BMJ Open

Industrially produced trans fat in popular foods in 2015-2016 in 15 countries of the former Soviet Union: a market basket investigation

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023184
Article Type:	Research
Date Submitted by the Author:	25-Mar-2018
Complete List of Authors:	Stender, Steen; Kobenhavns Universitet Det Natur- og Biovidenskabelige Fakultet, Nutrtion excersize and sports
Keywords:	Coronary heart disease < CARDIOLOGY, NUTRITION & DIETETICS, Health policy < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, central asia trans fat

1

24-03-2018

Industrially produced *trans* fat in popular foods in 2015-2016 in 15 countries of the former Soviet Union: a market basket investigation

Steen Stender, Professor, MD, DMSci

Steen Stender, Department of Nutrition, Exercise and Sports, University of Copenhagen, Rolighedsvej 26, Frederiksberg,1958 DK, Denmark

Correspondence to: S Stender stender@nexs.ku.dk

Telephone number: +45 35333162

Telephone number: +45 40258601

Key Words: Coronary heart disease, Health policy, Nutrition, Soviet Union, *Trans* fat

Word count: 3669 den 22-03-2018

ABSTRACT

Objective: To minimize the intake of industrial *trans* fat (I-TF) and thereby decrease the risk of coronary heart disease (CHD), several countries have implemented a legislative restriction on I-TF in foods. The objective of this study was to investigate the presence of I-TF in biscuits/cakes/wafers in 15 countries of the former Soviet Union. These countries all have a high coronary mortality compared with countries in Western Europe.

Design: Three large supermarkets were visited in each of the 15 capitals in 2015 or 2016. Pre-packaged biscuits/cakes/wafers were bought if the list of ingredients disclosed that the product contained more than 15 grams of total fat per 100 grams of product and if partially hydrogenated fat or a similar term, including margarine, refined fat or confectionary fat, were mentioned. Samples of the foods were subsequently analysed for total fat and TF.

Results: Some 994 products had more than 2% of total fat as I-TF (illegal in Denmark). In Armenia, 91 different products had a mean value (SD) of 21(11) % of the fat as I-TF. In Estonia, there were 8 products with 14(10) % of the fat as I-TF. The other 13 countries had values in between. In several countries a major part of the products were imported from Russia and Ukraine. Many of these products had a readable production date and a best before date. The mean shelf life (SD) of 673 packages was 218 (75) days. The % TF in the fat of products produced in Russia and in Ukraine in relation to the date of production declined significantly during the 2 years collection period.

Conclusions: The findings suggest that I-TF is used in popular foods in all 15 countries of the former Soviet Union. It opens a possibility for reduction of the high coronary mortality in these countries.

(Words 299) 20-03-2018

Strengths and limitation of this study

- A strength of this study is that the presence of industrial *trans* fat in popular foods for the first time is systematically investigated in each of the 15 countries of the former Soviet Union that all have a high coronary mortality and a population of nearly 300 million.
- A limitation of the study is that only pre-packaged biscuits/cakes/wafers were investigated for *trans* fat and no other food groups such as shortenings and margarines and no un-packaged foods such as baked goods, sweet rolls, pastries, buns and even un-packaged biscuits/cakes/wafers were investigated.
- Another limitation is that the average daily intake of *trans* fat was not measured in any subgroups of the populations, but instead was inferred from the availability of popular foods with high amounts of *trans* fat in large supermarkets.

.

INTRODUCTION

High amounts of *trans* fat (TF) in food originate from the industrial hydrogenation of edible oils. Compared to non-hydrogenated oils, fats containing industrially produced *trans* fat (I-TF) are solid at room temperature, have some technical advantages for food processing, and prolong the shelf life of products. TF are not synthesized in the human body and are not required in the diet, but is absorbed and metabolized. I-TF can be found at varying amounts in biscuits, pastries, margarine, spreads, confectionary creams and fillings and fried foods such as potato crisps. Fat from ruminants, for instance in dairy products, contains up to 5 % TF, which is considerably lower than the up to 50% TF that may be present in partially hydrogenated fat for human food.

Observational studies suggest an association between dietary intake of I-TF and increased risk of coronary heart disease (CHD).[1] The association is further corroborated by an association between plasma TF level and CHD.[2] Plausible mechanisms for the association involving an increase in plasma LDL-cholesterol [3] and unfavourable "beyond lipid effects" on inflammatory cells important for atherogenesis [4,5] suggest a causal relationship, even in the absence of randomized studies with hard clinical endpoints.[6] Based on the available evidence, Denmark in 2004 and some counties in New York State, USA in 2007, legally restricted the use of I-TF in the diet. The fewer CHD events, beyond temporal trends, in Denmark and in counties in New York State, strongly suggest that I-TF in the diet promotes CHD and a restriction in its use reduces CHD.[7–9] What remains to be explored is to what extent I-TF still is used in popular foods in countries with high rates of CHD-mortalities.

The 15 countries of the former Soviet Union all have an extraordinarily high rate of CHD mortality compared with countries in Western Europe.[10] In a systematic analysis[11], including 266 country-specific nutrition surveys, the average consumption of TF in 2010 in Central Asia and Eastern Europe that together include the 15 countries of the former Soviet Union, for adults of more than 20 years, were 0.9% of total energy intake; this corresponds to approximately 2.5 gram TF per day per adult and is less than the 1% of total energy percent recommended by WHO. The consumption level was found stable since 1990. Among the world's 21 different regions Central Asia and Eastern Europe were placed as number 5 and 8, respectively with the lowest average consumption of TF, suggesting that intake of TF is a minor health problem in these countries. However, the investigators mention the relatively limited data availability on TF consumption in most nations compared with other major dietary factors.[11] Furthermore a low average intake of TF in a nation does not exclude a high intake of TF in subgroups of the population if popular foods contain high amounts of I-TF.

Some recent Russian studies, with an abstract in English, mention the harmful effect on health of

dietary I-TF and pledge for a removal of I-TF from margarine "to preserve the health of Russia's population".[12,13] A study from Estonia reports about high I-TF in blended spreads, margarines and shortenings bought in the Estonian retail market in 2011.[14] Additionally, technical reports from the WHO find several types of street foods with high amounts of TF in Dushanbe, Tajikistan, April/May 2016, and in Bishkek, Kyrgyzstan, June/July 2016.[15] In a recent systematic review of TF intake and its dietary sources in general populations worldwide based on data from 29 different countries and a demonstration of a substantial reduction in I-TF in many countries, none of the countries of the former Soviet Union were included, either for the intake of the general population or for the study's secondary research question about the composition of fat in biscuits, because of the lack of data.[16] Apparently, there is a pronounced scarcity of newer internationally published data about I-TF in foods in most of these countries. The purpose of the present study was to investigate the presence of I-TF in popular foods in the 15 countries of the former Soviet Union by a newly developed market basket method.[17]

METHODS

Purchase of biscuits/cakes/wafers in supermarkets

By using Wikipedia and local tourist information offices, I identified three large supermarkets in each capital, preferably chain supermarkets with many large shops across the country.[18] Pre-packaged biscuits/cakes/wafers were chosen as the food to be investigated for TF in this study because these types of foods are frequently consumed, easily accessible, and transportable at ambient temperature. Furthermore, these foods traditionally contain I-TF-rich, partially hydrogenated vegetable oils as their major lipid ingredient, and I-TF has previously been found in these foods in high concentrations in some countries in Eastern Europe.[19–21] The packages of

biscuits/cakes/wafers were obtained in each supermarket by systematically examining the labels of the products. Packages were purchased if they met the following criteria on the list of ingredients:

- Total fat content was equal to or exceeded 15 g per 100 g of product.
- Listed one or more of the terms: partially hydrogenated fat, hydrogenated fat, hardened fat, refined fat, confectionary fat, deodorized fat, all-purpose fat or margarine.
- If the list of ingredients indicated *trans* fat > 0 g, these foods were also included.
- Packages with the term 'unhydrogenated fat' or 'fully hydrogenated fat' or 'no *trans* fat' were not purchased.

If the same package with the same barcode number was found in more than one of the three different supermarkets in the same capital, only the package with the most recent production date was included in the study. Each package was subsequently labelled with an ID-number, and duplicate samples each of approximately 50 grams of the product were taken for analysis. The barcode number, the name of the producer, the country of origin and, if available, the best before date as well as the production date were registered, and the empty packages were stored. The first 2 digits (sometimes three digits) in the barcode number identify the country or economic region numbering authority which assigned the manufacturer code. The Russian Federation uses the code 46, Ukraine the code 482. The manufacturer code is the next 5 digits followed by the product code, consisting of 5 digits. The last digit is the check digit.[22]

To investigate to what extent the presence of biscuits/cakes/wafers in the capital differed from other parts of the country, biscuits/cakes/wafers were obtained in 3 different supermarkets in each of 2 different regions of the 2 large countries Uzbekistan and Russia: in Uzbekistan also in the second largest city Samarkand (0.4 million inhabitants and approximately 300 km from Taskent) and in Russia also in Sct. Petersburg (5.2 million inhabitants and approximately 800 km from

Moscow). The third large country was Ukraine. In its capital Kiev, 3 other large supermarkets were visited (Kiev-2) in addition to the first 3 that were visited (Kiev-1).

Analysis of TF

The samples from the various countries were analysed successively and in the order they were obtained. Samples obtained in the first countries that we visited (Moldova, Belarus, Georgia, Armenia and Kyrgyzstan) were all analysed for TF. In the other countries that were visited later some packages were obtained with the same barcode number, and the same visual appearance and list of ingredients as on packages where the samples already were analysed. A sample from the newly found package was analysed only if the best before date on that package exceeded the best before date by more than 12 months on a package that already had been analysed. Otherwise, the TF values were carried forward to the new package.

For analysis the foods were homogenized, and the fatty acid content was analysed using gas chromatography on a 66-m highly polar capillary column, using a modification of the AOAC 006·06 method. All analytical work on samples was conducted by Microbac Laboratories in Warrendale, Pennsylvania, USA, an ISO-17025-certified laboratory. The measurement cannot distinguish I-TF from ruminant TF. If butter as a ruminant fat has been used in the product in addition to partially hydrogenated vegetable oil, some of the TF in the product may be derived from butter that on average contains a few percent of the fat as TF. In this paper, the term I-TF is used even though a minor portion in certain products may be TF derived from ruminant fat.

Statistical analysis

For each country, simple linear regression models were used to describe the change in %TF as a function of shelf life and over time recorded as date of production. The statistical software R was used (R Core Team, 2017).

RESULTS

The number of different packages obtained in 3 different supermarkets in each of the 15 capitals totalled 1332. Samples from 1068 packages were analysed, and TF values for each of the remaining 264 packages were taken from a similar package with an identical barcode but obtained earlier in another country and already analysed. Some 994 samples had more than 2% of the fat as TF. The different products in each country were ranked according to I-TF level, expressed as grams per 100 g of the product (figure 1). This latter value tells how much TF the consumer gets when 100 grams of the product is consumed. Each horizontal bar in each panel represents a product with more than 2% of the fat as TF. The number of these products is shown together with the number of packages, given in parenthesis that fulfilled the inclusion criteria. The products depicted in each panel are all different, but the same product may appear in the panels for two or more countries. The mean values of the percentages of fat that were I-TF in each of the countries, together with the SD and maximum values are shown. These percentages are of interest for food producers and for food authorities because they describe the fat that has been used in the food. In some products obtained in Armenia and in Kyrgyzstan about 50% of the fat was I-TF. (figure 1)

In the second largest city, Samarkand, in Uzbekistan, 29% of the packages were similar based on the barcode number to the packages found in the capital, Taskent. In Sct Petersburg, Russia, 30% were similar to the products found in Moscow, and in Kiev-2, the second region of collection, 48% were similar to the packages found in the first region of collection, Kiev-1. Even though most

products were different in the 2 different regions of the same country, the panels that reflect the availability of biscuits/cakes/wafers with more than 2% TF in the fat are rather similar. (figure 2) This suggests that the panels presented for each country in figure 1 represent a pattern found elsewhere in the country and not only in the capital.

For the 994 products with more than 2% of the fat as I-TF and obtained in the 15 capitals of the former Soviet Union, 52% were produced in Russia, 26% in Ukraine, 13% in own country, and 8 % in some other countries for instance Poland and Bulgaria. For the products produced in Russia, 85% were obtained outside Russia, in the other countries of the former Soviet Union combined, not including Ukraine and Lithuania. For the products produced in Ukraine, 69% were obtained outside Ukraine in the other countries, excluding Russia. The import patterns of pre-packaged biscuits/cakes/wafers differed from country to country exemplified by the patterns observed for Uzbekistan and Kazakhstan. (figure 3) The concentrations in grams of TF per 100 grams of product are in a panel for each of the two countries, depicted with each product placed together with other products from the same country of origin. The numbers and the mean values of the concentrations of TF in the fat of pre-packaged biscuits/cakes/wafers produced in Russia and in Ukraine but obtained in the various countries of the former Soviet Union are shown in table 1. All of the 75 different products with more than 2% of the fat as TF obtained in Moscow were produced in Russia, and 81 of the 83 different products obtained in Kiev-1 were produced in Ukraine. The same pattern was seen in Sct Petersburg, Russia and in Kiev-2, Ukraine, respectively. In each of the countries, except for the 3 Baltic countries and Belarus, a majority of the products were imported from Russia and Ukraine.

The analysis of shelf life was only conducted for packages produced in Russia and Ukraine, as they were the main exporters to most of the other countries, and in many of the products, especially from Russia, the length of the shelf life appeared on the package. This is in contrast to pre-packaged

biscuits/cakes/wafers produced in most countries in the more western part of Europe. These packages only have the best before date and the shelf life of the product is usually hidden for the final customer. Products with less than 2% TF in the fat had shelf lives of approximately 200 days, comparable to products with a much higher concentration of TF (table 2). The most frequent length of shelf life was 6 months, whether the products were produced in Russia or in Ukraine. The slope of the regression line between shelf life and % TF was significantly negative for Ukraine ($p<0.0001$) and significantly positive for Russia ($p<0.0001$). Taken together, the data did not support the conclusion that the higher the TF in the product, the longer the shelf life.

Since the samples of biscuits/cakes/wafers were collected between January 2015 and January 2017, and many were produced in Russia and in Ukraine, I have depicted the % TF of fat in the products in relation to the date of production to show a trend in the two countries in their use of fat with high amounts of TF in these products. The TF concentrations decrease significantly during the 2 years in Russia ($p<0.0001$) as well as in Ukraine ($p<0.0001$), but the decrease did not differ significantly between the 2 countries ($p=0.12$) (figure 4).

DISCUSSION

The principal findings

The findings of this study clearly demonstrate that in 2015-2017 I-TF was present in high concentrations in many different brands of biscuits/cakes/wafers in the countries of the former Soviet Union, except for the 3 Baltic countries. This information was not previously available and may be of relevance because of the high coronary mortality in these countries. In 3 of the countries- Russia, Ukraine and Belarus, by far most of the

products are produced in the country itself. For the other countries, except for the 3 Baltic countries, imports from food producers in Russia and Ukraine make up a major part of their products with high amounts of TF.

In all of the shops there were more packages that did not have the included terms on the label than packages that had one or more of the terms on the label. This suggests that there are plenty of biscuits/cakes/wafers without I-TF available in all of the 45 supermarkets. Of the 1332 packages that fulfilled the inclusion criteria and were bought, 335 (25%) of the products had less than 2% of the fat as TF. This suggests that the inclusion criteria are rather broad in relation to the presence of TF and/or that the terms on the list of ingredients do not always reflect the composition of the fat in the product.

The amounts of TF per 100 grams of product bought in Moscow did not exceed 6 grams (figure 1), whereas several products produced in Russia, but obtained in the other countries had more than 6 grams per 100 grams, for instance in Uzbekistan and Kazakhstan (figure 3). The same is seen for the average concentration of I-TF in products obtained in Russia, with mean value and SD of 10(6) %, compared with considerable higher values in Russian products obtained in the other countries (table 1). The same pattern is seen for products bought in Ukraine, compared with products produced in Ukraine but obtained in some of the other countries (table 1). The products obtained in Russia were bought more than a year later than the products produced in Russia but obtained in the other countries. This was also the case for the products obtained in Ukraine and outside Ukraine. Some of the difference may be due to a decrease in the use of I-TF during the time period.

I-TF has been used in foods such as biscuits/cakes/wafers also because it apparently prolongs the shelf life of the foods. This is presently not supported by the findings that 186 products produced in Russia with a shelf life of 6 months had TF concentrations of 11 (7)% mean value (SD) and 37 products with a shelf life of 12 months, also had TF concentrations of 11 (7)%. The corresponding values from products produced in Ukraine were for 86 products with shelf life of 6 months 20 (9) % and for 30 products with shelf life of 12 months 10 (6) %.

Based on the plasma concentrations of TF in nationally representative groups in 2010 in the USA (3 years after the introduction of mandatory labelling of TF on pre-packaged foods and in spite of a nearly 50% lower average TF-concentration in plasma), there is still a subgroup with very high TF concentrations, reflecting a subgroup in the general population with a high consumption of TF.[3] A vicious cycle apparently occurs: as long as foods with high amounts of I-TF are present in the shops, some consumers will buy them, and as long as they are bought, the foods will be available. This may also be the case in the countries of the former Soviet Union. Despite a decline in the average intake of I-TF in many countries in Western Europe,[16] in 2015 the WHO estimated that millions of Europeans still consume TF at levels that significantly increase their risk for CHD.[23] The findings reported in the present investigation confirm this estimate.

Strength and weaknesses of the study

Strengths of this study include that the same procedure was used to obtain popular foods in large supermarkets in all 15 countries of the former Soviet Union, and in the

3 largest countries in two different regions and that the samples were analysed for TF in the same laboratory. A limitation of this study is that no other food groups (such as shortenings and margarines) and no un-packaged foods (such as baked goods, sweet rolls, pastries, buns and even un-packaged biscuits/cakes/wafers) were investigated. However, the presence of high concentrations of I-TF in biscuits/cakes/wafers may be a sign that I-TF is used in other foods as well. Another limitation is that the average daily intake of I-TF was not measured in any subgroups of the populations, but instead was inferred from the availability of popular foods with high amounts of I-TF in large supermarkets. The competition for shelf space in supermarkets is usually fierce. If products do not sell, they are rapidly replaced by other products. A relevant subgroup for intake studies would be the consumers who buy pre-packaged biscuits/cakes/wafers.

Legislation and industrial *trans* fat in foods

In the USA in 2015, the FDA revoked the previous GRAS (Generally Recognized As Safe) status of partially hydrogenated oils - a step that may remove I-TF from food production in the USA before July 2018, and Canada is following suit.[24] In 2009, Austria and Switzerland introduced a legislative ban similar to the Danish ban from 2004, followed by Iceland in 2011, Hungary and Norway in 2014, and Latvia, Georgia, Lithuania, and Slovenia between 2016 and 2018. Other Eastern European countries including Russia may be on their way to I-TF legislation.[13,25,26] The European Union published a report in 2015, originally commissioned in 2011, that "indicates the need to continue and expedite work in this area by collecting more information".[27] In the

WHO-Europe investigation of the various ways to reduce I-TF in foods, it was concluded that establishing a legal limit for the content of I-TF in foods is potentially the only available option that reduces the risks associated with I-TF faced by all consumers and doing so may contribute to reducing inequalities. Such a policy is unique in its combination of efficacy, cost-effectiveness and low potential for negative impact. Removing I-TF from the food supply is possibly one of the most straightforward public health interventions for reducing risk of CHD.[23] If the trend of using fat with lower amounts of I-TF continues as observed (figure 4) or even accelerates among food factories in Russia and Ukraine that produce biscuits/cakes/wafers, the intake of I-TF in the 2 large countries may decrease, but in addition also the intake of I-TF among millions of inhabitants in the adjacent countries.[18]

Future research and implications for policy makers

It is of considerable interest whether coronary mortality and admission rates for CHD and stroke will change in countries that have recently introduced legislation that restricts the use of I-TF or intend to do so in the near future, in the same way as it was observed in Denmark and in certain counties in New York State.[6,9]

On the basis of data from 2009 to 2011, there is a fivefold difference in age-standardized mortality rates of CHD between some countries in Western Europe and countries in Central and Eastern Europe, with Central Asia having the highest rates.[10] Central Eastern Europe and Central Asia were the world regions with the highest current age-standardized cardiovascular mortality rates, which are more than twice

those of Latin America and the Caribbean.[29] The present study demonstrates a presence of I-TF in popular foods in the countries of the former Soviet Union with excessively high CHD mortality. Restriction of the use of I-TF in foods, either voluntarily by food producers or more efficiently by legislation, may be an easily implemented first strategy for the reduction of cardiovascular diseases in these countries.

REFERENCES

1. de Souza RJ, Mente A, Maroleanu A, et al. Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. *BMJ* 2015; 351: h3978. doi:101136/bmj.h3978.

2. Li H, Zhang Q, Song J, et al. Plasma trans-fatty acids levels and mortality: a cohort study based on 1999-2000 National Health and Nutrition Examination Survey (NHANES). *Lipids Health Dis* 2017; 16: 176.doi:10.1186/s12944-017-0567-6.

3. Yang Q, Zhang Z, Loustalot F, et al. Plasma *trans*-Fatty Acid Concentrations Continue to Be Associated with Serum Lipid and Lipoprotein Concentrations among US Adults after Reductions in *trans*-Fatty Acid Intake. *J Nutr* 2017; 147**:**896–07.

4. Nagasawa Y, Shinke T, Toh R, et al. The impact of serum trans fatty acids concentration on plaque vulnerability in patients with coronary artery disease: Assessment via optical coherence tomography. *Atherosclerosis* 2017 http://dx.doi.org/10.1016/j.atherosclerosis.2017.06.922.

5. Hirata Y, Takahashi M, Kudoh Y, et al. Trans-Fatty acids promote proinflammatory signaling and cell death by stimulating the apoptosis signal-regulating kinase 1 (ASK1)-p38 pathway. *J Biol Chem.* 2017; 292: 8174–85 doi: 10.1074/jbc.M116.771519.

6. Brouwer IA, Wanders AJ, Katan MB. *Trans* fatty acids and cardiovascular health: research completed? *Eur J Clin Nutr* 2013; 67:541–47.

7. Restrepo BJ, Rieger M. Denmark's Policy on Artificial Trans Fat and Cardiovascular Disease. *Am J Prev Med* 2016; 50: 69–76.

8. Restrepo BJ, Rieger M. Trans fat and cardiovascular disease mortality: Evidence from bans in restaurants in New York. *J. Health Econ* 2016; 45: 176–196

9. Brandt EJ, Myerson R, Perraillon MC, *et al*. Hospital Admissions for Myocardial Infarction and Stroke Before and After the Trans-Fatty Acid Restrictions in New York. *JAMA Cardiol* 2017; 2: 627–634.

10. Nichols M, Townsend N, Scarborough P, *et al*. Cardiovascular disease in Europe 2014: epidemiological update. *Eur Heart J* 2014; 35: 2950–59

11. Micha R, Khatibzadeh S, Shi P, *et al*. Global, regional, and national consumption levels of dietary fats and oils in 1990 and 2010: a systematic analysis including 266

country-specific nutrition surveys. *BMJ* 2014; 348: g2272 doi: 10.1136/bmj.g2272

12. Perova NV, Metel'skaia VA, Bo•tsov SA.[Trans isomers of unsaturated fatty acids increase the risk of atherosclerosis-related circulatory system diseases].[Article in Russian]. *Ter Arkh*. 2013; 85: 113-7.

13. Boytsov SA, Perova NV. To lower trans fatty acid intake as an important condition for the reduction of cardiovascular Mortality(Article) *Profilakticheskaya Meditsina* 2017; 20: 4-9.

14. Meremäe K, Roasto M, Kuusik S, *et al*. Trans Fatty Acid Contents in Selected Dietary Fats in the Estonian Market. *J Food Sci.* 2012; 77: 163-8.

15. FEEDcities project. The food environment description in cities in Eastern Europe and Central Asia - Tajikistan and Kyrgyzstan. Technical report, WHO Europe, June 2017 and August 2017 http://www.euro.who.int/__data/assets/pdf_file/0012/353001/FEED-TJK-report.pdf?ua=1 http://www.euro.who.int/__data/assets/pdf_file/0006/351996/FEED-KGZ-report-web-new.pdf?ua=1 (Accessed Mar 2018).

16. Wanders AJ, Zock PL, Brouwer IA. Trans Fat Intake and Its Dietary Sources in General Populations Worldwide: A Systematic Review. *Nutrients.* 2017; 9,840; doi:10.3390/nu9080840.

17. Stender S, Astrup A, Dyerberg J. A trans European Union difference in the decline in trans fatty acids in popular foods: a market basket investigation. *BMJ Open* 2012; 17;2 e000859. doi: 10.1136/bmjopen-2012-000859.

18. https://en.wikipedia.org/wiki/Post-Soviet_states#General_statistics (Accessed Mar 2018).

19. Kravic SZ, Suturovic ZJ, Svarc-Gajic JV, *et al*. Fatty acid composition including *trans*-isomers of Serbian biscuits. *Hem Ind* 2011; 65:139–46.

20. Stender S, Astrup A, Dyerberg J. Tracing artificial *trans* fat in popular foods in Europe: a market basket investigation. *BMJ Open* 2014; 4: e005218.

21. Stender S, Astrup A, Dyerberg J. Artificial trans fat in popular foods in 2012 and in 2014: a market basket investigation in six European countries. *BMJ Open*. 2016; 6: e010673.

22. https://da.wikipedia.org/wiki/International_Article_Number_(EAN) (Accessed Mar 2018).

23. Eliminating trans fats in Europe – A policy brief (2015)

http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/publications/2015/eliminating-trans-fats-in-europe-a-policy-brief-2015 (Accessed Mar 2018).

24. FDA Cuts Trans Fat in Processed Foods http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm372915.htm (Accessed Mar 2018).

25. Vučić V, Arsić A, Petrović S, *et al*. Trans fatty acid content in Serbian margarines: Urgent need for legislative changes and consumer information. *Food Chem* 2015; **185**: 437–40.

26. Wilczek MM, Olszewski R, Krupienicz A. *Trans*-Fatty Acids and Cardiovascular Disease: Urgent Need for Legislation. *Cardiology*. 2017; 138:254–58.

27. Report from the Commission to the European parliament and the Council regarding trans fats in foods and in the overall diet of the Union population. (03-12-2015) http://ec.europa.eu/dgs/health_food-safety/dyna/enews/enews.cfm?al_id=1650 (Accessed Mar 2018).

28. Inception Impact Assessment; Initiative to limit industrial trans fats intakes in the EU.

http://ec.europa.eu/smart-regulation/roadmaps/docs/2016_sante_143_trans_fats_en.pdf (Accessed Mar 2018).

29. Barquera S, Pedroza-Tobías A, Medina C, *et al*. Global Overview of the Epidemiology of Atherosclerotic Cardiovascular Disease. *Arch Med Res* 2015; **46**: 328–38

LEGENDS

Figure 1

Amounts of industrially produced *trans* fat (I-TF) in 100 grams of pre-packaged biscuits/cakes/wafers purchased in 2015-2016 in three supermarkets in each of the 15 capitals. Each bar in a panel represents a unique product bought in that capital, i.e., none of the products in a given panel had the same barcode number. N is the number of different products with more than 2% TF of the total fat content. (N) is the number of packages that fulfilled the inclusion criteria. X is the mean value and the standard deviation of the percentages of I-TF in the total fat of the N products, M is the maximal value of I-TF as percent of total fat. Each bar in all of the 15 panels has the same thickness, which means that the area of the bars is comparable between the different panels.

Figure 2

The legend for this figure is similar to the legend for Figure 1. The 4 upper panels show the result for 2 of the capitals and 2 other large cities in the same country. The lowest 2 panels give the results

from the purchase in 3 large supermarkets in Kiev (Kiev-1) and in 3 other large supermarkets in the same city (Kiev-2).

Figure 3

Amounts of industrially produced *trans* fat (I-TF) in 100 grams of pre-packaged biscuits/cakes/wafers, obtained in Uzbekistan and in Kazakhstan respectively with each product in the panel placed together with other products from the same country of origin.

Figure 4

Date of production and I-TF as percent of total fat for pre-packaged biscuits/cakes/wafers, produced in Russia or Ukraine, respectively, and obtained in the countries of the former Soviet Union between January 2015 and January 2017. Products obtained in Samarkand, Sct Petersburg and Kiev 2 were included if the products had their own *trans* fat analysis. Only products with more than 2% of the fat as I-TF were included.

Table 1

Country of origin for pre-packaged biscuits/cakes/wafers with more than 2% of the fat as I-TF obtained in the 15 countries of the former Soviet Union.

Table 2

Shelf life of pre-packaged biscuits/cakes/wafers produced in Russia or in Ukraine and obtained in the countries of the former Soviet Union between January 2015 and January 2017.

Footnotes

Contributors:

SS was responsible for the concept design of the study, for collection of food items, registration, and labelling, and writing the manuscript. SS is the manuscripts guarantor.

Funding: This work was supported by Jenny Vissings Foundation, University of Copenhagen and Department of Clinical Biochemistry, Copenhagen University Hospital, Gentofte, Denmark.

Role of the funding source

The sponsors of the study had no role in the study design, data collection, analysis, and interpretation, the writing of the report, or the decision to submit the article for publication. The corresponding author had full access to all the data for the study and had final responsibility for the decision to submit the manuscript for publication.

Competing interests:

None declared

Ethics approval

The study does not require an approval from the Ethics committee

Acknowledgements:

The authors acknowledge the support from Jenny Vissings Foundation, University of Copenhagen and the Department of Clinical Biochemistry, Copenhagen University Hospital, Gentofte, Denmark.

Copyright statement:

The Corresponding Author has the right to grant an exclusive licence on a worldwide basis to the BMJ Publishing Group LID Publishers and its licensees in perpetuity, in all forms, formats and media (whether known now or created in the future), to i) publish, reproduce, distribute, display and store the Contribution, ii) translate the Contribution into other languages, create adaptations, reprints, include within collections and create summaries, extracts and/or, abstracts of the Contribution, iii) create any other derivative work(s) based on the Contribution, iv) to exploit all subsidiary rights in the Contribution, v) the inclusion of electronic links from the Contribution to third party material where-ever it may be located; and, vi) licence any third party to do any or all of the above."

Transparency declaration:

Steen Stender (the manuscript's guarantor) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Data sharing statement:

The data used to construct figures 1-5, tables 1 and 2, and supplementary table A and B can be shared by e-mailing Steen Stender

Table 1 Country of origin for pre-packaged biscuits/cakes/wafers with more than 2% of the fat as *trans* fat (TF) obtained in the various countries.

Country	Capital	Produced in Russia					Produced in Ukraine				
			Trans fat per 100 gram fat						Trans fat per 100 gram fat		
		N	%*	Mean	SD	Max	N	%*	Mean	SD	Max
Armenia	Yerevan	50	55	18	9	51	33	36	26	14	51
Belarus	Minsk	20	20	15	9	36	5	5	23	6	32
Tadjikistan	Dushanbee	121	89	11	7	33	10	7	15	8	33
Kirgistan	Bishkek	58	72	15	8	30	15	19	29	13	50
Azerbaijan	Baku	58	68	12	7	35	15	18	13	1	33
Moldova	Chisinau	10	19	25	8	35	25	47	26	8	42
Ukraine	Kiev-1	0	0				81	98	14	10	42
*Ukraine **	*Kiev-2*	*0*	*0*				*62*	*97*	*14*	*11*	*45*
Uzbekistan	Taskent	50	65	10	8	35	20	26	16	10	33
*Uzbekistan **	*Samarkand*	*30*	*48*	*14*	*8*	*29*	*28*	*44*	*19*	*12*	*43*
Turkmenistan	Ashgabat	18	33	13	8	29	24	44	18	11	38
Georgia	Tblisi	23	49	16	10	36	8	17	21	9	35
Russia	Moscow	75	100	10	6	27	0	0			
*Russia **	*Sct Petersburg*	*105*	*100*	*9*	*5*	*27*	*0*	*0*			
Kazakhstan	Astana	28	56	11	8	29	12	24	11	6	19
Lithuania	Vilnius	0	0				9	28	11	8	33

25

Latvia	Riga	5	21	9	4	12	4	17	9	4	15
Estonia	Tallinn	1	13	29		29	2	25	19	17	31
15 countries together		517	52				263	26			
%* is the number of samples with more than 2% of the fat as TF as percent of all samples obtained in											
that country with more than 2% of the fat as TF.											
** the lines in italic present values for biscuits/cakes/wafers obtained by the same procedure but in											
extra regions of the same country.											
The values for the three regions are not included in the values in the bottom line.											

Table 2 Shelf life of pre-packaged biscuits/cakes/wafers produced in Russia and Ukraine

	Produced in Russia		**Produced in Ukraine**	
Trans fat as % of fat	< 2%	≥ 2%	<2%	≥ 2%
Number of samples*	140	453	24	220
Shelf life in days				
Mean (SD)	202(83)	219(70)	286(94)	215(85)
Range	59-547	46-396	123-366	46-546

* Samples obtained in Samarkand, Sct Petersburg, and Kiev-2 were included if these samples had their own *trans* fat analysis and a readable shelf life on the package

Figure 1

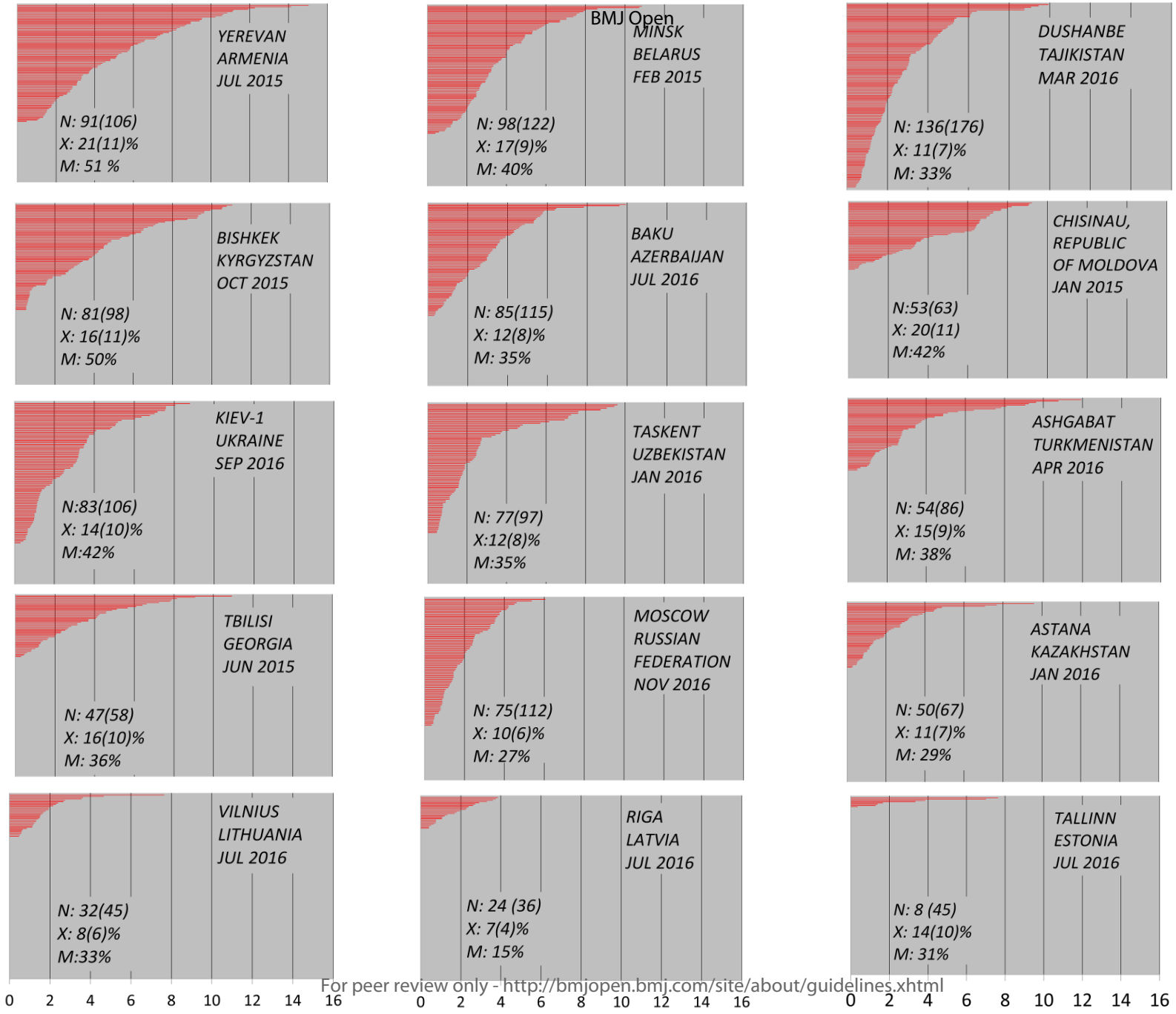


Figure 2

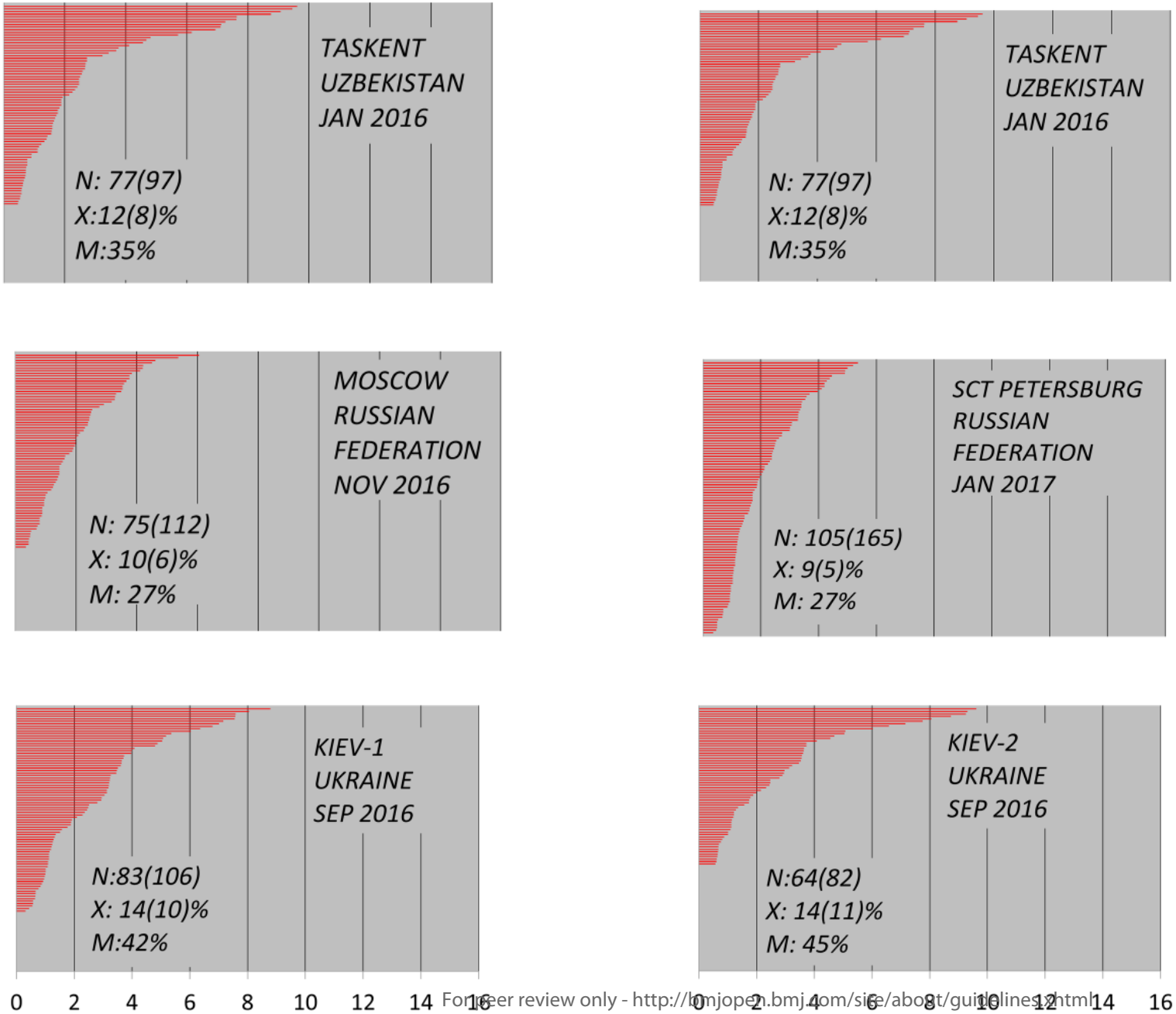


Figure 3

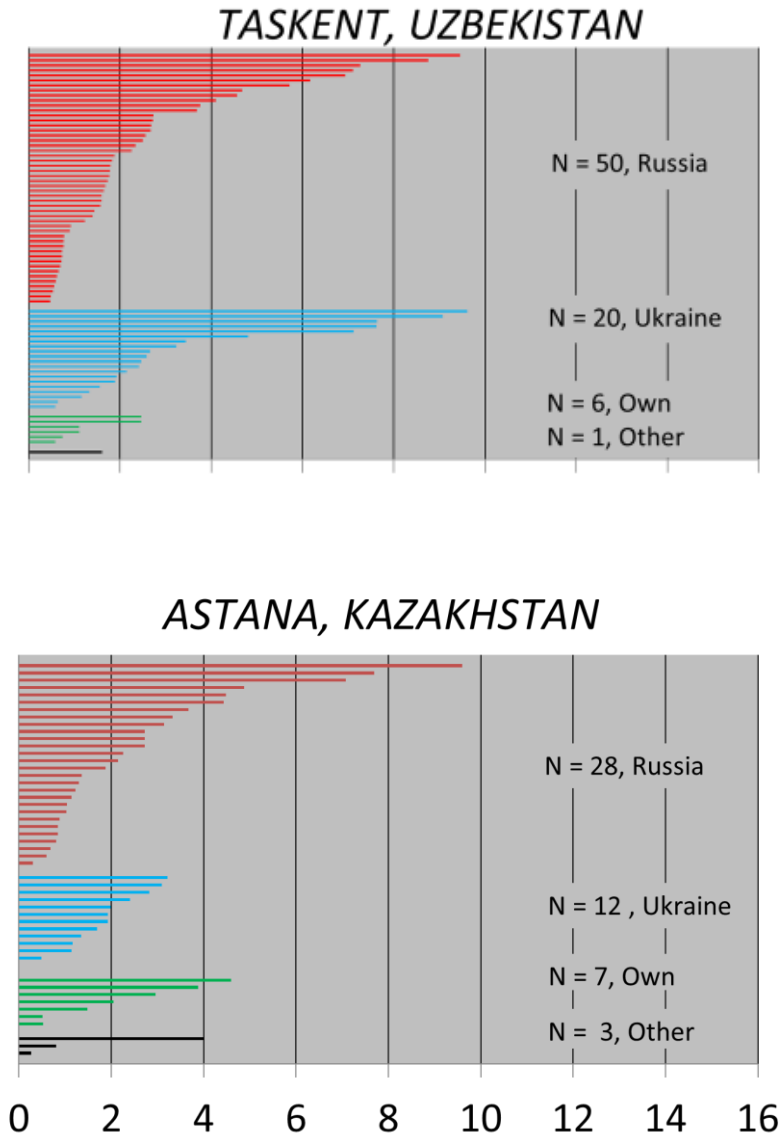
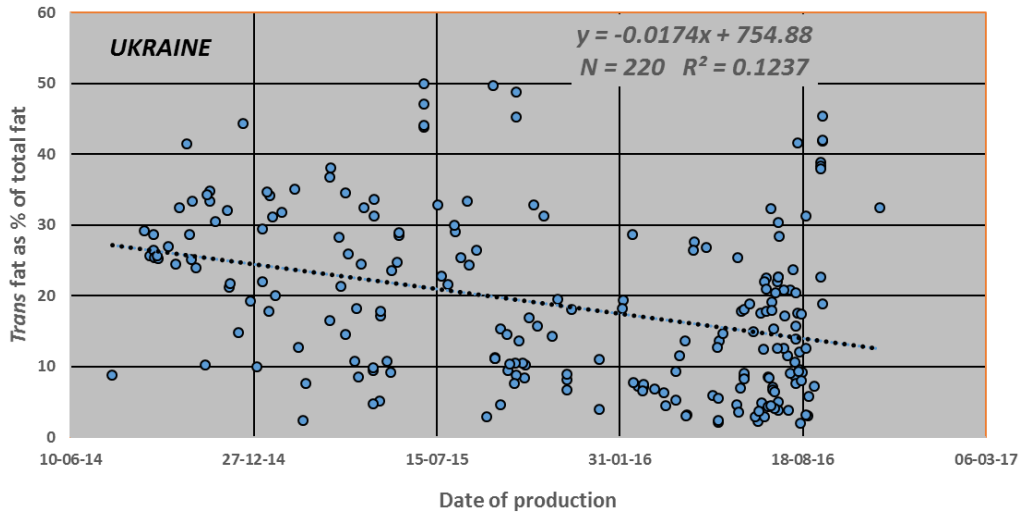
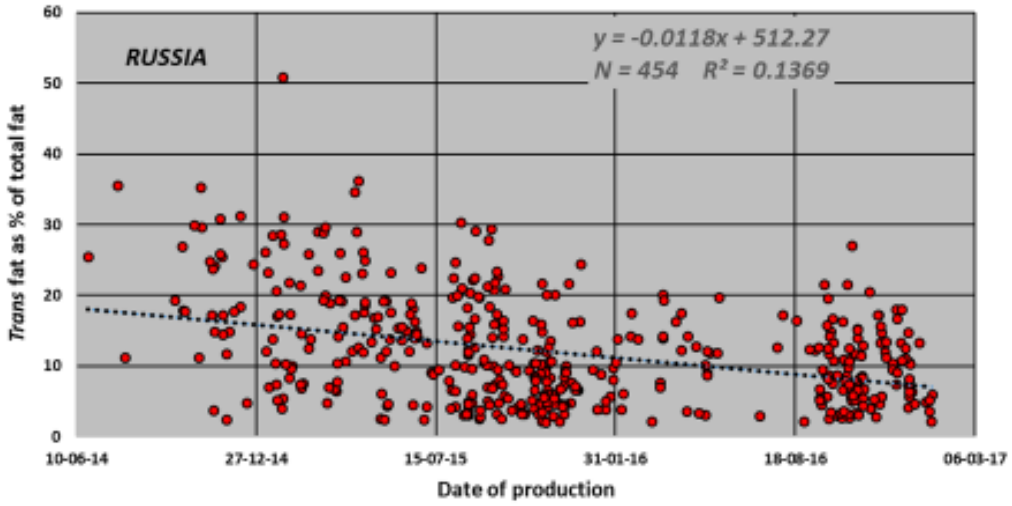


Figure 4


UKRAINE
y = -0.0174x + 754.88
N = 220 R² = 0.1237
Trans fat as % of total fat
0
10
20
30
40
50
60
10-06-14
27-12-14
15-07-15
31-01-16
18-08-16
06-03-17
Date of production


RUSSIA
y = -0.0118x + 512.27
N = 454 R² = 0.1369
Trans fat as % of total fat
0
10
20
30
40
50
60
10-06-14
27-12-14
15-07-15
31-01-16
18-08-16
06-03-17
Date of production

BMJ Open

Industrially produced trans fat in popular foods in 2015-2016 in 15 countries of the former Soviet Union: a market basket investigation

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023184.R1
Article Type:	Research
Date Submitted by the Author:	20-Sep-2018
Complete List of Authors:	Stender, Steen; Kobenhavns Universitet Det Natur- og Biovidenskabelige Fakultet, Nutrtion excersize and sports
Primary Subject Heading:	Health policy
Secondary Subject Heading:	Health policy, Global health, Public health
Keywords:	Coronary heart disease < CARDIOLOGY, NUTRITION & DIETETICS, Health policy < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, central asia trans fat

1

12-09-2018

Industrially produced *trans* fat in popular foods in 2015-2016 in 15 countries of the former Soviet Union: a market basket investigation

Steen Stender, Professor, MD, DMSci

Steen Stender, Department of Nutrition, Exercise and Sports, University of Copenhagen, Rolighedsvej 26, Frederiksberg, 1958 DK, Denmark

Correspondence to: S Stender stender@nexs.ku.dk

Telephone number: +45 35333162

Telephone number: +45 40258601

Key Words: Coronary heart disease, Health policy, Nutrition, Soviet Union, *Trans* fat

ABSTRACT

Objective: To minimize the intake of industrially produced *trans* fat (I-TF) and thereby decrease the risk of coronary heart disease (CHD), several countries have implemented a legislative restriction on I-TF in foods. The objective of this study was to investigate the presence of I-TF in biscuits/cakes/wafers in 15 countries of the former Soviet Union, all with a high coronary mortality compared with countries in Western Europe.

Design: Three large supermarkets were visited in the 15 capitals in 2015 or 2016. Pre-packaged biscuits/cakes/wafers were bought if the list of ingredients disclosed that the product contained more than 15 grams fat per 100 grams of product and if partially hydrogenated fat or a similar term, including margarine, refined fat or confectionary fat, were mentioned. Samples of the foods were subsequently analysed for total fat and TF.

Results: Some 994 products had more than 2% of total fat as I-TF (illegal in Denmark). In Armenia, 91 different products had a mean value (SD) of 21(11) % of the fat as I-TF. In Estonia, there were 8 products with 14(10) % of the fat as I-TF. The other 13 countries had values in between. In several countries a major part of the products were imported from Russia and Ukraine. Many of these products had a readable production date and a best before date. The mean shelf life (SD) of 673 packages was 218 (75) days. The % TF in the fat of products produced in Russia and in Ukraine in relation to the date of production both declined with approximately 10 % points during the 2 years collection period.

Conclusions: The findings suggest that I-TF is used in popular foods in all 15 countries of the former Soviet Union. It opens a possibility for some reduction of the high coronary mortality in these countries.

Strengths and limitation of this study

- A strength of this study is that the presence of industrially produced *trans* fat in popular foods for the first time is systematically investigated in each of the 15 countries of the former Soviet Union that all have a high coronary mortality and a population of nearly 300 million.
- A limitation of the study is that only pre-packaged biscuits/cakes/wafers were investigated for *trans* fat and no other food groups such as shortenings and margarines and no un-packaged foods such as baked goods, sweet rolls, pastries, buns and even un-packaged biscuits/cakes/wafers were investigated.
- A limitation is the assumption that if the inclusion words are not present in the list of ingredients, the product contains less than 2% of the total fat as *trans* fat.
- Another limitation is that the average daily intake of *trans* fat was not measured in any subgroups of the populations, but instead was inferred from the availability of popular foods with high amounts of *trans* fat in large supermarkets.

.

INTRODUCTION

High amounts of *trans* fat (TF) in food i.e. more than 5 % TF of total fat in the food originate from the industrial hydrogenation of edible oils. Compared to non-hydrogenated oils, fats containing industrially produced *trans* fat (I-TF) are solid at room temperature, have some technical advantages for food processing, and prolong the shelf life of products. TF are not synthesized in the human body and are not required in the diet, but is absorbed and metabolized. I-TF can be found at varying amounts in biscuits, pastries, margarine, spreads, confectionary creams and fillings and fried foods such as potato crisps. Fat from ruminants, for instance in dairy products, contains up to 5 % TF (R-TF), which is considerably lower than the up to 50% TF that may be present in partially hydrogenated fat for human food. In a double blind, randomised controlled trial in humans R-TF resulted in the same increase in LDL-cholesterol in blood compared with I-TF when the two different types of fat were given in the diet in same amounts.[1] It suggests that R-TF and I-TF have similar harmful effect on plasma lipoproteins**.**

Observational studies suggest an association between dietary intake of I-TF and increased risk of coronary heart disease (CHD).[2] The association is further corroborated by an association between plasma TF level and CHD.[3]

Plausible mechanisms for the association involving an increase in plasma LDL-cholesterol[4] and unfavourable "beyond lipid effects" on inflammatory cells important for atherogenesis[5] suggest a causal relationship, even in the absence of randomized studies with hard clinical endpoints.[6] Based on the available evidence, Denmark in 2004 and some counties in New York State, USA in 2007, legally restricted the use of I-TF in the diet. After the restrictions in Denmark there was a 4.3% lower yearly mortality rate due to CHD beyond temporal trends.[7] A similar decline of 4.5 % in CHD mortality rate was found in the counties with restriction in New York State compared with similar counties without restrictions.[8] Three and more years after the restriction in the counties, a hospital admission rate for myocardial infarction and stroke events combined was 6.2 % lower than in similar counties without restrictions.[9] These findings from 2 different groups of researchers, strongly suggest that I-TF in the diet promotes CHD and a restriction in its use reduces CHD. What remains to be explored is to what extent I-TF still is used in popular foods in countries with high rates of CHD-mortalities.

The 15 countries of the former Soviet Union all have an extraordinarily high rate of CHD mortality compared with countries in Western Europe.[10] (supplementary table A) In a systematic analysis, including 266 country-specific nutrition surveys, the average consumption of TF in 2010 in Central Asia and Eastern Europe that together include the 15 countries of the former Soviet Union, for adults of more than 20 years, were 0.9% of total energy intake.[11] This intake corresponds to approximately 2.5 gram TF per day per adult and is less than the 1% of total energy percent recommended by WHO. The consumption level was found stable since 1990. Among the world's 21 different regions Central Asia and Eastern Europe were placed as number 5 and 8, respectively with the lowest average consumption of TF, suggesting that intake of TF is a minor health problem in these countries. However, the investigators mention the relatively limited data availability on TF consumption in most of these nations compared with other major dietary factors.[11] Furthermore a low average intake of TF in a nation does not exclude a high intake of TF in subgroups of the population if popular foods contain high amounts of I-TF.

Some recent Russian studies, with an abstract in English, mention the harmful effect on health of dietary I-TF and pledge for a removal of I-TF from margarine "to preserve the health of Russia's population".[12,13] A study from Estonia reports about high I-TF in blended spreads, margarines and shortenings bought in the Estonian retail market in 2011.[14] Additionally, technical reports from the WHO find several types of street foods with high amounts of TF in Dushanbe, Tajikistan, April/May 2016, and in Bishkek, Kyrgyzstan, June/July 2016.[15] In a

recent systematic review of TF intake and its dietary sources in general populations worldwide based on data from 29 different countries and a demonstration of a substantial reduction in I-TF in many countries, none of the countries of the former Soviet Union were included, either for the intake of the general population or for the study's secondary research question about the composition of fat in biscuits, because of the lack of data.[16] Apparently, there is a pronounced scarcity of newer internationally published data about I-TF in foods in most of these countries. The purpose of the present study was to investigate the presence of I-TF in popular foods in the 15 countries of the former Soviet Union by a newly developed market basket method.[17]

METHODS

Purchase of biscuits/cakes/wafers in supermarkets

By using Wikipedia and local tourist information offices, three large supermarkets were identified in each capital, preferably chain supermarkets with many large shops across the country.[18] Pre-packaged biscuits/cakes/wafers were chosen as the food to be investigated for TF in this study because these types of foods are frequently consumed, easily accessible, and transportable at ambient temperature. Furthermore, these foods traditionally contain I-TF-rich, partially hydrogenated vegetable oils as their major lipid ingredient, and I-TF has previously been found in these foods in high concentrations in some countries in Eastern Europe.[19–21] The packages of biscuits/cakes/wafers were obtained in each supermarket by systematically examining the labels of the products. Packages were purchased if they met the following criteria on the list of ingredients:

- Total fat content was equal to or exceeded 15 g per 100 g of product.
- Listed one or more of the terms: partially hydrogenated fat, hydrogenated fat, hardened fat, refined fat, confectionary fat, deodorized fat, all-purpose fat or margarine.
- If the list of ingredients indicated *trans* fat > 0 g, these foods were also included.
- Packages with the term 'unhydrogenated fat' or 'fully hydrogenated fat' or 'no *trans* fat' were not purchased.

If the same package with the same barcode number was found in more than one of the three different supermarkets in the same capital, only the package with the most recent production date was included in the study. Each package was subsequently labelled with an ID-number, and duplicate samples each of approximately 50 grams of the product were taken for analysis. The barcode number, the name of the producer, the country of origin and, if available, the best before date as well as the production date were registered, and the empty packages were stored. The first 2 digits (sometimes three digits) in the barcode number identify the country or economic region numbering authority which assigned the manufacturer code. The Russian Federation uses the code 46, Ukraine the code 482. The manufacturer code is the next 5 digits followed by the product code, consisting of 5 digits. The last digit is the check digit.[22]

To investigate to what extent the presence of biscuits/cakes/wafers in the capital differed from other parts of the country, biscuits/cakes/wafers were obtained in 3 different supermarkets in each of 2 different regions of the 2 large countries Uzbekistan and Russia: in Uzbekistan also in the second largest city Samarkand (0.4 million inhabitants and approximately 300 km from Taskent) and in Russia also in Sct. Petersburg (5.2 million inhabitants and approximately 800 km from Moscow). The third large country was Ukraine. In its capital Kiev, 3 other large supermarkets were visited (Kiev-2) in addition to the first 3 that were visited (Kiev-1).

Analysis of TF

The samples from the various countries were analysed successively and in the order they were obtained. Samples obtained in the first countries that were visited (Moldova, Belarus, Georgia, Armenia and Kyrgyzstan) were all analysed for TF. In the other countries that were visited later some packages were obtained with the same barcode number, and the same visual appearance and list of ingredients as on packages where the samples already were analysed. A sample from the newly found package was analysed only if the best before date on that package exceeded the best before date by more than 12 months on a package that already had been analysed. Otherwise, the TF values were carried forward to the new package.(supplementary table B)

For analysis the foods were homogenized, and the fatty acid content was analysed using gas chromatography on a 66-m highly polar capillary column, using a modification of the AOAC 996·06

method. The method makes it possible to get total fat as triglycerides, which are then broken down into mono-, poly-, saturated- and total TF. Total TF may consist of up to 8 different *trans* fatty acids. All analytical work on samples was conducted by Microbac Laboratories in Warrendale, Pennsylvania, USA, The laboratory undergo annual proficiency testing and are audited every other year to ensure compliance of this method to ISO 17025.

From the peaks in the chromatogram TF is calculated as a fraction (percent) of total fat in the product. This value is primarily of interest for food producers, food chemists and food legislators. The total amount of fat in 100 grams of the product is determined from the chromatogram. When that value is multiplied by the TF-fraction the amounts of TF in gram per 100 grams of the product appear. This value is primarily of interest for the customers and for investigators of the health effect of intake of TF.

The measurement does not distinguish I-TF from R-TF. If butter as a ruminant fat has been used in the product in addition to partially hydrogenated vegetable oil, some of the TF in the product may be derived from butter that on average contains a few percent and maximum 5 % of the fat as R-TF. In this paper, the term I-TF is used even though a minor portion in certain products may be TF derived from ruminant fat. If butter is present in a product which have been analysed for TF in the present study and the value is higher than 5% TF of total fat, butter has diluted the I-TF in the product, because butter adds more non-TF than it adds TF. Values larger than 5% TF in the product are consequently minimum values for I-TF as percent of total fat. In products with less than 5% TF as percent of total fat that contain butter, the I-TF will be lower than the TF value. Such products are because of their low amounts of TF per 100 gram of product of less importance for the health of the consumer than products with much higher TF values and of less interest for health authorities and food scientists.

Patient and Public involvement

Patients and public were not involved in the present study**.**

Statistical analysis

For each country, simple linear regression models were used to describe the change in % TF of total fat as a function of shelf life and over time recorded as date of production. The statistical software R was used (R Core Team, 2017).

RESULTS

The number of different packages obtained in 3 different supermarkets in each of the 15 capitals totalled 1332. Samples from 1068 packages were analysed, and TF values for each of the remaining 264 packages were taken from a similar package with an identical barcode but obtained earlier in another country and already analysed. Some 994 samples had more than 2% of the fat as TF. The different products in each country were ranked according to I-TF level, expressed as grams per 100 g of the product (figure 1). This latter value tells how much TF the consumer gets when 100 grams of the product is consumed. Each horizontal bar in each panel represents a product with more than 2% of the fat as TF. The number of these products is shown together with the number of packages, given in parenthesis that fulfilled the inclusion criteria. The products depicted in each panel are all different, but the same product may appear in the panels for two or more countries. The mean values of the percentages of fat that were I-TF in the collection of different products from each of the countries, together with the SD and maximum values are shown. These percentages are of interest for food producers and for food authorities because they describe the fat that has been used in the food. In some products obtained in Armenia and in Kyrgyzstan about 50 % of the fat was I-TF. (figure 1)

In the second largest city, Samarkand, in Uzbekistan, 29% of the packages were similar based on the barcode number to the packages found in the capital, Taskent. In Sct Petersburg, Russia, 30% were similar to the products found in Moscow, and in Kiev-2, the second region of collection, 48% were similar to the packages found in the first region of collection, Kiev-1. Even though most products were different in the 2 different regions of the same country, the panels that reflect the availability of biscuits/cakes/wafers with more than 2 % TF in the fat are rather

similar (figure 2). This suggests that the panels presented for each country in figure 1 represent a pattern found elsewhere in the country and not only in the capital.

For the 994 products with more than 2% of the fat as I-TF and obtained in the 15 capitals of the former Soviet Union, 52% were produced in Russia, 26% in Ukraine, 13% in own country, and 8 % in some other countries for instance Poland and Bulgaria (table 1). For the products produced in Russia, 85% were obtained outside Russia, in the other countries of the former Soviet Union combined, not including Ukraine and Lithuania. For the products produced in Ukraine, 69% were obtained outside Ukraine in the other countries, excluding Russia. The import patterns of pre-packaged biscuits/cakes/wafers differed from country to country exemplified by the patterns observed for Uzbekistan and Kazakhstan. (figure 3) The concentrations in grams of TF per 100 grams of product are in a panel for each of the two countries, depicted with each product placed together with other products from the same country of origin. The numbers and the mean values of the concentrations of TF in the fat of pre-packaged biscuits/cakes/wafers produced in Russia and in Ukraine but obtained in the various countries of the former Soviet Union are shown in table 1. All of the 75 different products with more than 2% of the fat as TF obtained in Moscow were produced in Russia, and 81 of the 83 different products obtained in Kiev-1 were produced in Ukraine. The same pattern was seen in Sct Petersburg, Russia and in Kiev-2, Ukraine, respectively. In each of the countries, except for the 3 Baltic countries and Belarus, a majority of the products were imported from Russia and Ukraine.

The analysis of shelf life was only conducted for packages produced in Russia and Ukraine, as they were the main exporters to most of the other countries, and in many of the products, especially from Russia, the length of the shelf life appeared on the package. This is in contrast to pre-packaged biscuits/cakes/wafers produced in most countries in the more western part of Europe. These packages only have the best before date and the shelf life of the product is usually hidden for the final customer. Products with less than 2% TF in the fat had shelf lives of approximately 200 days, comparable to products with a much higher concentration of TF (table 2). The most frequent length of shelf life was 6 months, whether the products were produced in Russia or in Ukraine. The slope of the regression line between shelf life and % TF of total fat was significantly negative for Ukraine ($p<0.0001$) and significantly positive for Russia ($p<0.0001$). Taken together, the data did not support the conclusion that the higher the TF in the product, the longer the shelf life.

Since the samples of biscuits/cakes/wafers were collected between January 2015 and January 2017, and many were produced in Russia and in Ukraine, the % TF of total fat in the product was depicted in relation to the date of production. The TF concentrations decrease significantly during the 2 years in Russia ($p<0.0001$) as well as in Ukraine ($p<0.0001$), but the decrease did not differ significantly between the two countries ($p=0.12$) (figure 4). The products from Ukraine had a higher TF % in the start of the period than the products from Russia. Based on the slopes of the regression lines in figure 4, the decline for products from Ukraine was 12.4 % points and for products from Russia 8.4 % points during the 2 years period.

DISCUSSION

The principal findings

The findings of this study clearly demonstrate that in 2015-2016 I-TF was present in high concentrations i.e. more than 5 % I-TF in the total fat in many different brands of biscuits/cakes/wafers in the countries of the former Soviet Union, except for the 3 Baltic countries. This information was not previously available and may be of relevance because of the high coronary mortality in these countries. In 3 of the countries- Russia, Ukraine and Belarus, by far most of the products are produced in the country itself. For the other countries, except for the 3 Baltic countries, imports from food producers in Russia and Ukraine make up a major part of their products with high amounts of TF.

In all of the shops there were more different packages containing biscuits/cakes/wafers that did not have the inclusion terms on the label than packages that had one or more of the terms on the label. This suggests that there are plenty of biscuits/cakes/wafers without I-TF available in all of the 45 supermarkets. Of the 1332 packages that fulfilled the inclusion criteria and were bought, 335 (25%) of the products had less than 2% of the fat as TF. This suggests that the inclusion criteria are rather broad in relation to the presence of TF and/or that the terms on the list of ingredients do not always reflect the composition of the fat in the product. If the list of ingredients does not have the inclusion words the product may still have high amounts of TF and are not bought and analysed for TF.

The amounts of TF per 100 grams of product bought in Moscow did not exceed 6 grams (figure 1), whereas several products produced in Russia, but obtained in the other countries had more than 6 grams per 100 grams, for

instance in Uzbekistan and Kazakhstan (figure 3). The same is seen for the average concentration of I-TF in products obtained in Russia, with mean value and SD of 10(6) %, compared with considerable higher values in Russian products obtained in the other countries (table 1). The same pattern is seen for products bought in Ukraine, compared with products produced in Ukraine but obtained in some of the other countries (table 1). The products obtained in Russia were bought more than a year later than the products produced in Russia but obtained in the other countries. This was also the case for the products obtained in Ukraine and outside Ukraine. Some of the difference may be due to a decrease in the use of I-TF during the time period.(figure 4)

I-TF has been used in foods such as biscuits/cakes/wafers also because it apparently prolongs the shelf life of the foods. This is presently not supported by the findings that 186 products produced in Russia with a shelf life of 6 months had TF concentrations of 11 (7)% mean value (SD) and 37 products with a shelf life of 12 months, also had TF concentrations of 11 (7)%. The corresponding values from products produced in Ukraine were for 86 products with shelf life of 6 months 20 (9) % and for 30 products with shelf life of 12 months 10 (6) %.

Based on the plasma concentrations of TF in nationally representative groups in 2010 in the USA (3 years after the introduction of mandatory labelling of TF on pre-packaged foods and in spite of a nearly 50 % lower average TF-concentration in plasma), there is still a subgroup with very high TF concentrations, reflecting a subgroup in the general population with a high consumption of TF.[4] A vicious cycle apparently occurs: as long as foods with high amounts of I-TF are present in the shops, some consumers will buy them, and as long as they are bought, the foods will be available. This may also be the case in the countries of the former Soviet Union. Despite a decline in the average intake of I-TF in many countries in Western Europe,[16] in 2015 the WHO estimated that millions of Europeans still consume TF at levels that significantly increase their risk for CHD.[23] The findings reported in the present investigation confirm this estimate.

Strength and weaknesses of the study

Strengths of this study include that the same procedure was used to obtain popular foods in large supermarkets in all 15 countries of the former Soviet Union, and in the 3 largest countries in two different regions and that the samples were analysed for TF in the same laboratory. A limitation of this study is that no other food groups (such

as shortenings and margarines) and no un-packaged foods (such as baked goods, sweet rolls, pastries, buns and even un-packaged biscuits/cakes/wafers) were investigated. It is also assumed that if the inclusion words are not present in the list of ingredients, the product contains less than 2% of the total fat as TF. The presence of high concentrations of I-TF in biscuits/cakes/wafers may be a sign that I-TF is used in other foods as well. Another limitation is that the average daily intake of I-TF was not measured in any subgroups of the populations, but instead was inferred from the availability of popular foods with high amounts of I-TF in large supermarkets. The competition for shelf space in supermarkets is usually fierce. If products do not sell, they are rapidly replaced by other products. A relevant subgroup for intake studies would be the consumers who buy pre-packaged biscuits/cakes/wafers.

Legislation and industrially produced *trans* fat in foods

In the USA in 2015, the FDA revoked the previous GRAS (Generally Recognized As Safe) status of partially hydrogenated oils - a step that may remove I-TF from food production in the USA before July 2018, and Canada is following suit.[24] In 2009, Austria and Switzerland introduced a legislative ban similar to the Danish ban from 2004, followed by Iceland in 2011, Hungary and Norway in 2014, and initiated by Latvia, Georgia, Lithuania, Slovenia and other Eastern European countries including Russia between 2016 and 2018. [13,25,26,27] The samples for the present study were all collected in the various capitals in 2015 or 2016 (supplementary table A) before any of the legislative restrictions of I-TF in food were fully implemented in these countries. However, some of their food producers were most likely aware of future legislation. They may have commenced a gradual removal of I-TF from some of their products already in 2015 and 2016. This may be the explanation for the relatively few products with TF in Lithuania and in Latvia. The explanation is apparently not valid for Estonia that only had 8 products with more than 2 % of the fat as TF and has no internationally published plan for a legislation like the two other Baltic countries. (figure 1 and table 1)

The European Union published a report in 2015, originally commissioned in 2011, that "indicates the need to continue and expedite work in this area by collecting more information".[28,29] In the WHO-Europe investigation of the various ways to reduce I-TF in foods, it was concluded that establishing a legal limit for the content of I-TF in

foods is potentially the only available option that reduces the risks associated with I-TF faced by all consumers and doing so may contribute to reducing inequalities. Such a policy is unique in its combination of efficacy, cost-effectiveness and low potential for negative impact. Removing I-TF from the food supply is possibly one of the most straightforward public health interventions for reducing risk of CHD.[23] The trend over time of using fat with lower amounts of I-TF (figure 4) will probably accelerate in 2018 among food factories in Russia due to the Russian legislation [27] and among factories in Ukraine due to competition with Russian factories. The intake of I-TF in the two large countries may decrease, but in addition also the intake of I-TF among millions of inhabitants in the adjacent countries due to the large export of biscuits/cakes/wafers from Russia and Ukraine to these countries.[18] (table 1)

Future research and implications for policy makers

It is of considerable interest whether coronary mortality and admission rates for CHD and stroke will change in countries that have recently introduced legislation that restricts the use of I-TF or intend to do so in the near future, in the same way as it was observed in Denmark and in certain counties in New York State.[7,8,9]

On the basis of data from 2009 to 2011, there is a fivefold difference in age-standardized mortality rates of CHD between some countries in Western Europe and countries in Central and Eastern Europe, with Central Asia having the highest rates.[10] Central Eastern Europe and Central Asia were the world regions with the highest current age-standardized cardiovascular mortality rates, which are more than twice those of Latin America and the Caribbean.[30] These differences cannot be explained only by differences between the countries in the intake of I-TF. The present study demonstrates a presence of I-TF in popular foods in the countries of the former Soviet Union with excessively high CHD mortality. Restriction of the use of I-TF in foods, either voluntarily by food producers or more efficiently by legislation, may be an easily implemented first strategy for some reduction of cardiovascular diseases in these countries.

REFERENCES

1. Gebauer SK, Destaillats F, Dionisi F, *et al*. Vaccenic acid and trans fatty acid isomers from partially hydrogenated oil both adversely affect LDL cholesterol: a double-blind, randomized controlled trial. *Am J Clin Nutr* 2015;102(6):1339-46.

2. de Souza RJ, Mente A, Maroleanu A, *et al.* Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. *BMJ* 2015;351: h3978. doi:101136/bmj.h3978.

3. Li H, Zhang Q, Song J, *et al.* Plasma trans-fatty acids levels and mortality: a cohort study based on 1999-2000 National Health and Nutrition Examination Survey (NHANES). *Lipids Health Dis* 2017; 16: 176.doi:10.1186/s12944-017-0567-6.

4. Yang Q, Zhang Z, Loustalot F, *et al.* Plasma *trans*-Fatty Acid Concentrations Continue to Be Associated with Serum Lipid and Lipoprotein Concentrations among US Adults after Reductions in *trans*-Fatty Acid Intake. *J Nutr* 2017;147**:**896–07.

5. Hirata Y, Takahashi M, Kudoh Y, *et al*. Trans-Fatty acids promote proinflammatory signaling and cell death by stimulating the apoptosis signal-regulating kinase 1 (ASK1)-p38 pathway. *J Biol Chem*. 2017; 292: 8174–85 doi: 10.1074/jbc.M116.771519.

6. Brouwer IA, Wanders AJ, Katan MB. *Trans* fatty acids and cardiovascular health: research completed? *Eur J Clin Nutr* 2013;67:541–47.

7. Restrepo BJ, Rieger M. Denmark's Policy on Artificial Trans Fat and Cardiovascular Disease. *Am J Prev Med* 2016;50:69–76.

8. Restrepo BJ, Rieger M. Trans fat and cardiovascular disease mortality: Evidence from bans in restaurants in New York. *J. Health Econ* 2016;45:176–196.

9. Brandt EJ, Myerson R, Perraillon MC, *et al.* Hospital Admissions for Myocardial Infarction and Stroke Before and After the Trans-Fatty Acid Restrictions in New York. *JAMA Cardiol* 2017;2:627–634.

10. Nichols M, Townsend N, Scarborough P, *et al*. Cardiovascular disease in Europe 2014: epidemiological update. *Eur Heart J* 2014;35:2950–59.

11. Micha R, Khatibzadeh S, Shi P, *et al*. Global, regional, and national consumption levels of dietary fats and oils in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys. *BMJ* 2014; 348:g2272 doi:10.1136/bmj.g2272.

12. Perova NV, Metel'skaia VA, Boytsov SA. [Trans isomers of unsaturated fatty acids increase the risk of atherosclerosis-related circulatory system diseases].[Article in Russian]. *Ter Arkh* 2013;85:113-7.

13. Boytsov SA, Perova NV. To lower trans fatty acid intake as an important condition for the reduction of cardiovascular Mortality (Article). *Profilakticheskaya Meditsina* 2017;20:4-9.

14. Meremäe K, Roasto M, Kuusik S, *et al*. Trans Fatty Acid Contents in Selected Dietary Fats in the Estonian Market. *J Food Sci.* 2012;77:163-8.

15. FEEDcities project. The food environment description in cities in Eastern Europe and Central Asia - Tajikistan and Kyrgyzstan. Technical report, WHO Europe, June 2017 and August 2017 http://www.euro.who.int/__data/assets/pdf_file/0012/353001/FEED-TJK-report.pdf?ua=1 http://www.euro.who.int/__data/assets/pdf_file/0006/351996/FEED-KGZ-report-web-new.pdf?ua=1 (Accessed Sep 2018).

16. Wanders AJ, Zock PL, Brouwer IA. Trans Fat Intake and Its Dietary Sources in General Populations Worldwide: A Systematic Review. *Nutrients.* 2017;9:840; doi:10.3390/nu9080840.

17. Stender S, Astrup A, Dyerberg J. A trans European Union difference in the decline in trans fatty acids in popular foods: a market basket investigation. *BMJ Open* 2012;17:2 e000859. doi: 10.1136/bmjopen-2012-000859.

18. https://en.wikipedia.org/wiki/Post-Soviet_states#General_statistics (Accessed Sep 2018).

19. Kravic SZ, Suturovic ZJ, Svarc-Gajic JV, *et al*. Fatty acid composition including *trans*-isomers of Serbian biscuits. *Hem Ind* 2011;65:139–46.

20. Stender S, Astrup A, Dyerberg J. Tracing artificial *trans* fat in popular foods in Europe: a market basket investigation. *BMJ Open* 2014;4: e005218.

21. Stender S, Astrup A, Dyerberg J. Artificial trans fat in popular foods in 2012 and in 2014: a market basket investigation in six European countries. *BMJ Open* 2016;6: e010673.

22. https://da.wikipedia.org/wiki/International_Article_Number_(EAN) (Accessed Sep 2018).

23. Eliminating trans fats in Europe – A policy brief (2015) http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/publications/2015/eliminating-trans-fats-in-europe-a-policy-brief-2015 (Accessed Mar 2018).

24. FDA Cuts Trans Fat in Processed Foods http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm372915.htm (Accessed Sep 2018)

25. Vučić V, Arsić A, Petrović S, *et al*. Trans fatty acid content in Serbian margarines: Urgent need for legislative changes and consumer information. *Food Chem* 2015;185:437–40.

26. Wilczek MM, Olszewski R, Krupienicz A. *Trans*-Fatty Acids and Cardiovascular Disease: Urgent Need for Legislation. *Cardiology*. 2017;138:254–58.

27. Russia. Solnechnye Produkty ready to meet new trans-fat content standards http://www.blackseagrain.net/novosti/russia-solnechnye-produkty-ready-to-meet-new-trans-fat-content-standards (Accessed Sep 2018)

28. Report from the Commission to the European parliament and the Council regarding trans fats in foods and in the overall diet of the Union population. (03-12-2015) http://ec.europa.eu/dgs/health_food-safety/dyna/enews/enews.cfm?al_id=1650 (Accessed Sep 2018).

29. https://ec.europa.eu/info/consultations/open-public-consultation-initiative-limit-industrial-trans-fats-intakes-eu_en (accessed Sep 2018)

http://ec.europa.eu/smart-regulation/roadmaps/docs/2016_sante_143_trans_fats_en.pdf (Accessed Sep 2018).

30 Barquera S, Pedroza-Tobías A, Medina C, *et al.* Global Overview of the Epidemiology of Atherosclerotic Cardiovascular Disease. *Arch Med Res* 2015;46:328–38

LEGENDS

Figure 1

Amounts of industrially produced *trans* fat (I-TF) in 100 grams of pre-packaged biscuits/cakes/wafers purchased in 2015-2016 in three supermarkets in each of the 15 capitals. Each bar in a panel represents a unique product bought in that capital, i.e., none of the products in a given panel had the same barcode number. N is the number of different products with more than 2 % TF of the total fat content. (N) is the number of packages that fulfilled the inclusion criteria. X is the mean value and the standard deviation of the percentages of I-TF in the total fat of the N products, M is the maximal value of I-TF as percent of total fat. Each bar in all of the 15 panels has the same thickness, which means that the area of the bars is comparable between the different panels.

Figure 2

The legend for this figure is similar to the legend for Figure 1. The 4 upper panels show the result for 2 of the capitals and for 2 other large cities in the same country. The lowest 2 panels give the results from the purchase in 3 large supermarkets in Kiev (Kiev-1) and in 3 other large supermarkets in the same city (Kiev-2).

Figure 3

Amounts of industrially produced *trans* fat (I-TF) in 100 grams of pre-packaged biscuits/cakes/wafers, obtained in Uzbekistan and in Kazakhstan respectively with each product in the panel placed together with other products from the same country of origin.

Figure 4

Date of production and I-TF as percent of total fat for pre-packaged biscuits/cakes/wafers, produced in Russia or Ukraine, respectively, and obtained in the countries of the former Soviet Union between January 2015 and January 2017. Products obtained in Samarkand, Sct Petersburg and Kiev 2 were included if the products had their own *trans* fat analysis. Only products with more than 2% of the fat as I-TF were included.

Table 1

Country of origin for pre-packaged biscuits/cakes/wafers with more than 2% of the fat as I-TF obtained in the 15 countries of the former Soviet Union.

Table 2

Shelf life of pre-packaged biscuits/cakes/wafers produced in Russia or in Ukraine and obtained in the countries of the former Soviet Union between January 2015 and January 2017.

Contributors: SS was responsible for the concept design of the study, for collection of food items, registration, and labelling, and writing the manuscript. SS is the manuscripts guarantor.

Funding: This work was supported by Jenny Vissings Foundation, University of Copenhagen and Department of Clinical Biochemistry, Copenhagen University Hospital, Gentofte, Denmark.

Role of the funding source: The sponsors of the study had no role in the study design, data collection, analysis, and interpretation, the writing of the report, or the decision to submit the article for publication. The corresponding author had full access to all the data for the study and had final responsibility for the decision to submit the manuscript for publication.

Competing interests: None declared

Ethics approval: The study does not require an approval from the Ethics committee

Acknowledgements: The authors acknowledge the support from Jenny Vissings Foundation, University of Copenhagen and the Department of Clinical Biochemistry, Copenhagen University Hospital, Gentofte, Denmark.

Transparency declaration: Steen Stender (the manuscript's guarantor) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Data sharing statement: The data used to construct figures 1-4, tables 1 and 2, and supplementary table A and B can be shared by e-mailing Steen Stender

Table 1 Country of origin for pre-packaged biscuits/cakes/wafers with more than 2% of the fat as *trans* fat obtained in the various countries.

Country	Capital	All*			Produced in Russia			Produced in Ukraine					Produced in own country					Produced in other countries				
		Trans fat per 100 gram fat ≥2%			Trans fat per 100 gram fat			Trans fat per 100 gram fat					Trans fat per 100 gram fat					Trans fat per 100 gram fat				
		N	N	%**	Mean	SD	Max	N	%**	Mean	SD	Max	N	%**	Mean	SD	Max	N	%**	Mean	SD	Max
Armenia	Yerevan	91	50	55	17.6	8.8	50.8	33	36	25.5	13.8	50.0	7	8	20.0	8.0	29.6	1	1	10.2		10.2
Belarus	Minsk	98	20	20	14.8	8.5	35.5	5	5	22.5	5.6	32.0	71	72	17.4	8.9	39.9	2	2	6.8	5.4	10.6
Tadjikistan	Dushanbee	136	121	89	10.9	7.0	33.4	10	7	15·0	7.7	32.9	4	3	9.1	1.7	10.3	1	1	2.7		2.7
Kirgistan	Bishkek	81	58	72	14.9	8.3	30.3	15	19	28.6	12.9	49.7	4	5	7.0	4.1	12.2	4	5	2.8	0.6	3.6
Azerbaijan	Baku	85	58	68	12.3	7.3	34.6	15	18	12.9	1.3	33.4	1	1	3.1		3.1	11	13	12.5	5.5	21.6
Moldova	Chisinau	53	10	19	24.6	7.8	35.2	25	47	25.8	8.0	41.5	12	23	10.1	5.9	22.2	6	11	12.0	11.3	32.1
Ukraine	Kiev-1	83	0	0				81	98	13.8	9.7	41.6						2	2	4.2	1.0	4.9
*Ukraine ****	*Kiev-2*	*64*	*0*	*0*				*62*	*97*	*14.3*	*11.0*	*45.4*						*2*	*3*	*12.1*	*0.5*	*12.4*
Uzbekistan	Taskent	77	50	65	10.3	7.5	35.2	20	26	16.2	9.6	33.4	6	8	11.2	4.6	16.2	1	1	6.7		6.7
*Uzbekistan ****	*Samarkand*	*63*	*30*	*48*	*13.5*	*7.7*	*29.0*	*28*	*44*	*18.7*	*11.8*	*42.7*	*5*	*8*	*4.1*	*2.5*	*8.4*	*0*	*0*			
Turkmenistan	Ashgabat	54	18	33	13.3	8.0	28.9	24	44	18.4	10.6	38.0	0	0				12	22	12.6	7.3	26.2
Georgia	Tblisi	47	23	49	15.7	9.7	36.1	8	17	21.3	9.3	34.6	7	15	23.3	9.9	33.3	9	19	7.5	4.8	16.4
Russia	Moscow	75	75	100	9.5	5.6	27.0	0	0									0	0			
*Russia ****	*Sct Petersburg*	*105*	*105*	*100*	*9.3*	*5.0*	*27.0*	*0*	*0*									*0*	*0*			
Kazakhstan	Astana	50	28	56	11.3	7.6	29.1	12	24	10.9	5.6	19.3	7	14	12.0	8.7	28.7	3	6	11.8	7.6	20.5
Lithuania	Vilnius	32	0	0				9	28	10.5	8.3	32.5	9	28	8.4	4.6	15.7	14	44	6.7	3.8	14.0
Latvia	Riga	24	5	21	9.0	3.8	12.2	4	17	8.9	3.8	14.5	5	21	5.5	4.1	11.3	10	42	6.7	3.2	11.0
Estonia	Tallinn	8	1	13	29.2		29.2	2	25	19.3	17.0	31.3	0	0				5	63	8.0	3.6	11.3
15 countries together		994	517					263					133					81				

All* is the number in each country of different packages of biscuits/cakes/wafers with ≥ 2% trans fat per 100 gram fat

%** is the number of samples with more than 2% of the fat as trans fat as percent of all samples obtained in that country with more than 2% of the fat as trans fat

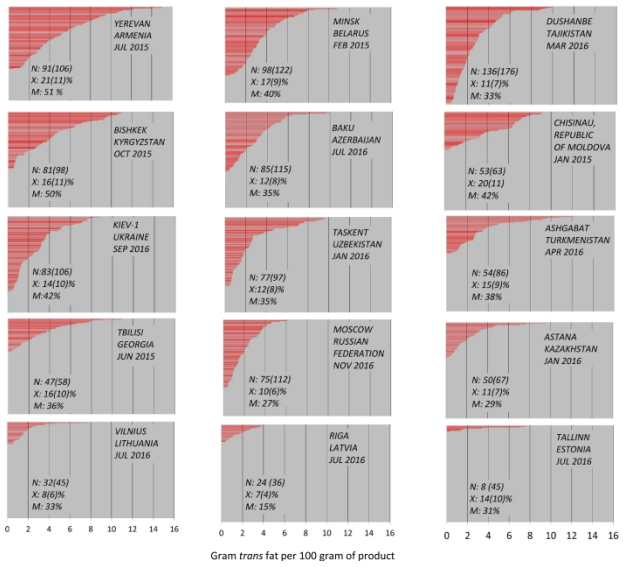
*** the lines in italic present values for biscuits/cakes/wafers obtained by the same procedure but in ekstra regions of the same country. The values are not included in the bottom line

Table 2 Shelf life of pre-packaged biscuits/cakes/wafers produced in Russia and Ukraine

	Produced in Russia		Produced in Ukraine	
Trans fat as % of fat	< 2%	≥ 2%	<2%	≥ 2%
Number of samples*	140	453	24	220
Shelf life in days				
Mean (SD)	202(83)	219(70)	286(94)	215(85)
Range	59-547	46-396	123-366	46-546

* Samples obtained in Samarkand, Sct Petersburg, and Kiev-2 were included if these samples had their own trans fat analysis and a readable shelf life on the package

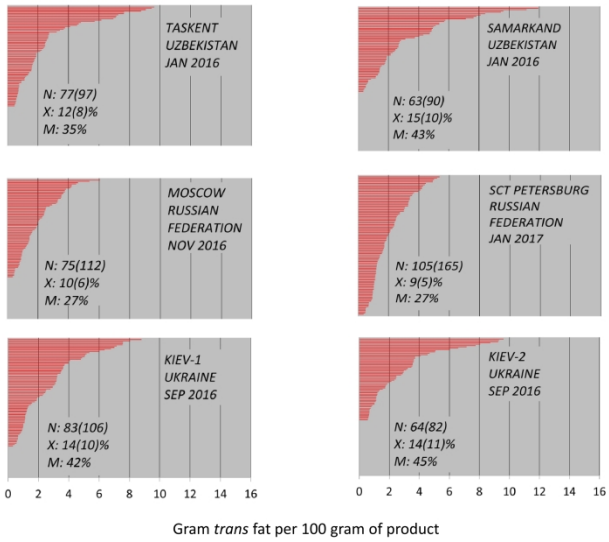
Figure 1



Amounts of industrially produced trans fat (I-TF) in 100 grams of pre-packaged biscuits/cakes/wafers purchased in 2015-2016 in three supermarkets in each of the 15 capitals. Each bar in a panel represents a unique product bought in that capital, i.e., none of the products in a given panel had the same barcode number. N is the number of different products with more than 2 % TF of the total fat content. (N) is the number of packages that fulfilled the inclusion criteria. X is the mean value and the standard deviation of the percentages of I-TF in the total fat of the N products, M is the maximal value of I-TF as percent of total fat. Each bar in all of the 15 panels has the same thickness, which means that the area of the bars is comparable between the different panels.

338x190mm (300 x 300 DPI)

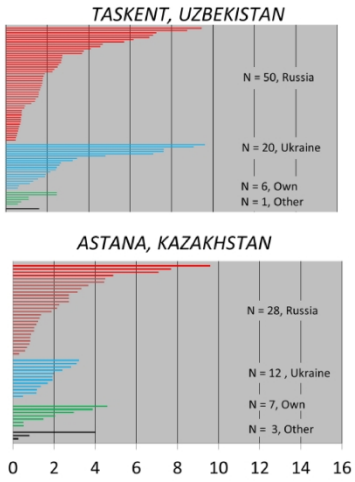
Figure 2



The legend for this figure is similar to the legend for Figure 1. The 4 upper panels show the result for 2 of the capitals and for 2 other large cities in the same country. The lowest 2 panels give the results from the purchase in 3 large supermarkets in Kiev (Kiev-1) and in 3 other large supermarkets in the same city (Kiev-2).

338x190mm (300 x 300 DPI)

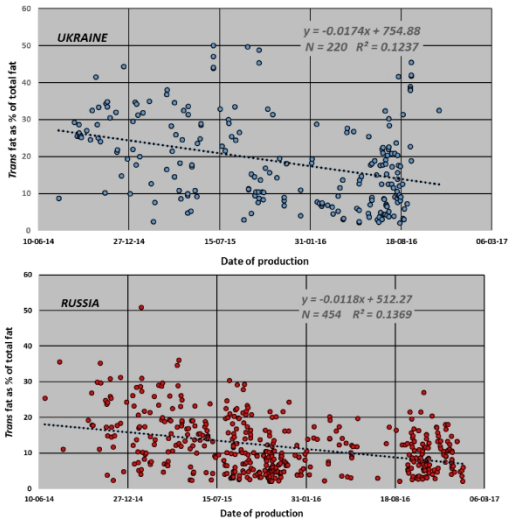
Figure 3



Amounts of industrially produced trans fat (I-TF) in 100 grams of pre-packaged biscuits/cakes/wafers, obtained in Uzbekistan and in Kazakhstan respectively with each product in the panel placed together with other products from the same country of origin.

338x190mm (300 x 300 DPI)

Figure 4



Date of production and I-TF as percent of total fat for pre-packaged biscuits/cakes/wafers, produced in Russia or Ukraine, respectively, and obtained in the countries of the former Soviet Union between January 2015 and January 2017. Products obtained in Samarkand, Sct Petersburg and Kiev 2 were included if the products had their own trans fat analysis. Only products with more than 2% of the fat as I-TF were included.

338x190mm (300 x 300 DPI)

Supplementary table A

Names and population of the countries of the former Soviet Union and the mortalities of coronary heart disease(CHD) and cardiovascular disease (CVD)

Country	Capital	Visit Month	Visit Year	Capital population millions*	Country population millions*	Year	CHD-mortality male per 100.000	CVD-mortality male per 100.000	Year	CHD-mortality female per 100.000	CVD-mortality female per 100.000
Republic of Moldova	Chisinau	Jan	2015	0.669	3.6	2014	528	790	2012	365	564
Belarus	Minsk	Feb	2015	1.893	9.5	2016	642	893	2009	284	428
Georgia	Tbilisi	Jun	2015	1.173	3.7	2016	80	325	2010	39	190
Armenia	Yerevan	Jul	2015	1.06	3.0	2016	352	525	2012	211	357
Kyrgyzstan	Bishkek	Oct	2015	0.871	5.5	2009	534	842	2010	373	588
Kazakhstan	Astana	Jan	2016	0.639	17.7	2016	265	810	2010	124	485
Uzbekistan	Taskent	Jan	2016	2.31	31.6	2016	454	858	2005	321	662
Tajikistan	Dushanbe	Mar	2016	0.779	8.6	2015	304	710	2004	186	504
Turkmenistan	Ashgabat	Apr	2016	1.031	6.7	2011	563	1017	1998	333	717
Estonia	Tallinn	Feb	2016	0.444	1.3	2016	256	501	2012	118	269
Latvia	Riga	Jul	2016	0.43	2.0	2014	355	655	2012	174	353
Lithuania	Vilnius	Jul	2016	0.537	2.9	2016	436	667	2010	239	383
Azerbaijan	Baku	jul	2016	2.182	9.8	2016	149	617	2007	93	489
Ukraine	Kiev	Sep	2016	2.847	42.5	2013	600	873	2012	356	533
Russian Federation	Moscow	Nov	2015	13.197	146.7	2016	501	915	2010	255	517
UK							111	205	2010	49	129
Denmark							67	174	2011	33	111

* Values from Wikipedia.[18] Mortalities from [10]

Supplementary table B:

Number of samples obtained from the various countries and the number and percentages of samples that were analysed for *trans* fat (TF)
Samples that were not analysed received their TF values from similar products that already have been analysed.

Country	Capital	All *samples			Samples with *trans* fat per 100 gram fat < 2%			Samples with *trans* fat per 100 gram fat≥2%		
			Analysed for TF			Analysed for TF			Analysed for TF	
		N-all	N	% of N-all	N<2%	N	% of N<2%	N≥2%	N	% of N≥2%
Armenia	Yerevan	106	106	100	15	15	100	91	91	100
Belarus	Minsk	122	122	100	24	24	100	98	98	100
Tadjikistan	Dushanbee	176	121	69	40	32	80	136	89	65
Kirgistan	Bishkek	98	98	100	17	17	100	81	81	100
Azerbaijan	Baku	115	58	50	30	19	63	85	39	46
Moldova	Chisinau	63	63	100	10	10	100	53	53	100
Ukraine	Kiev-1	106	84	79	23	19	83	83	65	78
*Ukraine **	*Kiev-2*	*82*	*40*	*49*	*18*	*7*	39	*64*	*33*	52
Uzbekistan	Taskent	97	63	65	20	12	60	77	51	66
*Uzbekistan **	*Samarkand*	*90*	*31*	*34*	*27*	*10*	37	*63*	*21*	33
Turkmenistan	Ashgabat	86	56	65	32	28	88	54	28	52
Georgia	Tblisi	58	58	100	11	11	100	47	47	100
Russia	Moscow	112	99	88	37	31	84	75	68	91
*Russia **	*Sct Petersburg*	*165*	*69*	*42*	*60*	*20*	33	*105*	*49*	47
Kazakhstan	Astana	67	44	66	17	15	88	50	29	58
Lithuania	Vilnius	45	34	76	13	11	85	32	23	72
Latvia	Riga	36	27	75	12	12	100	24	15	63
Estonia	Tallinn	45	35	78	37	28	76	8	7	88
15 countries and 3 extra regions		1669	1208	72	440	321	73	1226	887	72
15 countries without 3 extra regions		1332	1068	80	335	284	85	994	784	79

All* is the number in each country of different packages of biscuits/cakes/wafers that fulfilled the inclusion criteria
** the lines in italic present values for biscuits/cakes/wafers obtained by the same procedure but in extra regions in the same country

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

Manuscript ID bmjopen-2018-023184 entitled "Industrially produced trans fat in popular foods in 2015-2016 in 15 countries of the former Soviet Union: a market basket investigation
12-9-2018

	Item No	Recommendation
l Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **line 5**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **Lines 26-37**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **Lines 68-79**
Objectives	3	State specific objectives, including any prespecified hypotheses **Lines 102-103**
Methods		
Study design	4	Present key elements of study design early in the paper **Lines 107-120**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **Supplementary table A**
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants **Lines 107-120**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **Lines 145-166**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **Lines 145-151**
Bias	9	Describe any efforts to address potential sources of bias **Lines 157-166**
Study size	10	Explain how the study size was arrived at **Lines 22-25**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **Line 31**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **Lines 172-174**
		(*b*) Describe any methods used to examine subgroups and interactions **NA**
		(*c*) Explain how missing data were addressed **Lines 138-144, Supplementary table B**
		(*d*) If applicable, describe analytical methods taking account of sampling strategy **Lines 145-151**
		(*e*) Describe any sensitivity analyses **Lines 130-135**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **Table 1**
		(b) Give reasons for non-participation at each stage **NA**
		(c) Consider use of a flow diagram **NA**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **Lines 107-120**
		(b) Indicate number of participants with missing data for each variable of interest

Supplementary table B

Outcome data	15*	Report numbers of outcome events or summary measures **Figure 1 and Table1**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included **NA**
		(*b*) Report category boundaries when continuous variables were categorized **Line 31**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **NA**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **Lines 212-221**
Discussion		
Key results	18	Summarise key results with reference to study objectives **Lines 231-238**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias **Lines 271-282**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **Lines 320-322**
Generalisability	21	Discuss the generalisability (external validity) of the study results **Lines 260-268**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **Lines 434-435**

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Industrially produced trans fat in popular foods in 2015-2016 in 15 countries of the former Soviet Union: a market basket investigation

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023184.R2
Article Type:	Research
Date Submitted by the Author:	21-Nov-2018
Complete List of Authors:	Stender, Steen; Kobenhavns Universitet Det Natur- og Biovidenskabelige Fakultet, Nutrition Excercise and Sports
Primary Subject Heading:	Health policy
Secondary Subject Heading:	Health policy, Global health, Public health
Keywords:	Coronary heart disease < CARDIOLOGY, NUTRITION & DIETETICS, Health policy < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, central asia trans fat

1

19-11-2018

Industrially produced *trans* fat in popular foods in 2015-2016 in 15 countries of the former Soviet Union: a market basket investigation

Steen Stender, Professor, MD, DMSci

Steen Stender, Department of Nutrition, Exercise and Sports, University of Copenhagen, Rolighedsvej 26, Frederiksberg, 1958 DK, Denmark

Correspondence to: S Stender stender@nexs.ku.dk

Telephone number: +45 35333162

Telephone number: +45 40258601

Key Words: Coronary heart disease, Health policy, Nutrition, Soviet Union, *Trans* fat

ABSTRACT

Objective: To minimize the intake of industrially produced *trans* fat (I-TF) and thereby decrease the risk of coronary heart disease (CHD), several countries have implemented a legislative restriction on I-TF in foods. The objective of this study was to investigate the presence of I-TF in biscuits/cakes/wafers in 15 countries of the former Soviet Union, all with a high coronary mortality compared with countries in Western Europe.

Design: Three large supermarkets were visited in the 15 capitals in 2015 or 2016. Pre-packaged biscuits/cakes/wafers were bought if the list of ingredients disclosed that the product contained more than 15 grams fat per 100 grams of product and if partially hydrogenated fat or a similar term, including margarine, refined fat or confectionary fat, were mentioned. Samples of the foods were subsequently analysed for total fat and TF.

Results: Some 994 products had more than 2% of total fat as I-TF (illegal in Denmark). In Armenia, 91 different products had a mean value (SD) of 21(11) % of the fat as I-TF. In Estonia, there were 8 products with 14(10) % of the fat as I-TF. The other 13 countries had values in between. In several countries a major part of the products were imported from Russia and Ukraine. Many of these products had a readable production date and a best before date. The mean shelf life (SD) of 673 packages was 218 (75) days. The % TF in the fat of products produced in Russia and in Ukraine in relation to the date of production both declined with approximately 10 % points during the 2 years collection period.

Conclusions: The findings suggest that I-TF is used in popular foods in all 15 countries of the former Soviet Union. It opens a possibility for some reduction of the high coronary mortality in these countries.

Strengths and limitation of this study

- A strength of this study is that the presence of industrially produced *trans* fat in popular foods for the first time is systematically investigated in each of the 15 countries of the former Soviet Union that all have a high coronary mortality and a population of nearly 300 million.
- A limitation of the study is that only pre-packaged biscuits/cakes/wafers were investigated for *trans* fat and no other food groups such as shortenings and margarines and no un-packaged foods such as baked goods, sweet rolls, pastries, buns and even un-packaged biscuits/cakes/wafers were investigated.
- A limitation is the assumption that if the inclusion words are not present in the list of ingredients, the product contains less than 2% of the total fat as *trans* fat.
- Another limitation is that the average daily intake of *trans* fat was not measured in any subgroups of the populations, but instead was inferred from the availability of popular foods with high amounts of *trans* fat in large supermarkets.

.

INTRODUCTION

High amounts of *trans* fat (TF) in food i.e. more than 5 % TF of total fat in the food originate from the industrial hydrogenation of edible oils. Compared to non-hydrogenated oils, fats containing industrially produced *trans* fat (I-TF) are solid at room temperature, have some technical advantages for food processing, and prolong the shelf life of products. TF are not synthesized in the human body and are not required in the diet, but is absorbed and metabolized. I-TF can be found at varying amounts in biscuits, pastries, margarine, spreads, confectionary creams and fillings and fried foods such as potato crisps. Fat from ruminants, for instance in dairy products, contains up to 5 % TF (R-TF), which is considerably lower than the up to 50% TF that may be present in partially hydrogenated fat for human food. In a double blind, randomised controlled trial in humans R-TF resulted in the same increase in LDL-cholesterol in blood compared with I-TF when the two different types of fat were given in the diet in same amounts.[1] It suggests that R-TF and I-TF have similar harmful effect on plasma lipoproteins**.**

Observational studies suggest an association between dietary intake of I-TF and increased risk of coronary heart disease (CHD).[2] The association is further corroborated by an association between plasma TF level and CHD.[3]

Plausible mechanisms for the association involving an increase in plasma LDL-cholesterol[4] and unfavourable "beyond lipid effects" on inflammatory cells important for atherogenesis[5] suggest a causal relationship, even in the absence of randomized studies with hard clinical endpoints.[6] Based on the available evidence, Denmark in 2004 and some counties in New York State, USA in 2007, legally restricted the use of I-TF in the diet. After the restrictions in Denmark there was a 4.3% lower yearly mortality rate due to CHD beyond temporal trends.[7] A similar decline of 4.5 % in CHD mortality rate was found in the counties with restriction in New York State compared with similar counties without restrictions.[8] Three and more years after the restriction in the counties, a hospital admission rate for myocardial infarction and stroke events combined was 6.2 % lower than in similar counties without restrictions.[9] These findings from 2 different groups of researchers, strongly suggest that I-TF in the diet promotes CHD and a restriction in its use reduces CHD. What remains to be explored is to what extent I-TF still is used in popular foods in countries with high rates of CHD-mortalities.

The 15 countries of the former Soviet Union all have an extraordinarily high rate of CHD mortality compared with countries in Western Europe.[10] (supplementary table A) In a systematic analysis, including 266 country-specific nutrition surveys, the average consumption of TF in 2010 in Central Asia and Eastern Europe that together include the 15 countries of the former Soviet Union, for adults of more than 20 years, were 0.9% of total energy intake.[11] This intake corresponds to approximately 2.5 gram TF per day per adult and is less than the 1% of total energy percent recommended by WHO. The consumption level was found stable since 1990. Among the world's 21 different regions Central Asia and Eastern Europe were placed as number 5 and 8, respectively with the lowest average consumption of TF, suggesting that intake of TF is a minor health problem in these countries. However, the investigators mention the relatively limited data availability on TF consumption in most of these nations compared with other major dietary factors.[11] Furthermore a low average intake of TF in a nation does not exclude a high intake of TF in subgroups of the population if popular foods contain high amounts of I-TF.

Some recent Russian studies, with an abstract in English, mention the harmful effect on health of dietary I-TF and pledge for a removal of I-TF from margarine "to preserve the health of Russia's population".[12,13] A study from Estonia reports about high I-TF in blended spreads, margarines and shortenings bought in the Estonian retail market in 2011.[14] Additionally, technical reports from the WHO find several types of street foods with high amounts of TF in Dushanbe, Tajikistan, April/May 2016, and in Bishkek, Kyrgyzstan, June/July 2016.[15] In a

recent systematic review of TF intake and its dietary sources in general populations worldwide based on data from 29 different countries and a demonstration of a substantial reduction in I-TF in many countries, none of the countries of the former Soviet Union were included, either for the intake of the general population or for the study's secondary research question about the composition of fat in biscuits, because of the lack of data.[16] Apparently, there is a pronounced scarcity of newer internationally published data about I-TF in foods in most of these countries. The purpose of the present study was to investigate the presence of I-TF in popular foods in the 15 countries of the former Soviet Union by a newly developed market basket method.[17]

METHODS

Purchase of biscuits/cakes/wafers in supermarkets

By using Wikipedia and local tourist information offices, three large supermarkets were identified in each capital, preferably chain supermarkets with many large shops across the country.[18] Pre-packaged biscuits/cakes/wafers were chosen as the food to be investigated for TF in this study because these types of foods are frequently consumed, easily accessible, and transportable at ambient temperature. Furthermore, these foods traditionally contain I-TF-rich, partially hydrogenated vegetable oils as their major lipid ingredient, and I-TF has previously been found in these foods in high concentrations in some countries in Eastern Europe.[19–21] The packages of biscuits/cakes/wafers were obtained in each supermarket by systematically examining the labels of the products. Packages were purchased if they met the following criteria on the list of ingredients:

- Total fat content was equal to or exceeded 15 g per 100 g of product.
- Listed one or more of the terms: partially hydrogenated fat, hydrogenated fat, hardened fat, refined fat, confectionary fat, deodorized fat, all-purpose fat or margarine.
- If the list of ingredients indicated *trans* fat > 0 g, these foods were also included.
- Packages with the term 'unhydrogenated fat' or 'fully hydrogenated fat' or 'no *trans* fat' were not purchased.

If the same package with the same barcode number was found in more than one of the three different supermarkets in the same capital, only the package with the most recent production date was included in the study. Each package was subsequently labelled with an ID-number, and duplicate samples each of approximately 50 grams of the product were taken for analysis. The barcode number, the name of the producer, the country of origin and, if available, the best before date as well as the production date were registered, and the empty packages were stored. The first 2 digits (sometimes three digits) in the barcode number identify the country or economic region numbering authority which assigned the manufacturer code. The Russian Federation uses the code 46, Ukraine the code 482. The manufacturer code is the next 5 digits followed by the product code, consisting of 5 digits. The last digit is the check digit.[22]

To investigate to what extent the presence of biscuits/cakes/wafers in the capital differed from other parts of the country, biscuits/cakes/wafers were obtained in 3 different supermarkets in each of 2 different regions of the 2 large countries Uzbekistan and Russia: in Uzbekistan also in the second largest city Samarkand (0.4 million inhabitants and approximately 300 km from Taskent) and in Russia also in Sct. Petersburg (5.2 million inhabitants and approximately 800 km from Moscow). The third large country was Ukraine. In its capital Kiev, 3 other large supermarkets were visited (Kiev-2) in addition to the first 3 that were visited (Kiev-1).

Analysis of TF

The samples from the various countries were analysed successively and in the order they were obtained. Samples obtained in the first countries that were visited (Moldova, Belarus, Georgia, Armenia and Kyrgyzstan) were all analysed for TF. In the other countries that were visited later some packages were obtained with the same barcode number, and the same visual appearance and list of ingredients as on packages where the samples already were analysed. A sample from the newly found package was analysed only if the best before date on that package exceeded the best before date by more than 12 months on a package that already had been analysed. Otherwise, the TF values were carried forward to the new package.(supplementary table B)

For analysis the foods were homogenized, and the fatty acid content was analysed using gas chromatography on a 66-m highly polar capillary column, using a modification of the AOAC 996.06

method. Two procedures were modified in minor ways. The final step of the extraction/methylation calls for 1 mL of hexane to be added in accordance with AOAC.996.06. In the modified method, 4mL were added. This is done to alleviate the dilution that needs done at the instrument. The levels of the instrument are low and sample results are over range. This procedure cuts down on any possible error by only having one dilution.The method also calls for two forms of C11:0 undecanoic acid to be used as surrogate and internal standard. In the modified method C11:0 triundecanoin was used as an internal standard as per method and calculate it throughout the analysis alleviating the need for another form to be used as surrogate.

. The method makes it possible to get total fat as triglycerides, which are then broken down into mono-, poly-, saturated- and total TF. Total TF may consist of up to 8 different *trans* fatty acids. All analytical work on samples was conducted by Microbac Laboratories in Warrendale, Pennsylvania, USA, The laboratory undergo annual proficiency testing and are audited every other year to ensure compliance of this method to ISO 17025.

From the peaks in the chromatogram TF is calculated as a fraction (percent) of total fat in the product. This value is primarily of interest for food producers, food chemists and food legislators. The total amount of fat in 100 grams of the product is determined from the chromatogram. When that value is multiplied by the TF-fraction the amounts of TF in gram per 100 grams of the product appear. This value is primarily of interest for the customers and for investigators of the health effect of intake of TF.

The measurement does not distinguish I-TF from R-TF. If butter as a ruminant fat has been used in the product in addition to partially hydrogenated vegetable oil, some of the TF in the product may be derived from butter that on average contains a few percent and maximum 5 % of the fat as R-TF. In this paper, the term I-TF is used even though a minor portion in certain products may be TF derived from ruminant fat. If butter is present in a product which have been analysed for TF in the present study and the value is higher than 5% TF of total fat, butter has diluted the I-TF in the product, because butter adds more non-TF than it adds TF. Values larger than 5% TF in the product are consequently minimum values for I-TF as percent of total fat. In products with less than 5% TF as

percent of total fat that contain butter, the I-TF will be lower than the TF value. Such products are because of their low amounts of TF per 100 gram of product of less importance for the health of the consumer than products with much higher TF values and of less interest for health authorities and food scientists.

Patient and Public involvement

Patients and public were not involved in the present study**.**

Statistical analysis

For each country, simple linear regression models were used to describe the change in % TF of total fat as a function of shelf life and over time recorded as date of production. The statistical software R was used (R Core Team, 2017).

RESULTS

The number of different packages obtained in 3 different supermarkets in each of the 15 capitals totalled 1332. Samples from 1068 packages were analysed, and TF values for each of the remaining 264 packages were taken from a similar package with an identical barcode but obtained earlier in another country and already analysed. Some 994 samples had more than 2% of the fat as TF. The different products in each country were ranked according to I-TF level, expressed as grams per 100 g of the product (figure 1). This latter value tells how much TF the consumer gets when 100 grams of the product is consumed. Each horizontal bar in each panel represents a product with more than 2% of the fat as TF. The number of these products is shown together with the number of packages, given in parenthesis that fulfilled the inclusion criteria. The products depicted in each panel are all different, but the same product may appear in the panels for two or more countries. The mean values of the percentages of fat that were I-TF in the collection of different products from each of the countries, together with the SD and maximum values are shown. These percentages are of interest for food producers and for food

authorities because they describe the fat that has been used in the food. In some products obtained in Armenia and in Kyrgyzstan about 50 % of the fat was I-TF. (figure 1)

In the second largest city, Samarkand, in Uzbekistan, 29% of the packages were similar based on the barcode number to the packages found in the capital, Taskent. In Sct Petersburg, Russia, 30% were similar to the products found in Moscow, and in Kiev-2, the second region of collection, 48% were similar to the packages found in the first region of collection, Kiev-1. Even though most products were different in the 2 different regions of the same country, the panels that reflect the availability of biscuits/cakes/wafers with more than 2 % TF in the fat are rather similar (figure 2). This suggests that the panels presented for each country in figure 1 represent a pattern found elsewhere in the country and not only in the capital.

For the 994 products with more than 2% of the fat as I-TF and obtained in the 15 capitals of the former Soviet Union, 52% were produced in Russia, 26% in Ukraine, 13% in own country, and 8 % in some other countries for instance Poland and Bulgaria (table 1). For the products produced in Russia, 85% were obtained outside Russia, in the other countries of the former Soviet Union combined, not including Ukraine and Lithuania. For the products produced in Ukraine, 69% were obtained outside Ukraine in the other countries, excluding Russia. The import patterns of pre-packaged biscuits/cakes/wafers differed from country to country exemplified by the patterns observed for Uzbekistan and Kazakhstan. (figure 3) The concentrations in grams of TF per 100 grams of product are in a panel for each of the two countries, depicted with each product placed together with other products from the same country of origin. The numbers and the mean values of the concentrations of TF in the fat of pre-packaged biscuits/cakes/wafers produced in Russia and in Ukraine but obtained in the various countries of the former Soviet Union are shown in table 1. All of the 75 different products with more than 2% of the fat as TF obtained in Moscow were produced in Russia, and 81 of the 83 different products obtained in Kiev-1 were produced in Ukraine. The same pattern was seen in Sct Petersburg, Russia and in Kiev-2, Ukraine, respectively. In each of the countries, except for the 3 Baltic countries and Belarus, a majority of the products were imported from Russia and Ukraine.

The analysis of shelf life was only conducted for packages produced in Russia and Ukraine, as they were the main exporters to most of the other countries, and in many of the products, especially from Russia, the length of the shelf life in months or days appeared on the package together with the date of production. This is in contrast to

pre-packaged biscuits/cakes/wafers produced in most countries in the more western part of Europe. These packages only have the best before date and the shelf life of the product is usually hidden for the final customer. Products with less than 2% TF in the fat had shelf lives of approximately 200 days, comparable to products with a much higher concentration of TF (table 2). The most frequent length of shelf life was 6 months, whether the products were produced in Russia or in Ukraine. The slope of the regression line between shelf life and % TF of total fat was significantly negative for Ukraine ($p<0.0001$) and significantly positive for Russia ($p<0.0001$). Taken together, the data did not support the conclusion that the higher the TF in the product, the longer the shelf life.

Since the samples of biscuits/cakes/wafers were collected between January 2015 and January 2017, and many were produced in Russia and in Ukraine, the % TF of total fat in the product was depicted in relation to the date of production. The TF concentrations decrease significantly during the 2 years in Russia ($p<0.0001$) as well as in Ukraine ($p<0.0001$), but the decrease did not differ significantly between the two countries ($p=0.12$) (figure 4). The products from Ukraine had a higher TF % in the start of the period than the products from Russia. Based on the slopes of the regression lines in figure 4, the decline for products from Ukraine was 12.4 % points and for products from Russia 8.4 % points during the 2 years period.

DISCUSSION

The principal findings

The findings of this study clearly demonstrate that in 2015-2016 I-TF was present in high concentrations i.e. more than 5 % I-TF in the total fat in many different brands of biscuits/cakes/wafers in the countries of the former Soviet Union, except for the 3 Baltic countries. This information was not previously available and may be of relevance because of the high coronary mortality in these countries. In 3 of the countries- Russia, Ukraine and Belarus, by far most of the products are produced in the country itself. For the other countries, except for the 3 Baltic countries, imports from food producers in Russia and Ukraine make up a major part of their products with high amounts of TF.

In all of the shops there were more different packages containing biscuits/cakes/wafers that did not have the inclusion terms on the label than packages that had one or more of the terms on the label. This suggests that there

are plenty of biscuits/cakes/wafers without I-TF available in all of the 45 supermarkets. Of the 1332 packages that fulfilled the inclusion criteria and were bought, 335 (25%) of the products had less than 2% of the fat as TF. This suggests that the inclusion criteria are rather broad in relation to the presence of TF and/or that the terms on the list of ingredients do not always reflect the composition of the fat in the product. If the list of ingredients does not have the inclusion words the product may still have high amounts of TF and are not bought and analysed for TF.

The amounts of TF per 100 grams of product bought in Moscow did not exceed 6 grams (figure 1), whereas several products produced in Russia, but obtained in the other countries had more than 6 grams per 100 grams, for instance in Uzbekistan and Kazakhstan (figure 3). The same is seen for the average concentration of I-TF in products obtained in Russia, with mean value and SD of 10(6) %, compared with considerable higher values in Russian products obtained in the other countries (table 1). The same pattern is seen for products bought in Ukraine, compared with products produced in Ukraine but obtained in some of the other countries (table 1). The products obtained in Russia were bought more than a year later than the products produced in Russia but obtained in the other countries. This was also the case for the products obtained in Ukraine and outside Ukraine. Some of the difference may be due to a decrease in the use of I-TF during the time period.(figure 4)

I-TF has been used in foods such as biscuits/cakes/wafers also because it apparently prolongs the shelf life of the foods. This is presently not supported by the findings that 186 products produced in Russia with a shelf life of 6 months had TF concentrations of 11 (7)% mean value (SD) and 37 products with a shelf life of 12 months, also had TF concentrations of 11 (7)%. The corresponding values from products produced in Ukraine were for 86 products with shelf life of 6 months 20 (9) % and for 30 products with shelf life of 12 months 10 (6) %.

Based on the plasma concentrations of TF in nationally representative groups in 2010 in the USA (3 years after the introduction of mandatory labelling of TF on pre-packaged foods and in spite of a nearly 50 % lower average TF-concentration in plasma), there is still a subgroup with very high TF concentrations, reflecting a subgroup in the general population with a high consumption of TF.[4] A vicious cycle apparently occurs: as long as foods with high amounts of I-TF are present in the shops, some consumers will buy them, and as long as they are bought, the foods will be available. This may also be the case in the countries of the former Soviet Union. Despite a decline in the average intake of I-TF in many countries in Western Europe,[16] in 2015 the WHO estimated that millions of

Europeans still consume TF at levels that significantly increase their risk for CHD.[23] The findings reported in the present investigation confirm this estimate.

Strength and weaknesses of the study

Strengths of this study include that the same procedure was used to obtain popular foods in large supermarkets in all 15 countries of the former Soviet Union, and in the 3 largest countries in two different regions and that the samples were analysed for TF in the same laboratory. A limitation of this study is that no other food groups (such as shortenings and margarines) and no un-packaged foods (such as baked goods, sweet rolls, pastries, buns and even un-packaged biscuits/cakes/wafers) were investigated. It is also assumed that if the inclusion words are not present in the list of ingredients, the product contains less than 2% of the total fat as TF. The presence of high concentrations of I-TF in biscuits/cakes/wafers may be a sign that I-TF is used in other foods as well. Another limitation is that the average daily intake of I-TF was not measured in any subgroups of the populations, but instead was inferred from the availability of popular foods with high amounts of I-TF in large supermarkets. The competition for shelf space in supermarkets is usually fierce. If products do not sell, they are rapidly replaced by other products. A relevant subgroup for intake studies would be the consumers who buy pre-packaged biscuits/cakes/wafers.

Legislation and industrially produced *trans* fat in foods

In the USA in 2015, the FDA revoked the previous GRAS (Generally Recognized As Safe) status of partially hydrogenated oils - a step that may remove I-TF from food production in the USA before July 2018, and Canada is following suit.[24] In 2009, Austria and Switzerland introduced a legislative ban similar to the Danish ban from 2004, followed by Iceland in 2011, Hungary and Norway in 2014, and initiated by Latvia, Georgia, Lithuania, Slovenia and other Eastern European countries including Russia between 2016 and 2018. [13,25,26,27] The samples for the present study were all collected in the various capitals in 2015 or 2016 (supplementary table A) before any of the legislative restrictions of I-TF in food were fully implemented in these countries. However, some of their

food producers were most likely aware of future legislation. They may have commenced a gradual removal of I-TF from some of their products already in 2015 and 2016. This may be the explanation for the relatively few products with TF in Lithuania and in Latvia. The explanation is apparently not valid for Estonia that only had 8 products with more than 2 % of the fat as TF and has no internationally published plan for a legislation like the two other Baltic countries. (figure 1 and table 1)

The European Union published a report in 2015, originally commissioned in 2011, that "indicates the need to continue and expedite work in this area by collecting more information".[28,29] In the WHO-Europe investigation of the various ways to reduce I-TF in foods, it was concluded that establishing a legal limit for the content of I-TF in foods is potentially the only available option that reduces the risks associated with I-TF faced by all consumers and doing so may contribute to reducing inequalities. Such a policy is unique in its combination of efficacy, cost-effectiveness and low potential for negative impact. Removing I-TF from the food supply is possibly one of the most straightforward public health interventions for reducing risk of CHD.[23] The trend over time of using fat with lower amounts of I-TF (figure 4) will probably accelerate in 2018 among food factories in Russia due to the Russian legislation [27] and among factories in Ukraine due to competition with Russian factories. The intake of I-TF in the two large countries may decrease, but in addition also the intake of I-TF among millions of inhabitants in the adjacent countries due to the large export of biscuits/cakes/wafers from Russia and Ukraine to these countries.[18] (table 1)

Future research and implications for policy makers

It is of considerable interest whether coronary mortality and admission rates for CHD and stroke will change in countries that have recently introduced legislation that restricts the use of I-TF or intend to do so in the near future, in the same way as it was observed in Denmark and in certain counties in New York State.[7,8,9]

On the basis of data from 2009 to 2011, there is a fivefold difference in age-standardized mortality rates of CHD between some countries in Western Europe and countries in Central and Eastern Europe, with Central Asia having the highest rates.[10] Central Eastern Europe and Central Asia were the world regions with the highest current age-standardized cardiovascular mortality rates, which are more than twice those of Latin America and the

Caribbean.[30] These differences cannot be explained only by differences between the countries in the intake of I-TF. The present study demonstrates a presence of I-TF in popular foods in the countries of the former Soviet Union with excessively high CHD mortality. Restriction of the use of I-TF in foods, either voluntarily by food producers or more efficiently by legislation, may be an easily implemented first strategy for some reduction of cardiovascular diseases in these countries.

REFERENCES

1. Gebauer SK, Destaillats F, Dionisi F, *et al*. Vaccenic acid and trans fatty acid isomers from partially hydrogenated oil both adversely affect LDL cholesterol: a double-blind, randomized controlled trial. *Am J Clin Nutr* 2015;102(6):1339-46.

2. de Souza RJ, Mente A, Maroleanu A, *et al.* Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. *BMJ* 2015;351: h3978. doi:101136/bmj.h3978.

3. Li H, Zhang Q, Song J, *et al.* Plasma trans-fatty acids levels and mortality: a cohort study based on 1999-2000 National Health and Nutrition Examination Survey (NHANES). *Lipids Health Dis* 2017; 16: 176.doi:10.1186/s12944-017-0567-6.

4. Yang Q, Zhang Z, Loustalot F, *et al.* Plasma *trans*-Fatty Acid Concentrations Continue to Be Associated with Serum Lipid and Lipoprotein Concentrations among US Adults after Reductions in *trans*-Fatty Acid Intake. *J Nutr* 2017;147**:**896–07.

5. Hirata Y, Takahashi M, Kudoh Y, *et al*. Trans-Fatty acids promote proinflammatory signaling and cell death by stimulating the apoptosis signal-regulating kinase 1 (ASK1)-p38 pathway. *J Biol Chem.* 2017; 292: 8174–85 doi: 10.1074/jbc.M116.771519.

6. Brouwer IA, Wanders AJ, Katan MB. *Trans* fatty acids and cardiovascular health: research completed? *Eur J Clin Nutr* 2013;67:541–47.

7. Restrepo BJ, Rieger M. Denmark's Policy on Artificial Trans Fat and Cardiovascular Disease. *Am J Prev Med* 2016;50:69–76.

8. Restrepo BJ, Rieger M. Trans fat and cardiovascular disease mortality: Evidence from bans in restaurants in New York. *J. Health Econ* 2016;45:176–196.

9. Brandt EJ, Myerson R, Perraillon MC, *et al.* Hospital Admissions for Myocardial Infarction and Stroke Before and After the Trans-Fatty Acid Restrictions in New York. *JAMA Cardiol* 2017;2:627–634.

10. Nichols M, Townsend N, Scarborough P, *et al*. Cardiovascular disease in Europe 2014: epidemiological update. *Eur Heart J* 2014;35:2950–59.

11. Micha R, Khatibzadeh S, Shi P, *et al*. Global, regional, and national consumption levels of dietary fats and oils in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys. *BMJ* 2014; 348:g2272 doi:10.1136/bmj.g2272.

12. Perova NV, Metel'skaia VA, Boytsov SA. [Trans isomers of unsaturated fatty acids increase the risk of atherosclerosis-related circulatory system diseases].[Article in Russian]. *Ter Arkh* 2013;85:113-7.

13. Boytsov SA, Perova NV. To lower trans fatty acid intake as an important condition for the reduction of cardiovascular Mortality (Article). *Profilakticheskaya Meditsina* 2017;20:4-9.

14. Meremäe K, Roasto M, Kuusik S, *et al*. Trans Fatty Acid Contents in Selected Dietary Fats in the Estonian Market. *J Food Sci.* 2012;77:163-8.

15. FEEDcities project. The food environment description in cities in Eastern Europe and Central Asia - Tajikistan and Kyrgyzstan. Technical report, WHO Europe, June 2017 and August 2017 http://www.euro.who.int/__data/assets/pdf_file/0012/353001/FEED-TJK-report.pdf?ua=1 http://www.euro.who.int/__data/assets/pdf_file/0006/351996/FEED-KGZ-report-web-new.pdf?ua=1 (Accessed Nov 2018).

16. Wanders AJ, Zock PL, Brouwer IA. Trans Fat Intake and Its Dietary Sources in General Populations Worldwide: A Systematic Review. *Nutrients.* 2017;9:840; doi:10.3390/nu9080840.

17. Stender S, Astrup A, Dyerberg J. A trans European Union difference in the decline in trans fatty acids in popular foods: a market basket investigation. *BMJ Open* 2012;17:2 e000859. doi: 10.1136/bmjopen-2012-000859.

18. https://en.wikipedia.org/wiki/Post-Soviet_states#General_statistics (Accessed Nov 2018).

19. Kravic SZ, Suturovic ZJ, Svarc-Gajic JV, *et al*. Fatty acid composition including *trans*-isomers of Serbian biscuits. *Hem Ind* 2011;65:139–46.

20. Stender S, Astrup A, Dyerberg J. Tracing artificial *trans* fat in popular foods in Europe: a market basket investigation. *BMJ Open* 2014;4: e005218.

21. Stender S, Astrup A, Dyerberg J. Artificial trans fat in popular foods in 2012 and in 2014: a market basket investigation in six European countries. *BMJ Open* 2016;6: e010673.

22. https://da.wikipedia.org/wiki/International_Article_Number_(EAN) (Accessed Sep 2018).

23. Eliminating trans fats in Europe – A policy brief (2015) http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/publications/2015/eliminating-trans-fats-in-europe-a-policy-brief-2015 (Accessed Nov 2018).

24. FDA Cuts Trans Fat in Processed Foods https://experiencelife.com/article/u-s-trans-fat-ban-goes-into-effect/ (Accessed Nov 2018)

25. Vučić V, Arsić A, Petrović S, *et al*. Trans fatty acid content in Serbian margarines: Urgent need for legislative changes and consumer information. *Food Chem* 2015;185:437–40.

26. Wilczek MM, Olszewski R, Krupienicz A. *Trans*-Fatty Acids and Cardiovascular Disease: Urgent Need for Legislation. *Cardiology*. 2017;138:254–58.

27. Trans fat legislation in Republic of Russia http://rospotrebnadzor.ru/about/info/news/news_details.php?ELEMENT_ID=9510&sphrase_id=1487827 (accessed Nov 2018)

28. Report from the Commission to the European parliament and the Council regarding trans fats in foods and in the overall diet of the Union population. (03-12-2015) http://ec.europa.eu/dgs/health_food-safety/dyna/enews/enews.cfm?al_id=1650 (Accessed Nov 2018).

29. https://ec.europa.eu/info/consultations/open-public-consultation-initiative-limit-industrial-trans-fats-intakes-eu_en (accessed Nov 2018)

http://ec.europa.eu/smart-regulation/roadmaps/docs/2016_sante_143_trans_fats_en.pdf (Accessed Nov 2018).

30 Barquera S, Pedroza-Tobías A, Medina C, *et al.* Global Overview of the Epidemiology of Atherosclerotic Cardiovascular Disease. *Arch Med Res* 2015;46:328–38

LEGENDS

Figure 1

Amounts of industrially produced *trans* fat (I-TF) in 100 grams of pre-packaged biscuits/cakes/wafers purchased in 2015-2016 in three supermarkets in each of the 15 capitals. Each bar in a panel represents a unique product bought in that capital, i.e., none of the products in a given panel had the same barcode number. N is the number of different products with more than 2 % TF of the total fat content. (N) is the number of packages that fulfilled the inclusion criteria. X is the mean value and the standard deviation of the percentages of I-TF in the total fat of the N products, M is the maximal value of I-TF as percent of total fat. Each bar in all of the 15 panels has the same thickness, which means that the area of the bars is comparable between the different panels.

Figure 2

The legend for this figure is similar to the legend for Figure 1. The 4 upper panels show the result for 2 of the capitals and for 2 other large cities in the same country. The lowest 2 panels give the results from the purchase in 3 large supermarkets in Kiev (Kiev-1) and in 3 other large supermarkets in the same city (Kiev-2).

Figure 3

Amounts of industrially produced *trans* fat (I-TF) in 100 grams of pre-packaged biscuits/cakes/wafers, obtained in Uzbekistan and in Kazakhstan respectively with each product in the panel placed together with other products from the same country of origin.

Figure 4

Date of production and I-TF as percent of total fat for pre-packaged biscuits/cakes/wafers, produced in Russia or Ukraine, respectively, and obtained in the countries of the former Soviet Union between January 2015 and January 2017. Products obtained in Samarkand, Sct Petersburg and Kiev 2 were included if the products had their own *trans* fat analysis. Only products with more than 2% of the fat as I-TF were included.

Table 1

Country of origin for pre-packaged biscuits/cakes/wafers with more than 2% of the fat as I-TF obtained in the 15 countries of the former Soviet Union.

Table 2

Shelf life of pre-packaged biscuits/cakes/wafers produced in Russia or in Ukraine and obtained in the countries of the former Soviet Union between January 2015 and January 2017.

Contributors:

SS was responsible for the concept design of the study, for collection of food items, registration, and labelling, and writing the manuscript. SS is the manuscripts guarantor.

Funding: This work was supported by Jenny Vissings Foundation, University of Copenhagen and Department of Clinical Biochemistry, Copenhagen University Hospital, Gentofte, Denmark.

Role of the funding source

The sponsors of the study had no role in the study design, data collection, analysis, and interpretation, the writing of the report, or the decision to submit the article for publication. The corresponding author had full access to all the data for the study and had final responsibility for the decision to submit the manuscript for publication.

Competing interests:

None declared

Ethics approval

The study does not require an approval from the Ethics committee

Acknowledgements:

The authors acknowledge the support from Jenny Vissings Foundation, University of Copenhagen and the Department of Clinical Biochemistry, Copenhagen University Hospital, Gentofte, Denmark.

Transparency declaration:

Steen Stender (the manuscript's guarantor) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Data sharing statement:

The data used to construct figures 1-4, tables 1 and 2, and supplementary table A and B can be shared by e-mailing Steen Stender

---

Table 1. Country of origin for pre-packaged biscuits/cakes/wafers with more than 2% of the fat as *trans* fat obtained in the various countries.

Country	Capital	All* Trans fat per 100 gram fat ≥2%	Produced in Russia Trans fat per 100 gram fat					Produced in Ukraine Trans fat per 100 gram fat					Produced in own country Trans fat per 100 gram fat					Produced in other countries Trans fat per 100 gram fat				
		N	N	%**	Mean	SD	Max	N	%**	Mean	SD	Max	N	%**	Mean	SD	Max	N	%**	Mean	SD	Max
Armenia	Yerevan	91	50	55	17.6	8.8	50.8	33	36	25.5	13.8	50.0	7	8	20.0	8.0	29.6	1	1	10.2		10.2
Belarus	Minsk	98	20	20	14.8	8.5	35.5	5	5	22.5	5.6	32.0	71	72	17.4	8.9	39.9	2	2	6.8	5.4	10.6
Tadjikistan	Dushanbee	136	121	89	10.9	7.0	33.4	10	7	15·0	7.7	32.9	4	3	9.1	1.7	10.3	1	1	2.7		2.7
Kirgistan	Bishkek	81	58	72	14.9	8.3	30.3	15	19	28.6	12.9	49.7	4	5	7.0	4.1	12.2	4	5	2.8	0.6	3.6
Azerbaijan	Baku	85	58	68	12.3	7.3	34.6	15	18	12.9	1.3	33.4	1	1	3.1		3.1	11	13	12.5	5.5	21.6
Moldova	Chisinau	53	10	19	24.6	7.8	35.2	25	47	25.8	8.0	41.5	12	23	10.1	5.9	22.2	6	11	12.0	11.3	32.1
Ukraine	Kiev-1	83	0	0				81	98	13.8	9.7	41.6						2	2	4.2	1.0	4.9
*Ukraine ****	*Kiev-2*	*64*	*0*	*0*				*62*	*97*	*14.3*	*11.0*	*45.4*						*2*	*3*	*12.1*	*0.5*	*12.4*
Uzbekistan	Taskent	77	50	65	10.3	7.5	35.2	20	26	16.2	9.6	33.4	6	8	11.2	4.6	16.2	1	1	6.7		6.7
*Uzbekistan ****	*Samarkand*	*63*	*30*	*48*	*13.5*	*7.7*	*29.0*	*28*	*44*	*18.7*	*11.8*	*42.7*	*5*	*8*	*4.1*	*2.5*	*8.4*	*0*	*0*			
Turkmenistan	Ashgabat	54	18	33	13.3	8.0	28.9	24	44	18.4	10.6	38.0	0	0				12	22	12.6	7.3	26.2
Georgia	Tblisi	47	23	49	15.7	9.7	36.1	8	17	21.3	9.3	34.6	7	15	23.3	9.9	33.3	9	19	7.5	4.8	16.4
Russia	Moscow	75	75	100	9.5	5.6	27.0	0	0									0	0			
*Russia ****	*Sct Petersburg*	*105*	*105*	*100*	*9.3*	*5.0*	*27.0*	*0*	*0*									*0*	*0*			
Kazakhstan	Astana	50	28	56	11.3	7.6	29.1	12	24	10.9	5.6	19.3	7	14	12.0	8.7	28.7	3	6	11.8	7.6	20.5
Lithuania	Vilnius	32	0	0				9	28	10.5	8.3	32.5	9	28	8.4	4.6	15.7	14	44	6.7	3.8	14.0
Latvia	Riga	24	5	21	9.0	3.8	12.2	4	17	8.9	3.8	14.5	5	21	5.5	4.1	11.3	10	42	6.7	3.2	11.0
Estonia	Tallinn	8	1	13	29.2		29.2	2	25	19.3	17.0	31.3	0	0				5	63	8.0	3.6	11.3
15 countries together		994	517					263					133					81				

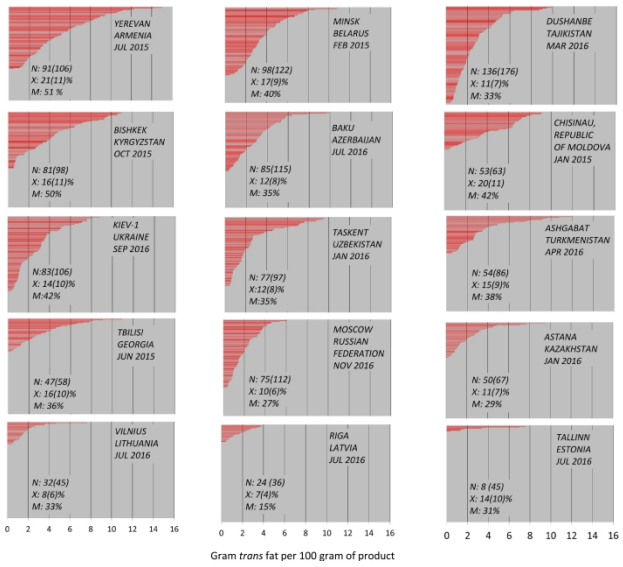
All* is the number in each country of different packages of biscuits/cakes/wafers with ≥ 2% trans fat per 100 gram fat
%** is the number of samples with more than 2% of the fat as trans fat as percent of all samples obtained in that country with more than 2% of the fat as trans fat
*** the lines in italic present values for biscuits/cakes/wafers obtained by the same procedure but in extra regions of the same country. The values are not included in the bottom line

Table 2. Shelf life of pre-packaged biscuits/cakes/wafers produced in Russia and Ukraine

	Produced in Russia		**Produced in Ukraine**	
Trans fat as % of fat	< 2%	≥ 2%	<2%	≥ 2%
Number of samples*	140	453	24	220
Shelf life in days				
Mean (SD)	202(83)	219(70)	286(94)	215(85)
Range	59-547	46-396	123-366	46-546

* Samples obtained in Samarkand, Sct Petersburg, and Kiev-2 were included if these samples had their own trans fat analysis and a readable shelf life on the package

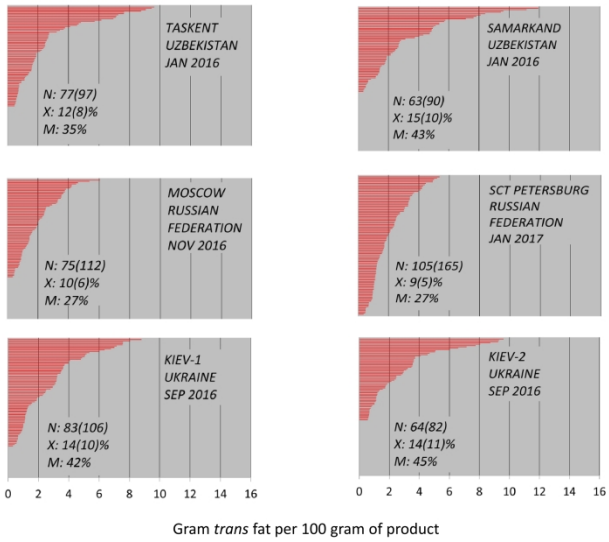
Figure 1



Amounts of industrially produced trans fat (I-TF) in 100 grams of pre-packaged biscuits/cakes/wafers purchased in 2015-2016 in three supermarkets in each of the 15 capitals. Each bar in a panel represents a unique product bought in that capital, i.e., none of the products in a given panel had the same barcode number. N is the number of different products with more than 2 % TF of the total fat content. (N) is the number of packages that fulfilled the inclusion criteria. X is the mean value and the standard deviation of the percentages of I-TF in the total fat of the N products, M is the maximal value of I-TF as percent of total fat. Each bar in all of the 15 panels has the same thickness, which means that the area of the bars is comparable between the different panels.

338x190mm (300 x 300 DPI)

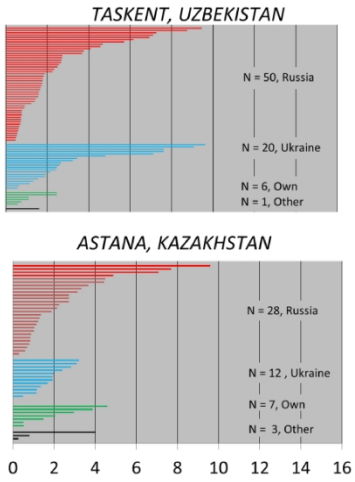
Figure 2



The legend for this figure is similar to the legend for Figure 1. The 4 upper panels show the result for 2 of the capitals and for 2 other large cities in the same country. The lowest 2 panels give the results from the purchase in 3 large supermarkets in Kiev (Kiev-1) and in 3 other large supermarkets in the same city (Kiev-2).

338x190mm (300 x 300 DPI)

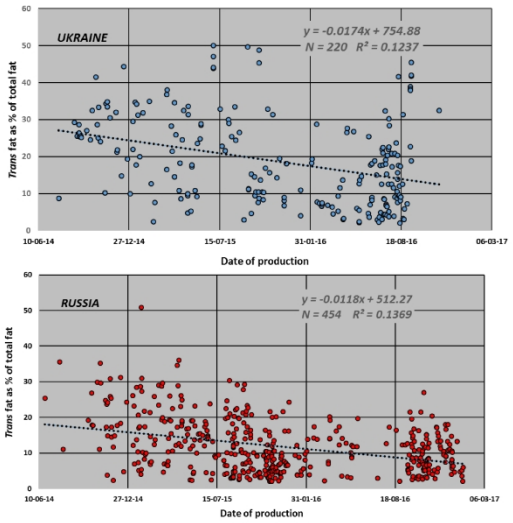
Figure 3



Amounts of industrially produced trans fat (I-TF) in 100 grams of pre-packaged biscuits/cakes/wafers, obtained in Uzbekistan and in Kazakhstan respectively with each product in the panel placed together with other products from the same country of origin.

338x190mm (300 x 300 DPI)

Figure 4



Date of production and I-TF as percent of total fat for pre-packaged biscuits/cakes/wafers, produced in Russia or Ukraine, respectively, and obtained in the countries of the former Soviet Union between January 2015 and January 2017. Products obtained in Samarkand, Sct Petersburg and Kiev 2 were included if the products had their own trans fat analysis. Only products with more than 2% of the fat as I-TF were included.

338x190mm (300 x 300 DPI)

Supplementary table A

Names and population of the countries of the former Soviet Union and the mortalities of coronary heart disease(CHD) and cardiovascular disease (CVD)

Country	Capital	Visit Month	Visit Year	Capital population millions*	Country population millions*	Year	CHD-mortality male per 100.000	CVD-mortality male per 100.000	Year	CHD-mortality female per 100.000	CVD-mortality female per 100.000
Republic of Moldova	Chisinau	Jan	2015	0.669	3.6	2014	528	790	2012	365	564
Belarus	Minsk	Feb	2015	1.893	9.5	2016	642	893	2009	284	428
Georgia	Tbilisi	Jun	2015	1.173	3.7	2016	80	325	2010	39	190
Armenia	Yerevan	Jul	2015	1.06	3.0	2016	352	525	2012	211	357
Kyrgyzstan	Bishkek	Oct	2015	0.871	5.5	2009	534	842	2010	373	588
Kazakhstan	Astana	Jan	2016	0.639	17.7	2016	265	810	2010	124	485
Uzbekistan	Taskent	Jan	2016	2.31	31.6	2016	454	858	2005	321	662
Tajikistan	Dushanbe	Mar	2016	0.779	8.6	2015	304	710	2004	186	504
Turkmenistan	Ashgabat	Apr	2016	1.031	6.7	2011	563	1017	1998	333	717
Estonia	Tallinn	Feb	2016	0.444	1.3	2016	256	501	2012	118	269
Latvia	Riga	Jul	2016	0.43	2.0	2014	355	655	2012	174	353
Lithuania	Vilnius	Jul	2016	0.537	2.9	2016	436	667	2010	239	383
Azerbaijan	Baku	jul	2016	2.182	9.8	2016	149	617	2007	93	489
Ukraine	Kiev	Sep	2016	2.847	42.5	2013	600	873	2012	356	533
Russian Federation	Moscow	Nov	2015	13.197	146.7	2016	501	915	2010	255	517
UK							111	205	2010	49	129
Denmark							67	174	2011	33	111

* Values from Wikipedia.[18] Mortalities from [10]

Supplementary table B:

Number of samples obtained from the various countries and the number and percentages of samples that were analysed for *trans* fat (TF)
Samples that were not analysed received their TF values from similar products that already have been analysed.

Country	Capital	All *samples			Samples with *trans* fat per 100 gram fat < 2%			Samples with *trans* fat per 100 gram fat≥2%		
			Analysed for TF			Analysed for TF			Analysed for TF	
		N-all	N	% of N-all	N<2%	N	% of N<2%	N≥2%	N	% of N≥2%
Armenia	Yerevan	106	106	100	15	15	100	91	91	100
Belarus	Minsk	122	122	100	24	24	100	98	98	100
Tadjikistan	Dushanbee	176	121	69	40	32	80	136	89	65
Kirgistan	Bishkek	98	98	100	17	17	100	81	81	100
Azerbaijan	Baku	115	58	50	30	19	63	85	39	46
Moldova	Chisinau	63	63	100	10	10	100	53	53	100
Ukraine	Kiev-1	106	84	79	23	19	83	83	65	78
*Ukraine **	*Kiev-2*	*82*	*40*	*49*	*18*	*7*	39	*64*	*33*	52
Uzbekistan	Taskent	97	63	65	20	12	60	77	51	66
*Uzbekistan **	*Samarkand*	*90*	*31*	*34*	*27*	*10*	37	*63*	*21*	33
Turkmenistan	Ashgabat	86	56	65	32	28	88	54	28	52
Georgia	Tblisi	58	58	100	11	11	100	47	47	100
Russia	Moscow	112	99	88	37	31	84	75	68	91
*Russia **	*Sct Petersburg*	*165*	*69*	*42*	*60*	*20*	33	*105*	*49*	47
Kazakhstan	Astana	67	44	66	17	15	88	50	29	58
Lithuania	Vilnius	45	34	76	13	11	85	32	23	72
Latvia	Riga	36	27	75	12	12	100	24	15	63
Estonia	Tallinn	45	35	78	37	28	76	8	7	88
15 countries and 3 extra regions		1669	1208	72	440	321	73	1226	887	72
15 countries without 3 extra regions		1332	1068	80	335	284	85	994	784	79

All* is the number in each country of different packages of biscuits/cakes/wafers that fulfilled the inclusion criteria
** the lines in italic present values for biscuits/cakes/wafers obtained by the same procedure but in extra regions in the same country

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

Manuscript ID bmjopen-2018-023184 entitled "Industrially produced trans fat in popular foods in 2015-2016 in 15 countries of the former Soviet Union: a market basket investigation
12-9-2018

	Item No	Recommendation
l Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **line 5**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **Lines 26-37**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **Lines 68-79**
Objectives	3	State specific objectives, including any prespecified hypotheses **Lines 102-103**
Methods		
Study design	4	Present key elements of study design early in the paper **Lines 107-120**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **Supplementary table A**
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants **Lines 107-120**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **Lines 145-166**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **Lines 145-151**
Bias	9	Describe any efforts to address potential sources of bias **Lines 157-166**
Study size	10	Explain how the study size was arrived at **Lines 22-25**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **Line 31**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **Lines 172-174**
		(*b*) Describe any methods used to examine subgroups and interactions **NA**
		(*c*) Explain how missing data were addressed **Lines 138-144, Supplementary table B**
		(*d*) If applicable, describe analytical methods taking account of sampling strategy **Lines 145-151**
		(*e*) Describe any sensitivity analyses **Lines 130-135**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **Table 1**
		(b) Give reasons for non-participation at each stage **NA**
		(c) Consider use of a flow diagram **NA**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **Lines 107-120**
		(b) Indicate number of participants with missing data for each variable of interest

Supplementary table B

Outcome data	15*	Report numbers of outcome events or summary measures **Figure 1 and Table1**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included **NA**
		(*b*) Report category boundaries when continuous variables were categorized **Line 31**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **NA**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **Lines 212-221**
Discussion		
Key results	18	Summarise key results with reference to study objectives **Lines 231-238**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias **Lines 271-282**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **Lines 320-322**
Generalisability	21	Discuss the generalisability (external validity) of the study results **Lines 260-268**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **Lines 434-435**

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Industrially produced trans fat in popular foods in 15 countries of the former Soviet Union from 2015-2016: a market basket investigation

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023184.R3
Article Type:	Research
Date Submitted by the Author:	12-Dec-2018
Complete List of Authors:	Stender, Steen; Kobenhavns Universitet Det Natur- og Biovidenskabelige Fakultet, Nutrition Excercise and Sports
Primary Subject Heading:	Health policy
Secondary Subject Heading:	Health policy, Global health, Public health
Keywords:	Coronary heart disease < CARDIOLOGY, NUTRITION & DIETETICS, Health policy < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, central asia trans fat

1

10-12-2018

Industrially produced *trans* fat in popular foods in 15 countries of the former Soviet Union from 2015-2016: a market basket investigation

Steen Stender, Professor, MD, DMSci

Steen Stender, Department of Nutrition, Exercise and Sports, University of Copenhagen, Rolighedsvej 26, Frederiksberg, 1958 DK, Denmark

Correspondence to: S Stender stender@nexs.ku.dk

Telephone number: +45 35333162

Telephone number: +45 40258601

Key Words: Coronary heart disease, Health policy, Nutrition, Soviet Union, *Trans* fat

ABSTRACT

Objective: To minimize the intake of industrially produced *trans* fat (I-TF) and thereby decrease the risk of coronary heart disease (CHD), several countries have implemented a legislative restriction on I-TF in foods. The objective of this study was to investigate the presence of I-TF in biscuits/cakes/wafers in 15 countries of the former Soviet Union that all have a high coronary mortality rate compared with countries in Western Europe.

Methods: Three large supermarkets in 15 capitals were visited in 2015 or 2016. Pre-packaged biscuits/cakes/wafers were bought if the list of ingredients disclosed that the product contained more than 15 grams of fat per 100 grams of product and if partially hydrogenated fat or a similar term, including margarine, refined fat or confectionary fat, were mentioned. Samples of the foods were subsequently analysed for total fat and TF.

Results: Some 994 products contained more than 2% total fat as I-TF (illegal in Denmark). In Armenia, 91 different products had a mean value (SD) of 21(11) % fat as I-TF. In Estonia, there were eight products with 14(10) % fat as I-TF. The other 13 countries had values between those of Armenia and Estonia. In several countries, a major portion of the products was imported from Russia and Ukraine. The mean shelf life (SD) of 673 packages was 218 (75) days. The % TF in the fat of the products produced in Russia and in Ukraine in relation to the date of production both declined by approximately 10 % points during the 2-year collection period.

Conclusions: The findings suggest that I-TF is used in popular foods in all 15 countries of the former Soviet Union. Therefore, these findings indicate a possible way for some reduction of the high coronary mortality rate in these countries.

Strengths and limitations of this study

- A strength of this study is that the presence of industrially produced *trans* fat in popular foods was systematically investigated for the first time in each of the 15 countries of the former Soviet Union that all have a high coronary mortality rate and a population of nearly 300 million people.
- A limitation of the study is that only pre-packaged biscuits/cakes/wafers were investigated for *trans* fat, and no other food groups, such as shortenings and margarines, and no un-packaged foods, such as baked goods, sweet rolls, pastries, buns and even un-packaged biscuits/cakes/wafers, were investigated.
- A limitation is the assumption that if certain words were not present in the list of ingredients, the product contained less than 2% total fat as *trans* fat.
- Another limitation is that the average daily intake of *trans* fat was not measured in any of the subgroups of the populations but instead was inferred from the availability of popular foods with high amounts of *trans* fat in large supermarkets.

INTRODUCTION

High amounts of *trans* fat (TF) in food i.e., more than 5% TF in total fat, in food originate from the industrial hydrogenation of edible oils. Compared to non-hydrogenated oils, fats containing industrially produced *trans* fat (I-TF) are solid at room temperature, have some technical advantages for food processing, and prolong the shelf life of products. TF are not synthesized in the human body and are not required in the diet but are absorbed and metabolized. I-TF can be found at

varying concentrations in biscuits, pastries, margarine, spreads, confectionary creams and fillings and fried foods such as potato crisps. Fat from ruminants, for instance, in dairy products, contains up to 5% TF (R-TF), which is considerably lower than the up to 50% TF that may be present in partially hydrogenated fat in human food. In a double blind, randomized controlled trial in humans, R-TF and I-TF resulted in the same increase in LDL cholesterol in blood when the two different types of fat were provided in the diet at the same amount.[1] This result suggests that R-TF and I-TF have similar harmful effects on plasma lipoproteins**.**

Observational studies suggest that there is an association between the dietary intake of I-TF and increased risk of coronary heart disease (CHD).[2] The association is further corroborated by an association between plasma TF levels and CHD.[3] Plausible mechanisms for the association involving an increase in plasma LDL cholesterol[4] and unfavourable "beyond lipid effects" on inflammatory cells that are important for atherogenesis[5] suggest a causal relationship, even with the absence of randomized studies with hard clinical endpoints.[6] Based on the available evidence, Denmark in 2004 and some counties in New York, USA, in 2007, legally restricted the use of I-TF in the diet. After the restrictions in Denmark, there was a 4.3% lower yearly mortality rate due to CHD beyond temporal trends.[7] A similar decline of 4.5% in the CHD mortality rate was found in the counties with restrictions in New York compared with that in similar counties without restrictions.[8] Three and more years after the restrictions were enacted in the counties, the combined hospital admission rate for myocardial infarction and stroke events was 6.2% lower than that in similar counties without restrictions.[9] These findings from 2 different groups of researchers strongly suggest that I-TF in the diet promotes CHD and that a restriction in its use reduces CHD. What remains to be explored is to what extent I-TF is still used in popular foods in countries with high rates of CHD mortality.

The 15 countries of the former Soviet Union all have an extraordinarily high rate of CHD mortality compared with that of countries in Western Europe.[10] (supplementary table A) In a systematic analysis, including 266 country-specific nutrition surveys, the average consumption of TF in 2010 in Central Asia and Eastern Europe, which together include the 15 countries of the former Soviet Union, for adults more than 20 years old, was 0.9% of total energy intake.[11] This intake corresponds to approximately 2.5 grams of TF per day per adult and is less than the 1% of the total energy recommended by the WHO. The consumption level was found to be stable since 1990. Among the 21 different regions in the world, Central Asia and Eastern Europe were placed at number 5 and 8, respectively, for the lowest average consumption of TF, suggesting that the intake of TF is a minor health problem in these countries. However, the investigators mention the relatively limited availability of data availability on TF consumption in most of these nations compared with the availability of data on other major dietary factors.[11] Furthermore, a low average intake of TF in a nation does not exclude a high intake of TF in subgroups of the population if popular foods contain high amounts of I-TF.

Some recent Russian studies, with an abstract in English, mentioned the harmful effect of dietary I-TF on health and pledged to remove I-TF from margarine “to preserve the health of Russia’s population”.[12,13] A study from Estonia reported on high I-TF in blended spreads, margarines and shortenings that were bought in the Estonian retail markets in 2011.[14] Additionally, technical reports from the WHO have found several types of street foods with high amounts of TF in Dushanbe, Tajikistan in April/May 2016 and in Bishkek, Kyrgyzstan, in June/July 2016.[15] In a recent systematic review of TF intake and its dietary sources in general populations worldwide based on data from 29 different countries and a demonstration of a substantial reduction in I-TF in many countries, none of the countries of the former Soviet Union were included for either the intake of the general population or for the secondary research question of the study about the composition

of fat in biscuits because of the lack of data.[16] Apparently, there is a pronounced scarcity of newer internationally published data on I-TF in foods in most of these countries. The purpose of the present study was to investigate the presence of I-TF in popular foods in the 15 countries of the former Soviet Union via a newly developed market basket method.[17]

METHODS

Purchase of biscuits/cakes/wafers in supermarkets

By using Wikipedia and local tourist information offices, three large supermarkets, preferably chain supermarkets with many large shops across the country, were identified in each capital.[18] Pre-packaged biscuits/cakes/wafers were chosen as the food to be investigated for TF in this study because these types of foods are frequently consumed, easily accessible, and transportable at ambient temperature. Furthermore, these foods traditionally contain I-TF-rich partially hydrogenated vegetable oils as their major lipid ingredient, and I-TF has previously been found in these foods in high concentrations in some countries in Eastern Europe.[19–21] The packages of biscuits/cakes/wafers were obtained in each supermarket by systematically examining the labels of the products. Packages were purchased if their list of ingredients met the following criteria:

- The total fat content was equal to or exceeded 15 g per 100 g of product.
- One or more of the following terms was listed: partially hydrogenated fat, hydrogenated fat, hardened fat, refined fat, confectionary fat, deodorised fat, all-purpose fat or margarine.
- If the list of ingredients indicated *trans* fat > 0 g, these foods were also included.
- Packages with the term ‘unhydrogenated fat,’ ‘fully hydrogenated fat’ or ‘no *trans* fat’ were not purchased.

If the same package with the same barcode number was found in more than one of the three different supermarkets in the same capital, only the package with the most recent production date was included in the study. Each package was subsequently labelled with an ID number, and duplicate samples of approximately 50 grams each of the product were taken for analysis. The barcode number, the name of the producer, the country of origin and, if available, the best before date as well as the production date were recorded, and the empty packages were stored. The first 2 digits (sometimes three digits) of the barcode number were used to identify the country or economic region numbering authority that assigned the manufacturer code. The Russian Federation uses the code 46; Ukraine, 482. The manufacturer code is the next 5 digits, followed by the product code, consisting of 5 digits. The last digit is the check digit.[22]

To investigate to what extent the presence of biscuits/cakes/wafers in the capital differed from other parts of the country, biscuits/cakes/wafers were obtained from 3 different supermarkets in 2 different regions of the 2 largest countries Uzbekistan and Russia: in Uzbekistan also in the second largest city Samarkand (0.4 million inhabitants and approximately 300 km from Taskent) and in Russia also in St. Petersburg (5.2 million inhabitants and approximately 800 km from Moscow). The third largest country was Ukraine. In its capital Kiev, 3 other large supermarkets were visited (Kiev-2) in addition to the first 3 that were visited (Kiev-1).

Analysis of TF

The samples from the various countries were analysed successively in the order they were obtained. The samples that were obtained from the first countries that were visited (Moldova, Belarus, Georgia, Armenia and Kyrgyzstan) were all analysed for TF. In the other countries that were visited later, some packages were obtained with the same barcode number, the same visual appearance and

list of ingredients as those on packages where the samples had already been analysed. A sample from the newly found package was analysed only if the best before date on that package exceeded the best before date by more than 12 months on a package that already had been analysed. Otherwise, the TF values were carried forward to the new package. (supplementary table B)

For analysis, the foods were homogenized, and the fatty acid content was analysed using gas chromatography on a 60-m highly polar capillary column via a modification of the AOAC 996.06 method. Two procedures were modified in minor ways. In the AOAC 996.06 method, the final step of the extraction/methylation called for 1 mL of hexane to be added. In the modified method, 4 mL was added. The 4mL of hexane makes it possible to achieve the detection limit needed for low fat samples. It provides extra volume for subsequent dilutions for high fat samples in order to achieve results that fall within the range of the detector. The extra volume also provides additional extract for further dilutions and subsequent analysis to optimize separation of cis/trans isomers or if more resolution is needed between other fatty acids. In addition, in the modified method, C11:0 triundecanoin was used as an internal standard. This method makes it possible to obtain total fat as triglycerides, which are then broken down into mono-, poly-, saturated and total TF. Total TF may consist of up to 8 different *trans* fatty acids. Microbac Laboratories in Warrendale, Pennsylvania, USA conducted all analytical work on the samples. The laboratory underwent annual proficiency testing and was audited every other year to ensure compliance of this method with ISO 17025.

From the peaks in the chromatogram, TF was calculated as a fraction (percent) of total fat in the product. This value is primarily of interest for food producers, food chemists and food legislators. The total amount of fat in 100 grams of the product was determined from the chromatogram. When that value was multiplied by the TF fraction, the amount of TF in grams per 100 grams of the product was calculated. This value is primarily of interest for the customers and for investigators studying the health effect of intake of TF.

The measurement does not distinguish I-TF from R-TF. If butter as a ruminant fat has been used in the product in addition to partially hydrogenated vegetable oil, some of the TF in the product may be derived from butter that on average contains a few percent and a maximum 5% fat as R-TF. In this paper, the term I-TF is used even though a minor portion in certain products may be TF derived from ruminant fat. If butter is present in a product that was analysed for TF in the present study and the value was higher than 5% TF of total fat, butter diluted the I-TF in the product because butter adds more non-TF than it adds TF. Values larger than 5% TF in the product are consequently the minimum values for I-TF as a percentage of total fat. In products that contain butter with less than 5% TF as a percentage of total fat, the I-TF will be lower than the TF value. Such products are less important for the health of the consumer than products with much higher TF values and of less interest for health authorities and food scientists because of their low amounts of TF per 100 grams of product.

Patient and Public involvement

Patients and the public were not involved in the present study**.**

Statistical analysis

For each country, simple linear regression models were used to describe the change in % TF of total fat as a function of shelf life and over time recorded as a function of the date of production. The statistical software R was used (R Core Team, 2017).

RESULTS

The number of different packages obtained in 3 different supermarkets in each of the 15 capitals totalled 1332. Samples from 1068 packages were analysed, and the TF values for each of the remaining 264 packages were taken from a similar package with an identical barcode but obtained earlier in another country and already analysed. Some 994 samples had more than 2% fat as TF. The different products in each country were ranked according to the I-TF level, which was expressed as grams per 100 g of the product (figure 1). This latter value tells how much TF the consumer receives when 100 grams of the product is consumed. Each horizontal bar in each panel represents a product with more than 2% fat as TF. The number of these products is shown together with the number of packages, given in parenthesis that met the inclusion criteria. The products depicted in each panel are all different, but the same product may appear in the panel for two or more countries. The mean values of the percentages of fat that were I-TF in the collection of different products from each of the countries, together with the SD and maximum values, are shown. These percentages are of interest for food producers and for food authorities because they describe the fat that has been used in the food. In some products obtained in Armenia and in Kyrgyzstan, approximately 50% of the fat was I-TF (figure 1)

In the second largest city, Samarkand, in Uzbekistan, based on the barcode number, 29% of the packages were similar to the packages found in the capital, Taskent. In St. Petersburg, Russia, 30% of the products were similar to the products found in Moscow. In Kiev-2, which was the second collection region, 48% were similar to the packages found in the first collection region, Kiev-1. Even though most of the products were different in the 2 different regions of the same country, the panels that reflect the availability of biscuits/cakes/wafers with more than 2% TF in fat are rather similar (figure 2). This result suggests that the panels presented for each country in figure 1 represent a pattern found elsewhere in the country and not only in the capital.

For the 994 products with more than 2% fat as I-TF and that were obtained in the 15 capitals of the former Soviet Union, 52% were produced in Russia, 26% in Ukraine, 13% in own country, and 8% in some other countries, such as Poland and Bulgaria (table 1). For the products produced in Russia, 85% were obtained outside of Russia and in the other countries of the former Soviet Union combined, not including Ukraine and Lithuania. For the products produced in Ukraine, 69% were obtained outside of Ukraine in the other countries, excluding Russia. The import patterns of pre-packaged biscuits/cakes/wafers that differed from country to country were exemplified by the patterns observed in Uzbekistan and Kazakhstan. (figure 3) The concentrations in grams of TF per 100 grams of product are shown in a panel for each of the two countries, and each product is placed together with other products from the same country of origin. The numbers and the mean values of the concentrations of TF in the fat of pre-packaged biscuits/cakes/wafers produced in Russia and in Ukraine but obtained in the various countries of the former Soviet Union are shown in table 1. All of the 75 different products with more than 2% fat as TF obtained in Moscow were produced in Russia, and 81 of the 83 different products obtained in Kiev-1 were produced in Ukraine. The same pattern was observed in St. Petersburg, Russia and Kiev-2, Ukraine. In each of the countries, except for the 3 Baltic countries and Belarus, a majority of the products were imported from Russia and Ukraine.

The analysis of shelf life was only conducted for packages produced in Russia and Ukraine, as they were the main exporters to most of the other countries, and in many of the products, especially from Russia, the length of the shelf life in months or days appeared on the package together with the date of production. This is in contrast to pre-packaged biscuits/cakes/wafers produced in most countries in the more western part of Europe. These packages only have the best before date, and the shelf life of the product is usually hidden from the final customer. Products with less than 2% TF in the fat had shelf lives of approximately 200 days, which is comparable to that of products with a much

higher concentration of TF (table 2). The most frequent length of shelf life was 6 months, whether the products were produced in Russia or in Ukraine. The slope of the regression line between shelf life and % TF of total fat was significantly negative for Ukraine ($p<0.0001$) and significantly positive for Russia ($p<0.0001$). Taken together, the data did not support the conclusion that the higher the concentration of TF in the product, the longer was the shelf life.

Since the samples of biscuits/cakes/wafers were collected between January 2015 and January 2017 and many were produced in Russia and in Ukraine, the % TF of total fat in the product was depicted in relation to the date of production. The TF concentrations decreased significantly during the 2 years in Russia ($p<0.0001$) as well as in Ukraine ($p<0.0001$), but the decrease did not differ significantly between the two countries ($p=0.12$) (figure 4). The products from Ukraine had a higher TF % at the start of the period than the products from Russia. Based on the slopes of the regression lines in figure 4, the decline for the products from Ukraine was 12.4 % points and for products from Russia 8.4 % points during the 2-year period.

DISCUSSION

The principal findings

The findings of this study clearly demonstrate that from 2015-2016, I-TF was present in high concentrations, i.e., more than 5% I-TF in the total fat, in many different brands of biscuits/cakes/wafers in the countries of the former Soviet Union, except for the 3 Baltic countries. This information was not previously available and may be of relevance because of the high coronary mortality rate in these countries. In 3 of the countries, Russia, Ukraine and Belarus, by far most of the products were produced in the country itself. For the other countries, except for the 3

Baltic countries, the imports from food producers in Russia and Ukraine made up a major portion of their products with high amounts of TF.

In all of the shops, there were more packages containing biscuits/cakes/wafers that did not have the inclusion terms on the label than packages that had one or more of the terms on the label. This result suggests that there are plenty of biscuits/cakes/wafers without I-TF available in all of the 45 supermarkets. Of the 1332 packages that met the inclusion criteria and were bought, 335 (25%) of the products had less than 2% fat as TF. This result suggests that the inclusion criteria are rather broad in relation to the presence of TF and/or that the terms on the list of ingredients do not always reflect the composition of the fat in the product. If the list of ingredients did not have the inclusion words, the product may still have had high amounts of TF but were not bought and analysed for TF.

The amount of TF per 100 grams of product that was bought in Moscow did not exceed 6 grams (figure 1), whereas several products that were produced in Russia but obtained in the other countries, such as Uzbekistan and Kazakhstan, had more than 6 grams per 100 grams (figure 3). The same was seen for the average concentration of I-TF in fat in products that were obtained in Russia, which had a mean value and SD of 10(6) %, compared with considerably higher values in Russian products that were obtained in the other countries (table 1). The same pattern was observed for products that were bought in Ukraine and compared with products that were produced in Ukraine but bought in some of the other countries (table 1). The products that were obtained in Russia were bought more than a year later than the products that were produced in Russia but obtained in the other countries. This same trend was also the case for the products that were obtained in Ukraine and outside of Ukraine. Some of the difference may be due to a decrease in the use of I-TF during the time period. (figure 4)

I-TF has also been used in foods such as biscuits/cakes/wafers because it apparently prolongs the shelf life of the foods. This fact is presently not supported by the findings of this study because 186 products that were produced in Russia with a shelf life of 6 months had TF concentrations of 11 (7)% mean value (SD) and 37 products with a shelf life of 12 months also had TF concentrations of 11 (7)%. The corresponding values for products that were produced in Ukraine were for 86 products with a shelf life of 6 months, 20 (9) % and for 30 products with a shelf life of 12 months, 10 (6) %.

Based on the plasma concentrations of TF in nationally representative groups in 2010 in the USA (3 years after the introduction of mandatory labelling of TF on pre-packaged foods and in spite of a nearly 50% lower average TF concentration in plasma), there is still a subgroup with very high TF concentrations, reflecting a subgroup in the general population with a high consumption of TF.[4] A vicious cycle apparently occurs: as long as foods with high amounts of I-TF are present in shops, some consumers will buy them, and as long as they are bought, the foods will be available. This cycle may also occur in the countries of the former Soviet Union. Despite a decline in the average intake of I-TF in many countries in Western Europe,[16] in 2015, the WHO estimated that millions of Europeans still consume TF at levels that significantly increase their risk of CHD.[23] The findings reported in the present investigation confirm this estimate.

Strength and weaknesses of the study

The strengths of this study include that the same procedure was used to obtain popular foods in large supermarkets in all 15 countries of the former Soviet Union and in two different regions in the 3 largest countries and that the samples were analysed for TF in the same laboratory. A limitation of this study is that no other food groups (such as shortenings and margarines) and no un-packaged foods (such as baked goods, sweet rolls, pastries, buns and even un-packaged biscuits/cakes/wafers)

were investigated. It was also assumed that if the inclusion words are not present in the list of ingredients, the product contained less than 2% total fat as TF. The presence of high concentrations of I-TF in biscuits/cakes/wafers may be a sign that I-TF is used in other foods as well. Another limitation is that the average daily intake of I-TF was not measured in any subgroups of the populations but instead was inferred from the availability of popular foods with high amounts of I-TF in large supermarkets. The competition for shelf space in supermarkets is usually fierce. If products do not sell, they are rapidly replaced by other products. A relevant subgroup for intake studies would be the consumers who buy pre-packaged biscuits/cakes/wafers.

Legislation and industrially produced *trans* fat in foods

In the USA in 2015, the FDA revoked the previous GRAS (Generally Recognized As Safe) status of partially hydrogenated oils, which was a step that may remove I-TF from food production in the USA before July 2018, and Canada is following suit.[24] In 2009, Austria and Switzerland introduced a legislative ban similar to the Danish ban from 2004, followed by Iceland in 2011, Hungary and Norway in 2014, and initiated by Latvia, Georgia, Lithuania, Slovenia and other Eastern European countries including Russia between 2016 and 2018. [13,25,26,27] The samples for the present study were all collected in the various capitals in 2015 or 2016 (supplementary table A) before any of the legislative restrictions of I-TF in food were fully implemented in these countries. However, some of their food producers were most likely aware of future legislation. The producers may have gradually started to remove I-TF from some of their products in 2015 and 2016. This removal may be the explanation for the relatively few products with TF in Lithuania and in Latvia. This explanation is apparently not valid for Estonia, which only had 8 products with more than 2% fat as

TF and has no internationally published plan for a legislation similar to the two other Baltic countries. (figure 1 and table 1)

The European Union published a report in 2015, which was originally commissioned in 2011, that "indicates the need to continue and expedite work in this area by collecting more information".[28,29] In the WHO-Europe investigation of the various ways to reduce I-TF in foods, it was concluded that establishing a legal limit for the content of I-TF in foods is potentially the only available option that reduces the risks associated with I-TF faced by all consumers and that doing so may contribute to reducing inequalities. Such a policy is unique in its combination of efficacy, cost-effectiveness and a low potential for negative impact. Removing I-TF from the food supply is possibly one of the most straightforward public health interventions for reducing the risk of CHD.[23] The trend of using fat with lower amounts of I-TF (figure 4) over time will probably accelerate in 2018 among food factories in Russia due to the Russian legislation [27] and among factories in Ukraine due to competition with Russian factories. The intake of I-TF in the two largest countries may decrease, along with the intake of I-TF among millions of inhabitants in the adjacent countries due to the large export of biscuits/cakes/wafers from Russia and Ukraine to these countries.[18] (table 1)

Future research and implications for policy makers

It is of considerable interest whether coronary mortality and admission rates for CHD and stroke will change in countries that have recently introduced legislation that restricts the use of I-TF or intend to do so in the near future in the same way these rates changed in Denmark and in certain counties in New York.[7,8,9]

On the basis of data from 2009 to 2011, there is a five-fold difference in age-standardized mortality rates of CHD between some countries in Western Europe and countries in Central and

Eastern Europe, with Central Asia having the highest rates.[10] Central Eastern Europe and Central Asia are the regions in the world with the highest current age-standardized cardiovascular mortality rates, which are more than twice those of Latin America and the Caribbean.[30] These differences cannot be explained only by differences in the intake of I-TF between the countries. The present study demonstrates that there is a presence of I-TF in popular foods in the countries of the former Soviet Union, which have excessively high CHD mortality rates. The restriction of the use of I-TF in foods, either voluntarily by food producers or more efficiently by legislation, may be an easily implemented primary strategy for some reduction of cardiovascular diseases in these countries.

REFERENCES

1. Gebauer SK, Destaillats F, Dionisi F, *et al*. Vaccenic acid and trans fatty acid isomers from partially hydrogenated oil both adversely affect LDL cholesterol: a double-blind, randomized controlled trial. Am J Clin Nutr 2015;102(6):1339-46.

2. de Souza RJ, Mente A, Maroleanu A, *et al.* Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. *BMJ* 2015;351: h3978. doi:101136/bmj.h3978.

3. Li H, Zhang Q, Song J, *et al.* Plasma trans-fatty acids levels and mortality: a cohort study based on 1999-2000 National Health and Nutrition Examination Survey (NHANES). *Lipids Health Dis* 2017; 16: 176.doi:10.1186/s12944-017-0567-6.

4. Yang Q, Zhang Z, Loustalot F, *et al.* Plasma *trans*-Fatty Acid Concentrations Continue to Be Associated with Serum Lipid and Lipoprotein Concentrations among US Adults after Reductions in *trans*-Fatty Acid Intake. *J Nutr* 2017;147**:**896–07.

5. Hirata Y, Takahashi M, Kudoh Y, *et al*. Trans-Fatty acids promote proinflammatory signaling and cell death by stimulating the apoptosis signal-regulating kinase 1 (ASK1)-p38 pathway. *J Biol Chem.* 2017; 292: 8174–85 doi: 10.1074/jbc.M116.771519.

6. Brouwer IA, Wanders AJ, Katan MB. *Trans* fatty acids and cardiovascular health: research completed? *Eur J Clin Nutr* 2013;67:541–47.

7. Restrepo BJ, Rieger M. Denmark's Policy on Artificial Trans Fat and Cardiovascular Disease. *Am J Prev Med* 2016;50:69–76.

8. Restrepo BJ, Rieger M. Trans fat and cardiovascular disease mortality: Evidence from bans in restaurants in New York. *J. Health Econ* 2016;45:176–196.

9. Brandt EJ, Myerson R, Perraillon MC, *et al.* Hospital Admissions for Myocardial Infarction and Stroke Before and After the Trans-Fatty Acid Restrictions in New York. *JAMA Cardiol* 2017;2:627–634.

10. Nichols M, Townsend N, Scarborough P, *et al*. Cardiovascular disease in Europe 2014: epidemiological update. *Eur Heart J* 2014;35:2950–59.

11. Micha R, Khatibzadeh S, Shi P, *et al*. Global, regional, and national consumption levels of dietary fats and oils in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys. *BMJ* 2014; 348:g2272 doi:10.1136/bmj.g2272.

12. Perova NV, Metel'skaia VA, Boytsov SA. [Trans isomers of unsaturated fatty acids increase the risk of atherosclerosis-related circulatory system diseases].[Article in Russian]. *Ter Arkh* 2013;85:113-7.

13. Boytsov SA, Perova NV. To lower trans fatty acid intake as an important condition for the reduction of cardiovascular Mortality (Article). *Profilakticheskaya Meditsina* 2017;20:4-9.

14. Meremäe K, Roasto M, Kuusik S, *et al*. Trans Fatty Acid Contents in Selected Dietary Fats in the Estonian Market. *J Food Sci.* 2012;77:163-8.

15. FEEDcities project. The food environment description in cities in Eastern Europe and Central Asia - Tajikistan and Kyrgyzstan. Technical report, WHO Europe, June 2017 and August 2017 http://www.euro.who.int/__data/assets/pdf_file/0012/353001/FEED-TJK-report.pdf?ua=1 http://www.euro.who.int/__data/assets/pdf_file/0006/351996/FEED-KGZ-report-web-new.pdf?ua=1 (Accessed Nov 2018).

16. Wanders AJ, Zock PL, Brouwer IA. Trans Fat Intake and Its Dietary Sources in General Populations Worldwide: A Systematic Review. *Nutrients.* 2017;9:840; doi:10.3390/nu9080840.

17. Stender S, Astrup A, Dyerberg J. A trans European Union difference in the decline in trans fatty acids in popular foods: a market basket investigation. *BMJ Open* 2012;17:2 e000859. doi: 10.1136/bmjopen-2012-000859.

18. https://en.wikipedia.org/wiki/Post-Soviet_states#General_statistics (Accessed Nov 2018).

19. Kravic SZ, Suturovic ZJ, Svarc-Gajic JV, *et al*. Fatty acid composition including *trans*-isomers of Serbian biscuits. *Hem Ind* 2011;65:139–46.

20. Stender S, Astrup A, Dyerberg J. Tracing artificial *trans* fat in popular foods in Europe: a market basket investigation. *BMJ Open* 2014;4: e005218.

21. Stender S, Astrup A, Dyerberg J. Artificial trans fat in popular foods in 2012 and in 2014: a market basket investigation in six European countries. *BMJ Open* 2016;6: e010673.

22. https://da.wikipedia.org/wiki/International_Article_Number_(EAN) (Accessed Nov 2018).

23. Eliminating trans fats in Europe – A policy brief (2015) http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/publications/2015/eliminating-trans-fats-in-europe-a-policy-brief-2015 (Accessed Nov 2018).

24. FDA Cuts Trans Fat in Processed Foods https://experiencelife.com/article/u-s-trans-fat-ban-goes-into-effect/ (Accessed Nov 2018)

25. Vučić V, Arsić A, Petrović S, *et al*. Trans fatty acid content in Serbian margarines: Urgent need for legislative changes and consumer information. *Food Chem* 2015;185:437–40.

26. Wilczek MM, Olszewski R, Krupienicz A. *Trans*-Fatty Acids and Cardiovascular Disease: Urgent Need for Legislation. *Cardiology*. 2017;138:254–58.

27. Trans fat legislation in Republic of Russia http://rospotrebnadzor.ru/about/info/news/news_details.php?ELEMENT_ID=9510&sphrase_id=1487827 (Accessed Nov 2018)

28. Report from the Commission to the European parliament and the Council regarding trans fats in foods and in the overall diet of the Union population. (03-12-2015) http://ec.europa.eu/dgs/health_food-safety/dyna/enews/enews.cfm?al_id=1650 (Accessed Nov 2018).

29. https://ec.europa.eu/info/consultations/open-public-consultation-initiative-limit-industrial-trans-fats-intakes-eu_en (Accessed Nov 2018)

http://ec.europa.eu/smart-regulation/roadmaps/docs/2016_sante_143_trans_fats_en.pdf (Accessed Nov 2018).

30 Barquera S, Pedroza-Tobías A, Medina C, *et al.* Global Overview of the Epidemiology of Atherosclerotic Cardiovascular Disease. *Arch Med Res* 2015;46:328–38

Contributors:

SS was responsible for the concept design of the study, for collection of food items, registration, and labelling, and writing the manuscript. SS is the manuscripts guarantor.

Funding: This work was supported by Jenny Vissings Foundation, University of Copenhagen and Department of Clinical Biochemistry, Copenhagen University Hospital, Gentofte, Denmark.

Role of the funding source

The sponsors of the study had no role in the study design, data collection, analysis, and interpretation, the writing of the report, or the decision to submit the article for publication. The corresponding author had full access to all the data for the study and had final responsibility for the decision to submit the manuscript for publication.

Competing interests:

None declared

Ethics approval

The study does not require an approval from the Ethics committee

Acknowledgements:

The authors acknowledge the support from Jenny Vissings Foundation, University of Copenhagen and the Department of Clinical Biochemistry, Copenhagen University Hospital, Gentofte, Denmark.

Data sharing statement:

The data used to construct figures 1-4, tables 1 and 2, and supplementary table A and B can be shared by e-mailing Steen Stender.

LEGENDS

Figure 1

Amounts of industrially produced *trans* fat (I-TF) in 100 grams of pre-packaged biscuits/cakes/wafers purchased from 2015-2016 in three supermarkets in each of the 15 capitals. Each bar in a panel represents a unique product that was bought in that capital. N is the number of different products with more than 2% TF in the total fat content. (N) is the number of packages that met the inclusion criteria. X is the mean value and the standard deviation of the percentages of I-TF in the total fat of the N products, M is the maximal value of I-TF as a percentage of total fat. Each bar in all 15 panels has the same thickness, which means that the area of the bars is comparable between the different panels.

Figure 2

The legend for this figure is similar to the legend for figure 1. The 4 upper panels show the results for 2 of the capitals and for 2 other large cities in the same country. The lowest 2 panels show the results from purchases from 3 large supermarkets in Kiev (Kiev-1) and from 3 other large supermarkets in the same city (Kiev-2).

Figure 3

Amounts of industrially produced *trans* fat (I-TF) in 100 grams of pre-packaged biscuits/cakes/wafers that were obtained in Uzbekistan and in Kazakhstan, with each product in the panel placed together with other products from the same country of origin.

Figure 4

Date of production and I-TF as a percentage of total fat for pre-packaged biscuits/cakes/wafers that were produced in Russia or Ukraine and obtained in the countries of the former Soviet Union between January 2015 and January 2017. Products obtained in Samarkand, St. Petersburg and Kiev 2 were included if the products had their own *trans* fat analysis. Only products with more than 2% fat as I-TF were included.

Table 1

Country of origin for pre-packaged biscuits/cakes/wafers with more than 2% fat as I-TF obtained in the 15 countries of the former Soviet Union.

Table 2

Shelf life of pre-packaged biscuits/cakes/wafers produced in Russia or in Ukraine and obtained in the countries of the former Soviet Union between January 2015 and January 2017.

Contributors:

SS was responsible for designing the study, collecting the food items, recording and labelling the samples and writing the manuscript. SS is the guarantor of the manuscript.

Table 1. Country of origin of pre-packaged biscuits/cakes/wafers with more than 2% fat as *trans* fat obtained in the various countries.

Country	Capital	All*		Produced in Russia				Produced in Ukraine					Produced in own country					Produced in other countries				
		Trans fat per 100 grams fat ≥2%			*Trans* fat per 100 grams fat					*Trans* fat per 100 grams fat					*Trans* fat per 100 grams fat					*Trans* fat per 100 grams fat				
		N	N	%**	Mean	SD	Max	N	%**	Mean	SD	Max	N	%**	Mean	SD	Max	N	%**	Mean	SD	Max
Armenia	Yerevan	91	50	55	17.6	8.8	50.8	33	36	25.5	13.8	50.0	7	8	20.0	8.0	29.6	1	1	10.2		10.2
Belarus	Minsk	98	20	20	14.8	8.5	35.5	5	5	22.5	5.6	32.0	71	72	17.4	8.9	39.9	2	2	6.8	5.4	10.6
Tajikistan	Dushanbe	136	121	89	10.9	7.0	33.4	10	7	15·0	7.7	32.9	4	3	9.1	1.7	10.3	1	1	2.7		2.7
Kyrgyzstan	Bishkek	81	58	72	14.9	8.3	30.3	15	19	28.6	12.9	49.7	4	5	7.0	4.1	12.2	4	5	2.8	0.6	3.6
Azerbaijan	Baku	85	58	68	12.3	7.3	34.6	15	18	12.9	1.3	33.4	1	1	3.1		3.1	11	13	12.5	5.5	21.6
Moldova	Chisinau	53	10	19	24.6	7.8	35.2	25	47	25.8	8.0	41.5	12	23	10.1	5.9	22.2	6	11	12.0	11.3	32.1
Ukraine	Kiev-1	83	0	0				81	98	13.8	9.7	41.6						2	2	4.2	1.0	4.9
*Ukraine ****	*Kiev-2*	*64*	*0*	*0*				*62*	*97*	*14.3*	*11.0*	*45.4*						*2*	*3*	*12.1*	*0.5*	*12.4*
Uzbekistan	Taskent	77	50	65	10.3	7.5	35.2	20	26	16.2	9.6	33.4	6	8	11.2	4.6	16.2	1	1	6.7		6.7
*Uzbekistan ****	*Samarkand*	*63*	*30*	*48*	*13.5*	*7.7*	*29.0*	*28*	*44*	*18.7*	*11.8*	*42.7*	*5*	*8*	*4.1*	*2.5*	*8.4*	*0*	*0*			
Turkmenistan	Ashgabat	54	18	33	13.3	8.0	28.9	24	44	18.4	10.6	38.0	0	0				12	22	12.6	7.3	26.2
Georgia	Tbilisi	47	23	49	15.7	9.7	36.1	8	17	21.3	9.3	34.6	7	15	23.3	9.9	33.3	9	19	7.5	4.8	16.4
Russia	Moscow	75	75	100	9.5	5.6	27.0	0	0									0	0			
*Russia ****	*St. Petersburg*	*105*	*105*	*100*	*9.3*	*5.0*	*27.0*	*0*	*0*									*0*	*0*			
Kazakhstan	Astana	50	28	56	11.3	7.6	29.1	12	24	10.9	5.6	19.3	7	14	12.0	8.7	28.7	3	6	11.8	7.6	20.5
Lithuania	Vilnius	32	0	0				9	28	10.5	8.3	32.5	9	28	8.4	4.6	15.7	14	44	6.7	3.8	14.0
Latvia	Riga	24	5	21	9.0	3.8	12.2	4	17	8.9	3.8	14.5	5	21	5.5	4.1	11.3	10	42	6.7	3.2	11.0
Estonia	Tallinn	8	1	13	29.2		29.2	2	25	19.3	17.0	31.3	0	0				5	63	8.0	3.6	11.3
15 countries together		994	517					263					133					81				

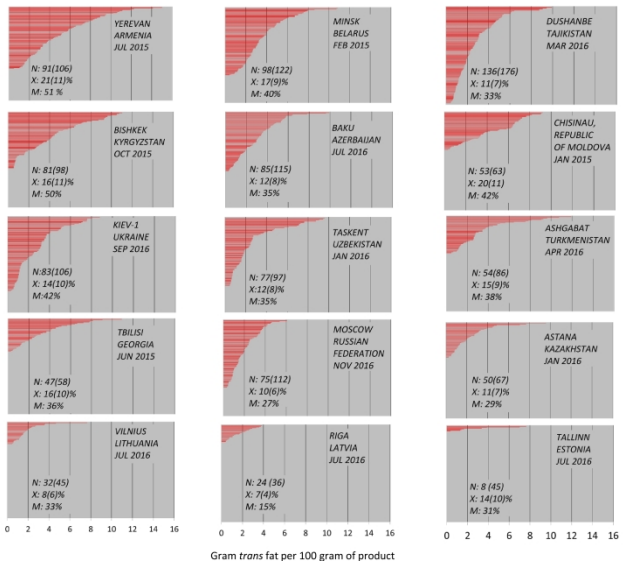
All* is the number of different packages of biscuits/cakes/wafers with ≥ 2% *trans* fat per 100 grams fat in each country
%** is the number of samples with more than 2% fat as *trans* fat as a percent of all samples obtained in that country with more than 2% fat as *trans* fat
*** the lines in italics indicate values for biscuits/cakes/wafers obtained by the same procedure but in extra regions of the same country. The values are not included in the bottom line

Table 2. Shelf life of pre-packaged biscuits/cakes/wafers produced in Russia and Ukraine.

	Produced in Russia		**Produced in Ukraine**	
***Trans* fat as % of fat**	< 2%	≥ 2%	<2%	≥ 2%
Number of samples*	140	453	24	220
Shelf life in days				
Mean (SD)	202(83)	219(70)	286(94)	215(85)
Range	59-547	46-396	123-366	46-546

* Samples obtained in Samarkand, St. Petersburg, and Kiev-2 were included if these samples had their own *trans* fat analysis and a readable shelf life on the package

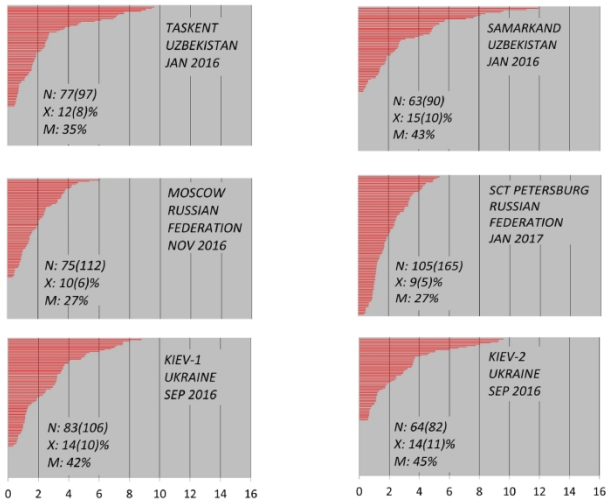
Figure 1



Amounts of industrially produced trans fat (I-TF) in 100 grams of pre-packaged biscuits/cakes/wafers purchased from 2015-2016 in three supermarkets in each of the 15 capitals. Each bar in a panel represents a unique product that was bought in that capital. N is the number of different products with more than 2% TF in the total fat content. (N) is the number of packages that met the inclusion criteria. X is the mean value and the standard deviation of the percentages of I-TF in the total fat of the N products, M is the maximal value of I-TF as a percentage of total fat. Each bar in all 15 panels has the same thickness, which means that the area of the bars is comparable between the different panels.

338x190mm (300 x 300 DPI)

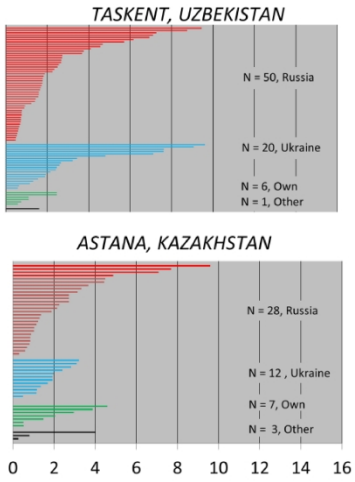
Figure 2



The legend for this figure is similar to the legend for figure 1. The 4 upper panels show the results for 2 of the capitals and for 2 other large cities in the same country. The lowest 2 panels show the results from purchases from 3 large supermarkets in Kiev (Kiev-1) and from 3 other large supermarkets in the same city (Kiev-2).

338x190mm (300 x 300 DPI)

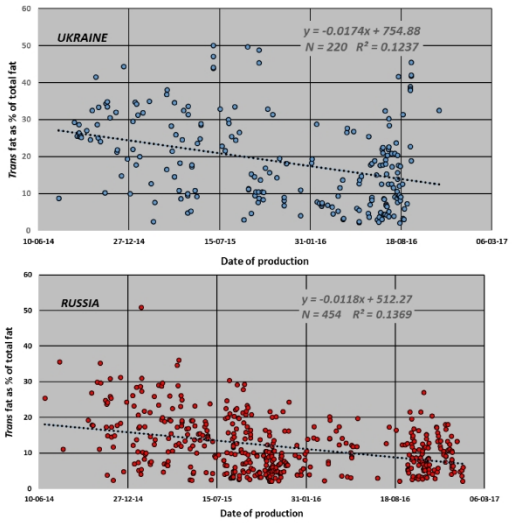
Figure 3



Amounts of industrially produced trans fat (I-TF) in 100 grams of pre-packaged biscuits/cakes/wafers that were obtained in Uzbekistan and in Kazakhstan, with each product in the panel placed together with other products from the same country of origin.

338x190mm (300 x 300 DPI)

Figure 4



Date of production and I-TF as a percentage of total fat for pre-packaged biscuits/cakes/wafers that were produced in Russia or Ukraine and obtained in the countries of the former Soviet Union between January 2015 and January 2017. Products obtained in Samarkand, Sct Petersburg and Kiev 2 were included if the products had their own trans fat analysis. Only products with more than 2% fat as I-TF were included.

338x190mm (300 x 300 DPI)

Supplementary table A

Names and population of the countries of the former Soviet Union and the mortalities of coronary heart disease(CHD) and cardiovascular disease (CVD)

Country	Capital	Visit Month	Visit Year	Capital population millions*	Country population millions*	Year	CHD-mortality male per 100.000	CVD-mortality male per 100.000	Year	CHD-mortality female per 100.000	CVD-mortality female per 100.000
Republic of Moldova	Chisinau	Jan	2015	0.669	3.6	2014	528	790	2012	365	564
Belarus	Minsk	Feb	2015	1.893	9.5	2016	642	893	2009	284	428
Georgia	Tiblisi	Jun	2015	1.173	3.7	2016	80	325	2010	39	190
Armenia	Yerevan	Jul	2015	1.06	3.0	2016	352	525	2012	211	357
Kyrgyzstan	Bishkek	Oct	2015	0.871	5.5	2009	534	842	2010	373	588
Kazakhstan	Astana	Jan	2016	0.639	17.7	2016	265	810	2010	124	485
Uzbekistan	Taskent	Jan	2016	2.31	31.6	2016	454	858	2005	321	662
Tajikistan	Dushanbe	Mar	2016	0.779	8.6	2015	304	710	2004	186	504
Turkmenistan	Ashgabat	Apr	2016	1.031	6.7	2011	563	1017	1998	333	717
Estonia	Tallinn	Feb	2016	0.444	1.3	2016	256	501	2012	118	269
Latvia	Riga	Jul	2016	0.43	2.0	2014	355	655	2012	174	353
Lithuania	Vilnius	Jul	2016	0.537	2.9	2016	436	667	2010	239	383
Azerbaijan	Baku	jul	2016	2.182	9.8	2016	149	617	2007	93	489
Ukraine	Kiev	Sep	2016	2.847	42.5	2013	600	873	2012	356	533
Russian Federation	Moscow	Nov	2015	13.197	146.7	2016	501	915	2010	255	517
UK							111	205	2010	49	129
Denmark							67	174	2011	33	111

* Values from Wikipedia.[18] Mortalities from [10]

Supplementary table B:

Number of samples obtained from the various countries and the number and percentages of samples that were analysed for *trans* fat (TF)
Samples that were not analysed received their TF values from similar products that already have been analysed.

Country	Capital	All *samples			Samples with *trans* fat per 100 gram fat < 2%			Samples with *trans* fat per 100 gram fat≥2%		
			Analysed for TF			Analysed for TF			Analysed for TF	
		N-all	N	% of N-all	N<2%	N	% of N<2%	N≥2%	N	% of N≥2%
Armenia	Yerevan	106	106	100	15	15	100	91	91	100
Belarus	Minsk	122	122	100	24	24	100	98	98	100
Tadjikistan	Dushanbe	176	121	69	40	32	80	136	89	65
Kyrgyzstan	Bishkek	98	98	100	17	17	100	81	81	100
Azerbaijan	Baku	115	58	50	30	19	63	85	39	46
Moldova	Chisinau	63	63	100	10	10	100	53	53	100
Ukraine	Kiev-1	106	84	79	23	19	83	83	65	78
Ukraine **	*Kiev-2*	*82*	*40*	*49*	*18*	*7*	39	*64*	*33*	52
Uzbekistan	Taskent	97	63	65	20	12	60	77	51	66
Uzbekistan **	*Samarkand*	*90*	*31*	*34*	*27*	*10*	37	*63*	*21*	33
Turkmenistan	Ashgabat	86	56	65	32	28	88	54	28	52
Georgia	Tiblisi	58	58	100	11	11	100	47	47	100
Russia	Moscow	112	99	88	37	31	84	75	68	91
Russia **	*Sct Petersburg*	*165*	*69*	*42*	*60*	*20*	33	*105*	*49*	47
Kazakhstan	Astana	67	44	66	17	15	88	50	29	58
Lithuania	Vilnius	45	34	76	13	11	85	32	23	72
Latvia	Riga	36	27	75	12	12	100	24	15	63
Estonia	Tallinn	45	35	78	37	28	76	8	7	88
15 countries and 3 extra regions	15	1669	1208	72	440	321	73	1226	887	72
countries without	3 extra regions	1332	1068	80	335	284	85	994	784	79

All* is the number in each country of different packages of biscuits/cakes/wafers that fulfilled the inclusion criteria
** the lines in italic present values for biscuits/cakes/wafers obtained by the same procedure but in extra regions in the same country

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

Manuscript ID bmjopen-2018-023184 entitled "Industrially produced trans fat in popular foods in 2015-2016 in 15 countries of the former Soviet Union: a market basket investigation
12-9-2018

	Item No	Recommendation
l Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **line 5**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **Lines 26-37**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **Lines 68-79**
Objectives	3	State specific objectives, including any prespecified hypotheses **Lines 102-103**
Methods		
Study design	4	Present key elements of study design early in the paper **Lines 107-120**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **Supplementary table A**
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants **Lines 107-120**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **Lines 145-166**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **Lines 145-151**
Bias	9	Describe any efforts to address potential sources of bias **Lines 157-166**
Study size	10	Explain how the study size was arrived at **Lines 22-25**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **Line 31**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **Lines 172-174**
		(*b*) Describe any methods used to examine subgroups and interactions **NA**
		(*c*) Explain how missing data were addressed **Lines 138-144, Supplementary table B**
		(*d*) If applicable, describe analytical methods taking account of sampling strategy **Lines 145-151**
		(*e*) Describe any sensitivity analyses **Lines 130-135**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **Table 1**
		(b) Give reasons for non-participation at each stage **NA**
		(c) Consider use of a flow diagram **NA**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **Lines 107-120**
		(b) Indicate number of participants with missing data for each variable of interest

Supplementary table B

Outcome data	15*	Report numbers of outcome events or summary measures **Figure 1 and Table1**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included **NA**
		(*b*) Report category boundaries when continuous variables were categorized **Line 31**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **NA**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **Lines 212-221**
Discussion		
Key results	18	Summarise key results with reference to study objectives **Lines 231-238**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias **Lines 271-282**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **Lines 320-322**
Generalisability	21	Discuss the generalisability (external validity) of the study results **Lines 260-268**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **Lines 434-435**

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.