shortenings but finally the hardened fish oils were out of the shortenings.

Monitoring a broader range of foods in Denmark started with the Transfair study in 1995, which aimed at estimating the intake of TFA all over Europe. The highest amounts were found in frying fat and French fries from two large burger chains (about 20% TFA of total fatty acids), popcorn (about 30% TFA of total fatty acids) and high amounts were also found in various types of cakes, cookies, biscuits and Danish pastry (generally up to 10% TFA of the total fatty acids).

A broader investigation of foods sold in Denmark (both imported and Danish produced foods) was carried out on 253 samples at the end of 2002 to the beginning of 2003, as a starting point for the new Danish regulation specifying a maximum of 2% industrially produced TFA of the fat from 1 January 2004. 70 samples contained more than 2% TFA of the fat, some of them with milk ingredients. Chocolate and confectionery products were almost free from TFA, but special types of sweets especially caramels contained high amounts with a maximum of 40% TFA of the fat. Two samples of fruit spreads contained 18% and 42% TFA and had also a high fat content of 9 and 4 g/100 g respectively. Some categories of industrial bakery products contained high amounts of TFA, like cookies with 24 out of 49 products with more than 2% TFA and wafers with up to 42% TFA. 5 out of 19 potato products contained more than 2% TFA, and 3 out of 4 products of ready to eat French fries contained more than 2% TFA with 9% of the fat as the highest amount. Of the 17 microwave oven popcorn 2 contained high amounts of TFA, 45% and 6% of the fat respectively.

After sufficient time has passed after 1 January 2004 a new broad investigation of the TFA content in Danish foods has been carried out. 143 imported and Danish produced foods have been taken in the period December 2004 to February 2005 in the same categories of foods in which significant amounts of TFA were found in the earlier investigation. Much fewer of the samples than in the earlier investigation contained more than 2% TFA of the fat and most of them with between 2 and 6% TFA, some even with milk ingredients. In a couple of potato and cake products a larger transgression was found, and steps have been taken by the relevant authorities to correct the content of TFA in these products.

In conclusion industrially produced TFA has over a period of 30 years been reduced to a level without significance for the intake of TFA in Denmark, not least helped by the regulation passed in 2003 of a maximum of 2% industrially produced TFA of the fat from the beginning of 2004.

## **Labeling of *trans* fatty acid content in food, regulations and what limits, pros and cons – the FDA view**

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With the scientific evidence associating *trans* fat intake with an increased risk of coronary heart disease, the U.S. FDA issued a final rule that requires the declaration of the amount of *trans* fat present in foods, including dietary supplements, on the nutrition label by January 1, 2006. The addition of *trans* fat to the nutrition label will lead to the prevention of 600 to 1,200 cases of coronary heart disease and 240-480 deaths each year saving \$900 million to \$1.8 billion per year in medical costs, lost productivity, and pain and suffering. For the purpose of nutrition labeling, *trans* fats are defined as the sum of all unsaturated fatty acids that contain one or more isolated (i.e. nonconjugated) double bonds in a *trans* configuration.

There are many issues that FDA has yet to resolve: 1) defining nutrient content claims for “free” and “reduced” levels of *trans* fat; 2) placing limits on the amount of *trans* fat in conjunction with saturated fat limits for nutrient content claims, health claims, and disclosure and disqualifying levels; 3) a daily value; and 4) a possible footnote or disclosure statement to enhance consumer understanding of cholesterol raising lipids. FDA issued an Advanced Notice of Proposed Rulemaking (ANPR) requesting comments on the unresolved issues. FDA will also be conducting consumer research to determine consumer understanding of various *trans* fat labeling possibilities. Comments to the ANPR, results of consumer research and current science will be used by FDA to resolve these issues and to determine future rulemaking for *trans* fat labeling.

## **Labelling of trans fatty acid content in food, regulations and what limits pros et cons - The Danish view**

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In June 2003 Denmark introduced a new regulation concerning trans fatty acids. As of 1 January 2004 the level of industrially produced trans fatty acids in oil and fats intended for the consumer, either alone or as an ingredient in foodstuffs, must not exceed 2 %. The rules do not apply to naturally occurring content of trans fatty acids in animal fats. The order further provides that only products containing less than 1 % of trans fatty acids of oil or fat in the final product may be claimed to be "free from trans fatty acids". The scientific background for this regulation is the risk assessment carried out by the Nutritional Council on the basis of the international scientific studies carried out in this area over the last decades. Following the first report from the Nutrition Council in the 1994, the Danish Authorities notified a proposal for a regulation of the content of trans fatty acids in oil and fats to the European Commission. At the time, the Commission found that the documentation for the adverse health effects of trans fatty acids compared to those of saturated fat did not justify Community rules for trans fatty acids specifically. In the light of the Commissions comments the Danish Authorities decided to await further studies but at the same time the Nutrition Council convinced the Danish margarine industry to reduce the trans fatty acids content in retail margarines.

After the second Nutrition Council report from 2001 the Danish authorities found that the new information confirmed the conclusion that there is a clearer correlation between trans fatty acids and in particular cardiovascular disease than between saturated acids and cardiovascular disease. Though there had been a remarkable reduction of trans fatty acids in margarine products there were still food products with high amounts of trans fatty acids. For certain groups of the population those products could constitute a health risk. Therefore, a new regulation was prepared and notified to the European Commission. In the process of examination the Commission and individual Member States raised questions concerning the proposal, which were taken into account before the regulation was finalised and entered into force.

The Danish Authorities would have preferred a regulation at Community level to protect consumers but we did not think that we could wait for the opinion of the scientific Committee. We are still confident that the order is in full compliance with our obligations under the treaty.

As an alternative to reducing the content of trans fatty acids in oils and fats in foodstuffs, mandatory labelling of trans fatty acids in food was considered. Today, according to the nutritional labelling directive such labelling is not allowed unless there is a claim concerning the content for of trans fatty acids.

The regulation has been into force now for two years and the first control results indicate that it has met its objective. It is our impression that trans fatty acids have been reduced without a decrease in the selection of foods or an increase in prices. There is also growing concern with trans fatty acids internationally. Scientific bodies around the world agree that the intake of trans fatty acids should be as low as possible, and there are indications that producers are increasingly substituting fat with content of trans fatty acids with other types. We remain confident that this development will eventually lead to an EC regulation on the content of trans fatty acids in food.

## **Is the quality and cost of food affected if industrially produced trans fatty acids are removed?**

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Since the start in Italy and Denmark more than ten years ago the application of fats and oils without trans fatty acids has increased all over the world. Today the food industry uses enormous resources to decrease the content of trans fatty acids in existing and new products.

In general if cost is not an issue almost anything is possible. But in this case cost is an issue and when evaluating the effect on cost and quality of food there are a number of parameters that are involved:

- The cost-effect of fat and oil (the amount of fat in the formulation)
- The quality-effect of fat and oil (does the ingredient have a major effect on the quality)
- The main quality parameter of the fat or oil (structure, stability etc.)
- The market position and price elasticity of the products (high price / high quality or the opposite).

Trans fatty acids in vegetable oils and fats are mainly formed by partial hydrogenation of unsaturated (liquid) oils and the main quality parameters that are affected are the structure (hardness, melting point, flavour release) and the oxidative stability.

The structure of the fat is an important parameter in products, as for example margarine, and the margarine industry was the first industry to react to the requirement of low or zero content of trans fatty acids. Today, most margarines in the European market have a lower content of trans fatty acids than 5 to 10 years ago and in most cases the change has been carried out without adverse effects for the consumer. The technologies used here are now implemented in a number of other industries. New technologies have been adopted to solve specific problems in, for example, the bakery and chocolate confectionery.

The cost effect of these changes is related to changes in process costs and in raw material costs. Of course there are examples of significant increases in cost for special products, but the market pressure on price sensitive products with high fat contents has minimised the increase of cost and as the process capacity in the industry is optimised to the new situation the difference will be even smaller. The cost of raw materials also depends upon supply and demand and only the future can tell how robust the market is, but some historical data can show us the effect up until now.

The other main functionality is oxidative stability. Polyunsaturated oils have a relatively low oxidative stability and new raw materials with a more stable composition are being developed. These products are, however, not yet available in large quantities, so it will take some time before the cost of these raw materials is comparable to the traditional raw materials known today.

## The scientific basis for TFA regulations – is it sufficient? A personal view

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In all dietary sources the quantitatively most important class of trans fatty acids (TFA) is the cluster of C 18:1 isomers. The position of the double bond varies between  $\Delta 6$  and  $\Delta 16$  of the fatty acid molecule, and the  $\Delta 9$ -11 isomers are the most common ones. In the Dutch and Finnish studies that showed the LDL cholesterol-increasing and HDL cholesterol-decreasing effects of high doses of TFA, the TFA were produced by partial hydrogenation of high-oleic acid sunflower oil. The product that contained high proportions of the  $\Delta 6$ -9 isomers has never been used in commercially available fats. Partially hydrogenated fish oils that contain high amounts of  $\Delta 9$  elaidic acid and in addition long-chain C20-22 trans isomers showed particularly strong effects on serum lipoproteins in a Norwegian study, whereas partially hydrogenated soybean oil with preponderance of the  $\Delta 10$  isomer showed weak and non-significant effects on HDL-cholesterol. The  $\Delta 11$  vaccenic acid, that comprises about one-half of trans isomers of ruminant animal fats, is to some extent desaturated to CLA by the  $\Delta 9$  desaturase. The effects on serum lipoproteins of vaccenic acid have not been studied separately. Thus there may be some differences between the effects of different trans isomers. On the basis of available evidence, however, all trans fatty acids should be considered similar in their metabolic and health effects.

Since the undesirable effects on lipoproteins of TFA were confirmed in 1990, attempts were started to reduce trans fatty acids in processed foods. Improved technology allowed the production of soft margarines with small amounts or without TFA. In the European TRANSFAIR study conducted in 1995-96 it was found that low-trans margarines had been generally produced without increasing the proportions of saturated fatty acids (SFA) in the products. The new margarines that were available in all of the 14 participating countries soon became market leaders.

In the TRANSFAIR study high amounts of TFA were found in certain industrial fats and processed foods such as French fried potatoes, fatty pastries, biscuits and wafers, and soup powders. During the recent ten years the amounts of TFA have been reduced to some extent in these products, but systematic studies of representative food samples have not been repeated after the TRANSFAIR study. The average intakes of TFA have been reduced from 1.5 en% to less than 1 en% in Norway and from 0.8 en% to 0.5 en% in Finland, apparently by reduced intakes of hydrogenated vegetable fats. When partially hydrogenated vegetable fats are replaced in processed foods, fats high in SFA like coconut oil and other palm kernel oils are often used. The desired texture in Danish pastries, croissants and wafers and the stability of fats used for biscuits or for repeated deep-frying are not achieved with unsaturated vegetable oils. Therefore it is anticipated that the present tendency to reduce TFA in industrial fats often leads to products that are higher in saturated fatty acids and may contain less unsaturated fatty acids than the previous products. In the food-based dietary guidelines it will be advised not to consume such foods on a daily basis, irrespective of their TFA content. In most if not all European countries the majority of TFA are derived from ruminant animal fats nowadays.

TFA are a minor dietary component but the foods that contain these fatty acids affect the balance between the "soft" (cis-MUFA + PUFA) and "hard" (SFA + TFA) fats in the diet. The recommended intake of "soft" fats is 2/3 and that of "hard" fats 1/3 of dietary fats. The problem of excessive consumption of "hard" fats should be managed with guidelines rather than regulations.

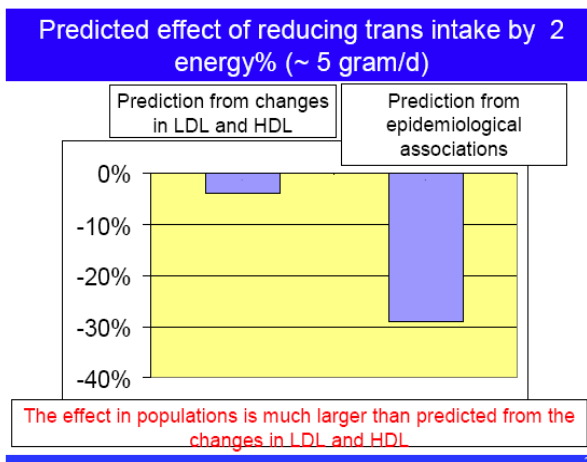
## The scientific basis for TFA regulations – is it sufficient?

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The evidence for adverse effects of trans fatty acids on health derives from two types of research: observational epidemiology, and trials with surrogate endpoints. There are no clinical trials of the effect of trans fatty acids on cardiovascular disease. The evidence for trans fat is therefore somewhat less complete than for saturated fats from dairy and meat: controlled clinical trials showed that replacement of such fats by high-linoleic acid vegetable oils markedly reduced coronary heart disease rates in middle-aged men [1-4].

We are therefore left with the epidemiology and the trials with surrogate endpoints such as blood lipids. The outcomes of the epidemiology and the trials are consistent, and together they form a sufficient basis for the present drive to reduce trans intake. The evidence that trans fats adversely affect health is in fact stronger than the evidence for adverse effects of food contaminants and pesticide residues, which rest largely on in vitro and animal studies. However, the two types of data on trans disagree on the extent of the effect. A decrease in trans intake of 2% of energy (5 g/d) would cause changes in LDL and HDL that reduce CHD risk by about 5%, but the epidemiological studies find a difference of almost 30% (Figure).

How can this discrepancy be explained? First, trans might act through more intermediates than LDL and HDL alone. We already know that trans fatty acids raise plasma triglycerides and lipoprotein(a), and there are also indications for an effect on inflammatory markers and on insulin resistance. Second, it is possible that the observational data contain residual confounding and that part of the effect of trans seen in the cohorts is due to other diet and lifestyle factors. This is hard to verify but it cannot be discarded. We need to study effects of trans fatty acids on risk factors other than blood lipids to resolve this discrepancy.



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## The Scientific Basis for TFA Regulation — Is it Sufficient?

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In reviewing the scientific basis for regulation of TFA, the philosophical criteria merit consideration. Absolute proof of safety of food additives is never possible because randomized studies of multiple doses that last lifetimes (or generations) and that are sufficiently large to detect small effects are impossible. Likewise, absolute proof that a food additive is harmful is typically not possible because multiple randomized trials with clinical endpoints may not be feasible or ethical. Thus, we are almost always operating in the middle of a spectrum ranging from certainty of no harm to certainty of harm. For food additives, which will be widely consumed by most members of the population, reasonable public health practice is to allow them only when available evidence suggests a position well toward the “certainty of no harm” end of the spectrum. This position is reflected, for example, by the U.S. Food and Drug Administration criterion of “reasonable certainty of no harm” and “Generally Regarded as Safe” (GRAS) for food additives. Because TFA from partially hydrogenated vegetable oils are artificial chemicals added to foods, they should be judged by these criteria.

A large body of evidence indicates unequivocally that when consumed at realistic levels TFA have adverse effects on blood lipids, whether compared to the original oils, carbohydrate, or saturated fat. Because of the known relation between blood lipids and risk of coronary heart disease, this body of evidence makes it impossible to conclude that there is reasonable certainty of no harm from consuming TFA. More recent evidence regarding intake of TFA and inflammatory markers, known to be related to type 2 diabetes and coronary heart disease, strengthen his conclusion. Hypothetically, it might be argued that TFA could have other, beneficial effects that counterbalance these adverse metabolic effects. However, prospective epidemiologic studies relating intake of TFA and incidence of coronary heart disease and diabetes add strong evidence that these metabolic changes are clinically important, and move the level of evidence close to certainty of harm, far beyond what is needed to forbid their use as a food additive.

At present TFA from partially hydrogenated fats are allowed in U.S. foods under the GRAS category. While this may have been appropriate at an earlier point in time, available evidence has led the U.S. Institute of Medicine to conclude that intake of total TFA should be as low as possible, and the U.S. Dietary Guidelines Committee concluded that intake should be below 1% of energy. Thus it is not possible to conclude that TFA are generally regarded as safe, and they should thus be disallowed from this category. This would have the appropriate effect of removing them from the food supply. If TFA are replaced largely by mix of unsaturated fatty acids, this will have a double benefit with enormous positive health consequences.

## Posters – List of Content

	Abstracts on page
P-1: <i>Nuernberg K, Nuernberg G, Ender K, Dannenberger D, Steinhart H.</i> Trans fatty acid isomers in different tissues of lamb.	27
P-2: <i>Dannenberger D, Nuernberg KN, Nuernberg G, Scollan N, Ender K.</i> Differences in C18:1 trans isomer distribution in muscle lipids of bulls fed concentrate and pasture.	28
P-3: <i>Weggemans RM, Trautwein EA, Rudrum M.</i> Health effects of ruminant and industrial trans-fatty acids	29
P-4: <i>Boué-Vaysse C, Combe N, Billeau C et al.</i> Trans Fatty Acid intake of a French women population in relation with coronary heart disease: the Aquitaine Study.	30
P-6: <i>DiRienzo M.</i> The Impact of Substitution of Low Linolenic Acid Soy Oil for Hydrogenated Soy Oil on Dietary Intakes of Fatty Acids by the US Population.	31
P-7: <i>Mizurini DM, Maia IC et al.</i> Thrombosis propensity in young and old rats related to trans fatty acids intake.	32
P-8: <i>Guimaraes DED, Silva APS et al.</i> Dietary Trans Fatty Acids and Adiposity in Young Rats.	33
P-9: <i>Guimaraes DED, Osso FS, Assumpcao RP et al.</i> Effects of Trans Fatty Acids on Glucose Homeostasis in Lactating Rats.	34
P-10: <i>Souza AS, Campos DBP, Pacheco LC et al.</i> Diets Containing Trans Fatty Acids Affect the Behaviour of Young Rats.	35
P-11: <i>Levart A, Zan M, Kompan D, Salobir J.</i> Variability of trans fatty acids content in milk produced in Slovenia.	36
P-12: <i>Rafecas M, Pascual JV, Codony R, Canela MA.</i> Evolution and final value of the content of “Trans” Fatty Acids in pig tissues: effect of diet and breed.	37
P-13: <i>Rafecas M, Ferrer C, Canela MA.</i> “Trans” Fatty Acids in poultry tissues: effect of diet TFAs in brain, liver and fat stores.	38

- P-14: *Chardigny J-M, Destailats F, Malpuech-Brugère C, Enjalbert F, German B, Bauman D, Combe N, Chaumont P, Francesca Gioffrida F, Bezelgues J-B, Cristiani I, Moulin J, Boirie Y, Dionisi F, Sébédio J-L.*  
Nutritional effect of trans fatty acids from hydrogenated fat versus milk fat in humans: the Transfact I protocol. 39
- P-15: *Gläser KR, Wenk C, Scheeder MRL.*  
Dose-response effects of feeding 18:1 trans fatty acids on body lipid composition of pigs and backfat firmness. 40
- P-16: *Dabadie H, Combe N, Peuchant E, Duhem K, Mendy F.*  
Influence of vaccenic and elaidic acids on lipidic parameters in healthy men in the frame of a study with balanced intakes of SFAs and PUFAs. 41
- P-17: *Chatziantoniou S, Paliou O, Triantafyllou D.*  
Fatty acid content of biscuits and crackers from Greek market. 42
- P-18: *Johansson L, Borgejordet Å, Pedersen JI.*  
Trans Fatty Acids in the Norwegian Diet. 43

**Trans fatty acid isomers in different tissues of lamb**

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The aim of this experiment was to investigate the effect of two dietary regimes on the fatty acid profile of total lipids, in muscle neutral lipid and phospholipid fraction and subcutaneous fat of lambs. The study exploited the use of Ag<sup>+</sup>-TLC and isothermal gas chromatography (125°C) to separate and measure C18:1*trans* isomers and Ag<sup>+</sup>-HPLC for individual CLA isomers in the muscle lipids of lambs. In total 13 male crossbred lambs (Black Head x Gotland) were divided into two feeding groups at 24 kg live weight. Lambs (n=6) were kept either on pasture (pasture grazing) or in stable (concentrate, n=7). The relative content of C18:1*trans*-11 (TVA) in total muscle lipids, phospholipids, triacylglycerols and subcutaneous fat was significantly increased by grass feeding compared to concentrate-fed. It is the most abundant C18:1*trans* isomer. Concentrate feeding caused a significantly higher proportion of C18:1*trans*-14, C18:1*trans*-15 and C18:1*trans*-16 compared to grass feeding. The proportion of CLA *cis*-9,*trans*-11 (1.9 % vs 1.1 in muscle, 2.5 vs 1.4 % in subcutaneous fat, phospholipid 0.7 vs 0.4 %) of lambs was significantly higher by grazing on pasture compared to concentrate feeding, respectively, when measured by gas chromatography. There is a positive correlation between TVA and CLA *cis*-9,*trans*-11  $r = 0.87$ .

In contrast to the GLC results the analysis of the CLA isomers by HPLC did not identify differences in the percentage of the most abundant isomer CLA *cis*-9,*trans*-11 between feeding groups. Keeping lambs on pasture caused only a significant decrease of the CLA *trans*-10,*cis*-12 and CLA *trans*-7,*cis*-9 proportion in the muscle fat.

### **Differences in C18:1 *trans* isomer distribution in muscle lipids of bulls fed concentrate and pasture**

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In a large study, sixty-four German Holstein and German Simmental bulls were randomly allocated to either an indoor concentrate system or periods of pasture feeding following by a finishing period on a concentrate containing linseed to enhance the contents of beneficial fatty acids in beef. As one part of the study the effects of feeding on C18:1 *trans* isomer distribution of *longissimus* muscle in beef bulls were examined. Due to the complexity of the methodology the measurements were only conducted for one breed, the German Holstein (n = 32). The analyses of C18:1 *trans* isomers were completed by the combination of Ag<sup>+</sup>TLC and isotherm GC on CP SIL 88 CB column (100m x 0.25 mm, Chrompack-Varian, USA).

The diet affected the distribution of individual C18:1 *trans* isomers in the lipids of the muscle tissue. The most abundant C18:1 *trans* isomer in the *longissimus* muscle was *trans*-vaccenic acid (C18:1 *trans*-11). Surprisingly, the concentration of C18:1 *trans*-11, and C18:1 *trans*-4, *trans*-5, and *trans*-9 were not affected by the diet. The concentration of C18:1 *trans*-11 tended to be higher in pasture-fed as compared to concentrate-fed bulls (102 vs 66.2 mg/100g fresh muscle), but the values did not reach statistical significance (p=0.12). However, pasture feeding decreased the concentrations of C18:1 *trans*-6/7/8, *trans*-10 in the muscle lipids. The concentrations of the C18:1 *trans*-13/14, *trans*-15, *trans*-16 isomers in the lipids of pasture-fed bulls were significantly increased.

Overall, pasture feeding results in a variation in the distribution pattern of C18:1 *trans* isomers as compared with concentrate-fed bulls, but the concentration of the sum C18:1 *trans* isomers in the muscle lipids of German Holstein bulls was not affected by the diet.

**Health effects of ruminant and industrial *trans*-fatty acids**

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*Trans*-fatty acids (TFA) intakes are associated with increased risk of developing coronary heart disease as compared to *cis*-unsaturated fatty acids. It is being suggested that TFA from ruminant sources are less detrimental for heart health than TFA from industrial sources. However, these comparisons have been based on the relative intake data (e.g. quintiles of intakes) rather than on the absolute intake data (e.g. g eaten per day). Therefore, we reviewed data describing the associations between intake of ruminant and industrial TFA and risk of coronary heart disease. There are no differences in risk of coronary heart disease between total, ruminant and industrial TFA over the range of intake (up to 2.5 g/d) where direct comparison is possible. At higher intakes (more than 3 g/d) total and industrial TFA increase risk of coronary heart disease but there is insufficient data available on ruminant TFA.

This analysis highlights the scarcity of data for comparing the health effects of ruminant versus industrial *trans*-fatty acids. In conclusion, in the intake range where direct comparison is possible there is no evidence for a differential effect of ruminant and industrial TFA intake on risk of coronary heart disease.

***Trans* Fatty Acid intake of a French women population in relation with coronary heart disease: the Aquitaine Study**

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**BACKGROUND :** In the 1990s, there was public health concern about epidemiological studies suggesting that dietary *trans* fatty acids (TFA) increase the risk of coronary heart disease (CHD). In the same time, there was no data in France concerning TFA intake level and their putative implication in CHD risk.

**OBJECTIVES :** Our study has been performed between 1996 and 1999, in South West of France, in 97 non-pregnant women, in order to determine the TFA intake level in Aquitaine country, and to assess the effect of dietary TFA on plasma concentration of lipoproteins involved in CHD risk.

**METHOD :** TFA consumption was determined from both seven-day diet record and TFA level in adipose tissue (AT) lipids (= biochemical indicator of long term TFA intake). TFA content of AT and plasma lipids were determined using a combination of capillary gas chromatography and silver nitrate thin-layer chromatography.

**RESULTS :** Our results showed that the mean TFA intake level for these Aquitaine women was 2.7g/d/pers. TFA were mainly provided by ruminant fats (64%), especially milk fats.

Finally, plasma concentrations of lipoproteins involved in CHD risk were not significantly correlated with either the amount of TFA consumed and stored in AT (2.3% of total fatty acids) or TFA percentage in plasma lipids (0.8% of total fatty acids).

**The Impact of Substitution of Low Linolenic Acid Soy Oil for Hydrogenated Soy oil on Dietary Intakes of Fatty Acids by the US Population.**

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Low linolenic acid soy oil (LLSO) has been developed as a substitute for hydrogenated vegetable oils to reduce intake of trans fatty acids while providing functionality in food applications. We assessed the dietary impact of substitution of LLSO for hydrogenated soy oil used in several food categories, such as fried foods, margarines, and snack foods. All substitutions were done at 100% market penetration. The impact on five fatty acids, palmitate, stearate, oleate, linoleate and linolenate, and on total trans fatty acids was assessed. The analysis showed that substitution of LLSO for current versions of hydrogenated soy oil resulted in a 45 % decrease in intake of trans fatty acids. Impacts on other fatty acids were small and dietarily insignificant. There was no decrease in intake of alpha linolenic acid associated with use of LLSO in place of hydrogenated soy oil, as might be expected from a soybean bred to be low in linolenic acid. This is due to the fact that LLSO substitutes for hydrogenated soy oils that are already low, or even devoid, in alpha linolenic acid.

### **Thrombosis propensity in young and old rats related to Trans Fatty Acids Intake**

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It is known that the profile of dietary fatty acids influence the blood coagulation process and may adversely affect hemostasis. The objective of this study was to investigate the influence of *trans* fatty acids on thrombosis propensity in young and old rats. Five different diets containing soy oil, palm oil, hydrogenated soy oil, canola oil, and fish oil, were fed to mothers during lactation and to the post-weaning male pups until day 45 (young rats) or 180 (old rats). Blood coagulation time was measured by APTT and PT tests. Platelets aggregation was induced by adding ADP, and  $EC_{50}$  was determined. The *t* test was used to compare the means ( $p < 0.05$ ). From young to old ages, APTT increased in the fish oil group, while PT increased in canola and fish oil groups and decreased in hydrogenated fat (*trans*) group. This indicates that hydrogenated fat leads to higher blood coagulability.  $EC_{50}$  decreased in rats fed *trans* and palm oil, showing a higher potential for platelet aggregation in old age. Our results indicate that *trans* intake adversely affects hemostasis, possibly leading to higher thrombosis propensity at old age, while fish and canola oil have favorable effects on thrombogenesis at this same age.

Support: CAPES, CNPq, ROCHE

\* in the aging phase

\* in this phase of the life.

## **Dietary *Trans* Fatty Acids and Adiposity in young rats**

### **EFFECTS OF DIETARY FAT SOURCES ON LIPID METABOLISM IN YOUNG MALE RATS**

Guimarães DED; Silva APS; Leandro MS; Mizurini DM; Tavares do Carmo MG; Instituto de Nutrição, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

The aim of this study was to compare the metabolic effects of diets based on hydrogenated fat (HF), palm oil (PO), canola oil (CO), and soy oil (SO) on young rats. Diets with these different lipid sources were fed to lactating rats and given to the male pups until the 45th day of life, when they were decapitated. The epididymal adipose tissue, the liver and the carcass were extracted to measure "in vivo" lipogenesis rate (LR) and lipid content. Food consumption, body weight, and the Lipase Lipoprotein (LPL) activity were also evaluated. Anova and Duncan's test were used to evaluate differences between groups ( $p < 0,05$ ). HF and PO groups showed increase in food consumption, body weight and liver, carcass and adipose tissue lipid contents, compared with CO and SO groups. Moreover, LR in liver and adipose tissue was significantly higher in PO and HF groups than CO and SO groups and there was no significant difference in the activity of LPL between groups. These results show that diets based on HF and PO can influence lipid metabolism in the young age, favouring lipid accumulation, with possible repercussions for the development of chronic not transmissible diseases.

**Effects of *Trans* Fatty Acids on Glucose Homeostasis in Lactating Rats**

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There are few studies about metabolic alterations in the maternal organism caused by dietary *trans* fatty acids (TFA). The aim of this study was to investigate the effects of maternal TFA consumption on glucose homeostasis and lipoprotein profile. Lactating rats were fed two different semisynthetic isocaloric diets containing 7% soy oil (control group) and 5% partially hydrogenated vegetable oil plus 2% soy oil (*trans* group). On day 14 of lactation, the rats were decapitated. Blood was collected for determination of glucose, insulin, total cholesterol, triacylglycerol, HDL-c, and total concentration of non esterified fatty acids (NEFA) using enzymatic kits. Glucose homeostasis was determined by hyperglycemic clamp technique. The *t* test was used to evaluate differences between groups ( $p < 0,05$ ). Dietary TFA caused alteration of the glucose homeostasis inducing a variable level of hyperinsulinemia and hypoglicemia with peaks over time. In the plasma, there was an increase in NEFA and insulin concentration and reduction in glucose and HDL-c concentration. These results suggest a possible insulin resistance caused by TFA in lactating rats, besides an alteration on maternal lipoprotein profile. Therefore it is important to alert the population to avoid excessive intake of TFA during lactation.

MÁXIMO PERMITIDO: 200 PALAVRAS

Está com 197 palavras.

### Diets Containing *Trans* Fatty Acids Affect The Behaviour of Young Rats

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It is known that dietary fatty acids influence spatial learning and memory. The aim of this study was to investigate whether dietary *trans* fatty acids affect the performance of young male rats in Morris water maze test. Three different diets containing soy oil (S), hydrogenated soy oil (*trans*), and fish oil (F), were fed to mothers during pregnancy and lactation and to the post-weaning male pups for 4 weeks. Group F presented important weight loss compared to S and *trans* ( $p < 0.0001$ ). There was a significant decrease in the scape latency for all diet groups in 20 trials during 4 days ( $p < 0.0001$ ). However, rats from F group had longer escape latency only in the second day of test ( $p < 0.05$ ). After 10 days, in a retention test, while S and F groups showed a short escape latency, it increased in the *trans* group from  $10.7 \pm 1.3$  to  $18.0 \pm 2.8$  s ( $p < 0.05$ ). The results suggest that fish oil diet caused alteration of the nutritional status, although the learning test performance was not affected. Furthermore, *trans* diet seems to impair long-term memory in young rats. Our results show that dietary *trans* fatty acids during perinatal period affect cognitive behavior in young rats.

Support: CAPES, CNPq.

## Variability of trans fatty acids content in milk produced in Slovenia

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Milk and dairy products can contribute non-negligible part to daily intake of *trans* fatty acids (TFAs) <sup>1</sup>. Since there are considerable differences in TFA content in milk produced in different European countries <sup>2</sup>, the aim of our study was to determine the TFA content and their seasonal variation in milk produced in Slovenia. Milk samples were collected in period from July 2003 to July 2004. Average yearly proportion of TFAs in analysed milk was 4.6% of total fatty acids. The obtained results are in agreement with available literature data for other European countries <sup>2</sup>. Large individual and seasonal variations in TFA content in milk were also observed. The highest TFA contents were found in summer milk and the lowest in winter milk ranging from 3.2 to 8.7% and 1.7 to 4.2% of total fatty acids, respectively. Mass fraction of *trans*-18:1 isomers varied from 76.7 to 85.4% of total TFAs. The most abundant *trans*-18:1 isomer was *trans*-vaccenic acid (*trans*-11-octadecenoic acid) which contributed from 1.1 up to 7.0% of total fatty acids and was positively correlated (R=0.86) with CLA content in milk samples. The obtained results are the first data on TFA content in milk produced in Slovenia. For assessment of TFA intake by Slovenian population further studies on TFAs content in milk produced in all management systems are needed.

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**“Trans” Fatty Acids in poultry tissues: effect of diet TFAs in brain, liver and fat stores**

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This poster presents the results of a study on the effect of the diet on the *trans* fatty acid (TFA) content in four poultry tissues: brain, liver, mesenteric and subcutaneous tissues. A sample of 60 male Ross chickens has been used, four diets being randomly assigned: a control (CTL) diet, a lard supplemented (LAR) diet, a linseed oil supplemented (LSO) diet and a sunflower oil supplemented (SFO) diet. Supplements were 60g/Kg. In general, we have found a TFA content, measured as a percentage of total fatty acid content, higher than that of the diet, except for the LAR diet. We have found a remarkable variety in the diet effect across tissues. In the fat stores, mesenteric and subcutaneous, the TFA content is similar to that of the diet (0.5%) for the LAR diet, and about one half of this for the other diets. The content in liver is similar for the CTL and LAR diet (about 0.25%), and almost irrelevant in the other two diets. Finally, in the brain the TFA content (about 0.47%) is independent of the diet.

**Evolution and final value of the content of “Trans” Fatty Acids in pig tissues: effect of diet and breed**

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This poster presents the result of a study on the change of *trans* fatty acids (TFA) content in response to four diets (76 days treatment) containing increasing amounts (0, 2, 4 and 8%) of a high linoleic acid blend. A sample of 112 pigs of four different breeds (Landrace, Large White, Duroc and a crossbreed Landrace × Duroc) has been used. In the first part of the study, the effects of diet and breed on the evolution of the fatty acid composition of backfat have been assessed from biopsy results. A decrease in TFA content is observed, except for the highly supplemented diet, where this decrease is compensated by an initial increase. The second part of the study deals with the final TFA content in four tissues (backfat, abdominal fat and the muscles *trapezius* and *longissimus thoracis et lumborum*). The results reveal a strong diet effect, although the TFA content is not linearly dependent on the TFA content of the diet. The breed effect is much weaker and nonsignificant. Results of the same experiments for other fatty acids are going to be published in a forthcoming paper in *Food Chemistry*.

**Nutritional effect of trans fatty acids from hydrogenated fat versus milk fat in humans: The Transfact I protocol**

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It is well established that trans fatty acids (TFA) from hydrogenated fat contribute to a higher cardiovascular risk, related to the increase in LDL-cholesterol and a decrease in HDL-cholesterol in healthy humans.

On the other hand, milk fat also contains TFA resulting from biohydrogenation of polyunsaturated fatty acids in the rumen. However, several major differences between both dietary sources are (i) the content of TFA and (ii) the position of the trans double bond on the carbon chain. The major TFA from partially hydrogenated vegetable oils are elaidic acid (trans-9-18:1) and trans-10-18:1, whereas vaccenic acid (trans-11-18:1) represents more than 45% of total trans fatty acids in milk fat. Moreover, vaccenic acid is bioconverted in vivo into rumenic acid, a conjugated linoleic acid isomer that may have beneficial effects on health.

In order to assess the respective effects of the trans fatty acids from both dietary origins (hydrogenated and milk), a randomized cross over clinical study will be started including 88 healthy volunteers for 2 X 3 weeks of treatment. During each experimental fat diet intake, subjects will consume about 3.3% of energy as TFA from one or the other source. Biological parameters related to cardiovascular risk will be determined.

The results are expected by mid 2006.

**Dose-response effects of feeding 18:1 *trans* fatty acids on body lipid composition of pigs and backfat firmness**Gläser KR, Wenk C and Scheeder MRLInstitute of Animal Sciences, Swiss Federal Institute of Technology Zurich (ETH),  
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Forty Large White pigs were fed from 30 kg to 103 kg body mass diets supplemented with 6 % of pure high-oleic sunflower oil (HO) or HO plus increasing amounts of partially hydrogenated rape seed oil (HR; 1.85 %, 3.70 %, 5.55 %), containing high levels of 18:1 *trans* fatty acid isomers. Increasing dietary 18: *trans* fatty acids resulted in a linear increase in 18:1 *trans* fatty acids and *cis*-9, *trans*-11 conjugated linoleic acid (CLA) in backfat (BF) as well as in neutral lipids (NL) and phospholipids (PL) of *M. long. dorsi*. Thus, the rate of bioconversion of *trans* vaccenic acid (TVA) into CLA and incorporation of 18:1 *trans* and CLA into pig adipose tissue was not limited up to 25 g total 18:1 *trans* fatty acids including 3.3 g of TVA per kg feed. In BF and NL the sum of saturated fatty acids (SFA) increased with increasing dietary amounts of HR, indicating inhibition of stearoyl-CoA-desaturase. In PL 18:1 *trans* fatty acids partly replaced SFA, obviously according to their physical properties. The firmness of backfat was also markedly increased ( $P < 0.05$ ) with increasing amounts of HR in feed.

## Influence of Vaccenic and Elaidic Acids on Lipidic Parameters in healthy men in the frame of a study with balanced intakes of SFAs and PUFAs

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Ruminant *trans* fatty acid (TFA), vaccenic acid, from dairy products do have different physiological effects from partially hydrogenated vegetable oils (PHVO) TFA, elaidic acid. Very few interventional studies have shown influence of these TFAs on lipidic parameters under “*ceteris paribus*” conditions. That was the aim of our study.

Nineteen male monks without dyslipidemia were given experimental diet (Diet 1) for 5 weeks: 36% fat, 13% SFA, 1.8% myristic acid, 4.7% palmitic, 1.6% stearic, 0.4% vaccenic, 0.6 % elaidic, 7% PUFA, 5.9 linoleic/alpha-linolenic ratio. This diet was compared to a isocaloric control diet (Diet 0): 35% fat, 12% SFA, 1.2% myristic acid, 5.4% palmitic, 2.2% stearic, 0.2% vaccenic, 0.8% elaidic, 7% PUFA, 3.5 linoleic/alpha-linolenic ratio. Samples were obtained at completion of each diets.

Diet 1 was associated with a decrease in total cholesterol, LDL-cholesterol, triglycerides, total/HDL-cholesterol ratio ( $p<0.01$ ), and with an increase in HDL-cholesterol ( $p<0.01$ ). Myristic and alpha-linolenic acids, EPA and DHA were increased in cholesteryl esters ( $p<0.05$ ). Vaccenic acid was increased by 64% ( $p<0.0002$ ) and was negatively correlated with rumenic ( $r=-0.62$ ;  $p=0.006$ ). Elaidic acid was decreased with Diet 1 by 20% ( $p<0.02$ ), and elaidic/vaccenic ratio was decreased by 53%. Elaidic acid from Diet 0 but not Diet 1 was positively correlated with triglycerides and total/HDL-cholesterol ( $r=0.54$ ;  $p=0.02$ ), and negatively correlated with HDL-cholesterol ( $r=-0.52$ ;  $p=0.03$ ). A one-year interventional study is in progress with the same diets and the assessment of membrane fluidity. Preliminary results showed that Diet 1 was associated with an increase in membrane fluidity ( $p<0.01$ ). The 18:1  $\square$  10t was increased with Diet 1 by 50% ( $p<0.0002$ ) but was not associated with any change in lipid profile.

These results show that under “*ceteris paribus*” conditions vaccenic and elaidic acids could not be associated with a same lipidic profile. The elaidic/vaccenic ratio could be taken into account.

## Fatty acid content of biscuits and crackers from Greek market

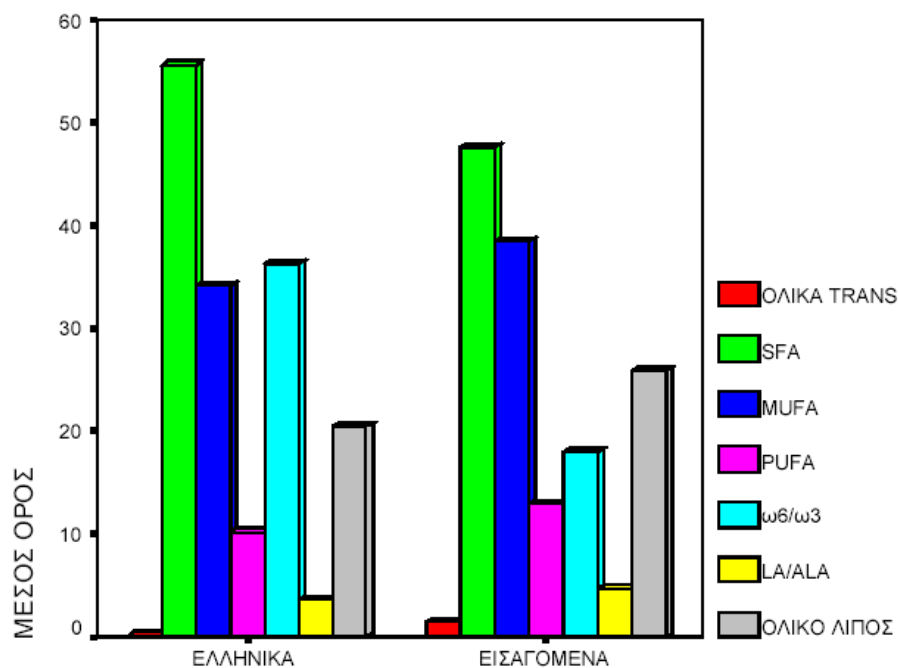
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The fatty acid composition including trans-isomers of 20 samples of biscuits (15 greek -5 foreign origin) and 10 samples of crackers obtained from the Greek market was determined using CP-SiL 88 column on gas chromatography. Results were statistically analyzed with ANOVA in order to find significant differences between samples according to their type or origin.

Total results of analyzed biscuits showed a higher mean content of biscuits in oleic acid (18:1) (33.52%) and total polyunsaturated fatty acids (PUFA) (10.97%), compared to results of similar research in other countries. A higher mean of TFA content was found in imported biscuits (9.5%) than in biscuits produced in Greece (0.9%).

Fatty acid profile of crackers showed a higher mean content in total TFA (0.47%) comparing with previous reported in other countries while Cream crackers were found to have a smaller trans fatty acid content (0.57%) in comparison to the results of similar research in other countries. Samples of Greek cream crackers were found to have a higher content of trans-linoleic acid (18:2 trans) (0.57%) than Greek plain crackers (0.14%). Greek industry shows to have some modification in production plan in order to produce products with less content in TFA.



## Trans fatty acids in the Norwegian diet

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**Background:** There was no up to date information about intake of trans fatty acids (TFA) in the Norwegian population.

**Material and methods:** Information on TFA content in foods in 2003 from a survey among Norwegian food industry and importers, is together with data from the Norwegian Food Composition data base used to estimate the content of trans fatty acids in food groups. Intake of TFAs is calculated on the basis of food intake reported in the national dietary survey among adults in 1997.

**Results:** Mean intake of TFAs was 1.6 gram/person/day, corresponding to 0.6 percent of total energy intake. Main contributors of TFAs were dairy products 50%, meat products 18%, buns and cakes 8% and bread products 7% of total trans fatty acid intake.

**Interpretation:** The calculated intake of trans fatty acids among Norwegians in 2003 was in accordance with the recommendation from WHO that intake of TFAs should be limited to less than 1 percent of energy intake. For several food groups the content of trans fatty acids have been reduced since 2003. Thus our calculations imply that intake of trans fatty acids no longer represents a public health concern for the Norwegian population.