Summaries of oral presentations of the First International Raw Milk Conference

RAW MILK
Health or Hazard?

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The symposium was held in conjunction with the
First International Conference on
Organic Food Quality and Health Research

The focus of the symposium was on: Understanding the health benefits and value of fresh, unprocessed whole milk, and management of the possible risks of bacterial diseases from raw milk consumption. The goal of the symposium was to present and discuss the contradictive aspects of (raw) milk in relation to animal and human health. New scientific research findings were presented about the different health and hazard aspects of raw milk examining the questions:

- What are the pros and cons of the consumption of raw milk?
- How can we handle the dangers (risks) of raw milk consumption for groups of people for whom raw milk might be very supportive for their personal health?

Milk has different faces: on one hand milk contains important food allergens; on the other hand, there is accumulating evidence that raw milk protects against asthma and atopic diseases. Milk can become a source of zoonotic bacteria and the public is advised to consume only heat treated milk (pasteurized, sterilized); while on the other hand drinking raw milk has been shown to be a contributing protective factor in development of the immune systems of babies and children. Milk is included in the recommended list of foods to be avoided because butter fat is a saturated fat; on the other hand milk from grass-based systems is a valuable and readily available source of long chain poly unsaturated fatty acids (CLA's) with Omega 3 and Omega 6 fatty acids occurring in ideal ratio (in whole milk).

Acknowledgements

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Prof. Dr. Ton Baars

Content

Take home messages of conference speakers

C.Braun: Farm children are strongly protected for asthma, allergies and atopic sensitisation. However, there is a protecting effect of consuming raw milk, which is found in all subgroups of children involved in the studies who consumed raw milk.

M.Perkin: The issue of what is influencing children in their risk of atopy and allergy is clearly multifactorial. Independent of being a farm child, raw milk appears to protect against eczema and hay fever. The significant effect is found in the group of atopic children. There is an ultimate paradox about living on a farm: children are healthy, although they do everything in contrast with official governmental advice.

T.Roos: In an oral provocation with different milks , young children with a clinical milk allergy can tolerate the raw milk of a low-input biodynamic farm in contrast to shop milk, that is, conventional, homogenised, and pasteurised milk. The patch and prick tests do not have the sensitivity to differentiate between reactions to the two types of milk.

W.Kneifel: With the consumption of raw milk there is always a risk of the transfer of harmful microbes such as EHEC or Salmonella. In the future, a tailored technology accompanied by suitable microbiological detection methods might support the production of a safe raw milk product.

R.Tschischkale: The German Vorzugsmilch-production is a legal way to sell raw milk. Although the milk is strictly controlled, negative campaigns in the press about zoonotic diseases and mainly EHEC strongly reduced the interest of the consumers, although EHEC was hardly found in Vorzugsmilch over the last 15 years.

T.Baars: EHEC-contamination in raw milk is a risk. EHEC was also found in the faeces of cows from an extensive biodynamic holding; however the bacteria were not found in their milk. Compared to 'normal' raw milk, farms delivering 'Vorzugsmilk' had much better control on the bacterial counts from all typical zoonotic diseases. Risks from this strictly controlled raw milk are very low; however, a zero-risk can never be attained.

M.Schmidt: the idea of food safety should be opened up to look first at the issue of freedom of choice and personal responsibility and, secondly, on ways of understanding the ecology of the involved zoonotic micro-organisms. At the farm level, cow sharing is a legal way to sell raw milk to families. There is an urgent need to expand the research on the apparent health benefits of raw milk and the increase of allergies and lactose intolerance.

C.Thijs: For mothers during pregnancy and breastfeeding, and for children thereafter, full fat milk products and especially those from organic origin may help to protect against atopic diseases, especially when fish intake is low.

G.Jahreis: Milk fat composition depends on the feeding of the cows. Biomarkers like CLAc9t11 are characteristic of the extensive grazing of cows. Trans-FAs in milk cannot be compared with industrial trans-FAs. Publicity encouraging the reduced consumption of all trans-fats, milk fats included, is misleading. In relation to health promotion, several FAs have an anti-inflammatory potential. Besides n3 FAs, CLAc9t11 and C18:1t11 also play a role in this regard.

D.Kusche: the intensification of organic dairy farming through copying conventional feeding practices (that is, through feeding of silages of grass and maize and elevated levels of concentrates) reduces the means of distinguishing between conventional and organic milk.

S.Mosler: Metabolomics is a powerful technology to elucidate the complex composition of milk. Metabolite profiles make it possible to differentiate milk from different origins and feeding protocols. Such multiplex bioanalytics will help us to better understand lactation and the determinants of milk composition and quality.

In advance: Studies on raw milk, asthma and allergies

Authors	Study population	Countries	Exposure	Main results
(1) Riedler et al. 2001	Rural farm and non-farm children (n=812), aged 6-12 years (ALEX study)	Austria, Germany, Switzerland	Milk directly produced or purchased on a farm	Consumption of farm milk during first year of life significantly inversely associated with asthma, hay fever, and atopy, independent of other farm exposures
(2) Waser et al. 2007	Rural farm and non-farm, Steiner Schools', and peri-urban children (n = 14 893) aged 5–13 years, (PARSIFAL-Study)	Sweden, Netherlands, Austria, Germany, Switzerland	Milk directly produced or purchased on a farm	Adj. OR and (95% CI) of farm milk consumption ever in life and asthma: 0.47 (0.61–0.88), rhinoconjunctivitis: 0.56 (0.43–0.73), sensitization to pollen: 0.67 (0.47–0.96), and food mix: 0.42 (0.19–0.92). Association observed in all subgroups, independent of farm-related co-exposures
(3) Bieli et al. 2007	ALEX (n = 576) and PARSIFAL (n = 1478) children with available DNA samples	Sweden, Netherlands, Austria, Germany, Switzerland	Milk directly produced or purchased on a farm	Association between farm milk and asthma varied between genotypes of CD14/-1721. Adj. OR (95%CI) AA: 0.81 (0.07–0.47); AG: 0.47 (0.26–0.86); and GG: 0.98(0.46–2.08). Similar patterns for symptoms of hay fever and pollen sensitization.
(4) Perkin and Strachan, 2006	Rural farm and non-farm children (n=4767), subsample (n = 879) with skin prick test	England	Unpasteurized milk (based on food frequency questionnaire)	Current unpasteurized milk consumption associated with less eczema adj. OR and (95% CI): 0.59 (0.40–0.87) and atopy: 0.42 (0.10–0.53), and higher production of whole blood stimulated IFN-g. Effect independent of farming status. No effect on asthma
(5) Barnes et al. 2001	Rural farm and non-farm and urban children aged 11-19 years (n=929)	Crete (Greece)	Unpasteurized milk products	Adj. OR and (95% CI) of atopy and unpasteurized farm milk consumption with and without simultaneous farm animal contact: 0.32 (0.13–0.78) and 0.58 (0.34–0.98), respectively
(6) Radon et al. 2004	Rural farm and non-farm young adults aged 18-44 years (n=321)	Northern Germany	Raw, unboiled farm milk	Raw milk consumption and atopy adj. OR and (95% CI): 0.65 (0.36–1.18). In those visiting animal houses before age 7 years raw milk consumption and atopy: 0.35 (0.17–0.74)5
(7) Wickens et al. 2002	Children living on farms or in small towns aged 7-10 years (n=293)	New Zealand	Unpasteurized milk ever, yogurt at least weekly before age 2 years	Adj. OR and (95% CI) for early yogurt consumption and hay fever 0.30 (0.1–0.7); any unpasteurized milk and atopic eczema: 0.2 (0.1–0.8). No significant association between unpasteurized milk consumption and asthma or atopy

(8) Remes et al. 2003	Rural farm and non-farm children aged 6-15 years (n=710)	Finland	Farm milk in infancy	Farm milk consumption not associated with atopy. No other allergic health outcomes reported
(9) Ege et al. 2008	922 farm and non-farm children, followed since pregnancy (PASTURE study)	Finland, France, Austria, Germany, Switzerland	Maternal consumption of boiled and unboiled farm milk during pregnancy	Maternal consumption of farm milk during pregnancy not related to IgE to seasonal allergens in cord blood of neonates. Boiled farm milk consumption during pregnancy positively associated with specific IgE to cow's milk: adj. OR and (95% CI): 1.78 (1.08–2.93)
(10) Pfefferle et al. 2010	PASTURE study	Finland, France, Austria, Germany, Switzerland	Skimmed and unskimmed farm milk, farm produced butter and yogurt during pregnancy	Maternal consumption of farm produced butter during pregnancy associated with increased IFN-g and TNF-a production in cord blood, farm produced yogurt inversely associated with these cytokines

Overview of epidemiological studies assessing the effect of farm milk consumption on asthma and allergic diseases; 1-8 Cross-sectional studies; 9-10 Cohort studies
(Taken from: C. Braun-Fahrländer und E. von Mutius, (2010) Can farm milk consumption prevent allergic diseases? Clinical and experimental allergy, 41, 29-35)

Can raw milk prevent allergic disease? Epidemiological evidence

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The research presented has been fulfilled in collaboration with European partners in a range of epidemiological studies. The studies compared 'children growing up at the farm' with 'rural children from the same villages, but not living at the farm'. In the Swiss, German, and Austrian studies it is the typical traditional family-farm. In the first studies (ALEX study) children were compared with respect to whether they were present in the stable during the 1st year of life, and whether they were drinking farm milk in their 1st year of life after weaning. In this first study the researchers came across the effect of the milk.
In a second study, the PARSIFAL study, besides these two groups of children, children going to Steiner schools also were included with a control group. Besides microbial exposures encountered in stables and barns, a number of dietary factors such as consumption of milk, butter and other dairy products, as well as margarine, eggs, meat, vegetables and fruits, have been studied. Among these dietary factors, milk and butter consumed from the farm as compared to shop-purchased milk and margarine, respectively, showed significant protective effects on allergic rhinoconjunctivitis, asthma, and atopic sensitisation among farmers and also among non-farmers' children purchasing these products directly from a farm. So, the milk effects were seen in farm children and in non-farm children. In addition to this milk effect and independent of the farm milk consumption, they found that if children consumed butter, produced on the farms or to some extent bought in a shop, there was also some protection. Moreover, and additionally to the milk effect, there is still an effect of 'being a farm child'.
The continental research was confirmed by the work of Michael Perkin in England, who showed comparable results about atopic sensitisation, which was lower in groups of children drinking farm milk. Of particular importance was the consistency of the findings across children from farming, rural non-farming, anthroposophic, and (sub)urban environments, indicating that farm milk consumption represents a route of exposure that is independent of concomitant exposures to microbial compounds present in animal sheds and farm homes. An inverse association between farm milk consumption and allergic health outcomes had also been reported other research teams from Germany, Britain, Crete and New Zealand. However, one study conducted in Finland did not confirm these findings.
In addition, a comprehensive clinical and genetic assessment of more than 10'000 farm and non-farm children has been performed, as well as laboratory analyses of milk samples of a subsample of GABRIEL participants.

Reference

C. Braun-Fahrländer and E. von Mutius (2010). Can farm milk consumption prevent allergic diseases? Clinical and experimental allergy, 41, 29-35

Take home message: Farm children are strongly protected for asthma, allergies and atopic sensitisation. However, there is a protecting effect of consuming raw milk, which is found in all subgroups of children involved in the studies who consumed raw milk.

UK unpasteurised milk research

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Compared to the alpine farming studies undertaken in Switzerland, Bavaria and Austria, farming in Britain is quite different. The farms are much more industrialised in terms of scale and, on average, three times bigger. Despite this we observed some remarkable associations with raw milk consumption. This lecture will review the farming protective effect from a hygiene-hypothesis perspective. The farming effect is not one single effect. The factors influencing childrens' risk of atopy and allergy are clearly multifactorial and complex.

Historical facts: in 1828 Bostock wrote: *"Summer catarrh[1] ... only occurs in the middle or upper classes of society, some indeed of high rank. I have made inquiry at the various dispensaries in London and elsewhere, and I have not heard of a single unequivocal case occurring amongst the poor."* So something appears to be different between the affluent and the poor. The environmental exposures a child experiences has changed very dramatically over the course of time. Blackley, another great physician, wrote in 1873: *"It would seem that hay-fever has, of late years, been considerably on the increase.....The persons most subjected to the action of the pollen belong to a class which furnishes the fewest cases of the disorder, namely, the farming class."* Observations made 150 years ago suggested that high allergen exposure did not result in allergy symptoms. What is it in the farm environment that seems to protect you? And more relevantly is there something of that farm environment that is applicable to children living outside the farm? What is it in the exposure at the farm that mediates the protection? At what age does it have to occur? In contrast to Switzerland, farming in the UK is on a bigger scale and pregnant woman do not work in the barns. Moreover, Britain does not have traditional buildings, where the animals are still kept underneath the same roof where the farm family is living. The British farming situation is missing this so-called 'intimacy' with the animals.
Looking at the history of the hygiene hypothesis, in a paper by Gerrard (1976) he compared some native Americans, a tribe called the Metis, with their caucasian neighbours in a nearby affluent town. He noted that the Metis' prevalence of parasite infection, such as worm infection, was much higher than their non-Metis neighbours. However, they were protected against atopic conditions. Gerrard wrote: *"It is suggested that atopic disease is the price paid by some members of the white community for their relative freedom from diseases due to viruses, bacteria and helminths[2] ".*
The hygiene hypothesis was formally proposed by Strachan (1989), who observed that risk of atopic disease decreased with an increased number of siblings in the family.

My farming study was undertaken in Shropshire, a county on the border of England and Wales. It is a very rural farming area with a good mix of different farming types. The study was in two stages. The first was a questionnaire study sent to all the primary schools in Shropshire. Three groups of children were identified: The first was farming children. The second was labourers' children, where the father was working on the farm but not living on the farm, (because we wanted to know if there was any protective effect from the farm which was carried home by the father). The third group was other rural children living in the countryside as a control group. The combined groups of children numbered over 5000 between 5-10 years of age. A significant number of children were drinking unpasteurised milk. The children were asked about the frequency of consumption[3] . In Britain it is not allowed to sell unpasteurised milk in a shop, but it is allowed to sell it at the farm gate or give it to your friends or associates and this practice is relatively

[1] Hay fever
[2] Helminth are no factor in a Western setting nowadays. There are hardly Western children carrying worms. Parasites are very protective against eczema and asthma, like in developing countries, where you donot see these allergic diseases.
[3] Although the status of the consumed milk was not controlled, the tradition in Britain is to drink fresh, only pasteurised milk (shop milk). At dairy farms, no one is boiling the milk before consumption.

common. We also attempted to identify if there was any evidence of a dose response effect. How often do you have to drink the unpasteurised milk to get the protection? The complexity of the problem is shown due to differences found between the farm children and the rural children drinking unpasteurised milk. Farm children have significantly less asthma and significantly less hay fever, whereas the children consuming unpasteurised milk had significantly less eczema as well as hay fever. It shows that no one single factor explains the farm protective effect. The farm environment and the consumption of raw milk both protected against hay fever (Figure 1).

	Model one OR (95% CI)*	p value	Model two OR (95% CI)†	p value
Farm status				
Controls	1.00 (Baseline)		1.00 (Baseline)	
Labourers' children	0.70 (0.41-1.18)	0.18	0.74 (0.44-1.26)	0.28
Farm children	0.51 (0.33-0.79)	0.002	0.58 (0.37-0.90)	0.02
Unpasteurised milk consumption				
None	1.00 (Baseline)		1.00 (Baseline)	
Infrequent	1.01 (0.63-1.63)	0.97	1.06 (0.66-1.72)	0.80
Frequent	0.50 (0.30-0.82)	0.006	0.61 (0.36-1.02)	0.06

Figure 1. Both the farming environment as well as the consumption of raw milk independently reduced the risk of hay fever

Additionally in this study the researchers also looked at the consumption of organic food. Here there was no effect found (OR = 0,94) in contrast to the unpasteurised milk consumption (OR = 0,20). There were more impressive effects found with the farm related foods (vegetables, eggs, meat). The consumption of unpasteurised milk had a strong effect in all three groups. What kind of allergic child is protected? Like in the Parsifal Study we showed a difference in protective effect of drinking unpasteurised milk on atopic (= skin prick sensitised) and non-atopic (= non IgE sensitised) disease. For the non-atopic conditions there was not much evidence of an effect. The dramatic protective effect of drinking unpasteurised milk is found in the atopic children. It means that this IgE is somewhere involved in this protective effect.
In conclusion: current consumption of unpasteurised milk[4] was inversely associated with current eczema symptoms and seasonal allergic rhinitis, but not with current asthma symptoms. This contrasts with the protective effect of farming status on asthma and allergic rhinitis, but not eczema. When both unpasteurised milk consumption and farming status were incorporated in the same model with current seasonal allergic rhinitis as the outcome, both remained statistically significant.
Fundamentally farming children are healthy, although they do everything in contrast with the official advice from the government regarding animal contact and drinking of unpasteurised milk. This is the ultimate paradox.

Take home message: The issue of what is influencing children in their risk of atopy and allergy is clearly multifactorial. Independent of being a farm child, raw milk appears to protect against eczema and hay fever. The significant effect is found in the group of atopic children. There is an ultimate paradox about living on a farm: children are healthy, although they do everything in contrast with official governmental advice.

4 In Britain the government advises not to give cow's milk to children under the age of one. It can be used in cooking or it can be used on cereals. Mothers breastfeed their children or give a formula milk up until one year. Cow's milk as a drink appears very late in the first year of life.

Testing milk allergic children in a double-blind placebo controlled trial

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Milk allergic children were tested with two types of milk. In a pilot study, ethical permission was obtained to test 20 allergic children. The final test was the provocation test with different milk to decide if a child was a real milk allergic child. The doctor (Thomas Roos) is a specialist for skin problems and allergies. Levels of IgE were measured, a prick and patch test was done, and finally the children were controlled for this double-blind oral milk provocation. In the past the practice was to compare a rice milk (as a placebo) with a shop (i.e., conventional) milk. For this testing, instead of rice milk, raw milk was used from an extensive grass-based biodynamic farm, which fed hay in winter with low levels of concentrates. This milk was compared with the shop milk, from conventional origin, pasteurised and homogenised. In the study we established the biggest differences between the milk types in terms of farm practices (biodynamic versus conventional) and in terms of processing (raw versus homogenised and pasteurised). Intermediate steps were not taken into account in this pilot study.

> **Case Tim:** The following is an example of a child in the study. A six year old boy with severe symptoms and a long history of allergy. He exhibits severe atopic dermatitis, angioedema and urticaria, and is multi-allergic. In the clinical testing at day 1 the child gets small, stepwise increasing amounts of the two milk samples, starting with ½ ml up to 50 ml on day 2. With the conventional milk the child immediately reacts with angioedema of his lips and perioral skin after 2 ml of milk. In contrast, the boy did not react to the raw milk, even in higher amounts. Currently this child still consumed raw, biodynamic milk and milk products, and also tolerates butter well. The boy's overall health status included a mild atopic eczema due to egg and soy protein allergy.

Until now only 12 children have been tested. In 7 of 11 children with a positive prick and patch test, there was an allergic reaction to the processed conventional milk after several millilitres of milk provocation, whereas all children could tolerate the biodynamic raw milk. Several of these children now consume biodynamic raw milk from their own region.

The biodynamic raw milk was controlled for its fatty acid composition. Furthermore the authorities sampled the raw milk monthly from the bulk tank for the control of zoonotic diseases due to its status as 'Vorzugsmilk-farm' (see contribution of Tschischkale).
The CLA level showed the typical curve of an organic low-input farm feeding hay in winter. CLAc9t11 is low in winter (5-6 mg/g fat), but increases after the cows are pastured. CLA-levels gradually rise until September, and sometimes October, (CLAc9t11 23-29 mg/g fat), but then the levels decrease as the cows turn to their winter diet of mainly hay. N3-levels are at a similar levels in summer and in winter (12 mg/g fat).
The monthly milk samples for the license of producing 'Vorzugsmilk' were required by the state to monitor the hygiene measurements of the farm milk. It showed that Campylobacter, Salmonella, Listeria, Staphylococci could not be detected over the last four years. E.coli were sometimes too high, especially during summer. Since there is no specific testing for EHEC, an additional sampling of milk from individual cows took place to test for zoonotic vectors, both in their milk and in their dung. Of the 70 individual milk samples, taken in March and October 2008 from all milking cows, only 1 had Listeria; Salmonella and Campylobacter were not found. VTEC/EHEC was not found in the 70 milk samples. 38 individual cow faeces samples were analysed for EHEC only in March 2008 and in 5-6 faeces samples VTEC/EHEC was found.

There are several possible explanations for the difference in allergic reaction to the two types of milk. Pasteurisation induces the formation of beta-lactoglobulin and alpha-lactalbumin aggregates, which promotes the allergic sensitisation by enhancing the antibody and cytokinin production. It might also be, that unpasteurised milk includes a range of probiotic bacteria, which supports the reaction of the immune system. Vital enzymes in milk are made inactive after pasteurisation. Also, the high quality of fatty acids in terms of CLAc9t11 and n-3 levels might play a role, although in the contribution of Braun and Perkin at this conference, it was shown that the main effect comes from the unboiled milk. However, the farms in both studies are mainly conventional farms.

Take home message: In an oral provocation with different milks , young children with a clinical milk allergy can tolerate the raw milk of a low-input biodynamic farm in contrast to shop milk, that is, conventional, homogenised, and pasteurised milk. The patch and prick tests do not have the sensitivity to differentiate between reactions to the two types of milk.

Raw milk quality vs. raw milk safety – Walking a tightrope

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Milk is a very complex fluid containing appr. 13% of dry matter content with 4% fat, 4.7% Lactose, 3.3% protein, 0.75% salts and minor components. Thus, milk may be utilised as substrate by almost every microorganism, including pathogens. However, some components or mixture of compounds in raw milk are responsible for the protecting effect in asthma/allergy prevention [Waser et al., 2006; Illi et al., subm. 2011]. It could be shown in different studies that only raw (that is, unpasteurized, unadulterated) milk causes these beneficial effects, but the reasons and the mechanisms are still obscure. On the other hand, raw milk consumption cannot be recommended due to a range of microbes among others toxin producing organisms like VTEC. The most relevant microorganisms mentioned in literature are verotoxin producing E.coli (1.7%), *Listeria* spp. (4.7%), *Salmonella* spp. and *Campylobacter* spp. (4.0%). Thus, raw milk may bear the hazard of being a vector for pathogens. Although only 1.5% of all food-related outbreaks in the EU are caused by consumption of milk and dairy products, even one single case of disease is already too much. Raw milk products should therefore not be given to very young or elderly people, and people with weak immune systems. One case of raw milk-related outbreak has been reported from Germany in 2008: 23 school pupils who visited a farm and drank raw milk became ill; they showed gastro-enteritis and 2 pupils even developed HUS, since the raw milk was contaminated with VTEC, and *Listeria monocytogenes* and *Campylobacter jejuni* were also found.

The major research interest is to understand where these microorganisms come from and how barriers can be built up in order to stop the transfer into the milk. Milk in the udder of healthy cows is potentially sterile; contaminations start at milking and continue during dairy processing. The first source of contamination may be the contact with the outer udder where a cocci-dominated, mostly non-harmful flora can be found. However, unhealthy cows could infect the milk with a mastitis flora like staphylococci. The second source of contamination are surfaces of the milking equipment or also the human skin. The surface-borne flora rather consists of *Pseudomonas* spp., Corynebacterium, Enterobacteria, Endospore formers and again *Staphylococcus* spp. The most problematic contamination-flora comes from the faeces of the cow where pathogens such as *E. coli*, enterococci, *Campylobacter* spp., *Listera monocytogenes*, *Salmonella* spp., *Mycobacterium* spp., as well as viruses are detected. Finally, contaminations can come from the air, where yeast and moulds, but also endospore formers and pseudomonades can be found. Therefore, in freshly-milked milk, levels of 1.000-5.000 KBE/ml can be found.

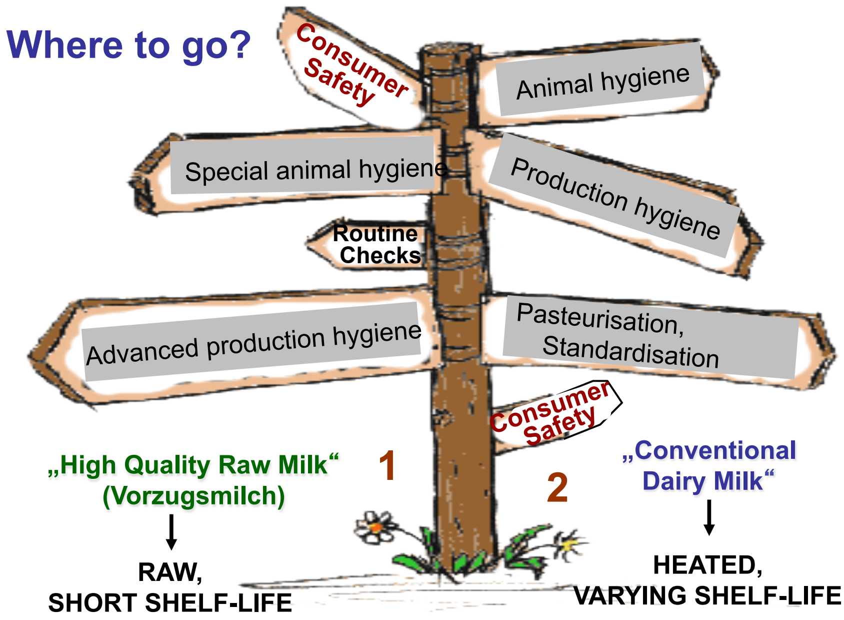

Figure 1. The complexity of (raw) milk safety in the future.

The central question is: Is it possible to produce raw milk that is totally safe for the consumers (Figure 1)? One way may be via the "Vorzugsmilch"-Track (Grade-A milk). This kind of milk underlies special animal hygiene and advanced production hygiene monitoring due to the extra attention and inspection by veterinarians. However, this milk has often a short time shelf-life. Moreover, the production of "Vorzugsmilch" is limited and not suitable for bulk production due to the following reasons: Infrastructural problems like the control of the herd, and the on-site packaging of the milk have to be faced. In addition, veterinary surveillance programmes have to be extended, whereas standardized microbial testing procedures are not rapid enough and inadequate for in-situ analyses. Also distribution logistics have to be addressed such as the maintenance of the cold chain and the short shelf-life. Last, but not least, the standard consumer is used to the sensory perceptions of pasteurised milk, and has to be trained again for the taste of raw milk.

Pasteurised milk is definitely safe in terms of pathogens, but the compounds of the raw milk, which possess the allergy protective effect, are no longer present. Pasteurisation and homogenisation induce unwanted changes in the milk composition such as protein denaturation effects. Another solution to obtain safe, but allergy-preventive milk ,could be to break down the milk into its components using modern technologies and separating out the compounds of interest. This is a challenge for the technologist. This technological solution has, in principle, already been performed by the dairy industry, when they were looking after several proteins with high functionality. It would be a challenge to select these compounds of interest within the raw milk, and then put the promising compounds together into a safe and decontaminated product. However, this is easier said than done. Although there are some hints that the asthma-/allergy-preventive effect is related to the protein, especially the whey protein fraction, it is still not clear, if also the casein, carbohydrate, fat or even combinations of diverse fractions are involved. Therefore, future research (including clinical studies) has to focus on the exact mechanisms. At the moment we are concentrating on different components of the whey fraction.
The whey fraction, however, is very diverse. There are several techniques nowadays to separate the proteins such as microfiltration, ultrafiltration, nanofiltration, ion exchange and adsorption chromatography. These methods could be used to produce certain compounds of interest. Thus, protein could be separated into its single components that may be used for the enrichment of pasteurized milk. Using this technique, safe but bioactive milk can be produced. During this process an in-situ monitoring system is needed in order to guarantee product safety (*"just in time"* monitoring). Safety relates not only to the milk as a whole, but also to the (mixture of) intrinsic components.
For further intervention studies a safe milk product is needed. This may be accomplished via a suitable primary production site, where high tech processes are implemented. The product then may be assessed for special target groups and for infants. A crucial point in "just-in-time" monitoring is the sampling method. You cannot control every produced product. A detection technology which can filter out the (un)wanted microbes is needed. This could be accomplished i.e. via immuno-magnetic separation technique. This method offers the possibility to enrich the desired microbes. By use of quantitative PCR techniques the number of living cells can be demonstrated. Within less than 1½ day the product safety may be approved. Nevertheless, there will never be a zero-risk, even if such techniques are more reliable compared to standard protocols. You have to bear in mind that pathogens such as *Salmonella* spp. and EHEC are hazardous even at very low concentrations. Therefore it will not be possible to guarantee 100% safety without including heating processes.
So, raw milk production and raw milk consumption is still walking on a tightrope. The challenge is to maintain the beneficial qualities of the product while getting rid of its risky side. Therefore we need modern, high tech solutions.

Take home message: With the consumption of raw milk there is always a risk of the transfer of harmful microbes such as EHEC or Salmonella. In the future, a tailored technology accompanied by suitable microbiological detection methods might support the production of a safe raw milk product.

Raw milk hazards and safety; Vorzugsmilch (Grade A raw milk) in Germany

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As a veterinarian, Tschischkale is a private consultant specializing in udder health, milk quality and milk hygiene for raw milk producers. Therefore he visits 8 members of the Vorzugsmilch-Verband (the German Association for producers of Grade A milk called Vorzugsmilch), about every month. In the 1950s and 60s there were still some 200-300 farmers producing this Vorzugsmilch. In his laboratory, quarter milk samples sent in from farmers and veterinarians are analysed for somatic cell counts, microbial spectrum, and antimicrobial testing. Farmers and vets are advised about treatment, but more importantly about prevention.

Vorzugsmilch – Grade A Milk – is raw milk, which fulfils very high standards that are high above the normal standards of production, concerning the health of cows, how they are kept, fed, and milked, and also milk hygiene, quality, and distribution. Production standards are regulated by law. The farmer bottles and distributes the milk by himself. State regulations concerning Vorzugsmilch were launched in 1931. About that time an Association for producers of Vorzugsmilch was founded. Before that there was the sale of milk mainly to the larger cities. In Prussia the first regulation for the selling of raw milk was issued, and was copied by the other German states. In that time the definition of Vorzugsmilch was that the milk should contain more than 3,0% fat, the total bacterial counts should be below <150.000/ml and Coliforms <30/ml. The absence of Brucellosis and Tuberculosis and illnesses that were present in that time was very important. In the countryside people drank a lot of raw milk, which was a vector of Tuberculosis. In 1987, EU-Milkhygiene-Guidelines (85/397/EWG) were formulated, reforming the milking hygiene laws present in the EU member states. The German interpretation was that it still gave farmers the possibility to produce and sell raw milk. In other European countries it was not legal to sell bottled raw milk. In 1989, there was a new Milk Directive (Milchverordnung) containing a renewed definition of Vorzugsmilch in Germany. It was only a slight adaptation of the previous guidelines for production. Since 1994 it is prohibited to sell raw milk in canteens of nurseries, public schools, senior homes and all other places with common catering. There was the first outbreak of E.coli, VTEC or EHEC (Entero Haemorrhagic *E.coli*). Although raw milk was not the reason for it, there was a huge media campaign about the dangers of EHEC in raw food. At the moment, individuals are allowed to buy Vorzugsmilch in shops or to buy raw milk directly at the farm. Farmers have to put a sign on their bulk tank as a warning, which says: 'raw milk, heat before consumption'. If people do not do so, this is their own responsibility.

The legal requirements for Vorzugsmilch acknowledge two different levels, 'm' as a minimum requirement and 'M', as a maximum requirement. There is a monthly testing of a bulk milk sample.

	m = at least 3 out 5 below the limit ,m'	M = allowed 2 out 5 between ,m' and ,M'
Total Bacterial Count/ml	20.000	50.000
Enterobacteriacae/ml	10	100
CPS = Coagulase+ Staph/ml	10	100
SCC = Somatic Cell Count/ml	200.000	300.000
Salmonella species/25 ml	0	0
Pathogenic microorganisms or their toxins, which could be harmful to humans are not allowed [1]		

[1.] *This could be Listeria, EHEC, Campylobacter, Query-Fever (Rickettsien)*

In the last 15 years we started to screen milk samples for EHEC in our laboratory. We examined about 100 milk samples per year from the Vorzugsmilch deliverers and up till now we only found one single case of EHEC. It shows that there is hardly any risk within this type of milk. In general the bacterial pressure is much higher in summer than in winter due to the higher temperatures and the greater possibility of bacteria multiplying.Too high levels of total bacterial counts or Enterobacteriaceae are in almost all cases not a problem of dirty cows, but of the milking equipment, pipes and bulk tank, which are not properly cleaned. The difficulty is that farmers have to be very constant, very rhythmic in what they are doing.

About the regulation of Vorzugsmilch: to start the production of Vorzugsmilch farmers need to go to their local veterinarian, who can advise them. The state-veterinarian gives the licence to produce. They can become a member of the 'Vorzugsmilch-Verband' (Association for Vorzugsmilch deliverers), but each farmer has to create his own delivery market. If approved by the authorities, farmers are then licensed to produce and distribute the Vorzugsmilch. The bottled milk has to be tested once a month at least. In the past every single cow had to be tested for its udder health status, but now only cows with an individual somatic cell count above 250.000/ml need to be checked for mastitis bacteria through the examination of quarter samples. Cows from which Vorzugsmilch is producedhave to be kept separately from the other cows. People who handle the milk and equipment have to be tested through faecal examination about their health status.

In the 1950s about 250 dairy farms produced and distributed Vorzugsmilch. In a way these producers were seen as snobbish, elitist. Farmers producing this milk earned a lot of money. In the 1980s there was a dramatic decline to about 30 farms, because the overall milk price was high and the distribution system with one litre bottles was too expensive and time consuming. In those days the prices for food went down and the price difference between Vorzugsmilch and other shop milk became too big. In the 1990s Vorzugsmilch came back: one dairy farmer started a delivery service for private households. Milk was delivered in 3 l bottles at the house door. About 100 farmers copied this way of delivery. In 1994 there was a media campaign concerning EHEC-outbreaks in food; however this was not created by raw milk. Nevertheless, to maintain their customers, farmers invested in expensive pasteurisation equipment. Therefore, they could now deliver pasteurised milk as well. Some of them found it easier to deal with pasteurised milk and milk products and ice cream instead of the raw Vorzugsmilch, and therefore stopped the production of this raw milk. As of 2010, there were 50 Vorzugsmilch producers and that number may decline. A small number of farmers (all organic) deliver raw milk only.

In Austria as well as Finland there is also a tradition of selling Vorzugsmilch. In the UK it is Grade A milk or blue top milk, which originally was also unpasteurised milk.

Take home message: The German Vorzugsmilch-production is a legal way to sell raw milk. Although the milk is strictly controlled, negative campaigns in the press about zoonotic diseases and mainly EHEC strongly reduced the interest of the consumers, although EHEC was hardly found in Vorzugsmilch over the last 15 years.

Dealing with EHEC risks in relation to health benefits of raw milk

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EHEC is one of the most problematic zoonotic diseases in relation to raw milk. The recent outbreak in the Hamburg area (State Niedersachsen) in June 2011 showed how risky EHEC can be. Over 30 people died and over 1200 people were hospitalised. In this case it had nothing to do with raw milk, but with uncooked vegetables and sprouts. The situation showed how explosive EHEC can be. New high-risk strains can develop rapidly (here O104:H4), and it seemed that human beings, rather than animals, were the bearers of this new, antibiotic-resistant strain.
The level of EHEC infection needed to become ill is low. People become ill because so-called Shiga-toxins are expelled by the bacteria, which belong to the species of E.coli. Even if the bacteria are killed, the toxins still might be active in milk. Additionally there is a risk of kidney failure, the so-called HUS-syndrome. The incubation of the EHEC is between 2-5 days. Until now we were dealing in most cases with O157 strains in relation to raw milk outbreaks.
The answer to EHEC contamination by officials is to increase hygienic practices (e.g., washing hands), heating of the food (milk included), the avoidance of eating salads, sprouts, etc., and avoidance of contact with farm- and zoo animals. Also, water can be a source of suspicion as people and water can bear EHEC and therefore risks sources are being re-evaluated. A cross-contamination through toilets from bearers is a possible vector for the spread of EHEC.
Besides a technological approach to find solutions, it is necessary to look for an ecological approach, in which the environmental prerequisites become clear, and how pathways of contamination can be avoided. Last but not least, it would be interesting to know if animals can become resistant against strains of EHEC without becoming EHEC bearers.

We are constantly dealing with risks and the deliberation of risks. This can be done for economical as well as for health reasons. However, an attitude of zero-risk tolerance in combination with an obligation for pasteurisation completely blocks the search for alternatives and essentially provides a prescription of what is acceptable to eat. This raises the issue of freedom in food choices.

Several studies have shown that the so-called 'Vorzugsmilk' or 'grade-A raw milk' in Germany has a much better hygienic standard than 'normal' raw milk sent to creameries and being pasteurised. These studies showed that, due to the attention to hygienic handling and processing of raw milk, in combination with a strict control system of raw milk for sale, there is already a huge reduction of risk factors. Total germs and other zoonotic diseases are low or even absent in 'Vorzugsmilk' in comparison to 'normal' raw milk.
Risks for Campylobacter, Listeria and Salmonella coming from 'Vorzugsmilk' can be negligible. In meat products, mainly chicken, the challenge of these zoonotic risks are much higher. From our own sampling at one of the biodynamic farms that was delivering the raw milk to test allergic children, neither of these germs was found. In 6 of 37 samples of cow's faeces, EHEC was detected, although it was never found in the raw milk itself. If E.coli only is measured at a farm it will only give a first indication whether EHEC also might be found, but you cannot rely on this. EHEC has to be tested separately. In the US, in beef before slaughter, the animals are put on a strict hay diet for about a month, which strongly reduced the challenge of EHEC expelling. The reduction was 80% (per oral communication from Michael Schmidt).
STEC, VTEC, EHEC as one of the E.coli strains are part of the digestion system of the cow. The O157 strain was detected for the first time in the 1980s and was called the 'hamburger disease', because the consumption of undercooked meat was the vector for the disease.
In literature there is no evidence that EHEC will not be found in organic cattle. The EHEC contamination in beef cattle was the same for organic and conventional cattle, although it was identified that EHEC levels increased as cows got more concentrates. There is a seasonal pattern, showing more EHEC cases during the summer months when cows are grazed and their faeces are more watery, and also when temperatures outside are higher. The number of HUS patients is higher during summer and autumn.

We analysed 27 well-described EHEC outbreaks found in case reports and health documents. We

were interested in the certainty about an EHEC cause. Therefore some criteria were developed, but the main elements were the logic of pattern recognition (f.i. case and control; dose and effect) and finally the possibility of detecting the same genetic type of germs in ill people as well as in the suspect food through genetic fingerprinting. Genetic typing is not always easy, because of the incubation time of the germs.
Raw milk dangers seem to be less in comparison to dangers from human to human contact, animal contact, dirty toilets, and unhygienic circumstances (such as sandboxes for children).

Take home message: EHEC-contamination in raw milk is a risk. EHEC was also found in the faeces of cows from an extensive biodynamic holding; however the bacteria were not found in their milk. Compared to 'normal' raw milk, farms delivering 'Vorzugsmilk' had much better control on the bacterial counts from all typical zoonotic diseases. Risks from this strictly controlled raw milk are very low; however, a zero-risk can never be attained.

Fighting for fundamental food rights

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I am here as a farmer, not as a scientist. The urgently needed scientific debate has been compromised through political actions. As a result, a constructive dialogue about the merits of raw milk for human consumption has been prevented. We have been witnessing this trend for the last 25 years in North America. Currently we are facing in the US the enforcement of the absolute no risk policy as it relates to raw milk. I have been involved in this battle for 17 years and the following comments have been made over and over again by Health Officials, Government agencies and the dairy lobby:

- Drinking raw milk is like playing Russian roulette.
- Drinking raw milk is tantamount to manslaughter.

It is a very overheated political debate, which currently prevents a constructive scientific dialogue about the real risks and how we can manage risks in a down-to-earth approach. It also prevents any debate about the obvious health effects associated with raw milk.
The discussion should not be about eliminating risks, which is impossible and would be clearly discriminating against raw milk. The approach has to be to minimize risks and initiate proper research of the claimed benefits.
This poster (Figure 1) expresses: "Liberty and the pursuit of raw milk freedom". The passionate raw milk debate in North America in fact has triggered in part the non-violent food-freedom resistance. In the meantime over 90% of the food on the shelves in North American supermarkets are already contaminated with GMOs, which indicates that the agricultural supply industry is definitely taking control over our food production. To quote Henry Kissinger: "Control oil and you control nations, control food and you control the people."
The debate about food and food safety is not any more within the scientific community or amongst the farmers. It has become a political power play. The industry itself has a tight grip on Government policy-making and therefore has almost everything under control, except those who start to resist and openly challenge the system. Raw milk itself has become an interesting component in the food freedom movement.

The traditional way of farming is on the way out. What we have now across the continent are more and more dairy farms with thousands of cows or feedlots with 50,000 cattle. Through the concentration of animal production there is an emerging pathogen pressure, caused amongst other factors by the uncontrolled use of therapeutical antibiotics. It is suspected that these industrial farms are also unintentionally breeding new forms of highly dangerous pathogens in a manner unseen before. If you are able to visit these huge farms you notice the lack of people. You see the animals and you see trucks and tractors driving around, but you will also observe the lack of human contact with the animals. The food production in North America is based on technology and utmost rationality. Across North America the separation of crop production and animal production is part of the industrial-based farming concept. The feed is coming from somewhere else, done by different corporations supplying cattle operations. Manure distribution and waste management become logistical and environmental problems of greatest concerns.
In contrast, at our own farm it is part of our holistic management that people understand where their food is coming from. They become co-owners. They can become co-workers and also enjoy taking part in harvesting, in building and in seasonal celebrations.
The centralisation of food production will ultimately lead to a total collapse of the small farms. Due to this a new desert is rapidly increasing in North America. Vast stretches of agricultural lands are deserted. One used to find a homestead on every 40 hectares (100 acres); now nobody lives there anymore.
Government Health Units promote health policies based on: "everything has to be sterile". Their concept of "food safety" disregards completely the nutritional aspect. However by now we know that we need enough bacteria to build-up our immune system.

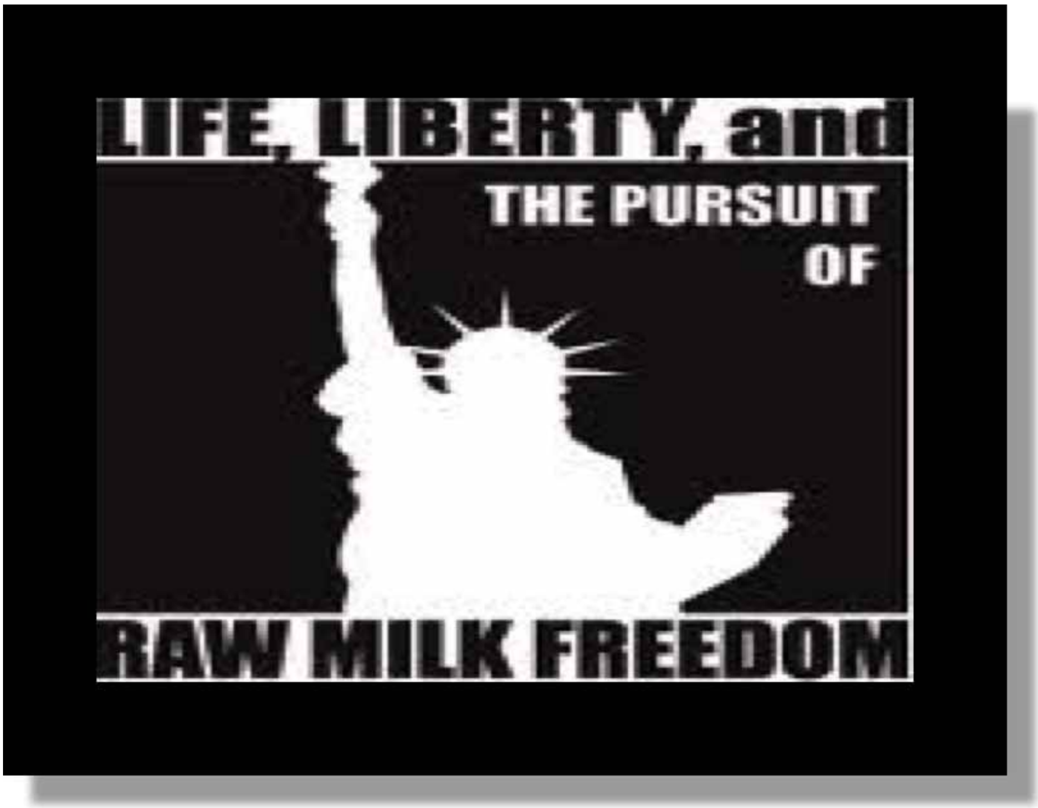

Figure 1. A documentary on the emerging food rights battle won the Food Oscars Award in New York

Figure 2. Families are connected with the farm through 'cow sharing'

In the shallow North American scientific debate, officials state: "... there is no proof of the nutritional benefit of drinking raw milk compared to pasteurized milk".
No study has been conducted on this subject in North America in the last 50 years.
In 2010 we decided at Glencolton Farms to conduct a small experiment by comparing a raw milk-fed calf versus a store milk-fed calf. The following observations have been the most obvious preliminary findings after the calves were butchered with 5 months. Both calves received the same feed except the difference of the daily ration of 8 litres of the different milks.
Pasteurized calf compared to the raw milk calf: Overall, the development of the pasteurized calf slowed down after two months. Lacklustre behaviour and lack of alertness during the last 3 months. The smell in the pasteurized calf pen was disgusting. Heart size 40% less than the raw milk calf. Very pale colouring of liver and kidneys. The digestive track was filled with foul smelling brown and runny soup compared to the other calf with solid and healthy smelling substance.

The testicle of the pasteurized calf was 30% smaller in size, hair loss and no shine of coat.
It is easy to dispute the findings based on just two calves. However it confirmed similar findings done in a similar, but widely- ignored study years ago. It would be crucial and very important to conduct a repeat study under the supervision of the scientific community.
In Canada, raw milk is banned. Half of the United States are also banning the sale and distribution of raw milk. A very strong involvement of the dairy industry to maintain the ban on raw milk has resulted in many violent attacks on individual farmers.
More and more a sometimes violent battle is unfolding similar in nature to drug raids across North America. On a positive note: after 17 years battling with the Ontario provincial government we unexpectedly won. The trial was about the right to cow sharing. Last year, after this highly public trial, we finally legalized "cow-sharing"in at least one Province in Canada.

Take home message: The idea of food safety should be opened up to look first at the issue of freedom of choice and personal responsibility and, secondly, on ways of understanding the ecology of the involved zoonotic micro-organisms. At the farm level, cow sharing is a legal way to sell raw milk to families. There is an urgent need to expand the research on the apparent health benefits of raw milk and the increase of allergies and lactose intolerance.

Fatty acids in breast milk and organic dairy, and the risk of atopic disorders

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Atopic disorders are diseases based on IgE mediated allergy. It includes hay fever and other airway allergies like asthma, and a food allergy that often manifests itself as eczema. The prevalence of atopic diseases has risen in the last 30-40 years throughout the Western world. Studies in other countries made clear that this increase is a real increase.
The hygiene hypothesis has been put forward as an explanation for this increase, and this hypothesis is also relevant to explain the protective effects of raw milk or farm milk. However, there is an additional hypothesis, involving fatty acids (FAs). The background is that over the last decades the FA composition of our diet has dramatically changed, with a presumed shift in the ratio between omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids (PUFAs). There has been an increase in the use of oils high in linoleic acid (n-6) in margarines and cooking oils, and on the other hand a decrease of n-3-PUFAs due to lower intake of green vegetables and fish.

n-6 and n-3 FA and inflammation
N-3 and n-6 FAs are precursors for biologically active compounds. Linoleic (LA) (n-6) and alpha-linolenic acid (ALA) (n-3) are considered essential FAs. From these precursors, long chain polyunsaturated fatty acids (LC-PUFAs) are synthesised by the human body, such as arachidonic acid (AA) (n-6), and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), both n-3 LC-PUFAs. Apart from endogenous syntheses, these LC-PUFAs can also be derived from our diet, for instance AA from red meat and eggs, and EPA and DHA mainly from fatty fish, hence the name "fish FAs". These LC-PUFAs are precursors for endogenous synthesis of eicosanoid mediators of inflammation, like prostaglandins (PGE2 and 3) and leukotrienes (LTB4 and 5). The mediators produced from AA have a stronger pro-inflammatory activity than those from the n-3 LC-PUFAs. The hypothesis is that the intake of FAs from our diet determines the balance between the more or less pro-inflammatory mediators, and that a shift in the direction of n-6 increases the risk of allergy.

Breast milk and fish fatty acids
Several studies have looked to the FA composition of breast milk, and a high proportion of n-3 LC-PUFAs is associated with a lower risk of eczema and wheeze in the child at subsequent follow-up. Also, numerous dietary studies have confirmed that high fish intake during pregnancy is associated with a lower risk of atopy in the child, and this is generally attributed to the high n-3 contents of fish. However, randomised trials with fish oil supplementation have not been convincing until now, neither for prevention nor cure of atopic diseases.

Milk and ruminant fatty acids
Dietary studies have also shown that use of whole-fat milk by children is associated with a lower risk of atopy compared with low fat milk, and the same holds for butter vs margarine. This is not clearly predicted by the FA balance hypothesis, and therefore other FAs in milk products may also be relevant, like conjugated linoleic acid (CLA). In cow's milk as well as human milk the most abundant CLA isomer is CLAc9t11 (rumenic acid, RA). It is produced by bacterial hydrogenation of C18:1t11 (trans-vaccenic acid, tVA), the main trans-fatty acid in cow's milk as well as in human milk. There is some endogenous production of RA in the human mammary gland, but about 90% is derived from maternal diet, mainly cow's milk, but also from the fats in meat from ruminating cattle.
In rodents, dietary supplementation trials have shown effects on markers of allergic inflammation. RA supplementation reduced the production of the inflammatory mediators PGE2 and LTB4, and the production of IgE and airway hyper responsiveness. The combined supplementation of RA and tVA also reduced IgE production as well as airway and skin inflammatory responses, with a synergistic effect of RA and tVA combined.

KOALA Birth Cohort Study
In the KOALA study we evaluated the role of FAs in the development of eczema and allergic sensitisation. The FAs composition in breast milk was taken as a marker of FA supply to the child

during pregnancy and lactation. Effects of both n-3 LC-PUFAs ("fish FAs") and tVA and RA as markers of "ruminant FAs" were evaluated.
Pregnant women were recruited among people with conventional lifestyles from the general population via obstetric practices. A second group was recruited among women with alternative lifestyles to increase the contrast of interesting exposures such as low vaccination and antibiotic use, and use of organic foods. Data were collected through repeated questionnaires from pregnancy on, including a food frequency questionnaire at 34 weeks of pregnancy. Children were followed-up by parental questionnaires, with a high follow-up rate of over 90% in the first two years. In a subsample of participants, home visits were done by research nurses. They collected a breast milk sample at 1 month postpartum, and blood at age 1 and 2 years to test for allergic sensitisation. At age 2 they also examined the child for eczema using the UK working party criteria for atopic dermatitis.

Results: eczema and allergic sensitisation
The parental questionnaires indicated that eczema was present during the first year in 19% of the children, and this increased to 30% in the first 2 years. Half of these cases could be confirmed at the home visit at age 2 years, the other half may have resolved at that time, or may have been over-reported by parents. Allergic sensitisation was present in 13% of the children tested at age 1 year. Specific IgE against cow's milk, hen's eggs and peanuts were measured. At age 2 years, 5 airway allergens were added, and 25% of the children tested positive for at least one of the food or airway allergens. These percentages may seem high, but are fairly comparable to other studies in westernised countries.

Results: fatty acids in breast milk
The different n-3 LC-PUFAs in breastmilk were highly correlated and summed up as 'fish FAs'. TVA and RA were also highly correlated. The correlation between RA and fish FAs was very low, confirming their distinct dietary sources and reflecting different dietary patterns of the mothers.

Results: fatty acids in breast milk and atopic outcomes
We found a negative association between atopic outcomes in the child in the first year of life and fish FAs in breast milk. At age 2 years the results were very similar for eczema (odds ratio 0,43), and was confirmed for atopic dermatitis using the UK working party criteria. Also wheeze in the first year of life showed a similar decreasing trend although the number of children was too small for further analysis.
In a multivariate regression analysis with both n-3 LC-PUFAs and tVA in the model, both groups of FAs were independently negatively associated with eczema, with very similar odds ratios, indicating that the effect sizes were in same order of magnitude. For RA, the results were fairly similar.
Results for allergic sensitisation were in the same order of effect sizes and also independent for the two types of FAs. However the associations between FAs and allergic sensitisation were only present at age 1 year, not at age 2 years.
This could be due to the first year being a critical period for allergic sensitisation, or could reflect the period when the child is dependent on breast milk, whereas after age 1 year the child's own dietary intake takes over and its enzyme system is fully developed to synthesize n-3 LC-PUFAs from its precursor ALA.

In a separate study within the KOALA study, we evaluated the associations with organic food choice and atopic disease. We asked the mothers to indicate whether they choose foods within food groups (e.g. dairy products, vegetables) mostly from conventional origin, mostly from organic origin (>90%) or in between. This was done for maternal diet at the end of pregnancy, and for the child's diet at age of 2 years. A lower risk of eczema and allergic sensitisation in the child was found when dairy products in the child's diet were from organic origin, and an even stronger protective association was found for the mother's diet during pregnancy. This finding is specific for dairy products since the origin of other food groups was not associated with eczema or allergic sensitisation in the children.
During breast feeding the lowest levels of RA in breast milk was found in mothers who do not use dairy products at all, while the highest levels were found in mothers who use dairy products from mainly organic origin (Figure 1). This suggests that RA from organic dairy are implicated in

the protection against atopic diseases. It also confirms that breast milk composition is dependent on maternal diet, and hence the protective effects of breastfeeding may differ according to maternal diet.

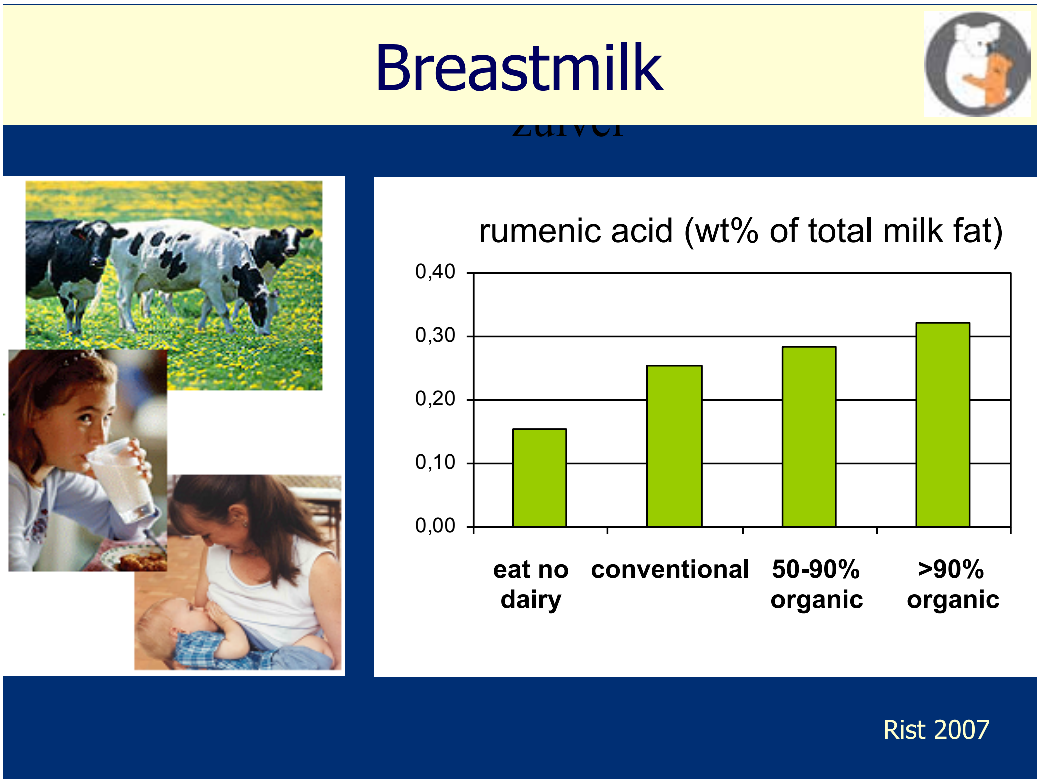

Figure 1. CLAc9t11 is increased in breast milk depending on the percentage of organic milk produce consumed by the mother.

Summary and conclusions

The results from the KOALA study confirms earlier studies indicating the fish fatty acids in breast milk are protective against eczema and allergy. Moreover, we find similar effects for ruminant fatty acids. This confirms experimental studies in rodent animals that showed that the effects of RA and tVA on atopic manifestations are causal. Dairy products from organic origins used by mother during pregnancy and lactation, and by the child thereafter, were associated with a lower risk of eczema and sensitisation, and this may be explained by the high levels of RA and tVA in dairy products from organic origin.

Acknowledgements and further information

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Take home message: For mothers during pregnancy and breastfeeding, and for children thereafter, full fat milk products and especially those from organic origin may help to protect against atopic diseases, especially when fish intake is low.

Fatty acids of organically produced milk and its beneficial effects in human nutrition

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Milk lipids have a lot of fatty acids (FAs), about 200. These include: short chain FAs, which are very important for the nutrition of children; medium chain FAs; and branched chain FAs. BCFAs are found especially in organic milk. C18:1 is present in different isomers, trans and cis. The poly unsaturated FAs, like linoleic acid, alpha linolenic acid (ALA) and conjugated linoleic acids (CLA) are also present. The content of FAs in the cow's feed is important. The microbes in the rumen influence the metabolic pathway of FA destruction. Finally there are endogenous enzymes, which influence the metabolites of FAs. The most important CLA is c9t11. A marker for milk of alpine regions is CLAt11c13. Extensive pasture-based feeding without concentrates results in several isomers. Intensive feeding leads to a dominance of CLAt7c9 and CLAt10c12. To analyse the CLAs,HPLC is used and one needs a 200m length of the column to separate cis and trans isomers. The balance of isomers in milk is very stable and not affected by heating or other processing. Milk is very rich in saturated FAs (SFA), and low in monounsaturated FAs (MUFA). This ratio can be changed by feeding, for example of linseed- or rapeseed-cake. There is a German dairy that pays farmers an additional price for a better FA profile, depending on the ratio SFA and MUFA, especially oleic acid (C18:1c9). This change in ratio also increases the levels of n3-PUFAs. However, there is at the same time an increase of trans-FA (tFA). Authorities will try to reduce the level of tFA in food, because they believe tFA in the human diet is 'bad' and PUFA in the diet is 'good'. However, it is not that simple and it is also incorrect. From this point of view organic milk will be bad, because of its high content of tFA. It is very important to fight against this interpretation. Consider that C18:1t11 (vaccenic acid, VA) can be converted in humans to CLAc9t11 though enzymes. So, if humans consume milk with high levels of VA, it can be converted to CLAc9t11, which has similar positive effects as n3 FA, namely the anti-inflammatory effect. Most diseases are increased by inflammatory factors. Anti-inflammatory nutritional factors will prevent diseases like rheumatism, atherosclerosis, etc. These FA are incorporated in the cell membrane and the signalling processes inhibit, for instance, the building of cytokines. Cows fed indoors with conserved fodders and concentrates have low concentrations of VA. Also there is a decrease of CLA. In organic milk where cows are grazed, the levels are much higher. C18:1t10 is normally very low in milk. When the level of C18:1t10 rises in comparison to C18:1t11, cows are fed improperly due to the high levels of concentrates in the diet (Figure 1). Besides the grazing policy and the other fodders, levels depend on the height above sea level where cows are kept. CLA levels are different among the ruminants. In ewe's milk it is very high (> 6%). CLA products are also offered in shops, but they only have two isomers: CLAc9t11 and CLAt10c12. In rat studies the animals were given soybean oil with a good level of CLAc9t11. If rats were given CLAt10c12, there was no fat in the body and all the fat was concentrated around the liver, the so-called fat liver syndrome.

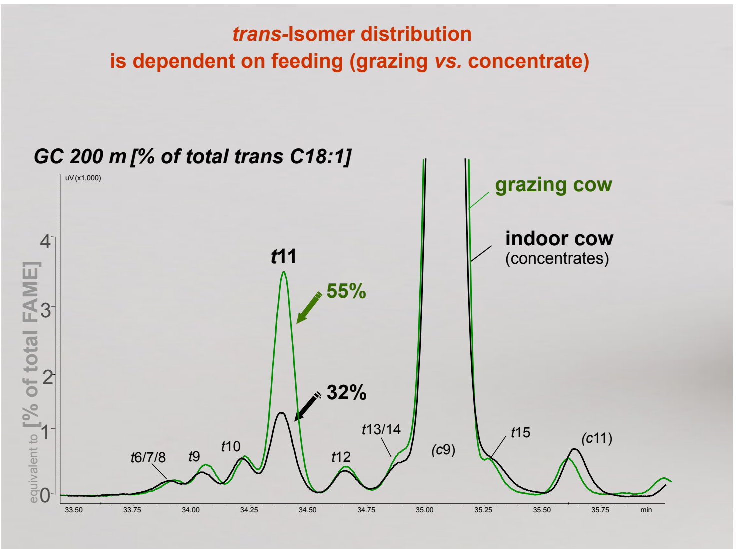

Figure 1. Trans-FAs are important elements of grazing cows; differences in C18:1-isomers of grazing cows and cows fed indoors with concentrates.

Through in-vitro studies in Jena with cell cultures and mouse models, single FAs, not complex triglycerides, were tested. In the cell studies there was a decrease in cytokine expression due to CLA c9t11, especially at higher concentrations. This isomer also decreased the level of arachidonic acid (AA n6), which is known as a pro-inflammatory FA. In mice studies, the effects of different FAs were measured. With linoleic acid, the highest airway inflammation was found, while CLA c9t11 significantly decreases airway sensitisation. So, CLAc9t11 reduces inflammation, which is a beneficial effect of this CLA.
Humans consume milk as a whole product. In studies of African Maassai it was measured that those people have a high level of VA in their red blood cells. The highest level was found in Bulgarian mountain farmers who lived from their ewe´s milk. The ratio of C18:1t9 (from margarine) to C18:1t11 (from milk) (= C18:1t9/C18:1t11) is extremely low in those herdsman, Bulgarian and Maassai, but extremely high in the German control group. So, analysis of the red blood cells expresses what kind of fat was digested. There are special markers in milk for extensive farming: CLAc9t11 is more than 80% of the total CLA, the second highest percentage comes from CLAt11c13.

Take home message: Milk fat composition depends on the feeding of the cows. Biomarkers like CLAc9t11 are characteristic of the extensive grazing of cows. Trans-FAs in milk cannot be compared with industrial trans-FAs. Publicity encouraging the reduced consumption of all trans-fats, milk fats included, is misleading. In relation to health promotion, several FAs have an anti-inflammatory potential. Besides n3 FAs, CLAc9t11 and C18:1t11 also play a role in this regard.

Fatty acid and anti-oxidant profiles of biodynamic and conventional milk from low- and high-input systems in summer and winter.

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The study design was done against the backdrop of the on-going debate over the product quality of organic food. At the start of our trials there were no consistent parameters to distinguish between organic and conventional quality. The IFOAM guidelines mention the 'principle of health' as one of the elements of organic production. Consumers are not aware of the scientific debate, but they think that organic food has a higher quality and health benefits for them. Therefore we designed a two factorial comparison system at farm level. We compared the milk fat quality of four farming systems, based on the factor origin: conventional (C) versus biodynamic (B) and based on intensification level: low input (LI) and high input (HI) systems. Each group consisted of six farms and we sampled bulk milk in two seasons bimonthly: during the grazing period and in winter, when the cows were fed only conserved fodders. Records were kept about the cow's actual diet at sampling. We analysed fatty acids in milk fat and anti-oxidants.
During summer both LI systems were mainly based on pasture: B through grazing, C often through feeding of fresh grass in the barn. Both HI systems were characterised by the feeding of grass silage and maize silage, and a reduced intake of fresh grass. The concentrate intake differed between the groups: very low in BLI to balance the energy-intake of the animals and high in CHI to stimulate the milk production.
In winter both LI system groups completely switched to a diet basically of hay. At CLI farms the milk is used for hard cheese production. In BLI systems it is also a matter of principle to feed dairy cows hay.

In a reflected discriminant analysis the greatest separation was between BLI and CHI, whereas CLI and BHI showed overlapping on the main, horizontal axis of the 1st reflective component. Further separation of these last two groups was possible due to the second, vertical axis.
The complexity of the contrast between BLI and CHI was reflected in a combination of FAs, which are inter-linked in the groups.
If the fodder intake was passively correlated with these axes, it could be shown that the higher percentage of grass in the cow's diet and, to a lesser extent, the higher percentage of hay in the ration were connected with the BLI group, whereas the percentage of high levels of concentrates, maize silage and grass silage was connected with the CHI group.
It can be concluded that there is a consistent difference found between the four system groups, as well as for origin and intensity. The BLI system showed the highest relevant FA concentrations related to health topics. The systems BHI and CLI were in an intermediate position in between BLI and CHI. The most important factor separating the systems in summer was the ratio of grass versus the total mixed ratio of concentrates, maize silage and grass silage. Finally the intensity of the system is very important both in C and in B for a high milk fat quality, and the intensification of organic systems can be seen as a threat for the authenticity of organic milk. Grass based systems are very important for the future of organic dairy production.

Take home message: The intensification of organic dairy farming through copying conventional feeding practices (that is, through feeding of silages of grass and maize and elevated levels of concentrates) reduces the means of distinguishing between conventional and organic milk.

Comparative cow milk metabolomics: a GC-MS pilot study on effects of roughage, farming style and season

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The complex composition of milk is of growing interest for dairy scientists and farmers. The enormously large number of biomolecular constituents of milk and their huge concentration of differences relate to the numerous aspects and effectors of lactational biology such as feed conditions and well-being of the cow as well as nutritional and regulatory needs for the development of the calf. Recent progress in bioanalytics has made it possible to approach the entire composition, measuring a wide range of substances in parallel by means of omics technologies and, thereby, assessing profile data. Separating numerous subfractions of milk and applying state-of-the-art bioanalytics to each, about 270 proteins have been detected by proteomics, about 200 fatty acids by lipidomics, and about 220 metabolites by metabolomics, so far. These numbers will further increase, since many substances already found are still unidentified and not yet in the compound databases required to identify constituents. In a pilot approach, we explored the potential of gas chromatography mass spectrometry-based metabolomics to differentiate distinct conditions of milk production. We extracted milk in a single step with methanol, assessing broad metabolite profiles in order to compare milk samples from two studies. First, a cross-over study in one herd with two types of hay feed, and second, the study of Daniel Kusche on four groups, differing in origin and intensity, with six herds in each group and sampling throughout summer and winter.

In the cross-over study, cows were fed with hay of permanent pasture, which was dominated by grasses and herbs or with hay of resown leys, which was dominated by grass and red clover. After depletion of lactose, about 80 metabolites could be identified, mainly sugar derivatives, organic acids and amino acids. Most of the metabolites showed level variations correlated with the feed, with 14 of these significantly differentiating the two roughage types. The interpretation of the metabolite profile differences is still fragmented, since we do not know yet enough about their meaning with regard to the physiology of the cow, the calf and the rumenal microbes in the calf. Remarkably, feeding hay of ley to cows elevated the precursors of fat synthesis, which could be seen as supporting metabolic processes related to growth and energy storage in the calf.

In the second study, the two-factorial comparison of systems, B vs C and LI vs HI, 36 metabolites were identified without prior lactose depletion. Variations were greater than in the hay study. Summer and winter milk were clearly distinguished, with profile differences being largest in summer. 15 metabolites significantly discriminated farming styles, while 11 separated input types. With regard to physiological meaning, feeding mainly hay in winter and grazing on pasture in summer might be characterized as supportive for 'integration and structure formation', while less roughage and more concentrates favor 'growth and energy storage'. A combination of 10 analytically robust metabolites, differentiating either one or both factors with high significance, proved to be strongly indicative for each of the four groups in summer and winter, respectively. Such indicator sets rather than single biomarkers will become important and useful in future research.

Take home message: Metabolomics is a powerful technology to elucidate the complex composition of milk. Metabolite profiles make it possible to differentiate milk from different origins and feeding protocols. Such multiplex bioanalytics will help us to better understand lactation and the determinants of milk composition and quality.