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3 Consumption of dairy products of biodynamic origin is

4 correlated with increased contents of rumenic and *trans*-

5 vaccenic acid in the breast milk of lactating women

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1 **Abstract**

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

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

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1 **Introduction**

2 Organic production of food excludes the use of synthetic inputs, such as synthetic
3 fertilizers and pesticides, preventive veterinary drugs, genetically modified seeds and
4 breeds, most preservatives, additives and irradiation (<http://www.ifoam.org/sub/faq.html>).

5 A subgroup of organic farms - the biodynamic ones – are structured as integrated units and
6 allow for animal and mineral products to be used to stimulate production and for the
7 cosmic rhythms to be considered, which in their view results in improvements of plant
8 production and animal husbandry (<http://www.demeter.net/>). The type of production can
9 affect the composition of foods, which has been particularly well studied in the case of the
10 lipid composition of cow's milk. Several studies have shown that cow's milk from animals
11 kept under the guidelines of organic production (Jahreis, Fritsche et al. 1997; Bergamo,
12 Fedele et al. 2003) or grass-fed (Jutzeler van Wijlen and Colombani 2010) - as often
13 happens in the organic animal husbandry - contains higher contents of conjugated linoleic
14 acid (CLA) isomers than conventional milk. Similarly, the meat from ruminants contains
15 higher amounts of CLA if they are grass-fed (Dannenberger, Nuernberg et al. 2004).

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

1 2006). A very recent quantitative review of interventional studies showed that CLA can
2 affect the cholesterol distribution in blood negatively, i.e. increase a risk factor for
3 cardiovascular diseases (Brouwer, Wanders et al. 2010); in how far this effect is restricted
4 to a particular isomer composition and to the high amounts of CLA of the used
5 supplements requires further investigations.

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

17 Interestingly, the observed effects on health of the ruminant fatty acids were often distinct
18 from those of the *trans* fatty acids typical for the industrially hydrogenated vegetal fats,
19 such as elaidic acid (*trans*9-C18 : 1). These other *trans* fatty acids - often present in high
20 amounts in commercial foods other than dairy, such as margarines and pastry - seem to
21 negatively affect health by augmenting the risk for diseases of the circulatory system
22 (Stender and Dyerberg 2004; Mozaffarian, Katan et al. 2006) and for type 2 diabetes
23 (Odegaard and Pereira 2006). A quantitative review of interventional studies showed that
24 *trans* fatty acids from hydrogenated vegetal fats are associated with an increased ratio of
25 LDL to HDL cholesterol (Brouwer, Wanders et al. 2010). In contrast, five epidemiological
26 studies showed no significant association between ruminant *trans* fatty acids intake and

1 heart diseases risk, even though one study revealed a non-significant positive and one a
2 non-significant negative association (Brouwer, Wanders et al. 2010). The health risks
3 associated with consumption of *trans* fatty acids from industrially hydrogenated vegetal
4 fats led several governments to limit their total amount in the aliments. In Denmark
5 (Stender and Dyerberg 2004) and in Switzerland (Richter, Albash Shawish et al. 2009) this
6 limit has been set to 2 % of the total fat content.

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

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

24 Since dietary ruminant products - milk and meat - are the major sources of CLA for
25 humans, we have hypothesized that the amount of the CLA and TVA in the milk of
26 breastfeeding women could be augmented by increasing the dietary amount of organic

1 ruminant products, which are known to be particularly rich in these fatty acids (see above).
2 In our previous work (Rist, Mueller et al. 2007), the contents of CLA and its main
3 precursor *trans*-vaccenic acid (TVA) were analysed in milk samples from 310
4 breastfeeding mothers, who participated in the KOALA Birth Cohort Study. The
5 participants were stratified according to the consumption of conventional or organic
6 products (milk and ruminant meat). The content of rumenic acid, the main CLA, and of
7 TVA increased while going from a conventional to a moderately organic and to a strict
8 organic diet. In contrast, the amounts of elaidic acid – the most common *trans* fatty acid in
9 industrially hydrogenated vegetal fats – were lower in the organic groups.

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

16

1 **Material and Methods**

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

1 variances); a difference between two groups was considered to be statistically significant if
2 $P \leq 0.05$. All statistical analyses were done in SPSS 12.0 for Windows (www.spss.com).
3 The food frequency questionnaire on the habitual consumption was included in a self-
4 administered questionnaire in week 34 of the pregnancy (Kummeling, Thijs et al. 2005).
5 The food frequency questionnaire consisted of approximately 160 food items, for which
6 frequency of consumption and portion size were to be estimated. Furthermore, we have
7 asked for information concerning the origin (conventional, organic and biodynamic) of
8 various food groups (meat, eggs, vegetables, fruit, milk and milk products, bread and dried
9 products) that were habitually consumed. Since biodynamic foods are expensive and
10 difficult to find, we only asked whether subjects used 'any' foods of biodynamic origin
11 within each food group.

12

13 **Results and Discussion**

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

24 Table 1 shows that the content of rumenic acid was higher in the breast milk of women
25 consuming biodynamic dairy products (0.323% of total fat, $P < 0.001$) than in those with a
26 conventional diet (0.254%). This is in agreement with our previous data on the influence of

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

15 A profile comparable to the one of CLA was found in the case of the TVA. Again the
16 highest level was detected in the milk of the women consuming biodynamic dairy products
17 (0.584%, $P<0.001$ compared with the conventional dairy group), followed by the group of
18 women consuming other (non-biodynamic) organic dairy products (0.545%). The contents
19 of TVA in the conventional milk (0.479%) and in the no-dairy products (0.464%) groups
20 were the lowest and comparable to each to other. That the results for TVA parallel those of
21 rumenic acid was to be expected since the former is a precursor of the latter.

22 Some previous studies have shown that although substantial differences might exist
23 between intake of some specific fatty acids in different dietary groups, the differences in
24 actual status are often smaller than expected (Rosell, Lloyd-Wright et al. 2005; Welch,
25 Shakya-Shrestha et al. 2010). This applies to our results, since even when statistically
26 significant, the differences were moderate. The physiological relevance of such apparently

1 moderated differences should however not be underestimated. Indeed an inverse correlation
2 between the levels of CLA in the milk of the women participating in this study and a
3 pathological situation – atopic diseases and eczema - could already be demonstrated (Thijs,
4 Muller et al. 2011).

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

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

25 The available data on the influence of *trans* and ruminant fatty acids on children's
26 overweight and obesity (Racine, Watras et al. 2010) indicate that children of women who

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

10

11 **Conclusions**

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

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

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 4
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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	17 5	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

6 Data are shown as mean ± SEM.
 7 ^a including organic but not biodynamic dairy
 8 ^b not using dairy or avoiding dairy
 9 * P<0.001, compared with the conventional dairy users

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 11