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Max Rubner Conference 2013

Health Aspects of Milk and Dairy Products

October 7-9, 2013
Karlsruhe, Germany

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Speaker Abstracts

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Speaker Abstracts

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Milk and dairy products – dietary recommendations and current intakes

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Milk and dairy products (e.g. yoghurt, cheese, whey products) are fundamental elements of traditional Western diets. Major constituents of milk/dairy products include protein, fat, carbohydrates, vitamins, minerals and trace elements. Fermented milk products further contain a range of beneficial bacterial products generated during fermentation. Traditionally, milk/dairy products make a substantial contribution to the overall intake of proteins, vitamins B2 and B12 as well as of calcium and zinc, and thereby contribute to overall diet quality.

Dietary recommendations of many national nutritional institutions in the Western world reflect the high nutritional value of these foods. Within Europe, the recommendation for the intake of milk/dairy products ranges from 250 g/d (Germany) to 568 g/d (United Kingdom). Similarly high recommendations are made in the Dietary Guidelines for the Americans 2010 (USA) and in the Australian Dietary Guidelines. All recommendations emphasize the intake of low-fat rather than full-fat dairy products to benefit from the higher nutrient density.

Recent European national consumption surveys give an overview of the current intake of milk/dairy products. Northern European countries, such as Finland, continue to have a high intake (FINDIET 2007, adults: F 367 g/d; M 445 g/d); similar high intakes were reported for the Netherlands (DNFCS 2007-2009, adults: F 296 g/d; M 334 g/d). Significantly lower intake levels were reported for France (INCA2 2006-2007, adults: F 217 g/d; M 208 g/d), Germany (NVS II 2006-2008, adults: F 197 g/d, M 202 g/d), and Italy (INRAN-SCAI 2005-2006, adults: F 183 g/d; M 157 g/d). The overall trend for the consumption of milk/dairy products suggests a decline in the consumption of milk in Western countries, while the intake of cheese is increasing.

Based on the current consumption level of milk/dairy products in Germany which provide 12 % of total energy intake, milk/dairy products contribute 40 % of calcium intake, 27 % of the intake of vitamins B2 and B12, 19 % of the protein intake and 17 % of the zinc intake. However, besides calcium, the total intake of these nutrients already exceeds dietary recommendations. Similar contributions of milk/dairy products to the total intake of micronutrients have been reported for other Western countries including the Netherlands and France.

In conclusion, milk/dairy products are recommended by major nutrition institutions in the Western world. Although the intake of milk/dairy products is slightly decreasing, they still contribute a significant amount to the overall intake of key essential nutrients in human nutrition.

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The effect of feeding and milking system on nutritionally relevant compounds in milk

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Our view on milk as a healthy food has changed over recent years. Whereas it's high quality protein and valuable source of calcium have always been appreciated and it is a recognised source of other nutritionally desirable functional compounds including bioactive peptides, vitamins, antioxidants, minerals, phytoesterols. The casein and whey proteins in milk are ideal for the needs of mammalian neonates, and they, along with bioactive peptides, originating from their digestion, are associated with antimicrobial, antiviral, opioid, anticarcinogenic, antihypertensive, antithrombotic and immunomodulating actions.

On the other hand, our attitude towards its fat contribution is now questioned and tends to be dictated by the predominance of saturated fatty acids (SFA), which tend to trigger alarm bells with respect to our health. However, milk also contains unsaturated fatty acids (many unique to ruminant milk and meat) including oleic acid, conjugated linoleic acid (CLA), omega 3 fatty acids (n-3) such as alpha linolenic acid, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and to a lesser extent docosahexaenoic acid (DHA); these have been linked to a reduced risk of CVD, certain cancers, obesity, and type 2 diabetes and enhanced immune system function. Switching to semi-skimmed or even skimmed milk and low fat dairy products might well achieve the goal of reducing our intake of SFA but this also reduces our consumption of these beneficial fatty acids, some of which are already lower than recommended. Milk composition does vary and research has given us an insight into how we can manipulate the relative proportion of nutritionally relevant compounds in milk, including individual fatty acids; which might be a more prudent approach.

Milk fat composition is a direct consequence of digestive physiology of cattle and sheep. The microbial populations in the rumen might well allow them to utilise fibrous forages and by-product feeds unsuitable for our diet (in contrast to mono-gastric livestock like pigs and poultry that are in direct competition with us for food/feed) but less than 95% of dietary PUFA leaves the rumen intact and becomes incorporated into milk and meat. The high SFA content of milk, beef and lamb is a consequence of this hydrogenation process or loss of PUFA during digestion, although if not fully implemented, it also generates a range of isomers of CLA and their precursors, many of which have proved to be nutritionally relevant.

As with so many traits, milk composition is under both genetic and environmental control. As a general rule the acid profile and antioxidant content of milk are largely influenced by livestock feeding, in contrast, its protein composition is considered to be predominantly under genetic control. In reality however, the overall composition can be manipulated by breeding, feeding and diseases control of the dairy cows, sheep and goats and this paper discusses aspects of livestock management that can be exploited to enhance the content of nutritionally relevant compounds in milk.

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Current examples of the effect of milk processing on nutritionally relevant milk components

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The lecture will cover the following topics:
(1) Thermal treatment and novel preservation technologies of milk
(2) Lactose hydrolysis and glucose isomerisation in milk and whey
(3) Enzymatic crosslinking of milk proteins and prececal digestibility

(1) Extended shelf life (ESL) milk has a durability of about 3 weeks under chill chain conditions and fills the gap between high-temperature short-time (HTST)-heated milk, which typically is assigned a shelf life of 10 days and ultra-high temperature (UHT)-heated milk, which can be stored for a few months at room temperature. In a study of the Max Rubner-Institut, different drinking milk samples from 17 German dairies were analysed. HTST-heated products showed lower contents of non-denatured lactoferrin, serum albumin and immunoglobulin in comparison with raw milk, while the average content of the main whey proteins did not differ significantly. The contents of non-denatured whey proteins in ESL milk are highly dependent on the manufacturing technique. In UHT-heated milk, heat sensible proteins like lactoferrin, serum albumin and immunoglobulin were not detectable. The furosine content of HTST-heated and ESL milk types was between 5 and 20 mg/100 g protein, but ten times as high in UHT-heated milk. As long as the milk samples were stored at 4-6°C, no vitamin losses were detectable, and comparable concentrations of vitamins were found in the different types of drinking milk. In addition, novel preservation techniques for drinking milk will be compared in relation to whey protein denaturation. An exemplary manufacturing process for drinking milk with a high content of non-denatured whey proteins will be presented.

(2) During the processing of lactose free milk, transgalactosylation occurs apart from hydrolysis, and leads to the formation of galacto-oligosaccharides (GOS). Up to 5% of the lactose is transformed in GOS, depending on the type of β-galactosidase (β-GAL) used. In further studies, the potential of β-GAL and glucose isomerase (GLI) to enhance the sweetening power out of lactose and to generate GOS and lactulose was studied.
UF-permeates of skim milk, sweet whey, acid whey and lactose solutions were incubated with β-GAL and GLI. Lactose hydrolysis was 96-99%, glucose isomerisation about 50%. On a scale from 0 to 5, the intensity of sweetness increased from 1 to 3. The use of these food ingredients in the manufacture of dairy products and other foodstuffs may lead to significant reduced total sugar contents. Applying the bi-enzymatic system to 400 g/l lactose solutions led to synthesis of about 200 g/l prebiotic GOS. Several products, such as 6-galactobiose, allolactose and 6-galactosyllactose were identified. In addition, up to 30 g/l lactulose was formed. Besides the enhancement of sweetening power, the novel bi-enzymatic process provides a potential health effect by the generation of GOS and lactulose.

(3) Crosslinking of food proteins by transglutaminase improves especially techno-functional properties like water binding, gel forming and heat stability. In order to examine whether crosslinking reduces protein digestibility, the prececal digestibility of caseinate was studied in Goettingen miniature pigs. For *in vivo* investigations four boars with a T-cannula at the ileum were each given a semi-synthetic test meal containing 30 g of native or crosslinked caseinate, which were labelled with the stable isotope ^{15}N. The protein digestibility was determined from ^{15}N recovered in the ileal chyme. The indigestible markers chromic oxide and polyethylene glycol 4000 were added to the test meals in order to compensate for the chyme lost and to determine the flow-rate of the liquid phase of the digesta. Neither the quantity nor the dry matter of the chyme showed significant differences during the 33 hour collection period after feeding the test animals. Furthermore, the kinetics of the digesta-flow were similar. The calculated protein digestibilities were 92.3% for caseinate and 91.9% for crosslinked caseinate, which are not significantly different.

Likewise, the quantities of endogenous nitrogen, i.e. the nitrogen secreted into the gastrointestinal tract during digestion, were not significantly different after the two test meals. The results of this study indicate that crosslinking of caseinate by transglutaminase changes neither the normal physiological process of digestion nor the protein digestibility.

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Natural presence of steroid hormones in bovine milk

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Hormones are chemicals produced by the endocrine system of animal organisms to act as messengers that coordinate their physiological activities. There are different types of hormones (peptides, amino acids ...) but one of the groups we hear about more often is the steroid hormones, which are derived from cholesterol. Steroid hormones are produced in the adrenal cortex, testis, ovary, and some peripheral tissues and they are responsible for many important vital functions such as growth, sexual development and reproduction. This hormone group includes mineralocorticoids, glucocorticoids, progestagens, androgens and estrogens. Sex steroid hormones, i.e. progestagens, androgens and estrogens, are a group of steroids that play a number of important physiological roles and have interesting effects in animals. Due to the ability of these compounds to improve weight gain and feed efficiency in meat producing animals, and their capacity to regulate reproduction and/or oestrous cycle, natural sex steroid hormones and/or synthetic compounds with similar effects have been used in animal husbandry for several decades.

The hormones in general and more precisely the sex steroids are an essential part of life. They are naturally produced in the bodies of animals, including humans, and they control important body functions. However, exogenous steroidogenically active compounds may interfere in the hormonal endogenous equilibrium affecting health and natural body development. The Directive 96/22/EC prohibited the use in stockfarming of certain substances having a hormonal or thyrostatic action, in order to protect consumer's health. However, endogenous hormones cannot be completely avoided in food of animal origin, since they are part of animal metabolism and they support life. All foodstuff of animal origin may contain steroid hormones and metabolites, but their concentration varies with the kind of food, species, gender, age and physiological stage of the animal. Bovine milk contains considerable quantities of hormones and is of particular concern. As a result of modern farming and animal breeding, today's milk originates from genetically improved dairy cows such as Holstein, which are pregnant during most of their lactation period. In this research, liquid chromatography tandem mass spectrometry (LC-MS/MS) methodology has been applied for the quantification of several sex hormones in milk. Raw milk samples from pregnant and non-pregnant cows have been analysed. As well, a series of baby formulas have been analysed, confirming the presence of hormones in these baby foods. Basing on the obtained data, it could be concluded that maximum daily intakes for hormones are not reached with milk ingestion. However and although dairy products are an important source of hormones, other products of animal origin must be considered for intake calculations.

The author has nothing to disclose.

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Oligosaccharides in milk from various species: components, quantities, and functional significance

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Since the discovery of human milk oligosaccharides (HMOs) more than 60 years ago, research has faced major challenges including (i) the development of methods to identify and characterize these components, (ii) the need to use HMO fractions for functional studies since single HMOs were not available, and thus (iii) the low availability of large quantities of single HMOs for animal and human studies. In the last 10 years, there has been a tremendous progress in all these areas. Based upon in vitro experiments, animal studies and a few association studies in humans, many functions of HMOs have been proposed. Recent animal studies support HMOs functions shown in vitro. Concomitantly with these observations, progress in biotechnology today allows the production of at least some of the major HMOs to be potentially added to infant formula or food in general. To be able to decide which compound should be used in which concentrations or combinations, studies are needed regarding absorption, metabolism and physiological functions in infants.

Currently, there is also a great interest in characterizing milk from various animal species to potentially be used for the isolation of milk oligosaccharides for commercial purposes. However, there are remarkable differences between human milk (HM) and animal milk (AM) from bovine, sheep, goat, pig, horse, camels and others. In HM the total amount of oligosaccharides various between 10-15 g/L in mature milk with a much higher amount in colostrum. In milk from all other species (with the exception of elephants) the total amount in mature milk is rather low. Major differences also occur in the complexity of the structures with about 200 different components characterized in HM and 35 to 45 in AM. About 10 of the structures in milk from various species are present in HM. In general, the ratio of acidic (sialylated) oligosaccharides compared to neutral oligosaccharide in AM is much higher compared to HM (about 90/10 in AM versus 30/70 in HM.

Strikingly, only in HM the pattern of oligosaccharides depends on the mothers Lewis blood group specificity and secretor/non-secretor status which leads to 4 different HMOs pattern characterized by very specific components being present or not. HMOs are mainly characterized by type 1 structures (Galβ1-3GlcNAc-linkages)). Milk of other species including apes, monkeys, bovine, goat either contains primarily type 2 oligosaccharides (Galβ1-4GlcNAc-linkages). It seems likely that type I HMOs exert specific effects in breast-fed infants. Here, we will focus on the potential of milk oligosaccharides to influence the microbial composition in the gastrointestinal tract, to prevent the adhesion of pathogens to the epithelium, and to influence inflammatory processes after intestinal absorption. In addition, an increasing number of publications indicate a link between the blood group and secretor status of an individual and the risk for diseases, such as inflammatory gastrointestinal diseases. Thus, not only the HMO content but also their pattern of specific structures may be relevant for health.

As currently supplemented infant formula with GOS/FOS are not similar to milk oligosaccharides, neither in their structures nor in their functions, the next decade will focus on supplementing oligosaccharides isolated from milk of animal species or produced by various methods. Hence, the following important questions need to be addressed:

- Do single milk oligosaccharides affect the microbial composition and/or activities more efficient than a mixture of various components and how can health effects be investigated in humans?
- Which specific oligosaccharides have a direct impact on intestinal or tissue target cells, e.g. on cell maturation, cell surface glycosylation or brain functions?
- Are milk oligosaccharides differently metabolised in term and preterm infants and what is the underlying mechanism?

- Are there specific oligosaccharides or their precursor which are preferentially absorbed and transported into target cells?
- Are milk oligosaccharides promising components to improve the overall health of other populations than infants, e. g. regarding gastrointestinal diseases or inflammatory and infectious diseases?

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Supported by the German Research Foundation (Ru 529/7-3 and Ku 781/8-3)
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The proteome of human and bovine milk

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Milk is the single source of nutrients for the newborn mammal. During evolution, the composition of milk of different mammals has been adapted to fulfill the needs of the newborn. Milk not only provides nutrients, but also serves as a medium for transfer of host defense components to the newborn. The host defense proteome of milk from different mammalian species is expected to reveal signatures of evolution. The goal of my work is to compare the proteome of both human and bovine milk, focusing on host defense proteins.

For studying the qualitative and quantitative differences, milk samples is divided in two protein fractions: milk serum and milk fat globule membrane. For qualitative experiments, the proteins are separated using SDS-PAGE, followed by in-gel digestion of the proteins. For quantitative experiments, the samples are prepared using on-filter cleanup & digestion. The peptides obtained after both sample preparation methods are studied using LC/MSMS (FT-MS Orbitrap). Proteins are identified and quantified using MaxQuant software, using the human and bovine Uniprot database.

The results on the host defense proteome of this study have been published (Hettinga et al, 2011). In short, 268 proteins in human milk and 269 proteins in bovine milk were identified. Of these, 44 from human milk and 51 from bovine milk are related to the host defense system. Proteins involved in the mucosal immune system (immunoglobulin A, CD14, lactoferrin, and lysozyme) were present in high concentrations in human milk. On the other hand, antimicrobial proteins (5 cathelicidins and lactoperoxidase) were abundant in bovine milk. These differences in the host defense proteome are thought to be related to developmental differences between human and bovine newborns.

This first detailed comparison of the human and bovine milk proteome is an important first step in understanding the function of milk in immune system development. However, to better understand the implications of these proteomics findings, it is necessary to further characterize the milk proteome. In my presentation, I will discuss the techniques we use, the results we have obtained up till now, and my plans for further characterization of the milk proteome.

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Cow milk consumption: a life history approach

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Mammalian milk is produced to feed infants, and each mammal species' milk composition reflects the unique requirements and feeding ecology of that species. Milk is consumed only during infancy and it functions to support the rapid growth and development typical of this life history phase. For most mammals, milk contains lactose, and the digestive enzyme required to digest lactose, lactase, is produced only during infancy, and is down-regulated by the time of weaning.

Humans are unusual insofar as many consume the milk of different species, most often bovines, and consume it well beyond the traditional age of weaning. This is reflected in the high frequency of lactase persistence found in some poulations (e.g. Europeans, nomadic populations of Asia and Africa, South Asians) that have a long history of animal domestication and milk usage. Life history variation in lactase activity is a major axis of population genetic variation in our species, and hence a life history approach is particularly useful when considering the adaptive significance of bovine milk in human diets. Life history traits are those related to the pace of growth,age-specific body size, age at sexual maturation, quantity and size of offspring, pace of reproduction, and lifespan, among others, and life history theory makes predictions about trade-offs among these traits based on organisms' ecological context and phylogenetic constraints. Two questions arise: (1) Does ongoing bovine milk consumption by humans alters life history trajectories, and (2), if so, what is the adaptive significance of such alterations?

Despite evidence for strong positive selection for lactase persistence among milk-using populations, the fitness benefits associated with dairy consumption beyond the weaning period are poorly understood. Hypotheses have included an overall increase in high quality food, or calcium in the diet, or lactose's ability to substitute for Vitamin D, but evidence providing strong support for any of these is scant. Alternatively, given milk's unique evolutionary function as a food to support the growth and development of nursing infants, and the fact that bovine infants grow rapidly to large body sizes, it is possible that consumption of bovine milk by post-weaning age human children could enhance growth and accelerate sexual maturation. This could result in earlier or more successful reproduction, and ultimately enhanced fitness among milk drinkers.

A mechanism by which bovine milk consumption could affect these life history traits is via insulin-like growth factor I (IGF-I), which has gained attention as an important mediator of life history traits across a wide array of taxa. IGF-I is centrally involved in the two fundamental life history processes: growth and reproduction, and may act as a signaling mechansim for environmental resource availablity. Milk contains IGF-I, and while there is debate about whether this IGF-I exerts independent effects among milk consumers, circulating IGF-I levels rise with milk consumption.

In this paper I outline the potential life history effects of bovine milk consumption, drawing on data from large cross-sectional national surveys in the United States and an ongoing cohort study in Maharastra, India. The research in India provides a useful counterpoint to U.S. or European studies as milk has a deep history there but milk consumption occurs against a very different dietary and ecological backdrop. These data may shed light on the adaptive significance of lactase persistence and ongoing milk consumption.

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Milk intake and cancer risk

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The association between milk and dairy product intake and the risk of certain cancers has been evaluated in observational studies. Intake has been characterized as dairy products in total, as well as milk, cheese, and fermented products as separate exposures. In addition, intake of certain nutrients of which dairy products are rich sources (e.g., calcium) has also received attention in some epidemiologic studies. No interventions have evaluated the feeding of milk or dairy products on cancer risk in humans, although some relevant nutrient-based interventions have been conducted.

In 2007, the World Cancer Research Fund and American Institute for Cancer Research published their second report on effects of diet and nutritional factors on cancer risk (WCRF/AICR, 2007). The Report included a systematic literature review of epidemiologic data on dairy product intake and 15 categories of cancer. For 12 of the categories, the number of cohort studies was small, and precluded formal meta-analyses; but, for colorectal, prostate, and bladder cancer, there were sufficient data. The Report suggested that there was reasonably consistent evidence from cohort studies that milk protects against colorectal cancer. The Report also indicated that there was limited evidence that dairy products and milk were associated with prostate cancer and that milk protects against bladder cancer.
Since 2007, results of several additional meta-analyses and large cohort studies of dairy products and cancer have been published. A recent systematic review and meta-analysis updated the colorectal cancer evidence up to May 2010. High intakes, as compared to low intakes, of milk and total dairy products were associated with a statistically significant reduction in colorectal cancer risk (Aune et al, Ann Oncol, 2012). When stratified by cancer site, the inverse associations were only present for colon cancer.

Another meta-analysis of prospective cohort studies examined the association between dairy product consumption and breast cancer risk (Dong et al, Breast Cancer Res Treat, 2011). Eighteen prospective cohort studies were eligible for analysis. The summary relative risk of breast cancer for the highest intake of total dairy food compared with the lowest was 0.85 (95% CI: 0.76–0.95), and for milk consumption was 0.91 (95% CI: 0.80–1.02), suggesting that increased consumption of total dairy food, but not milk, may be associated with reduced risk of breast cancer.
Mao et al (Nutr Cancer, 2011) reported the results of a meta-analysis of 6 cohort and 13 case-control studies evaluating the association between milk consumption and bladder cancer. When compared with the lowest category of milk intake, high milk intake was associated with decreased risk of bladder cancer (OR, 0.84; 95% CI 0.71–0.97). Restricted to the 6 cohort studies, the pooled relative risk was 0.88 (95% CI 0.76–1.00). The overall results suggest that milk may be associated with reduced bladder cancer risk; however, the confounding and bias inherent in case-control designs make interpretation of the results more difficult.

In populations with high intakes of milk and dairy products (e.g., USA, Australia, and much of Europe), intakes of certain nutrients are closely linked to dairy intake. For example, milk and dairy products are a major dietary source of calcium in these populations. Subsequently, calcium intake serves as a useful intake marker of dairy consumption. Studies of dietary calcium and colorectal cancer further support a protective effect of milk, whereas for prostate cancer, diets high in calcium tend to be associated with higher risk (WCRF/AICR, 2007). A systematic review of randomized controlled trials assessing effectiveness of calcium supplementation in prevention of colorectal cancer or adenomatous polyps reported a statistically significant 18% reduction in risk of adenoma recurrence (RR 0.82, 95% CI 0.69–0.98) and a non-significant reduction in risk of advanced adenomas (RR 0.77, 95% CI 0.50–1.17) with calcium use. In contrast, there was no significant effect of calcium on risk of colorectal cancer (RR 1.08, 95% CI 0.87–1.34), although studies were of relatively short duration (Cooper et al, Health Technol Assess, 2010).

In summary, results of recent meta-analyses further support the inverse association between intake of milk and dairy products and colon and bladder cancer, and dairy products and breast cancer. Nonetheless, the complex composition of milk and dairy products and the heterogeneity of cancer as multiple diseases make this a challenging area of study.

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Dietary patterns and mortality in the Iowa Women's Health Study (IWHS) with special reference to dairy products and milk fat

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For many years, associations of both foods and nutrients with chronic disease outcomes have been examined one by one, with strikingly inconsistent results across studies. The theory of food synergy suggests that this single item approach is likely to be misleading. "Food synergy" is that idea that foods, which almost all originate from living organisms, are highly complex, non-random mixtures of thousands of compounds that affect health as individual units in ways that cannot be predicted from the behaviour of the individual compounds found in the food. Furthermore, nutritional epidemiology has clearly shown that intakes are substantially correlated over either foods or nutrients. Dietary patterns reflecting these correlations exist and are strongly related to future chronic disease. A recent randomized clinical trial of the Mediterranean-type diet strongly supported this concept by finding reduced incident cardiovascular disease over 5 years in at risk older Spanish men and women (1). We recently showed that the A Priori Diet Quality Score predicted lower death rates over 22 years in 29,634 women in the IWHS, initially 55-69 years old and free of heart disease, diabetes, and cancer (2). Although dietary data suffer notoriously from within person variation, this a priori score had a substantial correlation (r=0.55) across 18 years in these women. The incidence density/100 person years for total death over 22 years followup, adjusted in Poisson regression for age, energy intake, marital status, education, residence, hormone replacement therapy, physical activity, and smoking, was 36.9, 34.6, 32.5, 30.2, respectively, across the increasing score quartiles. Study of dietary patterns promises to reveal much about diet and risk for chronic disease.
Nevertheless, people eat individual foods in their dietary pattern. A predictive diet pattern can also be used to better understand individual foods, by serving as a control for correlations of the studied food for the rest of the diet. In this presentation the focus will be on the mortality patterns for the 12 dairy and dairy containing foods that were queried in the IWHS: skim or low fat milk, whole milk, cream, sour cream, ice milk, ice cream, yogurt, cottage cheese, cream cheese, other cheese, butter, and pizza. Mortality patterns will be examined holding constant the A Priori Diet Quality Score. In this way, any associations found for dairy products can be judged to be above the known strong predictive capability of the a priori score. A general preliminary finding is that dairy foods, eaten in various forms, but not eaten in excess, are associated with reduced total mortality, independent of the a priori score.

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Milk intake and risk of cardiovascular diseases/hypertension

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Background The consumption of milk may influence the risk of hypertension, cardiovascular diseases (CVD) and total mortality, but findings have been conflicting.

Objective To examine the associations between milk, and the risk of hypertension, fatal and non-fatal cardiovascular disease (CVD)(including coronary heart disease (CHD) and stroke) and total mortality via meta-analyses of prospective cohort studies.

Design We conducted two main meta-analyses to study the association between 1) milk and risk of hypertension, and 2) to study the association between fatal-and non-fatal CVD, CHD, strokes and all-cause mortality. We systematically searched the literature using MEDLINE, EMBASE and Scopus. Random-effects meta-analyses were performed with generalized least squares for trend estimation of summarized dose-response data. Milk as the main dairy product was pooled together.

Results 9 prospective cohort studies were found comprising 57,256 individuals with 15,367 incident hypertension cases accrued during a follow-up time of 2-15 years. Intake of milk (6 studies) was inversely and linearly associated with a lower risk of hypertension (up to intake of ~600 ml per day for milk). The pooled relative risk (RR) for intake per 200 ml per day was 0.96 (95% CI: 0.93-0.99) for milk, without statistical heterogeneity.

Among 17 prospective studies, there were 2,283 CVD, 4,391 CHD, 15,554 stroke and 23,949 mortality cases. A modest inverse association was found between milk intake and risk of overall CVD (4 studies; RR=0.94 per glass/day (200 ml/d), 95%CI: 0.89-0.99). Milk intake was not associated with risk of CHD (6 studies, RR per glass/day=1.00, 95%CI: 0.96-1.04), stroke (6 studies; RR=0.87, 0.72-1.05) and total mortality (8 studies; RR per glass/day= 0.99, 0.95-1.03).

Conclusions These dose-response meta-analyses of prospective studies indicates that with increasing milk intake per glass a day there is a borderline significantly inverse association with incident hypertension and CVD, but no significant association with risk of total mortality or with CHD or stroke when analysed separately.

The presentation will focus on the association between milk and hypertension and CVD/all-cause mortality from two recently published meta-analyses. In addition, more recent work on the associations between milk and CVD in the large prospective cohort studies with interesting new ideas for further research will be presented.

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Milk intake and allergic diseases

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There are many studies showing that children raised in a farming environment are protected from the development of asthma, hay fever and allergic sensitization. The most important factors explaining these protective effects have been identified as exposure to animal sheds early in life and consumption of unprocessed cow's milk. This protective effect of unprocessed cow's milk consumption has been shown in alpine areas as well as in the UK and in Poland. The effect was seen for asthma, atopic sensitization and hay fever. In the latest large cross-sectional survey of farm children and controls, the GABRIEL study, the protective factors in cow's milk have been investigated. The GABRIEL study included a phase I with a screening questionnaire and a phase II with an extensive questionnaire of a random selection of the phase I population in four rural areas (Bavaria, Baden Wurttemberg, Tyrol and Switzerland). The extensive questionnaire assessed whether the child consumed milk from a farm and whether this consumption was exclusive. Furthermore, the questionnaire assessed whether this farm milk was boiled or not. The protective association with asthma, atopy and hay fever was only seen if children drank unboiled farm milk. In a subsequent nested case control, the phase III study, additional measurements were performed in milk samples collected from participants' homes. In all milk samples the milk heating status was determined by objective enzymatic measurement (ALP/LPO). Furthermore, total fat content, whey proteins and total viable bacterial count, microbiological subgroups and somatic cell counts were performed. As expected, total fat and total bacteria counts increased in unprocessed farm milk samples. Also, detectable microbiological subgroups such as *pseudomonades*, *staphylococci*, *lactobacilli*, and psychrotrophic bacteria were clearly more abundant in unheated and unprocessed farm milk samples. However, we found no significant association between total fat content, total bacterial count, microbiological subgroups and health outcomes, nor did we see associations between somatic cell counts and lactose levels with allergic diseases in the children. But we detected an inverse association between asthma and whey proteins, in particular *alpha-lactoglobulin*, *beta-lactoglobulin* and bovine serum albumin. However, given the correlation between all whey proteins we could not disentangle with certainty the most important protective whey protein. In summary, there is evidence that the consumption of unprocessed cow's milk protects from the development of asthma, allergy and hay fever in children. This protection is also found among non-farm children consuming the unprocessed milk. This association is only significant for unheated farm milk. Viable bacterial counts and whey proteins are associated with heating status. Only the whey proteins are also related to asthma but not the bacterial counts or the microbiological subgroups.

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Impact of milk consumption on body composition

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Many classes of milk and dairy products contain components that classify them as a nutrient-dense food (necessary nutrients:energy) and as such its consumption during a hypoenergetic period is advisable in order to obtain many of the necessary nutrients while on a limited energy intake. Apart from this fact, milk, and dairy products in general, contain components – protein and calcium – that appear to exert important regulatory influences on the pattern of tissue weight loss during energy restriction. Evidence-based analyses support the conclusion that a higher consumption of milk and dairy products during a hypoenergetic period promotes a greater loss of body fat and a greater retention of lean tissue. This pattern of body composition change is what we have referred to as better 'quality' weight loss. It appears that this pattern of weight loss is enhanced in persons who were, prior to the weight loss intervention, low- or no-dairy consumers and also when the intake of calcium during the hypoenergetic period exceeds 1000mg/d. The reasons for these observations are not entirely clear but potential mechanisms have been proposed.

Evidence from our lab has shown that consumption of dairy and in particular whey protein has a body composition-enhancing effect in exercise training situations. In these studies both acute post-exercise feeding of whey protein shows a highly stimulatory role of whey protein on muscle protein synthesis. This observation may explain, in part, why studies of chronic whey protein feeding during a period of training demonstrate a positive effect on muscle mass gains. The mechanism for the marked stimulatory action of whey protein on muscle protein synthesis is likely attributable to the rapid rate at which it is digested, the high leucine content of the protein, and potentially also to other peptide components that appear in circulation after whey protein consumption.

In combination, we have found that a highly potent combination of conditions – higher dietary protein from dairy sources, higher calcium intakes, in combination with aerobic and resistive exercise – during a hypoenergetic period facilitates a very high quality pattern of weight loss. In fact, under these conditions we have observed that women and men lose weight that is comprised entirely of body fat and particularly from the visceral region. Simultaneously, most of these people will actually gain muscle mass. This pattern of weight loss completely reverses the normal trend seen in weight loss in which muscle is lost, as well as fat, and metabolic rate declines. We view such a pattern of high quality weight loss as highly advantageous and deserving of more in-depth study.

Mechanisms of how dairy and whey protein exert their influence on changes in body composition during weight loss as well as under conditions of weight gain (resistance training) will be discussed in addition with new data on how whey protein exerts an effect during weight loss.

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Milk intake and bone health

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The acquisition and maintenance of bone mass and strength is influenced by environmental factors, including physical activity and nutrition. Several milk nutrients play a major role for the building of bone structure resistant to usual mechanical loading, and attenuating its deterioration with aging. Among milk micronutrients, calcium (Ca) and inorganic (i) phosphate (P) are the two main constituents of the bone mineral ($[Ca_{10}(PO_4)6(OH)_2)]$) that strengthens the organic matrix mechanical resistance. Sone contains about 99% and 80% of the whole body Ca and P, respectively. The Ca/P mass ratio in bone is 2.2, close to that measured in human milk. Among milk macronutrients, proteins play a major role for bone health. Protein supply from foods is required to promote bone formation. As for any other organs, amino acids are required for the synthesis of intracellular and extracellular bone proteins, and other nitrogen-containing compounds. Besides this role as “brick supplier”, proteins, through their amino acid constituents can influence Ca-P economy and bone metabolism. Thus, dietary proteins stimulate the formation of IGF-1 from hepatic cells, the main source for this circulating growth factor. IGF-1 is anabolic for bone. Protein intake in association with Ca, to the extent the vitamin D status is adequate, exerts a specific impact on bone health throughout life. ln adulthood, insufficient supply of these nutrients contributes to the decline in bone mass and strength and thereby increases the fragility fracture risk. These fractures are the main complication of the osteoporosis disease, the prevalence of which dramatically rises after the menopause and, with aging in both women and men. Several large-scale epidemiologic studies have documented a positive relationship between protein intake and area bone mineral density, as assessed by osteodensitometry. This relationship is particularly pronounced when the Ca intake is sufficient. ln agreement with this notion, relatively high protein intake reduces the risk of fragility fractures. Furthermore, in patients with hip fracture, the positive impact of casein as compared to an isocaloric placebo supplementation has been documented in a randomized controlled trial. Thus, a significant and substantial attenuation of the bone loss that follows hip fracture was recorded. Moreover, the consumed isocaloric protein supplement that normalized the low intake frequently observed in elderly patients experiencing hip fracture increased the IGF-1 circulating level. The muscle strength was also improved, an effect that can weil be explained by the essential role played by amino acids and IGF-1 on skeletal muscle mass and strength. ln institutionalized elderly women, we recently reported that the consumption of soft white cheese, thus increasing the intake of both proteins and calcium, reduced biochemical markers of bone resorption while other markers reflecting bone formation tended to increase. This positive uncoupling effect could be explained by the inhibition the dairy product exerts on parathyroid hormone (PTH), an endocrine factor that enhances osteoclastic bone resorption and by the Stimulation of osteoblastic bone formation by IGF-1. ln 60 years old postmenopausal women, an inhibition of bone resorption was also documented after increasing milk consumption and thereby Ca and protein intakes. Other studies showed that enhancing the protein intake stimulates the intestinal Ca absorption, but not bone resorption, while reducing plasma PTH Ievei. These observational and interventional studies point to an inhibitory influence of Ca and protein intakes on bone resorption, bone loss, and fragility fractures. Preclinical studies in appropriate animal models of human osteoporosis fully supported the key role of dietary protein on calcium economy, bone mass and strength. Protein intake is also an important nutrient for bone acquisition lndeed, from birth to maturity, it enhances the positive influence of physical activity on bone mineral mass and structure development in a manner that should confer greater resistance to mechanical strain. Thus, Ca and proteins, which are both particularly available in dairy products, represent essential nutrients for the early prevention of osteoporosis du ring peak bone mass acquisition as weil as for the reduction of postmenopausal skeletal resorption leading to bone loss and in the management of elderly patients with osteoporotic fractures.

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Milk/dairy fat and health – evidence from intervention studies

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Epidemiological studies have found that a high intake of dairy products, such as milk, cheese, cream, butter and yogurt is associated with a reduced risk of developing the metabolic syndrome. Moreover, consumption of dairy products was negatively related with the development of cardiovascular disease (CVD) and type 2 diabetes mellitus in case-control studies and prospective cohort studies. It is not very likely that these relationships can be explained by the lipid composition of dairy, also because the observed associations between dairy and health parameters are not restricted to full-fat dairy products alone. Dairy products also contain high amounts of calcium, which is well known for its beneficial blood pressure effects. In addition, calcium is known for its intestinal fat binding capacity, inducing the formation of insoluble calcium soaps thereby reducing intestinal fat absorption and increasing fecal fat excretion. By this, calcium may positively affect lipoprotein metabolism. However, intervention studies have not convincingly shown that this mechanism is important at realistic intakes of calcium.

Dairy products are also an important source of dietary protein. It has been shown that replacing dietary carbohydrates by proteins favorably reduced the total:HDL cholesterol ratio. One of the most important characteristics of dietary protein is probably its potential of modifying body composition in relation to weight loss. Because of its satiating properties, dietary protein, energy intake may decrease and as a result fat mass, whilst sparing fat free mass thereby inducing a shift in body composition. Direct effects of dairy on energy expenditure are not likely.

However, dairy products are complex food products and their metabolic effects may not be related to single dairy constituents alone. By far most of the nutritional intervention studies have been carried out in the fasted state. However, evaluation of postprandial responses is of great importance, since people spend most of the day in a postprandial state. In a recent study, we have investigated the effect of milk and two of its major constituents (protein and calcimu) on postprandial responses in sixteen overweight men. Our results indicated that the intake of low-fat milk with a fat-containing meal enhanced postprandial triacylglycerol and insulin responses and may blunt glucose increases. The milk also beneficially affected postprandial sICAM-1 and sVCAM-1 (two adhesion molecules) concentrations, but negatively those of TNFα. The protein fraction may be the most important determinant for these effects. However, other nutrients may be involved as well, since milk tended to induce even larger reductions in adhesion molecules, other nutrients may be involved as well. Calcium did not change any of the postprandial responses.

It is evident that dairy plays a prominent role in the diet of many people and is a rich source of many nutrients. Intervention studies have found both positive and negative effects of dairy products on a wide variety of health parameters. Long-term randomized controlled intervention studies are however required to give more definite answers on the health aspects of dairy products.

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Role of mTOR as a key regulator of metabolism and immunity

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The mammalian target of rapamycin (mTOR) is an evolutionarily conserved serine-threonine kinase that is known to sense the environmental and cellular nutrition and energy status. Diverse mitogens, growth factors and nutrients stimulate the activation of the two mTOR complexes mTORC1 and mTORC2 to regulate diverse functions such as cell growth, proliferation, development, memory, longevity, angiogenesis, autophagy, and innate as well as adaptive immune responses. Dysregulation of the mTOR pathway is frequently observed in various cancers and in genetic disorders such as tuberous sclerosis complex or cystic kidney disease. In this presentation I will give an overview of the current understanding of mTOR signalling and its roles in metabolism and immunity. mTORC1 centrally regulates cellular metabolism to promote the biosynthesis of building blocks and macromolecules fundamentally essential for cell growth and proliferation, including proteins, lipids and nucleic acids. In addition, mTOR has essential functions in cells of the immune system to control the immune response to pathogens and tumors, and to maintain tissue homeostasis by regulating the expression of inflammatory mediators such as cytokines and chemokines. Increased knowledge of the function of mTOR in cellular processes will potentially expand the clinical applicability and efficacy of mTOR inhibition in various disease settings.

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Milk: an endocrine signalling system activating mTORC1 – does it impair human health?

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Regular consumption of milk and dairy products, staples of Western diet, is a novel human behaviour introduced by the Neolithic revolution and maximized by widespread refrigeration technology that may have long-term adverse effects on human health. To date, nutrition science and medicine regard milk as a recommendable food supplying valuable proteins, vitamins and calcium.

However, milk is not "just food" but is the anabolic endocrine signalling system of mammals promoting appropriate neonatal growth and metabolic programming. Accumulating evidence demonstrates that milk represents a most sophisticated materno-neonatal species-specific signalling system activating the nutrient-sensitive kinase *mechanistic target of rapamycin complex 1* (mTORC1) of the milk recipient. Thus, milk is not only a simple donor of calories, proteins, lipids, vitamins and minerals but fulfils a more important biologic function: the activation of mTORC1 promoting cell growth, cell division, lipid and nucleotide biosynthesis, gene expression, thus boosting anabolism and inhibiting autophagy. It is just conceivable that bovine milk in comparison to human milk elicits a much higher magnitude of mTORC1 signalling as *Bos taurus* duplicates birth weight four times faster than *Homo sapiens*.

The overlooked functionality of milk resides in two major signal transduction systems: 1) the transfer of mTORC1-activating amino acids, especially of leucine, and 2) the transfer of exosomal secretory microRNAs, especially of micro-RNA-21. Amino acids via direct and indirect activation of mTORC1 enhance translation initiation, whereas micro-RNA-21 may inhibit the expression of important cell cycle suppressors (*PTEN, Sprouty, PDCD4*) in a virus-like mode of metabolic transfection further enhancing mTORC1 signalling (Melnik BC *et al.* (2013) Nutr J 12: 103).

Recent evidence of molecular medicine supports the view that persistently increased mTORC1 signalling significantly contributes to the pathogenesis of diseases of civilization like acne, obesity, type 2-diabetes, cancer and neurodegenerative disorders. Epidemiological evidence confirms the relationship between increased milk consumption and acne, overweight and prostate cancer. Data from animal experiments support the association between increased mTORC1 signalling and early onset of beta-cell apoptosis (diabetes) and pathological changes resembling Alzheimer´s disease.

Whereas human breast milk is the ideal food for infants allowing appropriate postnatal growth and species-specific metabolic programming, persistently high mTORC1 signalling elicited by current infant formula feeding as well as continued cow´s milk consumption during adolescence and adulthood may substantially promote the development and early onset of mTORC1-driven diseases of civilization. A deeper understanding of milk´s signalling functions promoting milk´s evolutionary mission, i.e., mTORC1-driven postnatal growth and metabolic programming, may help to curtail the epidemics of diseases of civilization.

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Milk and Linear Growth: Programming of the IGF-1 axis

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There is convincing evidence that cow's milk is stimulating linear growth and that the IGF-1 axis plays a central role in mediating this effect. The most convincing effects are seen in low-income countries, where the diet of young children are often cereal based with very little animal source protein. However, there are also convincing observational studies from high income countries showing that even in well-nourished populations there seem to be a specific effect of milk on linear growth. The effect seems to be strongest during early life when growth velocity is highest. Data from NHANES analysed by Wiley suggest that there is no effects during middle childhood and then an effect again in adolescence when linear growth velocity increases again.

It is not clear which factors in milk is responsible for the stimulation of linear growth. It could be the high protein quality (high PDCAAS and DIAAS), effects of specific amino acids, peptides, the content of growth promoting type II minerals e.g. potassium, magnesium and phosphorus, or a combination of these factors. Several studies have suggested that the stimulating effect on IGF-1 is mainly caused by casein, while whey seems to stimulate insulin. Insulin is an anabolic hormone which also has an enhancing effect on early growth. Animal studies suggest that lactose could also have a beneficial effect on growth, because of beneficial luminal effects in the GI tract. However, this is most relevant in low income countries where many young children have environmental enteropathia.

The interaction between milk intake, IGF-1 levels and stature later in life is complex and not yet fully understood. Breastfed infants have considerable lower levels of IGF-I, also during late infancy, and are slightly shorter than infants receiving infant formula in late infancy. Protein content is likely to explain a considerable part of this difference, since differences in protein content in infant formula is reflected in differences in IGF-I levels. However, several studies suggest that there is a programming of the IGF-axis. Breastfed infants and infants with low IGF-I levels seems to have higher IGF-I levels and be taller later in childhood. The sensitive age window for this programming is not known. Reference values for IGF-I show low values at birth which gradually increase through infancy and childhood and peaks at puberty. Thus, the high growth velocity during infancy is not reflected in this pattern. Newer studies suggest that after an increase in IGF-1 levels during the first few months after birth there is a decrease until around 6-9 months and then an increase again which continues until puberty. The implications of this pattern for growth and programming of the IGF-1 axis is not known. Girls have considerably higher IGF-I values than boys before puberty, despite their lower linear growth, suggesting different interactions between diet, IGF-1 and growth in boys and girls. The complexity is underlined by new studies showing that variants of the IGF-1 gene are regulating IGF-1 levels.

A high intake of cow's milk protein early in life is a risk factor for later obesity and it is possible that some of this effect involves regulation of IGF-I. There are some data suggesting that IGF-I levels stimulate accretion of both body lean mass and body fat mass, but the effect of cow's milk on body composition is not yet fully understood.

In high income countries high intake of cow´s milk, high growth velocity and certain patterns of change in body composition in early life are associated with increased risks of developing obesity and non-communicable diseases later in life. In low income countries intake of animal source foods and especially cow's milk during the complementary feeding period seems to optimise growth, prevent stunting and optimize later development. Understanding the complex interaction between intake of cow's milk and the IGF-1 axis, and how it influences linear growth and body composition is a high priority, as it most likely will improve our understanding of how to optimize growth, development and later health.

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Poster Abstracts

Part 1: Milk composition/constituents

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Poster 1

Fatty acids and protein composition of milk produced under different feeding regime - “We eat what we feed”-

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Milk production nowadays is based on a variety of different philosophies: Economic efficiency is the goal on farms feeding Total Mixed Rations (TMR) with over 1000 kg of concentrate per year to generate highest possible yields. Conventional Farms try to hold the balance between tradition and high-yield production by feeding cows not only concentrate, but also hay and fresh cut grass. Full Pasture Farms focus on sustainability and feed their dairy cows on pasture from their own resources, resulting in a lower amount of milk yield, but increasing the longevity of their livestock. And the Organic Farms mainly focus on organic production of their feed allowing concentrate as long as it has been produced organically.

Currently, a consumer can only distinguish between conventional and organic milk. All other differences in the production are not transparent and the impact of the feeding regime on the composition of the milk are mainly unknown. Therefore, milk, produced under different feeding conditions was analyzed for their fatty acid and protein profile.

Earlier studies on the fatty acid composition showed an increase in Omega 3, CLA, branched chain fatty acids and a decrease in LDL-raising saturated fatty acids in milk that was produced by pasture feeding [1][2][3]. In this study we could confirm those results.

However, the impact of the feeding on the protein composition could not be demonstrated so far, due to a lack of specific quantification methods for the different milk proteins. Milk contains a few quantitatively dominant proteins and over 100 different minor proteins They can be regrouped into caseins, whey proteins, and proteins associated with the milk fat globule membrane. So far, a method for the absolute quantification of individual proteins was lacking. Consequently, a mass spectrometry-based method (selected reaction monitoring) was developed, which allows the simultaneous quantification of twenty major dairy proteins in different milk varieties.

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Poster 2

Organic cow´s milk and cheese products contain higher amounts of alpha-linolenic acid, ruminant trans-fatty acids and conjugated linoleic acids, especially trans11,cis13-CLA compared to conventional products.

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In recent feeding studies conjugated linoleic acids (CLA) and vaccenic acid (*trans*11 C18:1; *t*11) were increased by organic cow´s milk production. Especially, the highly increased *trans*11,*cis*13-CLA isomer was evaluated as appropriate marker of organic milk production.

The aim of this study was to compare the fatty acid profile with focus on *trans*-fatty acids (TFA), conjugated linoleic acids (CLA), alpha-linolenic acid (ALA) and tocopherol content regarding the labeled production system (organic, org vs. conventional, conv) in milk and cheese products available on the German market.

Material and Methods: We analysed cow´s milk samples (org: n=23; conv: n=34) and cheese samples (org: n=46; conv: n=92) from various supermarkets and grocery stores, particularly in Thuringia. The fatty acid methyl esters (FAME) were determined by using two gas chromatography methods (GC; 60 m & 200 m *cis*- & *trans*-C18:1) and the CLA isomer distribution was analysed by Ag^{+}-HPLC.

Results: The org cow´s milk samples contained higher amounts of n-3 PUFA (mainly ALA), ruminant (R) TFA (mainly *t*11) and total CLA (mainly *c*9,*t*11-CLA) compared to conv milk. The CLA isomers differed regarding the *t*11,*c*13-CLA portion (org 6% vs. 3% of ∑CLA). Similar results were found for the cow´s cheese samples. Irrespective of the production system, the highest CLA content was found in mountain/alpine cheese (1.7%) compared to Gouda (0.8% ∑FAME). In general, the major R-TFA was t11. The *t*11 portion of total *trans*-C18:1 differed regarding the production system in milk (org 38% vs. conv 28%) and cheese samples (53% vs. 37%). The tocopherol content in org milk and cheese was significantly 25% and 40% higher compared to conv samples, respectively. In all samples ALA and *t*11 correlated with *c*9,*t*11-CLA. The org milk and cheese products were generally 36% - 46% more expensive, respectively. None of the org milk samples has simultaneously lower contents of the three markers ALA, *t*11 and *c*9,*t*11-CLA and vice versa. In cheese samples only 4% of the org samples had lower contents of the respective average of these markers and 8% of conv samples had higher values.

In principle, the label of the analysed samples indicates the fatty acid profile of the respective production system. In organic cow´s milk and cheese products higher portions of tocopherols, ALA, *t*11 and *c*9,*t*11-CLA can be expected. The *t*11,*c*13-CLA was 2-fold higher and was confirmed as appropriate indicator of organic milk production.

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Poster 3

Traceability of organic food – analytical authentication of processed dairy products

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Objective

Recently, analytical parameters obtained by stable isotope and fatty acid analysis have been established for the authentication of organic drinking milk in Germany. The aim of the present work was to confirm the proposed threshold values for $\delta^{13}C$ and the α-linolenic acid (C18:3ω3) content in milk fat to be applicable with processed dairy products. Moreover, procedures for the authentication of reconstituted products require including not only the fat component but protein as well. Such laboratory methods being capable of differentiating between organic and conventional products are intended to be applicable at the retail level in order to improve the capability of protecting consumers and organic milk producers against potential fraud.

Materials and Methods

During a period of 18 months, 3 conventionally and 3 organically produced brands of German full cream milk were collected at retail every 4 weeks (n=120). Milk fat and protein were subjected separately to stable isotope analysis of carbon. Moreover, a variety of processed organic dairy products were obtained during a period of 2 years (n=56 in total) with a focus on cream cheese and curd (n=39). The extracted milk fat was analyzed for fatty acids (C18:3ω3) and carbon isotopes ($\delta^{13}C$). From a selection of 17 cream cheese and curd samples $\delta^{13}C$ was analyzed also in protein. The fatty acid composition of milk fat (g/100 g of fatty acids) was analyzed by GC of FAME (60-m Sil88 column). The stable isotope ratio of carbon ($\delta^{13}C$, in ‰ against PDB) was determined by IRMS of CO_2 after combustion of the samples in an elemental analyzer.

Results

The analysis of milk samples revealed a very close correlation of r=0.99 between δ^{13}Cfat and $\delta^{13}C_{protein}$, while the $\delta^{13}C$ level in protein on average was 2.6‰ higher than in fat. Consequently, $\delta^{13}C$ in both fat and protein can be used to authenticate organic milk products by a maximum level of 26.5‰ in fat and -23.5‰ in protein. Milk fat extracted from both soft and semi-hard cheeses, butter, cream, sour cream, buttermilk, yoghurt and low-fat milk always showed an α-linolenic acid (C18:3ω3) content above the minimum level of 0.50% and a stable isotope ratio of carbon ($\delta^{13}C$) below the maximum level of 26.5‰ required for organic milk according to our previous examination of German drinking milk (n=246). Results beyond these thresholds were were obtained for whey, because of its special lipid composition, as well as both Italian ice creams and cheeses. Analyses of cream cheese and curd lipids revealed that also 7 out of 39 samples did not comply with these two thresholds. An additional analysis of δ13C in the protein fraction showed that these reconstituted products apparently contained a combination of organic skim milk and conventional or imported organic cream. The inherent correlation between δ13C in fat and protein indicated their different origins by an actual deviation between $\delta^{13}C_{protein}$ and $\delta^{13}C_{fat}$ of less than 1.0‰.

Conclusions

This study showed that the previous C18:3ω3 and δ13C thresholds derived from fresh milk are generally applicable to processed dairy products from Germany. Non-organic constituents can be identified by correlating $\delta^{13}C$ in protein and fat, which may provide evidence of fraud.

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Poster 4

Comparative milk proteomics and metabolomics: milk-compositional differences between extensive conventional versus organic farming systems with regard to forage-to-concentrate ratio

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High milk yield requires a correspondingly high supply of metabolisable energy, ME. In extensive conventional dairy cattle farming systems, concentrates rich in non-fibrous carbohydrates are a common means to meet the enhanced ME demand during lactation. The resulting low forage-to-concentrate ratio constitutes a major difference to extensive organic farming. High energy input shortens rumenation time, enhances the portion of intestinally resorbed glucose, increases levels of volatile fatty acids in rumen, and changes, both, their profile and, subsequently, the mode of supplying the biosynthetic processes in mammary-glandular epithelium with energy and precursor compounds.

In order to investigate differences in milk composition due to low vs high forage-to-concentrate ratios in extensive system, an on-farm research approach assessed conventional milk (CON yield: 7,300 kg/y, ME: 248 MJ/d) and organic one (ORG yield: 4,800 kg/y, ME: 182 MJ/d) at 4 periods, from early to late in lactation. CON cows were fed concentrates and green cobs, supplemented with fresh green in-doors and on paddock. ORG cows were fed hay plus little concentrates in winter, while grazing on permanent pasture all summer. For each farming system, 6 matched herds (breeds in both: Brown Swiss and German Brown cattle) were combined, sampling 6-7 matched cows per herd (CON total: n=42; ORG total: n=37) and storing pooled samples at -25 °C. Milk serum proteins were analysed by nLC-ESI-Orbitrap tandem mass spectrometry, followed by MaxQuant lable-free quantitation. Metabolites were analysed by J-resolved 1H-NMR sprectroscopy and GC mass spectrometry. Accurate comparability of relative quantitations was achieved by standardisation to sets of analytes with low variability within each method.

In total, 32 proteins and 43 metabolites, exhibited significant effect ($p<0.05$) with a median absolute change of 32.4%, Q10: 11.4% and Q90: 71.3%. Merging the data from all periods and methods, many hierarchical clusters of closely correlated milk constituents were found, reflecting distinct adaptational states in response to the two experimental conditions.

At the first level of differentiation, the low forage-to-concentrate ratio of conventional milk expectably coincides with elevated glycolysis intermediates, phosphate, phosphocreatine and glycerophosphorylcholine. The high flow through tricarboxylic acid cycle for synthesis purposes is reflected in increased isocitrate dehydrogenase plus lactotransferrin, delivering the Fe-ions required by it, and in fumarate depletion. The recruitment of dietary and body proteins for fueling the high milk protein synthesis manifests in elevated leucine and urea. A shift towards metabolic acidosis is evident by increased lactate and hemopexin. Correspondingly, organic milk displays higher levels of hippurate, beta-hydroxybutyrate and acetate, reflecting the high forage-to-concentrate ratio. Consequently, uridinediphosphate, lactose and beta-1,4-galactosyltransferease, as well as carnitine conjugates, ethanolamine, choline and apolipoprotein E are elevated. Furthermore, glycosylation precursors differ by higher xylose, mannose, glucuronate and N-acetylglucosamine in CON, while ORG exhibits higher levels of fucose, activated sugars and N-actylglucosamine-1-phosphotranferase.

Conclusively, forage-to-concentrate ratio affects milk composition to a large extend. The aspect of concommittant metabolic stress should be considered with regard to animal well-being. In addition, the consequences for differences in oligosaccharide composition deserves more attention with regard to further processing by fermentive microbes, and, last not least, consumer health.

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Poster 5

Genetic variability of lipoprotein lipase gene (LPL) and preliminary association study with milk fatty acids in Italian Mediterranean river buffalo

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Introduction

Lipoprotein lipase is a enzymatic glycoprotein responsible for hydrolysis of triacylglycerol cores (TG) of chyromicrons and low density lipoproteins. It is associated with the luminal side of capillaries and arteries where it hydrolyzes TG to produce free fatty acids. These free fatty acids are assimilated by muscle and adipose tissue. Therefore LPL regulate energy balance, fat deposition and growth traits. The gene coding for such protein has been deeply investigated in several species, including ruminants. For instance in bovine species the cDNA has been completely determined (Senda et al., 1987) and SNP were recently associated with milk fat content (Marchitelli *et al.*, 2013). In goat a missense mutation at position 17 of the signal peptide has been associated with fat content and tended to affect the milk dry weight basis (Badaoui *et al.*, 2007). Milk yield and composition are of great economic importance for the dairy industry of southern Italy where river buffalo is mainly reared for the cheese making as mozzarella PDO (Protected Denomination of Origin – Reg. EC 510/2006). The identification of genes associated with phenotypic differences for this trait could allow for the implementation of gene-assisted selection programs. The aim of this study was to characterize the LPL *locus*, to search for genetic markers and to test preliminary associations between the milk fatty acid content and the genetic variability in the Italian Mediterranean river buffalo.

Material and methods

A total of 22 buffalo cows were included in the study. The animals were at the third calving, on average at 110-120 days in milking (DIM) and belonging to the same experimental farm. Biological samples of blood and milk were collected and used to isolate DNA and fat respectively. Genomic DNA was amplified using primers designed on the homologous bovine sequence. Two amplicons corresponding to the fragments promoter/exon 1 (850 bp, sense: 5'-gcatttcttgatgagtttgagga-3', antisense: 5'-gctcagagccagcagaa-3') and exon 3/exon 4 (~2000 bp, sense: 5'-acaggaatgtatgagagttgg-3', antisense: 5'-cagacttcctgcaataccag-3') were amplified, purified and sequenced. The same amplicons were investigated for SNP discovery. A method based on PCR-RFLP was developed as tool for the quick genotyping of the population for the SNP (g.107A>G). Milk fat was isolated according to standard protocol described in literature and analyzed for the fatty acids (FA) profiles via gas chromatography (GC). Data were analyzed by using a general linear model ($y_{ijk} = \mu + Month_j + LPL_k$) carried out with Jump ver. 6 software. Results on the LPL genotype effects were presented as least square means ± SE and linear contrasts were tested between the genotypes.

Results and conclusion

DNA regions spanning from the promoter to exon 4 of the river buffalo LPL gene were amplified and sequenced twice in both directions for 10 buffalo cows. The comparison of the sequences showed 11 SNP in total. In particular, 6 polymorphic sites were detected at the level of the intron 3, whereas the analysis of the region including the promoter and the exon 1 showed a total of 5 SNP. All these nucleotide mutations falls into no coding regions of the gene, with the exception of the SNP g.107G>A detected at the exon 1 (numbering starts from the first nucleotide of the exon 1). Such transition (g.107G>A) is a silent mutation and it creates a site for the endonuclease restriction DdeI (5'...C↓TNAG...3'). Therefore, a fast PCR-RFLP protocol was set up for the genotyping of the animals. The digestion of the PCR product (850 bp) is characterized by 6 fragments of 349-163-139-103-91-5 bp for the homozygous AA. The same amplicon is restricted into 5 fragments (452-163-139-91-5 bp) in presence of guanine at the homozygous status.

The restriction pattern of the heterozygous samples showed a combined pattern with 7 DNA fragments.
Fatty acid desaturation indices (DI) were calculated from milk fatty acid profiles of 22 river buffaloes, all reared in the same farm to avoid differences due to environmental conditions, diet, calving, DIM, etc... The g.107G>A LPL genotype was significantly associated with the total desaturation index (P=0.01). The genotype GA showed an over-dominance effect with an average DI approximately 2% higher than GG buffaloes. However further investigation is necessary to confirm these data on a wider population.
The genetic variability of LPL gene in river buffalo seems to be higher compared to the data reported in literature for other ruminant species (Marchitelli *et al.*, 2013; Crepaldi *et al.*, 2013; Badaoui *et al.*, 2007). In particular, the SNP found in the LPL promoter regions appear to be very interesting. Considering the importance that any mutation in the regulatory region can potentially have an effect on the transcriptional regulation (either enhance or repress), further studies will be necessary in the future to evaluate their real influence on milk composition. This result represents for the river buffalo species an important starting point in the study of genes involved in the regulation of milk fatty acids composition in mammary gland.

Acknowledgment
This work was financially supported by the Italian Ministry for Agriculture and Forestry Policy - MiPAAF (INNOVAGEN project).

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Poster 6

Occurrence of fatty acids and steroid hormones in bovine milk and infant formulas

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Milk is one of the most consumed foods worldwide, source of many nutrients that are necessary for the neonate. It plays also an important role during humans' adult life, helping them to meet their nutritional requirements. Among those nutrients, fatty acids (FA) are important components with many functions in humans. Milk fat contains approximately 400 different FA, being the most complex fat of all natural fats. Derived from two sources, the feed and the microbial activity of rumen, milk fat composition is highly variable. The saturated FA are the most important portion from a quantitative point of view (approx. 75%). The short-chain saturated FA (C4:0-C10:0), produced by rumen bacteria, are present in a relative high percentage and not present in other kind of fat. They are easily absorbed and rapidly digested by humans, avoiding fat deposition. In bovine milk there are also mono-unsaturated and poly-unsaturated FA (18:2 omega-6 and 18:3 omega-3) with potential antiatherogenic, antiobesity or anticarcinogenic role. In the mono-unsaturated part, oleic acid (C18:1,n-9) is the principal FA of the group. Vaccenic acid is a positional and geometric isomer of oleic acid and the predominant trans isomer in ruminant fats. It is the precursor of the main isomer of conjugated linoleic acid (CLA), rumenic acid, which has many biological funcions. Additionally, the polyunsaturated Omega-6 and omega-3 are essential FA for humans, and they are especially important during gestation and also for neonates.

Besides nutrients, milk also contains endogenous hormones which are unavoidable due to the animal origin of this food. The hormones in general and more precisely the sex steroids are an essential part of life. They are naturally produced in the bodies of animals, including humans, and they control important body functions. However, exogenous steroidogenically active compounds may interfere in the hormonal endogenous equilibrium affecting health and body development. The Directive 96/22/EC prohibited the use in stockfarming of certain substances having a hormonal or thyrostatic action, in order to protect consumer's health. However, all foodstuff of animal origin may contain steroid hormones and metabolites, but their concentration varies with the kind of food, species, gender, age and physiological stage of the animal.

The reported values on FA composition in food packages are sometimes scarce and limited to the most important ones and hormone content is not declared at all. Both steroid hormone and FA composition of milk and dairy products are highly variable, but very important for human health. In this research, raw milk samples from pregnant and non-pregnant cows and a series of baby formulas from Spain have been analysed. The obtained data for fatty acids and sex steroid hormones in these foods is presented, contributing to the existing knowledge and to the evaluation of daily intakes for both groups.

The author has nothing to disclose.

Poster 7

Monitoring milk for recombinant bovine somatotropin (ab)use

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Recombinant bovine somatotropin (rbST) can be used to enhance milk production in dairy cows. This is licensed in several countries, like for instance the United States, but banned in the European Union. To enable screening of milk for rbST (ab)use, we have developed different screening methods. Detection of rbST itself in milk, however, is difficult due to the very low concentrations, its similarity with the endogenous hormone bST and the short half-life. Therefore, these screening methods focus on analysing one or multiple biomarkers instead of rbST itself. Biomarkers analysed are for instance rbST-induced antibodies and IGF-1, as they are both altered during rbST treatment. These methods range from fast and easy-to-use field tests using e.g. lateral flow devices, to methods, which require more advanced equipment, like the microsphere-based Luminex method. These different biomarker based screening methods for rbST (ab)use, which we are developing, will be presented.

Acknowledgements: This project was financially supported by the Dutch Ministry of Economic Affairs

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Poster 8

Identification and quantification of milk oligosaccharides from different mammals

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The aim of this work was to characterize oligosaccharides (MOS) from goat, sheep, mare and camel milk in relation to those from human and bovine milk. Because of their assumed beneficial prebiotic and anti-infective effects, MOS are substances of particular interest in human nutrition. However, MOS are not available as dietary ingredients. Therefore MOS from bovine and non-bovine milk may be an attractive source for potential application in human nutrition. For this reason, raw milk samples from different mammals were analyzed.

The milk samples were defatted by centrifugation at 4°C and the proteins were removed by ultrafiltration (NMWCO 10kDalton). Size exclusion chromatography was used to separate the MOS from lactose, monosaccharides and other undesired analytes. The identification and quantification of MOS was performed by high-pH anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) and parallel on-line electrospray ion-trap mass spectrometry (IT-MS). This analytical system allows a direct characterization of the composition and structure of the MOS fractions without derivatization.

The results demonstrate that milk samples are species-specific in relation to the type and content of MOS. As expected, the highest amount of MOS was found in human milk. The MOS content in sheep, mare, goats and camel milk was approximately 20 times smaller and more similar to the amount in cow milk. Nevertheless, the structure and amount of oligosaccharides in milk of different origin can vary significantly.

To provide a better understanding of the relation between structure and possible health benefits of MOS, further studies will deal with the prebiotic and anti-inflammatory properties of oligosaccharides from different species.

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Poster Abstracts

Part 2: Health effects

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Poster 9

Cheese consumption and blood lipids; a systematic review and meta-analysis of randomized controlled trials

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Aims
Cheese may have a different effect on lipids and lipoproteins than expected from the saturated fat content. We performed a systematic review and meta-analysis of randomized controlled trials (RCTs) to examine the effect of cheese consumption on blood lipids and lipoproteins in healthy populations.

Methods
A systematic search in MEDLINE, EMBASE, Scopus, Cababstracts, Cochrane Controlled Trials Register, Clinicaltrials.gov was performed to identify RCTs of cheese supplementation in human adults with total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides as a primary or secondary outcome (published until September 2013). A quantitative meta-analysis was performed if more than four RCTs with a comparable control treatment were available. Within person-differences of lipids with corresponding standard errors caused by the cheese compared to the control treatment were pooled (random effects model, STATA 11.0).

Results
We identified 15 RCTs, published between 1978 and 2012. We pooled four RCTs comparing the effect of cheese intake to butter with a similar fat content on plasma levels of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides. The amount of cheese used in these trials was rather large, ranging between 120 and 205 g/d. This is approximately equivalent to 3 to 5 cheese servings per day. Intake of cheese (weighted mean difference: 142.6 g/d) reduced total cholesterol significantly by -0.27 mmol/l (95% CI: -0.36 to -0.18), LDL-C by -0.21 mmol/l (95% CI: -0.29 to -0.13), and HDL-C by -0.05 (95% CI: -0.08 to -0.02) compared to butter. The pooled effect on triglycerides was 0.004 (95% CI: -0.058 to 0.065). No heterogeneity was observed (all I2=0%). Cheese was also compared with tofu (n=4 RCTs), fat-modified cheese (n=3), CLA-rich cheese (n=3), milk (n=2), fish (n=1), egg white (n=1). Trials that compared cheese with tofu or fat-modified cheese suggest that differential effects of the products can mainly be attributed to the differences in fatty acid content of the diets. Comparisons with CLA-rich cheese were of limited value because those studied the effects of CLA (and not cheese). Too few trials with milk, egg white, and fish were available to draw conclusions.

Conclusions
Based on a limited number of trials, cheese appears less hypercholesterolemic than butter with a similar fat content. Differences in plasma lipids based on cheese compared with tofu and fat-modified products are likely to be caused by the different fat content of the total diets.

Supported by an unrestricted grant from the Global Dairy Platform, the Dairy Research Institute, and Dairy Australia.

Poster 10

^{13}C-labeled oligosaccharides from mother's milk are differentially excreted in breastfed infants' urine

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Human milk oligosaccharides (HMO) have received much attention due to their beneficial effects observed *in vitro*, such as prebiotic, anti-infective, anti-inflammatory and other properties. However, *in vivo* investigations on the metabolism and functions of HMO are very rare. The few data available indicates that HMO are absorbed to a low extent and excreted in the urine without noteworthy modifications, whereas the major proportion is passed to the colon undigested.

Via intrinsic ^{13}C-labeling of HMO during their biosynthesis in the mammary gland of ten lactating women, we were able to follow the fate of ^{13}C-labeled oligosaccharides from their secretion via milk to the excretion in urine of their breastfed infants. As there are indications for structure-specific differences in bioactivity and absorption of HMO, we determined tracer enrichment in more than ten individual neutral oligosaccharide species by means of a novel and rapid matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) based approach.

Whereas the time pattern of isotopomer enrichment in milk with two peaks during 36 h could be seen uniformly in all subjects and oligosaccharide species, the time curves for the appearance of labeled compounds in infants' urine varied strongly between individuals and single oligosaccharide species, with a tendency of higher excretion of larger, fucosylated structures.

Our data suggests that neutral HMO might be processed and/or utilized differentially after absorption from the gut, as deduced from their structure-dependent variation in the extent of tracer enrichment and in the retention times in the infant's organism. Future investigations *in vivo* should take this into account when elucidating HMO metabolism and functions in infants.

(supported by the German Research Foundation, DFG Ru 529/4-2, Ku 781/2-2 and the "Studienstiftung des deutschen Volkes" (V.D)

Poster 11

Oligosaccharides in term and preterm milk - determination of Lewis and secretor specific pattern and quantitation of major components

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Background
Human milk oligosaccharides (HMO) are considered to play an important physiological role for the infant [Kunz et al Ann Rev Nutr 2000, Bode Glycobiology 2012]. Of particular interest are (a) the Lewis and secretor specific HMO pattern, (b) changes during lactation and (c) differences between term and preterm milk.
Our **objectives** were to investigate whether

(i) the Lewis-related blood group status of lactating mothers reflects Lewis and secretor specific HMO in milk without ambiguity
(ii) changes of the HMO pattern and of single HMO occur during the first weeks of lactation in preterm and term milk

Methods
Milk samples from mothers after preterm (n = 21) or term (n=7) delivery were collected on days 3, 7, 21 and 56 postpartum. Milk (50 µl) was centrifuged before subjected to porous graphitized carbon cartridges [Blank et al Anal Bioanal Chem 2011]. Further analysis was done by HPAEC-PAD with modifications as described previously [Kunz et al J Chromatogr 1996].

Results and Discussion
HPAEC-PAD analysis of the structural diversity of HMOs revealed the characteristic Lewis blood group and secretor specific pattern. It was observed that the Lewis status determined by blood analysis was not always identical to the Lewis and secretor pattern found after the analysis of milk. For example, in milk samples from 9 women allocated as Lewis negative after a blood test only 2 revealed a typical Lewis negative HMO pattern. The remaining milk samples were characteristic for Lewis a (n=3) or Lewis b (n=4). The quantitation of ten single HMO, i. e. of 2´Fucosyl-Lactose, Lacto-N-Tetraose, Lacto-N-neo-Tetraose, Lacto-N-Fucopentaose I, II and III, Difucosyl-Lactose, 6´and 3`Sialyl-Lactose and Disialyl-Lacto-N-Tetraose, revealed various changes during the first weeks of lactation.

Conclusion
As the Lewis and secretor specificity of HMO may play an important role for the infant´s health a reliable assignment using the HMO pattern is required. Blood analysis did not fulfil these criteria, at least not for Lewis negative blood samples. For future clinical studies using single HMO or mixtures data are needed with regard to the total amount of individual components in term and preterm milk. Published data vary significantly probably due to different methods used.
There is no conflict of interest of any author.

(supported by the German Research Foundation, DFG Ru 529/4-2, Ku 781/2-2)

Poster 12

Model calculations on the impact of toddler formula on nutrient intake of young children

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Special formula products for toddlers are commercially available in Germany and are promoted for the special dietary needs of young children between one and three years of age. These products contain less protein than cow's milk and are enriched with all vitamins and minerals in significant amounts, especially with those that are not highly present in cow's milk. The nutrient content of toddler formula is thus significantly different from that of cow's milk.

Against this background, our aim was to assess the possible impact of toddler formula on the nutrient intake of young children. To this end, we performed model calculations on the basis of the VELS study , calculating energy and nutrient intake from the normal diet, including cow's milk, in comparison with that from the following two scenarios:

A) consumption of toddler formula instead of cow's milk in equivalent amounts;
B) consumption of toddler formula instead of cow's milk, assuming that all children consume 480 ml per day, based on the highest amount found to be recommended by the manufacturers.

For this purpose, a toddler formula was selected on the basis of its availability and representativeness for formula products on the market. The nutrient content data of that product were inserted into the food composition databank used for calculating nutrient intakes of participants included in the VELS study [1].

Energy, macro- and micronutrient intakes were calculated for children between one and three years, using PASW Statistics Version 18. Intake values calculated for each of the scenarios were compared with those from normal diet (baseline) as well as with reference values for this age group and, in the case of micronutrients, with upper intake levels derived by the European Food Safety Authority (EFSA).

Data show that children included in the VELS study achieved through normal diet, including cow's milk, reference values for macronutrients. The intake of mono- and disaccharides was, however, higher than the levels considered tolerable within a healthy diet. Furthermore, the median intake values for vitamins and minerals were at or above respective reference values, except for iron, iodine, calcium (only girls), vitamin D and folate.

Assuming that toddler formula is consumed instead of cow's milk (scenario A), intakes of protein and saturated fatty acids would decrease and those of unsaturated fatty acids increase. With respect to micronutrients, intakes of zinc, copper, manganese and of the vitamins B1, B2, B6, A, and C would increase considerably so that even higher proportions of children would meet or exceed the reference values. Also, the intakes of iron, iodine, vitamin D and folate would increase, but the median intake of iodine and vitamin D would still be below the reference values. Finally, the intake of calcium would undesirably decrease because the toddler formulae often contain less calcium than cow's milk.
Assuming that all children consume toddler milk in an amount of 480 ml per day (scenario B), the median intake values of vitamin A, zinc and copper would even exceed the respective upper levels of intake (UL).

Altogether, consumption of toddler formula instead of cow's milk would increase the median intake values of iron, iodine, vitamin D and folate. However, it would also lead to median intake values of other micronutrients that exceed by far the reference intake values and in some cases even the upper intake levels. According to the Federal Institute

for Risk Assessment (BfR), from a nutritional and physiological point of view, toddler formulae are not necessary and do not offer an advantage to semi-skimmed milk, which is recommended for children from one year onwards.

[1] VELS: Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln (Food consumption survey to determine food intake by infants and young children for the estimation of the acute toxicity risk from pesticide residues)

Poster 13

Trans-palmitoleic acid arises endogenously from dietary vaccenic acid

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Trans-palmitoleic acid (C16:1*t*9, alternatively named *trans*-16:1n-7), a *trans* fatty acid (*t*FA) that is assumed to be exclusively diet-derived, has been linked to the beneficial metabolic effects of dairy fat consumption. Recently, plasma phospholipid C16:1*t*9 was cross-sectionally associated with improved plasma triglycerides and lower fasting insulin, and prospectively with lower incidence of type 2 diabetes in elderly [1]. In the present work, we assessed the putative endogenous and intracellular conversion of supplemented vaccenic acid (C18:1*t*11), naturally occurring in dairy fat, to C16:1*t*9. For this purpose, we re-evaluated fatty acid data 1) obtained from human serum following ingestion of C18:1*t*11 and 2) from human peripheral blood mononuclear cells (PBMC) after incubation with C18:1*t*11, respectively. Both studies have been previously published in their entirety [2, 3].

In the human study, the participants consumed a ruminant-fat free diet supplemented with 2.9 g/d C18:1*t*11 and 2.9 g/d C18:1*t*12, or a C18:1*c*9-rich control-supplement, daily over six weeks. In the *in-vitro* approach, PBMC were incubated with 11 µM C18:1*t*11 for 24 h. Serum and PBMC fatty acid distribution including *t*FA were analysed by combining two GC methods, (i) for total fatty acid methyl esters (FAME) (column: DB-225 MS: 60 m × 0.25 mm i.d. 0.25 µm film thickness; Agilent Technologies, USA) and (ii) for hexa- and octadecenoic acid methylesters with *cis*- and *trans*-configuration (column: CP-select: 200 m × 0.25 mm i.d. 0.25 µm film thickness; Varian, Netherlands).

Ingestion of C18:1*t*11 resulted in 8-fold elevated serum levels of C18:1*t*11, compared to both baseline and control group after intervention ($p < 0.001$ each). This increase was accompanied by a significant increase in C16:1*t*9 (5-fold, $p < 0.001$ each). Since the diet was free of C16:1*t*9, and a strong correlation was observed between both fatty acids ($R2 = 0.808$,

$p < 0.001$), it is most likely that C16:1t9 arose from C18:1*t*11, due to chain shortening by two C-atoms. The conversion rate of C18:1*t*11 to C16:1*t*9 was, on average, 17% (range 10% to 30%). Likewise, C18:1*t*12 and the respective C16:1*t*10 showed up in serum, what supports the assumption of an endogenous partial ß-oxidation of the supplemented fatty acids.

In PBMC, the percentage of C18:1*t*11 increased within the cellular lipids from 0.12 ± 0.02% to 17.1 ± 3.7% of total FAME ($p = 0.006$ compared with DMSO-ctrl.). In parallel, C16:1*t*9 increased 25-fold, from 0.01 ± 0.01% to 0.27 ± 0.04% ($p < 0.001$).

We conclude that endogenous C16:1*t*9 is not exclusively diet-derived but may also be produced by partial (peroxisomal) ß-oxidation of dietary C18:1*t*11.

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Poster 14

Differences in protein digestion and generation of bioactive peptides dependent on milk processing

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Milk is a highly valuable source for proteins and bioactive peptides. Through milk processing a multitude of dairy products are generated, having a different physiological impact on our body. These differences may arise in consequence to transformation procedures, such as fermentation, heat treatment, or homogenization and have influence the digestive processes. Whether potentially bioactive peptides are present in a given product, if they resist the digestion process, or if they are generated during digestion, is the key for a better understanding of the physiological effects after dairy product consumption and prerequisite for the development of new and healthy milk products.
In this study, the protein and peptides of different dairy products were analyzed at different steps of in vitro digestion. All major milk proteins were degraded to smaller peptides after the gastric phase, with the exception of beta-lactoglobulin. For some products a product dependent shortened fragment was identified underlining differences induced by the technological transformation. Also, it could be observed that alpha-s1 casein was faster degraded to smaller peptides compared to beta-lactoglobulin, by summing up all generated peptides according to their number of amino acids. Moreover, for each product a specific peptide profile including beta-lactoglobulin and alpha-S1-casein peptides was generated. Dipeptidyl peptidase IV inhibitory peptides, playing a role in satiety regulation and therefore being interesting for product development, were quantified and compared between products. A better knowledge of the mechanisms needed for the generation of important bioactive peptides is a first step towards the appreciation of dairy products and needed to develop new products with increased health beneficial properties.

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Poster 15

The effect of dairy product intake on overweight and central obesity incidence in representative sample of Polish girls aged 13-21 Years. The GEBAHEALTH Project.

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Aim of the study: Comparison of overweight and central obesity incidence in Polish girls consuming and not-consuming dairy products.

Material and Methods: The research involved 1107 girls from a cohort of girls aged 13-21 years representative of the national population. A total of 2104 individuals were randomly selected from the PESEL data base. Interviews were conducted with 52.6% initial group. To assess food intake a validated Food Intake Variety Questionnaire (FIVeQ; Niedźwiedzka & Wądołowska 2008) was used. Respondents were divided into 2 groups as consuming (yes) or not-consuming (no) dairy products as follows: consuming milk/milk beverages in an amount greater than 7 glasses/week (yes/no) and, separately, cottage cheese and cheese in an amount greater than 2 tablespoons/week (yes/no). Height, weight and waist circumference were evaluated using the Polish standards (Kułaga et al. 2011, 2008). The overweight incidence was determined using the international classification of BMI for youth (Cole et al. 2000), and the central obesity on the basis of the waist-to-height ratio (WHtR). The following cut-offs were used: for height, weight and waist circumference – above 90 percentile, BMI>25 kg/m2 and WHtR≥0.5.

Results: The girls who consumed milk/milk beverages had a significantly higher average height (about 0.9 cm; $p<0.05$), and lower average: BMI (about 0.6 kg/m2; $p<0.01$), waist circumference (about 1.9 cm; $p<0.05$) and WHtR (about 0.01; $p<0.01$). The girls who consumed cheese had lower average: weight (about 1.7 kg; $p<0.01$), BMI (about 0.6 kg/m2; $p<0.01$), waist circumference (about 1.9 cm; $p<0.01$) and WHtR (about 0.01; $p<0.01$). Among girls consuming cottage cheese no differences were found in mean values of weight, height, waist circumference, BMI and WHtR ($p>0.05$). These results have been partially confirmed in distributions and logistic regression analysis. Significantly less girls consuming milk/milk beverages had central obesity compared to girls not-consuming milk/milk beverages (7.0% vs. 12.0%, respectively; $p=0.042$). Significantly less girls consuming cheese had weight above 90 percentile compared to girls not-consuming cheese (11.0% vs. 16.3%, respectively; $p=0.015$). Among girls consuming cheese the odds ratio (OR) of incidence of weight above 90 percentile was 0.63 (95%CI 0.44-0.90; $p<0.01$), in comparison to normal weight girls (10-90 percentile; OR=1.00).

Conclusions: Positive effect of consumption of milk and milk beverages and cheese on a body weight of Polish girls aged 13-21 years was observed. The consumption of regular-fat dairy products as well as high-fat dairy products was conducive to smaller incidence of overweight and central obesity in Polish girls.

The study was financed within the framework of NCN project no. N N404 068540.

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Poster 16

Genetic variants in bovine α_{s1}- and β-casein result in differences in the allergenic potential of epitopes demonstrated by microarray-immunoassay

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The caseins belong to the major allergens of cow's milk. Within these proteins, a noticeable genetic variation has been identified in productive and endangered cattle breeds. The genetic variants are characterized by amino acid exchanges or deletions of peptide fragments. Their importance in human nutrition, especially regarding to the allergenic potential, has not yet been adequately exploited.

In this study, we investigated the influence of the genetic variants in α_{s1}- and β-casein on the IgE-binding properties of epitopes. Moreover, we determined differences in IgE-binding to peptides resulting from in vitro gastrointestinal digestion of common casein variants. For this purpose, a set of 77 commercially synthesized peptides, covering the previously identified sequential IgE-binding epitopes of α_{s1}- and β-casein, were tested by means of peptide microarray for IgE-binding by using sera from humans with cow's milk allergy. In addition, α_{s1}- and β-casein peptides, which were shown to resist gastrointestinal digestion and harbor IgE-binding epitopes, were included.

In the α_{s1}-casein variants A, B, C, E and I, the amino acid substitutions and deletion influenced the immunoreactivity of 4 epitopes. Furthermore, modifications in IgE-binding could also be demonstrated for 4 variant specific peptides originating from gastrointestinal digestion. However, a marked heterogeneity in IgE-binding between individual sera was observed. Variation in IgE-binding was further detected for 2 digested peptides of the β-casein variants A^1, A^2 and B.

The results of our investigations showed that genetic variants in cow's milk differ in their allergenic properties and therefore provide a new approach for the identification of an alternative and suitable protein source for cow's milk allergic patients.

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Poster 17

Calcium deficiency and absence of vitamin D alter bone, calcium and fat absorption, but not body weight

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Vitamin D and/or calcium previously considered as involved only with bone health are now known to have broader metabolic functions. Milk is a good source of calcium and in some countries, because of fortification, of vitamin D as well. Calcium and vitamin D deficiency is associated with disturbances of energy, carbohydrate and fat metabolism, whereas high vitamin D and calcium intakes have been reported to facilitate weight and fat loss of subjects on a hypocaloric diet. Also, PTH concentrations are said to be positively and 25(OH)-vitamin D concentrations to be negatively related to body weight, but it is unclear whether these findings are consequences or causes of overweight. Moreover, because of conflicting findings on the effect of supplemental vitamin D on plasma concentrations of 25(OH)-vitamin D, there is no consensus on the differentiation between low vitamin D status and hypovitaminosis D. We have reported how calcium and vitamin D deficiency altered plasma 25(OH)-vitamin D levels, calcium retention and bone density and quality[§]. We now report the effect of the deficient diet on fat absorption without significantly altering body weight in a model with experimentally induced osteomalacia[§]. Twenty adult female minipigs were switched from a standard regimen with 9 g calcium and 2000 IU vitamin D3 per kg to one with 6 g calcium and 6500 IU vitamin D3 (control diet) or one with 2 g calcium per kg and no vitamin D(deficient diet). Plasma 25(OH)-vitamin D levels of minipigs on the control diet rose progressively for 10 months from a base value of 74.8 ± 10.8 (SEM) nmol/L to 255.4 ± 40.2 nmol/L. Thereafter they plateaued for 3 months and then decreased to 206.1 ± 10.8 nmol/L at the end of the study (15 months). Plasma 25(OH)-vitamin D levels (nmol/L) in animals on the deficient diet dropped significantly from a base value of 60.2 ± 11.4 to 20.5 ± 8.2 after 5 months and to 15.3 ± 3.4 after 10 months. Plasma levels of 1,25(OH)2-vitamin D were persistently higher and of PTH transiently higher than in controls. After 15 months the animals on the deficient diet were in negative calcium balance (-3.65 ± 2.23 g/7d), having lost significantly more bone mineral density (-51.2 ± 14.7 mg/cm3) than the controls (-2.3 ± 11.8 mg/ cm3) whose calcium balance was positive (1.32 ± 1.26 g/7d; $p<0.08$). The bone moisture content of the animals on the deficient diet also was higher. Animals on the deficient diet had lost an insignificant amount of weight although they absorbed slightly but significantly more fat (379.8 ± 1.4 g/7d) than the controls (370.9 ± 2.8 g/7d; $p<0.009$) whose body weight remained constant. Our findings support the hypothesis that a diet deficient in calcium and vitamin D may affect energy metabolism in that it increases fat absorption. They do not support observations of a higher body weight gain. However an increase of fat mass at the expense of lean mass or an accumulation of abdominal fat at the expense of peripheral fat cannot be excluded.

[§] Scholz-Ahrens KE, Glüer, C-C, Bronner, F et al., 2013. Modulation of Vitamin D Status and Dietary Calcium Affects Bone Mineral Density and Mineral Metabolism in Göttingen Minipigs. ISRN Rheumatology Volume 2013, Article ID 460512, 12 pages, http://dx.doi.org/10.1155/2013/460512.

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Poster 18

A frequency of dairy products consumption and prevalence of hypertension

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Aim of the study: Assess the occurrence of high blood pressure (BP) in women with different frequency of consumption of dairy products.

Material and methods: The study involved 541 women aged 19-58 with BMI≥18.5 kg/m^2. Arterial BP was measured among 255 women. High BP was classified as SBP≥130 mmHg or DBP≥85 mmHg. Food consumption was determined using the Food Frequency Questionnaire (FFQ10). We calculated the odds ratio (OR) of an occurrence of hypertension depending from the frequency of dairy products consumption. The values were adjusted by BMI, age and parental hypertension. As reference groups (OR=1.00) women with normal blood pressure were taken.

Results: Among women 23% had a high SBP and 23% DBP. 23% of the women were overweight and 50% had high body fat. OR of high SBP and DSP were 3 and 4.8-times higher in women with OW (OR=3.04, 95% confidence interval (CI) 1.65, 5.61 and OR=4.76, 95% CI 2.57, 8.80, respectively), 6 and 4.8-times higher in women aged >40 years (OR=6.11, 95% CI 2.83, 13.20 and OR=4.39, 95% CI 2.05, 9.39), 1.9 and 2.6-times higher in women with parental hypertension (OR=1.87, 95% CI 1.01, 3.46 and OR=2.61, 95% CI 1.43, 4.77). Women with regular consumption of flavored milk beverages compared to women with occasional consumption had 61% lower OR of high SBP (OR=0.39, 95% CI 0.21, 0.72, adjusted by BMI OR=0.35). Women with regular consumption of milk dessert and cream had 2.7 and 1.9-times higher chance of high DBP (OR=2.71, 95% CI 1.24, 5.94, adjusted by BMI OR=2.90 and OR=1.94, 95% CI 1.05, 3.59, respectively). A high frequency of consumption of dairy products decreased the OR of high SBP adjusted by BMI by 60% (OR=0.40, 95% CI 0.19, 0.85). For DBP adjusted on age, OR increased 2.1-times for women from the upper tercile (T3) of dairy products consumption in comparison to women from the bottom tercile (T1) (OR=2.12, 95% CI 1.04, 4.29).

Conclusions: SBP adjusted by BMI was decreasing with a regular frequency of dairy products consumption, especially low fat milk beverages. DBP adjusted by age was increasing with a high regular consumption of dairy products, especially milk desserts.

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Poster 19

A frequency of dairy products consumption and prevalence of high body fat and weight changes in women aged 19-55

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Background: Literature review showed the evidences for the existence of an inverse relation between calcium and body fat or weight. The best dietary sources of calcium are dairy products, but some of them like rennet cheese apart the high calcium contain a lot of fat and energy.

Aim of the Study: An assessment of the influence of frequency of the consumption of dairy products on the prevalence of high body fat (>30%), overweight (BMI>25.0 kg/m2) and recent weight gain in women (>4kg).
MATERIAL AND METHODS: The study involved 539 women aged 19-55 with BMI≥18.5 kg/m^2, living in north-east Poland. The women were classified into 2 groups: normal weight (NW: BMI=18.5-24.9 kg/m^2) and overweight (OW: BMI≥25.0 kg/m^2). Body fat was calculated based on measurements of skin-folds thickness according to the Durnin & Womersley equation. Women were divided into 2 groups: normal body fat (≤30% of weight) and high body fat (>30%). Women were asked about birth weight, changing in weight and prevalence of parental overweight according to the Ruderman algorithm (1998). Changes in women´s weight were described as: no change in weight (1), loss of weight (2) and gain of weight >4kg (3). Food consumption was determined using the Food Frequency Questionnaire (FFQ10), which is a modification of the validated Food Intake Variety Questionnaire (FIVeQ). Dairy products were divided into 8 groups: milk and natural milk beverages (1), cream (2), flavored milk beverages (3), fresh cheese (4), flavored cream cheese (5), rennet cheese (6), butter (7) and milk desserts like pudding, ice-cream etc. (8). The frequencies of the dairy consumption were divided into 2 groups: occasional (1-2 times per week and less) and regular (at least 3-times per week). We calculated the odds ratio of an occurrence of high body fat, high BMI and weight gain in the last 6 months depending from the frequency of dairy products consumption. The values were adjusted by age, birth mass and parental overweight. As reference groups (OR=1.00) women with normal body fat, normal BMI and losing weight in the last 6 months were taken, respectively.

Results: OR of high body fat was increasing in women aged >40 years (OR=6.98, 95% confidence interval (CI) 4.01, 12.2), gaining weight in the last 6 months and after 18 years of life (OR=1.71, 95%CI 1.06, 2.78 and OR= 2.86, 95%CI 1.95, 4.20, respectively). The OR values were higher after adjusting by parental overweight and birth mass. Birth mass and parental overweight were not significantly important independent factors. OR of high BMI were increasing in women with parental overweight (OR= 2.53, 95%CI 1.67, 3.83), aged>40 (OR= 3.61, 95%CI 2.28, 5.71) and weight gain after the age of 18 years (OR=3.12, 95%CI 1.84, 5.30). Low birth mass was a cause of increasing OR values, but it was not significantly important as an independent factor. Weight gain in the last 6 months had no significant effect on OR values of overweight occurrence. OR of high body fat was increased with regular consumption of milk desserts (OR=1.83, 95%CI 1.11, 3.02), flavored cream cheese (adjusted by age OR=1.81, 95%CI 1.10, 2.99) and cream (adjusted by parental overweight OR=1.55, 95%CI 1.03, 2.33). OR of gaining weight was 2-times higher in women with regular consumption of milk desserts (OR=2.32, 95%CI 1.17, 4.61) and 41% lower in women with regular consumption of milk and natural milk beverages (OR=0.59, 95%CI 0.35, 0.99). There was no significant difference in the frequency of dairy product consumption between women with normal BMI and overweight. Consumption of rennet cheese, fresh cheese, flavored milk beverages and butter showed no significant differences in women with various body fat and weight.

Conclusions: A higher prevalence of high body fat and gain of weight in the last six months were noticed in women with more frequent consumption of dairy desserts, cream and cheese cream. While high frequency of consumption of milk and natural milk beverages led to losing weight in the last six months.

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