Consumption of dairy products of biodynamic origin is correlated with increased contents of rumenic and trans-vaccenic acid in the breast milk of lactating women

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Consumption of dairy products of biodynamic origin is correlated with increased contents of rumenic and *trans*-vaccenic acid in the breast milk of lactating women

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Abstract

Our previous work showed that incorporation of organic dairy products in the maternal diet may lead to increased contents of the conjugated linoleic acid isomers (CLA) in human breast milk, which might positively affect infant's health. Now, the effect of biodynamic - a special form of organic - dairy products in the diet on the CLA content in human breast milk has been analysed. The content of rumenic acid (the main CLA) in breast milk was higher in the women consuming biodynamic products (n=64, 0.323% of total fat, $P<0.001$) than in the women with a conventional diet (n=175, 0.254%), with the group of women consuming other dairy products including organic (but not biodynamic) showing an intermediate value (n=44, 0.279%). The contents of the CLA-precursor *trans*-vaccenic acid (TVA) paralleled those of rumenic acid, whereas the opposite was found for those of elaidic acid that often occurs in high concentrations in partially hydrogenated fats. Both the higher contents of CLA and TVA as well as the lower level of elaidic acid in the milk of women consuming biodynamic products might conceivably contribute to a better infant's health.

Keywords: conjugated linoleic acid, *trans*-vaccenic acid, human milk, biodynamic nutrition

Introduction

Organic production of food excludes the use of synthetic inputs, such as synthetic fertilizers and pesticides, preventive veterinary drugs, genetically modified seeds and breeds, most preservatives, additives and irradiation (htpp://www.ifoam.org/sub/faq.html). A subgroup of organic farms - the biodynamic ones – are structured as integrated units and allow for animal and mineral products to be used to stimulate production and for the cosmic rhythms to be considered, which in their view results in improvements of plant production and animal husbandry (http://www.demeter.net/). The type of production can affect the composition of foods, which has been particularly well studied in the case of the lipid composition of cow's milk. Several studies have shown that cow's milk from animals kept under the guidelines of organic production (Jahreis, Fritsche et al. 1997; Bergamo, Fedele et al. 2003) or grass-fed (Jutzeler van Wijlen and Colombani 2010) - as often happens in the organic animal husbandry - contains higher contents of conjugated linoleic acid (CLA) isomers than conventional milk. Similarly, the meat from ruminants contains higher amounts of CLA if they are grass-fed (Dannenberger, Nuernberg et al. 2004).

CLA are a special subclass of fatty acids which according to data from *in vitro* experiments and animal tests are likely to have a positive impact on human health [for reviews see (Bhattacharya, Banu et al. 2006; Jutzeler van Wijlen and Colombani 2010)]. These fatty acids may improve body fat composition, i.e. on the proportion of fat tissue to muscle mass (Belury 2002; Corl, Mathews Oliver et al. 2008), blood lipid profile, bone composition as well as oxidative and inflammatory status. They may as well positively affect immune-system and body weight (Watras, Buchholz et al. 2007; Whigham, Watras et al. 2007) and contribute to lower rates of diabetes, atherosclerosis and cancer (see reviews mentoipned above). Nevertheless, there are some contradicting observations from studies on the influence of CLA on health, in particular on body weight and lipid profile, with some effects appearing to be isomer-specific (Tricon, Burdge et al. 2006; Tricon and Yaqoob

2006). A very recent quantitative review of interventional studies showed that CLA can affect the cholesterol distribution in blood negatively, i.e. increase a risk factor for cardiovascular diseases (Brouwer, Wanders et al. 2010); in how far this effect is restricted to a particular isomer composition and to the high amounts of CLA of the used supplements requires further investigations.

There are still only a few published studies concerning the effects of CLA ingestion on children's health. We have previously reported that higher levels of the CLA-isomer rumenic or *cis*-9, *trans*-11-octadecadienoic acid and its precursor *trans*-vaccenic or *trans*11-C18 : 1 acid, TVA (often called ruminant fatty acids) in the mother milk correlate with lower rates of atopic manifestations in the children (Thijs, Muller et al. 2011). Furthermore, it could be shown that a diet including strictly organic dairy – and therefore likely to have higher contents of rumenic acid and TVA – was correlated with reduced incidence of infant eczema in small children (Kummeling, Thijs et al. 2008). Finally, a supplementation study revealed that CLA is able to decrease body fatness in children who were overweighted or obese even though no clear-cut change in the plasma lipids or glucose was observed (Racine, Watras et al. 2010).

Interestingly, the observed effects on health of the ruminant fatty acids were often distinct from those of the *trans* fatty acids typical for the industrially hydrogenated vegetal fats, such as elaidic acid (*trans*9-C18 : 1). These other *trans* fatty acids - often present in high amounts in commercial foods other than dairy, such as margarines and pastry - seem to negatively affect health by augmenting the risk for diseases of the circulatory system (Stender and Dyerberg 2004; Mozaffarian, Katan et al. 2006) and for type 2 diabetes (Odegaard and Pereira 2006). A quantitative review of interventional studies showed that *trans* fatty acids from hydrogenated vegetal fats are associated with an increased ratio of LDH to HDL cholesterol (Brouwer, Wanders et al. 2010). In contrast, five epidemiological studies showed no significant association between ruminant *trans* fatty acids intake and heart diseases risk, even though one study revealed a non-significant positive and one a non-significant negative association (Brouwer, Wanders et al. 2010). The health risks associated with consumption of *trans* fatty acids from industrially hydrogenated vegetal fats led several governments to limit their total amount in the aliments. In Denmark (Stender and Dyerberg 2004) and in Switzerland (Richter, Albash Shawish et al. 2009) this limit has been set to 2 % of the total fat content.

Although most of the available clinical evidence on effects of *trans* fatty acids on health originates from studies with adults, some evidence suggest that this type of fat might as well have implications for both fetal and infant's health as well (Bauer and Waldrop 2009). The International Study of Asthma and Allergies in Childhood (ISAAC) showed a positive association between the consumption of *trans* fatty acids - but not of dairy products - and the prevalence of symptoms of asthma, rhinoconjunctivitis and eczema (Asher, Stewart et al. 2010).

The different effects of both types of *trans* fatty acids – characteristic for industrially partially hydrogenated and ruminant fats - on health already led to differences in the restrictions defined in several countries. Since the two types of fat contain in part similar species of *trans* fatty acids – just in different proportions - the definition of the restrictions is not straightforward. In Denmark, ruminant *trans* fatty acids have been excluded from the restrictions (Brouwer, Wanders et al. 2010) and in Switzerland no limit has been imposed on ruminant products, only on vegetal fats (Richter, Albash Shawish et al. 2009), whereas the US Food and Drug administration included not-conjugated ruminant *trans* fats such as TVA in its labelling rules for *trans* fatty acids, but exempted CLA (Brouwer, Wanders et al. 2010).

Since dietary ruminant products - milk and meat - are the major sources of CLA for humans, we have hypothesized that the amount of the CLA and TVA in the milk of breastfeeding women could be augmented by increasing the dietary amount of organic ruminant products, which are known to be particularly rich in these fatty acids (see above). In our previous work (Rist, Mueller et al. 2007), the contents of CLA and its main precursor *trans*-vaccenic acid (TVA) were analysed in milk samples from 310 breastfeeding mothers, who participated in the KOALA Birth Cohort Study. The participants were stratified according to the consumption of conventional or organic products (milk and ruminant meat). The content of rumenic acid, the main CLA, and of TVA increased while going from a conventional to a moderately organic and to a strict organic diet. In contrast, the amounts of elaidic acid – the most common *trans* fatty acid in industrially hydrogenated vegetal fats – were lower in the organic groups.

A considerable part of the women who donated a milk sample in the present study were consuming dairy products from biodynamic production. We have now analysed the data on CLA, TVA and elaidic acid available for the milk of these women in comparison with the corresponding data of the women who consumed conventional and other organic (but not biodynamic) milk; a German version of this manuscript will be published elsewhere (Simões-Wüst, Rist et al. 2011).

Material and Methods

Breast milk samples were donated by breastfeeding participants in the KOALA study, a prospective birth cohort study described in detail elsewhere (Kummeling, Thijs et al. 2005). Briefly, 312 women with varying lifestyles (conventional and alternative) were enrolled, each donating one sample of breast milk, one month post partum. The study was approved by the Medical Ethical Committee of Maastricht University/ Academic Hospital Maastricht, Maastricht, The Netherlands. Collection of milk, storage and processing of the milk samples have been described elsewhere (Rist, Mueller et al. 2007). Lipids were extracted from 0.2 ml milk samples, transmethylated and the fatty acids methyl esters (duplicate samples) analysed in by GC-FID and Ag+-HPLC as previously described in detail (Rist, Mueller et al. 2007). For the GD-FID analysis, an Agilent 6890 Gas Chromatograph (Agilent Technologies, Waldbroon, Germany) – equipped with a split/splitless injector at 230 °C, a flame ionisation detector at 260 °C, an autosampler and a CP SIL 88 column (100 m, 0.25 mm, 0.2 μm film thickness; Varian, Darmstadt Germany) – was used. Conjugated fatty acid isomers were separated using Ag+-HPLC-DAD. The system consisted of an isocratic Merck-Hitachi L-6000 A HPLC pump equipped with a Waters 717 autosampler (Waters, Eschborn, Germany) and a Waters 996 diode-array detector operated at wavelength between 210.4 and 395.4 nm. Three ChromsSpher 5 lipid columns (250 mm x 4.6 mm, 5 μm) were used in series with a 50 mm x 4.6 mm pre-column of the same column material (Varian). Millenium software (Version 3.20, Waters) was used for data analysis. The normal distribution of fatty acids was visually tested using Q-Q plots, indicating that no transformation was needed. Mean wt % of the various fatty acids were computed for groups of subjects classified by the origin of dairy products, using Student's T-test to assess differences between the groups (not assuming equality of variances); a difference between two groups was considered to be statistically significant if $P \leq 0.05$. All statistical analyses were done in SPSS 12.0 for Windows (www.spss.com).

The food frequency questionnaire on the habitual consumption was included in a self-administered questionnaire in week 34 of the pregnancy (Kummeling, Thijs et al. 2005). The food frequency questionnaire consisted of approximately 160 food items, for which frequency of consumption and portion size were to be estimated. Furthermore, we have asked for information concerning the origin (conventional, organic and biodynamic) of various food groups (meat, eggs, vegetables, fruit, milk and milk products, bread and dried products) that were habitually consumed. Since biodynamic foods are expensive and difficult to find, we only asked whether subjects used 'any' foods of biodynamic origin within each food group.

Results and Discussion

A total of 310 women could be considered in this analysis, since fatty acid determinations were complete for 310 of the 312 participants. The majority of them (n=175) practiced a conventional diet, whereas 64 consumed at least some biodynamic dairy products and 44 other dairy products (including organic but excluding biodynamic). Twenty-seven women did not consume dairy products at all. The questionnaires focused on dairy and ruminant meat products, since these constitute the major dietary sources of CLA for humans. Whereas a considerable number of women consumed dairy products of biodynamic origin (n=64), only half of them ate also biodynamic meat (n=32) and even fewer women ate only biodynamic meat but no dairy (n=12). Therefore only the data on biodynamic dairy are shown.

Table 1 shows that the content of rumenic acid was higher in the breast milk of women consuming biodynamic dairy products (0.323% of total fat, $P<0.001$) than in those with a conventional diet (0.254%). This is in agreement with our previous data on the influence of organic versus conventional dairy on the level of CLA in human breast milk. The average CLA content in breast milk from women consuming organic dairy products other than biodynamic was intermediate (0.279%). Although this value was not statistically different from any of the other groups, a tendency for a difference between it and the one of the breast milk of women consuming conventional milk dairy products was apparent (P=0.053). The level of rumenic acid in the no dairy use group was 0.230%, which is lower – but not statistically significantly (P=0.101) – than the value obtained by the users of conventional dairy products. Since TVA can be converted to rumenic acid in humans (Turpeinen, Mutanen et al. 2002; Mosley, McGuire et al. 2006), we believe that in this group rumenic acid was probably newly synthesized from TVA, which is present in low amounts in industrially partially hydrogenated fats (Brouwer, Wanders et al. 2010). Alternatively, some participants might have acquired CLA from other sources than dairy such as meat or pastry, even though the option for not ingesting dairy products is likely to be associated with consequent vegetarianism or even veganism.

A profile comparable to the one of CLA was found in the case of the TVA. Again the highest level was detected in the milk of the women consuming biodynamic dairy products (0.584%, P<0.001 compared with the conventional dairy group), followed by the group of women consuming other (non-biodynamic) organic dairy products (0.545%). The contents of TVA in the conventional milk (0.479%) and in the no-dairy products (0.464%) groups were the lowest and comparable to each to other. That the results for TVA parallel those of rumenic acid was to be expected since the former is a precursor of the latter.

Some previous studies have shown that although substantial differences might exist between intake of some specific fatty acids in different dietary groups, the differences in actual status are often smaller that expected (Rosell, Lloyd-Wright et al. 2005; Welch, Shakya-Shrestha et al. 2010). This applies to our results, since even when statistically significant, the differences were moderate. The physiological relevance of such apparently moderated differences should however not be underestimated. Indeed an inverse correlation between the levels of CLA in the milk of the women participating in this study and a pathological situation – atopic diseases and eczema - could already be demonstrated (Thijs, Muller et al. 2011).

Table 1 also shows how the level of elaidic acid differs among the various groups. The content of elaidic acid in the biodynamic milk group was significantly lower than in the conventional milk group (0.487% versus 0.606%, $P<0.001$), and somewhat lower than in the other (non-biodynamic) organic milk group (0.526%). These data suggest an inverse association between consumption of biodynamic dairy and consumption of industrially produced, partially hydrogenated fat. The presence of some elaidic acid in diets comprising high amounts of biodynamic dairy products is however not surprising, since this industrially partially hydrogenated fat is present in a variety of food products, such as pastry and fast food. Moreover, this fatty acid is also present – though in much smaller amounts – in ruminant products.

It is known that *trans* fatty acids of industrially partially hydrogenated fat are likely to contribute to pathological conditions (see introduction). In our previous work, we showed that a low elaidic acid/TVA-ratio in human milk is associated with a diet rich in dairy products and/or meat or dairy of organic origin, whereas a diet low in these products (and probably higher in *trans* fatty acids of industrial origin) leads to a high ratio (Mueller, Thijs et al. 2010). Therefore we suggested seeing this ratio as an indicator of the origin of the *trans* fatty acids – industrially partially hydrogenated or ruminant ingested by the mother. The present observation that milk from women consuming dairy products of biodynamic production exhibits the lowest elaidic acid/TVA ratio (Table 1) suggests that these women obtain their *trans* fatty acids more often from ruminant products.

The available data on the influence of *trans* and ruminant fatty acids on children's overweight and obesity (Racine, Watras et al. 2010) indicate that children of women who consume dairy products of biodynamic origin might be at least in part protected from these diseases. Similarly, previous observations on the effects of *trans* and ruminant fatty acids on children's tendency to develop atopic diseases and eczema (Thijs, Muller et al. 2011) suggest that children of the women who consume dairy products of biodynamic origin are less likely to suffer from these diseases than children of women who do not. Given that a biodynamic nutrition is often associated with a lifestyle influenced by anthroposophy, our data may explain why children who grow up in families with a anthroposophic lifestyle have a reduced risk of atopic diseases compared to those in families with conventional lifestyles (Alm, Swartz et al. 1999; Alfven, Braun-Fahrlander et al. 2006).

Conclusions

The data show that consumption of biodynamic milk and milk products is correlated with the production of breast milk with increased levels of CLA and TVA as well as with a lower level of elaidic acid, when compared with breastfeeding women consuming only conventionally produced milk and other dairy products. Although the differences point in the same direction as those previously detected between the breast milk composition of women consuming organic and conventional dairy products, they are greater and indicate a possible difference between the biodynamic group and the group of women consuming other milk (including organic but excluding the biodynamic). Both the higher contents of CLA and TVA as well as the lower level of elaidic acid might conceivably contribute to a better infant's health.

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Table 1. Consumption of biodynamic dairy products affects the levels of rumenic acid, *trans*-vaccenic acid and elaidic acid in human milk (n=310).

Group	n	rumenic acid (% of total milk fat)	TVA (% of total milk fat)	elaidic acid (% of total milk fat)	elaidic acid/ TVA
biodynamic dairy users	64	0.323 ±0.013*	0.584±0.026*	0.487±0.023*	0.83
other organic dairy users [a]	44	0.279 ±0.016	0.545±0.031	0.526±0.038	0.97
conventional dairy users	175	0.254 ±0.005	0.479±0.015	0.606±0.019	1.27
no dairy use [b]	27	0.230 ± 0.015	0.464±0.036	0.596±0.065	1.28

Data are shown as mean ± SEM.

[a] including organic but not biodynamic dairy

[b] not using dairy or avoiding dairy

* P<0.001, compared with the conventional dairy users