

# Milk and Diabetes

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Jürgen Schrezenmeir, PhD, and Alexandra Jagla, PhD

*Institute of Physiology and Biochemistry of Nutrition, Federal Dairy Research Center, Kiel, GERMANY*

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Type 1 diabetes is based on autoimmunity, and its development is in part determined by environmental factors. Among those, milk intake is discussed as playing a pathogenic role. Geographical and temporal relations between type 1 diabetes prevalence and cow's milk consumption have been found in ecological studies. Several case-control studies found a negative correlation between frequency and/or duration of breast-feeding and diabetes, but this was not confirmed by all authors. T-cell and humoral responses related to cow's milk proteins were suggested to trigger diabetes. The different findings of studies in animals and humans as well as the potential underlying mechanisms with regard to single milk proteins (bovine serum albumin,  $\beta$ -lactoglobulin, casein) are discussed in this review. In contrast to type 1 diabetes, the etiology of type 2 diabetes, characterized by insulin resistance is still unclear. In a population with a high prevalence of type 2 diabetes, the Pima Indians, people who were exclusively breastfed had significantly lower rates of type 2 diabetes than those who were exclusively bottlefed. Studies in lactovegetarians imply that consumption of low fat dairy products is associated with lower incidence and mortality of diabetes and lower blood pressures. In contrast, preference for a diet high in animal fat could be a pathogenic factor, and milk and high fat dairy products contribute considerably to dietary fat intake. Concerning milk fat composition, the opposite effects of various fatty acids (saturated fatty acids, *trans*-fatty acids, conjugated linoleic acid) *in vitro*, in animals and in humans have to be considered.

## Key teaching points:

- Type 1 diabetes is an autoimmune disease which is in part determined by environmental factors
- Case-control studies on infant feeding practices and detection of immune reactions against cow's milk proteins led to the hypothesis that cow's milk consumption is causally related to diabetes development; however, the findings supporting this theory have not been confirmed by other authors
- Intervention studies in humans seem to be necessary to define clearly a potential risk of CM consumption in infants
- The etiology of type 2 diabetes, characterized by insulin resistance and hyperinsulinemia, is still unclear
- Bottle-feeding in infancy has been discussed as being involved and certain constituents of CM could be related to diabetes development in adults
- On the other hand, lactovegetarian diets seem to be protective with respect to the development of the metabolic syndrome, and certain fatty acids have been shown to have beneficial effects on glucose tolerance in diabetic animals

## MILK AND TYPE 1 DIABETES: INTRODUCTION

Diabetes is prevalent in about 3% to 5% of the population in industrial countries. The major forms of diabetes, type 1 and type 2 diabetes, contribute at about 10 percent and 90 percent, respectively.

In type 1 diabetes the insulin secreting pancreatic  $\beta$ -cells are

destroyed by autoreactive T-lymphocytes and macrophages and autoantibodies occur against surface antigen p69, glutamate decarboxylase (GAD), cytoplasmic islet cell antigen (ICA) and insulin (IAA). Type 1 diabetes is associated with other autoimmune diseases in about 15% of cases [1]. The association of these diseases is entitled "syndrome of pluriglandular insufficiency" and comprises affections of the thyroid (Graves' disease, M. Hashimoto) of the stomach (atrophic gastritis), of

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Abbreviations: BB rat= bio-breeding rat, BLG= $\beta$ -lactoglobulin, BSA=bovine serum albumin, CM=cow's milk, GAD=glutamate decarboxylase, GLUT-2=glucose transporter-2, HLA=human leukocyte antigen, IAA=insulin autoantibodies, ICA=islet cell antibodies, ICA69=islet cell antigen 69 (=p69), IgA, IgG=immunoglobulins, NOD-mouse=non-obese diabetic mouse, PCA=parietal cell antibodies, Tep69=T-cell epitope 69.

Address reprint requests to: Prof. Dr. Jürgen Schrezenmeir, Institute of Physiology and Biochemistry of Nutrition, Federal Dairy Research Center, Hermann-Weigmann-Straße 1, D-24103 Kiel, GERMANY.

the skin (vitiligo) and of the adrenals (M. Addison) and other organs. Whether type 1 diabetes will develop or not is determined by genetic and environmental factors. The genetic impact is estimated to be about 30% to 40% on basis of studies in monozygotic twins. The genetic susceptibility is mediated in part by genes in the human leukocyte antigen (HLA) region, and it was shown to highly correlate with the absence of aspartate at position 57 of HLA DQB and the presence of arginine in position 52 of DQA [2–4]. Geographic variations and temporal trends in the incidence of type 1 diabetes and studies on migrants moving from an area with low incidence to an area with rather high incidence revealed that environmental factors seem to have the higher impact on disease development (60% to 70%). Besides virus infections (coxsackie, cytomegalie and so on) dietary factors, particularly milk intake, have been discussed as being involved.

## MILK CONSUMPTION AND FREQUENCY OF TYPE 1 DIABETES

### Epidemiological and Ecological Findings

Geographical and temporal relations between type 1 diabetes's prevalence and cow's milk (CM) consumption have been found in ecological studies [5–7]. Scott [5] evaluated data about the consumption of unfermented milk products from 13 countries and reported a significant positive correlation ( $r=0.86$ ) with diabetes incidence.

Dahl-Jorgensen *et al.* [6] found a similar close correlation

( $r=0.96$ ), comparing age-standardized incidence rates of diabetes in children 0 to 14 years of age and data on fluid CM consumption in 12 countries. However, these calculations included milk consumption in individuals of all ages and did not refer to infant diet. A negative relationship ( $r=-0.53$ ) was found between breast-feeding (avoidance of cows milk consumption) until the age of three months and diabetes risk referring to data from 18 countries [5].

Correlation of incidence rates in children 0 to 14 years of age with cow's milk and cheese consumption in nine regions of Italy revealed a strong association between diabetes and fluid milk consumption ( $r=0.84$ ), but none to cheese consumption [7]. The authors state that the high incidence of type 1 diabetes in Sardinia and in other countries worldwide cannot be explained simply by the quantity of fluid cow's milk consumed. Interestingly, though milk consumption in Sardinia is less than half that in Finland, both regions have a similar incidence of diabetes in individuals 0 to 14 years of age [7–9]. Borch-Johnsen *et al.* hypothesized that variations in diabetes incidence within one genetically stable population might reflect changes in the exposure to one or more diabetogenic or protective factor. Indeed, the collection data in two regions in Norway and Sweden over about 40 years revealed an inverse correlation between diabetes in childhood and breast-feeding frequency [10].

### Case-Control Studies

Case-control studies have been carried out to analyse if there is a direct association of type 1 diabetes and exposure to

**Table 1.** Milk and Type 1 Diabetes

Pro	Contra
<ul style="list-style-type: none"> <li>● Increased incidence of diabetes in children not breast fed or &lt;4 month after birth [10–15]</li> <li>● Association between diabetes prevalence and cow's milk consumption [5–7]</li> <li>● Presence of antibodies against BSA and ABBOS peptide/homology of ABBOS with islet cell surface protein p69 [46,47,50]</li> <li>● Presence of autoantibodies binding <math>\beta</math>-casein A1 in (pre)diabetics [69,70]</li> <li>● Structural homology of <math>\beta</math>-casein with p69, carboxypeptidase and glucose transporter GLUT-2 of islet cells [70,71]</li> <li>● Increased levels of IgA antibodies to BLG (<i>and</i> BSA) and independent association of IgA antibodies to BLG (but <i>not</i> to BSA) and cow's milk with the risk of type 1 diabetes [37,40,42,62]</li> <li>● Cow's milk diet increased manifestation of autoimmune diabetes in animal models [5,31,34]</li> <li>● Immunization of NOD mice (but not healthy strains) with BSA or p69 generated crossreactive T-cell responses to T-cell epitope Tep69 <i>and</i> ABBOS (but not albumin) [54]</li> <li>● <math>\beta</math>-casein and <math>\beta</math>-casomorphin induce diabetes in NOD mice [74]</li> <li>● Only from <math>\beta</math>-casein A1, not from <math>\beta</math>-casein A2, casomorphin-7 is released which stimulates macrophages; diabetes induction by <math>\beta</math>-casein A1 is inhibited by naloxone, an opiate antagonist [74,75,77]</li> </ul>	<ul style="list-style-type: none"> <li>● Meat and soy protein also increase the manifestation of type 1 diabetes [5,34,38]</li> <li>● Antibodies to BSA are not specific for type 1 diabetes (also other autoimmune diseases) [56]</li> <li>● Relationship between anti-BSA, anti-ABBOS and anti-p69/ICA69 is questionable—epiphenomenon?</li> <li>● Anti-BSA is only a reflection of disturbances in foreign/own recognition [51,56]</li> <li>● Injection of the BSA peptide ABBOS into young mice even reduces diabetes incidence [54]</li> <li>● No correlation between type 1 diabetes and frequency and duration of breastfeeding [28]</li> <li>● Type 1 diabetes incidence was also increased after introduction of solid food before the 4<sup>th</sup> month [14]</li> <li>● In some studies milk/BSA did not increase diabetes incidence in NOD mice [55]</li> <li>● Bovine IgG has shown even to be protective in NOD mice [55]</li> </ul>

BLG:  $\beta$ -lactoglobulin, BSA: bovine serum albumin, GLUT-2: glucose transporter-2, IgA: immunoglobulin A.

CM. The finding of an increased incidence of type 1 diabetes in individuals who have not been breast fed or ceased with breast feeding within the first four months after birth emerges from several studies [10–16]. A meta-analysis of 13 studies by Gerstein [17] revealed a 1.5 times higher risk of developing diabetes for people who were exposed to CM based nutrition before four months of age. A second meta-analysis of 17 studies by Norris and Scott [18] revealed a similar relation between early infant diet and insulin-dependent diabetes risk. A summary of all studies indicated a moderate effect for exposure to breast-milk substitutes (odds ratio=1.38) and cow's milk-based substitutes (OR=1.61) before three months of age. This rather weak association was attributed to potential bias due to differences in the participation rates of cases and controls.

It was questioned whether the exposure to some component of CM was a pathogenic factor or whether breast-feeding was protective. A multivariate analysis of data from a large case-control study in Finland (*Childhood Diabetes in Finland, DiMe*) revealed that the observed association between diabetes and breast-feeding was completely explained by its correlation with CM exposure, implying that, in fact, the exposure to CM is responsible for the development of disease [19,20]. On the other hand, the possibility was discussed that CM protein itself did not explain the relationship, but a higher weight gain in infants fed infant formula compared to breast milk might be a confounding factor [20,21].

However, there is also evidence questioning a causal implication of CM: The negative correlation between frequency and/or duration of breast-feeding and diabetes was not confirmed by other authors [22–27] nor were even positive associations seen [28–30]. In addition, an increase in diabetes incidence was likewise induced by the implementation of solid foods within the first four month after birth [14]. Similarly, Fort *et al.* [22] reported that there was no difference in the age of introduction of solid food between diabetic and nondiabetic children. Simiatycki *et al.* [23] demonstrated some indication of elevated risk for children in Montreal who had not been breast-fed, but only in univariate analyses; in multivariate analyses, psychological and social factors turned out to have a greater impact. A study carried out in Ireland and Scotland [27] demonstrated a small and nonsignificant reduction in risk among breast-fed children only after adjustment for social class (odds ratio=0.76).

A study in northern Sardinia, which is characterized by an ethnically homogenous population at high risk of type 1 diabetes, demonstrated that a larger proportion of the diabetic children rather than the control children had been breast-fed, and the risk of type 1 diabetes among children who had not been breast-fed was below unity (odds ratio=0.41). Overall, the data suggested a slight increase in the risk of type 1 diabetes with longer duration of breast-feeding (odds ratio 1.10 per month). Although a larger proportion of control children rather than diabetic children had been given CM-derived formula and

solid food before the age of three months, there was no time-risk relationship [30]. Not only a short duration of breast-feeding and early exposure to CM, but also solid foods were more significant risk factors (odd ratios about 14 to 15) when studies were carried out in individuals genetically predisposed to diabetes [13,14].

In a study carried out in Chile considering HLA DQA1 and DQB1 types as risk markers, early exposure was defined as the ingestion of food sources other than maternal milk before three months of age. Fewer children were exclusively breast fed in the diabetic group than in the control group. In addition, exposure to CM and solid foods occurred earlier in the diabetic group than in the control group [15].

Norris *et al.* compared feeding practices in children with elevated diabetes-associated autoantibodies against insulin, glutamic acid decarboxylase, or insulinoma-associated islet tyrosine phosphatases from a cohort of children between the ages of nine months and seven years, 18 with those of 153 unrelated autoantibody-negative controls. There were no differences in the proportion of cases and controls who were exposed to CM or foods containing CM or to cereal, fruit and vegetable, or meat protein by three months or by six months of age. Children with autoantibodies were breast-fed for a slightly longer duration than controls (median duration 10 vs. 8 months,  $p=0.07$ ), suggesting that early exposure to CM or other dietary protein is not associated with islet cell antibodies [25].

## Animal Experiments

Animal models like the NOD (non-obese diabetic) mouse or the BB (biobreeding) rat share many disease characteristics of humans with type 1 diabetes and are regarded as suitable means to identify pathogenic mechanisms of diabetes. Several studies on animal models revealed the significance of nutrition for the development of diabetes. Those animal models spontaneously develop diabetes with an incidence of 40% to 60% when fed a chow consisting of protein from different plant and animal sources [5]. Studies by Elliott and Martin [31] showed that addition of skim milk powder to a protein-free diet produced diabetes in BB rats with an incidence of 52%, whereas a semisynthetic amino acid diet reduced the incidence to 15%. The effect seemed to be established during the early postnatal period [32]. However, in another study, diabetogenic food components resulted in diabetes as late as puberty in the BB rat or even later in the NOD mouse [33].

The pathogenic role of milk ingestion in those animal models has been questioned since introduction of meat and soy protein increased autoimmune diabetes as well [5,34]. Furthermore, other authors did not confirm the effect of CM protein on diabetes frequency: Malkani *et al.* [35] reported that neither the addition of bovine serum albumin (BSA) to a milk protein-free diet nor the introduction of total milk protein increased the frequency of diabetes in BB/Wor rats.

## IMMUNE RESPONSES TO CM PROTEINS

T-cell and humoral responses related to the consumption of CM proteins were hypothesized to trigger diabetes. These immunological effects are in part ascribed to structural similarities between certain regions of the milk proteins and islet cell antigens. Due to this molecular mimicry the regulation of discrimination between “foreign” and “own” may be impaired in early infancy when the immune system is undergoing a learning process and when the gut is more permeable and ingestion of CM in infants may be followed by appearance of CM protein fractions in the blood and subsequent immunization against those proteins. Antibody as well as T-cell responses are later on directed against homologous  $\beta$ -cell antigens and mediate their destruction.

Vaarala *et al.* described induction of systemic humoral and cellular responses to CM proteins in infants fed with CM-based formula. The finding that T-cell response later declined was explained by induction of oral tolerance. Exposure to CM proteins after the age of nine months resulted in depressed cellular and humoral responsiveness to these proteins [36].

In contrast, it was shown that humoral immune responses to CM proteins were related not only to the age at introduction of supplementary milk feeding in infancy [38], but also to milk consumption in later childhood [38,39]. In a follow-up study in siblings of diabetic children the age of introduction of CM was not significantly related to antibody positivity and to diabetes development. After adjustment for age, gender, infant feeding, maternal age and education, high milk consumption in childhood (more than three glasses a day) was associated with more frequent emergence of autoantibodies than low consumption (fewer than three glasses a day) (adjusted odds ratio 3.97) [39].

Several studies investigated antibody as well as T-cell responses to distinct CM proteins in diabetics compared to controls to identify possible diabetogenic factors. Saukkonen *et al.* found significantly higher levels of IgA and also a tendency towards higher IgG antibodies to BSA in 104 newly diagnosed type 1 diabetes patients compared to 111 matched controls. However, when antibodies to BSA,  $\beta$ -lactoglobulin, whole CM and islet cell antibodies were studied as risk determinants of type 1 diabetes in a multivariate, logistic regression analysis, only IgA antibodies to  $\beta$ -lactoglobulin and to CM were independently associated with the risk, while anti BSA antibodies were not a significant risk factor [40]. In contrast, the same group reported recently indeed a significance of BSA and other CM proteins, when genetic risk determinants were included. Diabetic children had higher BSA IgG antibody levels than their age-, gender- and HLA-DQB1-matched controls. In 42 sibling pairs identical for HLA-DQB1 alleles, children with type 1 diabetes had higher median levels of BSA IgG, of  $\beta$ -lactoglobulin IgG and of CM formula IgG and IgA antibodies than the non-diabetic siblings [41].

The relation between islet cell specific antibodies (ICA and IAA) and antibodies to CM proteins was studied in type 1 diabetes and pre-type 1 diabetes in a population-based study with more than 650 children with newly diagnosed type 1 diabetes and more than 550 initially non-diabetic siblings [42]. There was no significant difference in CM antibodies between diabetic children with and without ICA or IAA. The siblings with ICA had higher CM-formula (IgA and IgM) antibody levels and BLG (IgA) antibody levels than the remaining siblings, but no such differences were found when comparing IAA-positive and negative siblings. Siblings positive for both ICA and parietal cell autoantibodies (PCA) had increased levels of antibodies against CM-formula, BLG and BSA.

There has been discussion that the enhanced cellular and humoral immune responses to dietary antigens observed in type 1 diabetes are not directly implicated in the pathogenetic process, but could rather be explained by a broken tolerance to dietary antigens due to regulatory defects in the gut immune system in general. The finding that a gut-specific adhesion molecule ( $\alpha 4\beta 7$ -integrin) was expressed in a remarkable population of  $\beta$ -cell-reactive T-lymphocytes was discussed as a link between the gut immune system and type 1 diabetes [43]. It was hypothesized that dysregulation of the gut immune system could result in an unspecific stimulation of recirculating lymphocytes, e.g., by CM proteins, which finally react with pancreatic antigens [44]. Furthermore, from the finding that siblings positive for both islet cell antigens and parietal cell antibodies had increased levels of antibodies to CM proteins it was concluded that there may be an enhanced transfer of nutritional antigens across the gut barrier due to mucosal damage in these subjects [42]. The findings and suggestions for the potential underlying mechanisms differ with regard to different CM proteins:

### Bovine Serum Albumin (BSA)

Elevated levels of anti BSA antibodies were detected in diabetic animals (NOD mice, BB rats) [45–47] and patients [47–49]. Karjalainen *et al.* [50] found elevated serum concentrations in IgG anti-BSA antibodies (but not of antibodies to other milk proteins) in 100% of diabetic subjects, the majority of which were specific to the 17 amino acid residue peptide “ABBOS” (amino acids 152–168). These antibodies were shown to cross-react with p69 (also named ICA69), a 69kD  $\beta$ -cell membrane protein, which is inducible by interferon  $\gamma$  and a target of autoantibodies in type 1 diabetes [47,50,51]. It was suggested that exposure of genetically susceptible individuals to BSA or ABBOS early in life could lead to immunization and development of memory cells for ABBOS. Any subsequent unrelated infection generating systemic interferon levels would induce transient p69 expression in the  $\beta$ -cells, exposing them to immune-mediated destruction [50].

BSA was further shown to induce cellular immunity. Significantly higher T-cell proliferation responses to BSA and

ABBOS were found in children with newly diagnosed type 1 diabetes than in non-diabetic children and normal adults [52,53]. ABBOS-specific T-cells of diabetic children were shown to bind to a homologous sequence in the p69 antigen, the so-called T-cell epitope p69 (Tep69) [53]. Karges *et al.* demonstrated, that immunization of nonobese diabetic (NOD) mice with BSA or ICA69 generated cross-reactive T-cell responses to both Tep69 and ABBOS [54].

The role of BSA, however, was questioned by several studies in animals and humans. Paxson *et al.* report, investigating NOD mice starting with test diets prior to conception, that there was no difference in diabetes incidence between mice fed a standard diet and those fed a milk-free diet [55]. Furthermore, addition of BSA to the milk-free diet resulted in no change in diabetes incidence. In the same study, a trend to a lower diabetes incidence was observed on a diet containing bovine IgG: seven months cumulative type 1 diabetes incidence in NOD mice was 93% (female) and 54% (male) under milk free diet, 78% and 17% under milk-containing standard chow, 93% and 17% under BSA supplementation and only 67% and 0% in the bovine IgG group. This might indicate that diets containing less immunoglobulin than human breast milk may induce diabetes; supplementation with bovine IgG, however, seems to be protective.

In contrast to the abovementioned studies, several authors did not confirm the findings of enhanced humoral or cellular responses to BSA or ABBOS, respectively. Only moderately elevated or even similar levels of IgG anti-BSA antibody levels were found in diabetic patients compared to controls [56–58]. Fuchtenbusch *et al.* pointed out the low, though significantly elevated, frequency (between 10% and 16%) of BSA antibodies found in their studies [59], as did others in adults and children (mean age 10.7 years) with type 1 diabetes [57,58]. These results limit the usefulness of BSA antibodies as clinical markers and their pathogenic impact in type 1 diabetes.

Anti-BSA antibodies were also demonstrated in ICA- and/or IAA-positive first degree relatives of diabetic patients and in other autoimmune diseases (thyroid disease, systemic lupus erythematosus, rheumatoid arthritis, Down's syndrome) [56,59,60]. Luhder *et al.* found no association between BSA antibodies (though elevated in diabetic patients) and type 1 diabetes-associated antibodies as cytoplasmic islet cell antibodies (ICA) or glutamate decarboxylase autoantibodies [61]. Atkinson *et al.* concluded from their findings that anti-BSA antibodies may reflect a general defect in the formation of immunologic tolerance associated with a predisposition to autoimmunity rather than immunity specific to  $\beta$ -cells [56].

Also, no T-cell responses to BSA and ABBOS were found in type 1 diabetics and first-degree relatives positive and negative for islet-cell antibodies [56]. Similarly, Vaarala *et al.* [62] failed to show a difference in cellular reactivity to BSA between patients and controls.

In addition, the relation between anti-BSA or anti-ABBOS and anti-ICA69 was questioned, since ICA69 expression is not restricted to islet cells and islet-cell p69 only shares two small regions of sequence homology with BSA, and these are distinct from the putative ABBOS region [51,60]. Furthermore, elevations of ICA69 antibodies in diabetic patients were not confirmed by all authors, possibly due to methodical problems [63,64].

In a recent study no cross-reactivity between BSA and ICA69 was found in human and animal experiments [65]. It was observed by means of Western blot assays that diabetic individuals, first degree relatives and normal controls showed similar levels (approximately 70%) of antibodies against ICA69, whereas considerably fewer (approximately 13%) had anti-BSA antibodies on Western blots, and individuals with antibodies to both proteins occurred only rarely (2% to 3%). In addition, in rats immunized with BSA, anti-BSA titres increased about 100,000-fold over preimmune levels, whereas anti-ICA reactive antibodies remained unchanged and similarly, in rats immunized with ICA69, anti-BSA antibodies were unchanged over preimmune levels.

### **$\beta$ -Lactoglobulin**

Data from the *Swedish Nationwide Case-Referent Study* and the *Childhood Diabetes in Finland Study (DiMe)* demonstrated immune responses to  $\beta$ -lactoglobulin in diabetic patients [37,40,42,62,66].

Increased levels of IgA antibodies to  $\beta$ -lactoglobulin were found and were shown to be an independent risk marker for type 1 diabetes [37,40,42,66]. Savilahti *et al.* [66] observed markedly higher levels of IgG and IgA antibodies to CM and IgG antibodies to  $\beta$ -lactoglobulin in patients fewer than 3 years old compared with the control subjects. Older groups of diabetic patients (3.0 to 6.9 and 7.0 to 14.9 years of age) had significantly higher levels of IgA antibodies to CM and  $\beta$ -lactoglobulin than the age-matched comparison groups of both unrelated control subjects and nondiabetic siblings, although the median values of the diabetic patients were closer to those of the comparison groups than in the youngest groups. Vaarala *et al.* [62] showed an enhanced cellular immune response to  $\beta$ -lactoglobulin in 55% of patients with newly diagnosed type 1 diabetes compared with 22% of healthy children. The median stimulation index to  $\beta$ -lactoglobulin was 3.3 in patients and 1.5 in healthy children. The finding that cellular responsiveness to  $\beta$ -lactoglobulin was not associated with HLA-DQB1 risk alleles implied that immune response to the protein did not reflect the accumulation of these HLA alleles in the patients with type 1 diabetes.

### **Casein**

Animal experiments produced contrasting results concerning the diabetogenic potential of casein. On the one hand, there are studies demonstrating a protective effect of whole casein as

a substituent for skim milk [5,67,68]; on the other hand, it was shown that introduction of  $\beta$ -casein as the only source of protein from before weaning leads to the development of diabetes in NOD mice [34].

Diabetes could be prevented or reduced in BB rats and in NOD mice by introducing hydrolyzed casein [5,34,67,68]. From the findings that partial hydrolysis of casein retained the diabetogenic effect of casein and injection of intact casein failed to induce diabetes, it could be hypothesized that the proteolysis of casein by intestinal enzymes and the release of immunologically active peptides from the protein is crucial for its potential diabetogenic activity [20,34].

In humans, elevated levels of antibodies to  $\beta$ -casein were demonstrated in patients with newly diagnosed type 1 diabetes [69,70]. Sardinians, however, had low  $\beta$ -Casein A1 antibody titers despite of a high diabetes incidence [70].

Cavallo *et al.* demonstrated cell-mediated immune responses to  $\beta$ -casein in patients with diabetes of recent onset compared to healthy controls; in contrast, no lymphocyte proliferation was seen in autoimmune thyroid disease, suggesting that this feature is specific for type 1 diabetes. The authors postulated a sequence homology between residues 63–67 of bovine  $\beta$ -casein and 415–419 of the  $\beta$ -cell-specific glucose-transporter GLUT-2 as the pathogenic mechanism [71] with regard to the finding that autoantibodies to GLUT-2 occurred in type-1 patients of recent onset [72]. Molecular mimicry between casein and other islet antigens (p69 and carboxypeptidase) is also a matter of discussion [71].

Ellis *et al.* also found cellular immune responses to  $\beta$ -casein in type 1 diabetes compared to autoantibody negative healthy control subjects. Strikingly, autoantibody negative relatives of the diabetic patients demonstrated almost identical T-cell reactivities [73]. The authors concluded that individuals genetically prone to autoimmunity may be deficient in forming tolerance to dietary antigens and that the role of  $\beta$ -casein as a causative factor in diabetes development remains unclear.

Elliott *et al.* [74,75] draw attention to the role of different genetic  $\beta$ -casein variants in the pathogenesis of diabetes. The authors showed that  $\beta$ -casein produced from milk from cows of the phenotype A<sup>1</sup>A<sup>1</sup> but not A<sup>2</sup>A<sup>2</sup> induced diabetes in NOD mice [74]. In a recent study, type 1 diabetes incidence in 0 to 14-year-old children from 10 countries or areas was compared with the national CM protein consumption with regard to milk protein polymorphism [75]. Total protein consumption did not correlate with diabetes incidence ( $r=+0,402$ ), but consumption of the  $\beta$ -casein A<sup>1</sup> variant did ( $r=+0,726$ ). Even more pronounced was the relation between beta-casein (A<sup>1</sup>+B) consumption and diabetes ( $r=+0,982$ ).

Diabetes induction in NOD mice by  $\beta$ -casein A<sup>1</sup> was inhibited by naloxone, an opiate antagonist [74]. Enzymatic digestion of  $\beta$ -casein (human and bovine) results in the release of several bioactive peptides. Casein fractions with opioid (casomorphins) or immunomodulatory properties are located in

a hydrophobic part of the  $\beta$ -casein molecule containing several proline residues [76]. The hydrophobic  $\beta$ -casein peptides have been shown to stimulate human macrophages [77]. Whether those bioactive peptides occur *in vivo* is still a matter of debate. However, the *in vivo* presence of precursors of the immunoreactive  $\beta$ -casomorphin-7 was demonstrated in animals and humans after milk ingestion [78,79]. Subcutaneous injection of  $\beta$ -casomorphin-7 was shown to induce diabetes in NOD mice [74]. Bioactive beta-casomorphin-7 has been shown to be released only from  $\beta$ -casein variants A<sup>1</sup> and B after *in vitro* digestion with intestinal enzymes, but not from the common A<sup>2</sup> variant or the corresponding human or goat caseins [75,80]. The immunostimulatory properties of this peptide were suggested to explain the relation between the consumption of these beta-casein variants and diabetes incidence.

## Insulin

Vaarala *et al.* [81] investigated whether exposure to CM formulas containing bovine insulin (BI) could lead to immunization against insulin. IgG antibodies to BI and human insulin (HI) were measured in children who received, during the first nine months of life, either a formula containing whole CM proteins or a formula containing hydrolyzed casein peptides. At six months of age the children who received CM formula had higher levels of IgG antibodies to BI than children who received either hydrolyzed casein formula or children who were exclusively breast-fed (median levels 0.480 *versus* 0.185). Antibodies to BI and HI showed a positive correlation and cross-reacted in inhibition studies. The high incidence of insulin-binding antibodies in young children with type 1 diabetes was assumed to be explained by oral immunization to BI present in CM. It was concluded, that exposure to BI, which differs from HI only by three amino acids, may break the tolerance to insulin.

However, several experiments in animals revealed a protective effect of oral administration of HI on diabetes incidence [82–85]. After feeding human insulin to 6-week-old NOD-mice for one month Ploix *et al.* [85] demonstrated a reduction in the severity of insulinitis and diabetes incidence due to the development of regulatory T-cells (secreting IL-4) which suppress the activity of autoreactive T-cell clones (secreting IL-2). The effect of oral insulin therapy is currently studied in two trials in diabetic children in the USA and France.

## CONCLUSION

The question whether milk is involved in the pathogenesis of type 1 diabetes still needs further clarification. Methodological problems of population studies have to be taken into account: Most of the breast-feeding studies that show a link between CM intake and diabetes are retrospective, relying on

long-time maternal dietary-recall, and therefore inaccuracies in diet quantification have to be considered, especially if data have been collected many years after the diagnosis of diabetes. This could, on the one hand, mean that the relation between milk consumption and diabetes is questionable; on the other hand, it has been suggested that the above mentioned meta-analyses even underestimate the association between early CM intake and diabetes, because breast-feeding data did enclose children who were only partly breast-fed (and therefore could have been at higher risk) and control groups consisted of volunteers from the whole population, instead of people genetically predisposed to diabetes [20]. Indeed, when analyzing children genetically at high risk, the inverse association between breast-feeding and diabetes became more significant [14,15]. From seventeen studies, fourteen relied on retrospectively collected infant diet data based on long-term maternal recall, which may be biased or inaccurate; three studies used existing infant diet records to assess exposure, thus lessening the possibility of recall bias or inaccurate data. The studies using existing records demonstrated little association compared with the studies relying on long-term recall. Studies in which the controls had a participation rate that was more than 20% lower than that of the cases showed a stronger diabetogenic effect of never being breast-fed (OR=1.58) than studies whose cases and controls had similar participation rates (OR=1.06). Thus, differences in the participation rates of cases and controls may have biased the results of these studies. This meta-analysis indicates that the weak association between infant diet and risk of diabetes mellitus may have methodologic explanations [18].

However, considering the fact that several prospective studies did not support the association between CM and type 1 diabetes, it remains to be determined if there is really a causal relationship.

To definitely answer this question, intervention studies in humans are required. Such an intervention study is conducted by Akerblom *et al.* [86]. In this *Trial to Reduce Type 1 Diabetes in the Genetically at Risk (TRIGR)*, children with MHC risk alleles and first degree relatives were recruited. The aim is to investigate if type 1 diabetes can be prevented by the avoidance of intact bovine protein during the first six months of life. For this reason a casein hydrolysate (Nutramigen®) is used as test formula, which showed itself to be protective against diabetes in NOD mice [54].

It is rather unclear what impact consumption of CM has beyond infancy. Whereas animal experiments suggest that diabetogenic diets can induce diabetes later in life, such studies are rare in humans. There are only few studies demonstrating a link between intake of CM and antibody levels in individuals several years of age [25,39].

Concerning potential mechanisms or components by which CM might induce diabetes, further research is necessary before any conclusions can be drawn.

## MILK AND TYPE 2 DIABETES: INTRODUCTION

In contrast to type 1 diabetes, the etiology of type 2 diabetes is still unclear. Type 2 diabetes is part of the so-called metabolic syndrome which beside diabetes comprises abdominal obesity, hypertension, dyslipoproteinemia (hypertriglyceridemia and low HDL) and precocious atherosclerosis and is characterized by insulin resistance and hyperinsulinemia. Whereas in type 1 diabetes islet cell destruction results in insulin dependence, type 2 diabetes may be treated with diet and later oral antidiabetics before a so-called secondary failure of the islets induces insulin requirements as well. It was therefore called Noninsulin Dependent Diabetes Mellitus (NIDDM). Since hyperinsulinemia and insulin resistance are the main features in type 2 diabetes, dietary factors inducing high insulin levels and decreasing insulin sensitivity may be regarded as diabetogenic.

## MILK INTAKE AND TYPE 2 DIABETES

### Infant Nutrition

Pettitt *et al.* [87] examined the association between breast-feeding and type 2 diabetes in a population with a high prevalence of this disorder, the Pima Indians. Infant-feeding practices, as assessed by a standard questionnaire for the first two months of life, were classified into three categories: exclusively breastfed, some breastfeeding or exclusively bottlefed. People who were exclusively breastfed had significantly lower rates of type 2 diabetes than those who were exclusively bottlefed in all age-groups. The odds ratio for type 2 diabetes in exclusively breastfed people, compared with those exclusively bottlefed, was 0.41 adjusted for age, gender, birthdate, parental diabetes and birthweight. Breast feeding as well reduced incidence of overweight resulting in hyperinsulinemia and insulin resistance. The authors, however, did not point to CM as a potential pathogenic factor, but to bottle feeding. This was shown to result in overfeeding and hyperinsulinemia [88–90], possibly due to the smaller efforts of suckling and to a constant fat content of bottle formula, whereas during breast feeding the infant has already become tired of suckling when the high caloric hind milk is secreted. Critical remarks have been made to this study, since the prevalence of type 2 diabetes is even 10- to 20-fold higher than in Europeans in those Pima who were breastfed, indicating that the relative contribution of bottle-feeding to the risk is rather small; furthermore, an inverse association between breastfeeding rates and type 2 diabetes has not been shown in other populations; for example, South Asians have a high prevalence of type 2 diabetes and very high breastfeeding rates [91].

It has been suggested, that the increased prevalence of type 2 diabetes could be a consequence of greater infant illness due

**Table 2.** Milk and Type 2 Diabetes

Pro	Contra
<p><b>Bottle-feeding in infants</b></p> <ul style="list-style-type: none"> <li>● Breast feeding reduces type 2 diabetes incidence in Pima Indians adjusted for age, gender, parental diabetes, birthweight [87]</li> <li>● Breast-feeding reduces incidence of overweight [88–90]</li> <li>● Bottle-feeding causes overfeeding and hyperinsulinemia (smaller effort, constant fat content) [88–90]</li> </ul> <p><b>Saturated Fat</b></p> <ul style="list-style-type: none"> <li>● High fat food leads to higher caloric intake, higher postprandial triglycerides and free fatty acids, higher insulin levels, insulin sensitivity (particularly in offspring of diabetics) [113]</li> </ul> <p><b>Trans-Fatty Acids</b></p> <ul style="list-style-type: none"> <li>● <i>Trans</i>-fatty acids affect insulin release from mouse islets [131]</li> </ul>	<p><b>Intake of (low-fat) dairy products</b></p> <ul style="list-style-type: none"> <li>● Diabetes incidence and mortality is low in lactovegetarians</li> <li>● Diabetes incidence and mortality is not correlated with milk or cheese consumption, but positively correlated with meat consumption (saturated fat and N-nitroso compounds) [100]</li> <li>● Introduction of skim milk to a vegetarian diet enhances decrease in blood pressure [101]</li> </ul> <p><b>CLA</b></p> <ul style="list-style-type: none"> <li>● CLA normalizes impaired glucose tolerance and improves hyperinsulinemia in the prediabetic (ZDF) rat [126]</li> </ul> <p><b>ACE Inhibitory Peptides from MP</b></p> <ul style="list-style-type: none"> <li>● ACE inhibitory peptides from MP reduce blood pressure in humans</li> <li>● ACE inhibition increases insulin sensitivity</li> </ul> <p><b>Calcium</b></p> <ul style="list-style-type: none"> <li>● Calcium intake from dairy products is inversely associated with blood pressure [105] and risk of stroke [106]</li> </ul>

CLA: conjugated linoleic acid

ZDF: Zucker Diabetic Fatty

ACE: Angiotensin converting enzyme

to problems of hygiene [91], referring to the theory that not only low birth weight [92] but also weight loss during the first year of life is associated with an increased risk of impaired glucose-tolerance and type 2 diabetes as well as a raised plasma concentration of 32–33 split proinsulin, which is interpreted as a sign of  $\beta$ -cell dysfunction [93]. The low birth weight, however, may result from maternal malnutrition, which has indeed been shown to be associated with obesity in the infants later life [94]. Low birth weight may be due to placenta insufficiency, too. Since nutrients like fat and proteins have impact on vasomotor activity, such dietary factors have to be considered in future investigations. For example, palmitate reduces nitric oxide release from endothelial cells, n3 PUFA's are transformed to vasodilatory prostaglandins, and ACE-inhibitory substances are released from CM proteins [95–99].

### Milk Consumption in Adults

Epidemiological data about a relationship between milk consumption and type 2 diabetes are rare. A large study on animal product consumption and mortality related to different diseases was carried out in a cohort of Seventh-day Adventists in California [100]. Adventists are prohibited from using tobacco, alcoholic beverages and pork and are discouraged from consuming other meats, fish, eggs and caffeine-containing beverages, but milk consumption is promoted. Therefore, their nutrition is characterized by a substantially lower consumption of meat and eggs, but higher consumption of milk, associated with a lower intake of total and saturated fat, but higher intake of polyunsaturated fats than that of non-Adventists. In Adventists, mortality and incidence of diabetes (as well as of other diseases) were lower than in the control group, whereas meat

consumption was positively associated with diabetes-related mortality in males.

## MILK INTAKE AND HYPERTENSION

Considering the association between type 2 diabetes and other features of the metabolic syndrome, the relationship between milk intake and hypertension is also of interest. It was shown that introduction of low-fat dairy products into a diet rich in fruits and vegetables, resulting in lower saturated and total fat than the common Western diet, can substantially lower blood pressure. In the *Dietary Approaches to Stop Hypertension (DASH)* trial the effects of dietary patterns on blood pressure were assessed in adults with or without hypertension (systolic blood pressure < 160 mm Hg, diastolic blood pressures of 80 to 95 mm Hg) [101]. Subjects received for eight weeks either a control diet, a diet rich in fruits and vegetables or a “combination” diet rich in fruits, vegetables and low-fat dairy products, with reduced saturated and total fat. Sodium intake and body weight were maintained at constant levels. The combination diet reduced systolic and diastolic blood pressure by 5.5 and 3.0 mm Hg more, respectively, than the control diet; the fruits-and-vegetables diet reduced systolic blood pressure by 2.8 mm Hg more and diastolic blood pressure by 1.1 mm Hg more than the control diet. Among the 133 subjects with hypertension (systolic pressure  $\geq$  140 mm Hg, diastolic pressure  $\geq$  90 mm Hg, or both), the combination diet reduced systolic and diastolic blood pressure by 11.4 and 5.5 mm Hg more, respectively, than the control diet; among the 326 subjects without hypertension, the corresponding reductions were 3.5 mm Hg and 2.1 mm Hg.

Strict vegetarians as well as lactovegetarians are thought to have lower blood pressures than the general population after adjustment for age, gender and body weight [102]. However, in a study by Sacks and Kass [103], intake of dairy products was associated with higher blood pressure in lactovegetarians. Intake of cheese and butter was correlated significantly with blood pressure, implying that the high fat content could be a trigger of hypertension. This could be explained by an inhibition of nitric oxide release from endothelial cells by palmitic acid [96]. In intervention studies, however, no impact of animal fat on blood pressure could be demonstrated. This was deduced from the findings that (1) a four-week meat containing diet (250 g/d) after two weeks strict vegetarian diet produced no increase in blood pressure and (2) a three-month low-fat lactovegetarian diet produced no decrease in blood pressure in non-vegetarians [103]. However, a direct effect on blood-pressure of a diet containing high fat milk products, but no meat, was not analyzed.

It was suggested that moderate intake of animal products in lactovegetarians might be a marker for a large intake of other potentially beneficial components of vegetable [103]. On the other hand, one could assume from the studies cited above that the reduction of fat (saturated fatty acids) decreases diabetogenic compounds and unveils protective effects of milk ingredients.

One such protective compound might be calcium. According to a meta-analysis of 23 population studies, dietary calcium intake is inversely associated with blood pressure [104]. In a cohort study in California, significantly less calcium intake from milk was reported in hypertensive *versus* normotensive men (but not women) and the association was independent of age and obesity. In a subsample of men from this cohort the effect of total dietary calcium intake from all dairy products was estimated from a 24-hour dietary recall. Again, hypertensive men consumed significantly less calcium than normotensives. In men, both systolic and diastolic blood pressure levels were inversely associated with calcium intake from dairy products. After controlling for age, obesity, and alcohol, diastolic blood pressure was negatively and significantly associated with total calcium intake from dairy products, and systolic blood pressure was similarly associated with whole milk calcium [105].

Abbott *et al.* [106], referring to data from the *Honolulu Heart Program*, examined the influence of dietary calcium and milk intake on thromboembolic stroke during 22 years of follow-up in older middle-aged men. Men who were nondrinkers of milk experienced stroke at twice the rate of men who consumed 16 oz/day or more. While the rate of stroke decreased with increasing milk intake, the decline in stroke risk with increased consumption was modest for those who consumed under 16 oz/d. Intake of dietary calcium was also associated with a reduced risk of stroke, although its association was confounded with milk consumption. Calcium intake from nondairy sources was not related to stroke, suggesting that

other constituents or covariates related to milk consumption may be important.

In this context the release of peptides from CM proteins inhibiting angiotensin converting (ACE) enzyme should be mentioned [97–99]. In *in vitro* and *in vivo* studies their effect was demonstrated. In a clinical trial hydrolysate of casein was shown to lower arterial blood pressure in humans [107].

There may be impact on carbohydrate metabolism, too. Since ACE inhibitory substances were shown to increase insulin sensitivity [108,109].

## MILK INTAKE AND GLUCOSE/INSULIN RESPONSE

The influence of milk intake (compared to a lactose-, glucose- or fructose-containing meal) on glucose and insulin responses was analysed by Aro *et al.* [110] with respect to the impact of different carbohydrates. The glucose response was significantly higher after the glucose containing meal and lower after the fructose meal as compared with the other meals. The insulin response was significantly higher after the lactose and glucose meals than after the milk and fructose meals. After the milk and lactose meals, the blood glucose responses were similar, whereas the insulin response was significantly lower after the milk meal. Since lactose apparently was similarly absorbed from the two meals, the difference in the insulin response was suggested to be due to different insulinogenic effects of the protein components or to differences in the physical properties of the respective meals.

In contrast, Gannon *et al.* [111] showed that the glucose response to milk can be predicted from the glucose response to the constituent carbohydrate lactose, whereas the observed insulin response was several-fold greater than expected from the glucose response.

In our own studies, glycemic response and insulin requirements and insulin secretion after glucose, milk, Continental (low fibre, low protein, high fat) and English breakfast (high fibre, high protein) of equal carbohydrate content were compared in healthy, type-1 and type-2 diabetic volunteers [112]. In type-1 diabetics, we found the highest insulin requirements after consumption of a continental breakfast. Ten percent less insulin was infused after milk and 30% less after an English breakfast. Type-2 diabetics showed no significant differences in insulin requirements between the three test meals. The endogenous insulin release as reflected by C-peptide levels, indeed, was much more pronounced after milk.

The glycemic response in healthy individuals had no relation to these insulin requirements. Continental and English breakfasts had similar glycemic effects, whereas milk produced only 30% of the blood glucose response observed after the continental breakfast and a more prompt and shorter-lasting insulin release.

## MILK FAT

With regard to their fat content, milk and moreover high fat dairy products contribute considerably to dietary fat intake. Preference for high fat meals (resulting in high levels of free fatty acids) has been considered to be a pathogenic factor [113]. According to epidemiological studies, consumption of high (saturated) fat diets is associated with insulin resistance, obesity and increased prevalence of diabetes [114,115]. It is rather difficult to quantify the contribution of milk fat to diabetes development, since milk consumption is variable and milk is usually not the only source of (animal) fat. High milk intake could be a sign of a general preference for animal fat and therefore be related to meat consumption in people at risk of developing type 2 diabetes.

Milk fat is very rich in saturated fatty acids (SFA, about 60%), predominantly palmitic, stearic and myristic acid. The major monounsaturated fatty acid is oleic acid, composing about 25%. Polyunsaturated fatty acids (linoleic acid and linolenic acid) occur in minor concentrations (about 2%). Furthermore, milk fat contains a considerable amount of *trans* fatty acids (TFA), predominantly *trans*-vaccenic acid (18:1 t11), originating from ruminant bacterial fermentation. The total TFA concentration in raw milk ranges between 1.5% and 6.5% [116,117]. In addition, TFA are generated during processing of milk products (heat treatment, fermentation).

Milk is furthermore an important source of conjugated linoleic acid (CLA). CLA is a mixture of positional (double bonds in positions 9 and 11 or 10 and 12) and geometric (*cis* or *trans*) isomers derived from linoleic acid, with 18:2 *c9,t11* and 18:2 *t9,c11* being the most biologically active forms. Milk is an important source of CLA, since it is generated during the hydrogenation of unsaturated fatty acids by bacteria in ruminants. The CLA concentration in milk fat was reported to vary between 0.2% and 1.8% in a study from Sweden [118] and depends largely on feeding conditions and processing parameters [119]. Anti-carcinogenic [120,121] immune-stimulatory [122] and anti-atherogenic [123] properties have been attributed to CLA.

Concerning the fatty acid composition of milk, different effects on serum lipoprotein levels have to be considered. Among SFA, lauric (12:0), myristic (14:0) and palmitic acid (16:0) were shown to elevate total and LDL cholesterol. Stearic acid (18:0), however, differs from the other saturated fatty acids, having a more or less neutral effect on blood lipids. The monounsaturated oleic acid (18:1) is regarded as protective concerning atherogenesis due to its positive effects on blood lipids [123]. TFA have been shown to elevate total and LDL cholesterol levels [124]. In contrast, CLA seems to be protective: Rabbits fed a semisynthetic diet (14% fat and 0.1% cholesterol) supplemented with 0.5% CLA had lower total and LDL-cholesterol and triglyceride levels and less atherosclerosis than rabbits fed the same diet without CLA [122].

Fatty acids have also been demonstrated to be implied in

diabetes pathogenesis. A diet enriched in SFA or TFA was shown to elevate postprandial insulin responses in obese patients with type 2 diabetes after a test meal, whereas glucose response remained unchanged [125]. A recent paper describes implications of CLA in glucose-tolerance [126]. CLA was shown to normalize impaired glucose tolerance and improve hyperinsulinemia in the pre-diabetic Zucker Diabetic Fatty (ZDF) rat. The insulin sensitizing effects were reported to be, at least in part, due to activation of the peroxysome proliferator activated receptor (PPAR) gamma. Feeding mice different concentrations of CLA (0.5% to 1.5% w/w), Belury and Kempa-Steczko [127] reported a lower weight gain compared to mice fed diets without CLA. Increasing dietary CLA was associated with reduced linoleate in hepatic phospholipids and increased oleate and decreased arachidonate in neutral lipids. The authors suggested that CLA may affect metabolic interconversion of fatty acids in liver and thereby modification in fatty acid composition and eicosanoid metabolism in extrahepatic tissues.

Since patients with type 2 diabetes often present with elevated free fatty acid levels, direct effects of certain fatty acids on pancreatic function were analysed by *in vitro* experiments. Zhou and Grill [128] showed that long-term exposure of pancreatic islets to free fatty acids (palmitate, oleate or octanoate) inhibited glucose-induced insulin secretion and biosynthesis *in vitro*. Increased plasma concentrations of fatty acids and ketones were suggested to be important factors behind the negative influences on  $\beta$ -cell function exerted by a diabetic state in type 2 diabetes [129]. It was further demonstrated that palmitate induced increases in islet triglyceride content associated with inhibition of  $\beta$ -cell function, and long-term exposure to palmitate also induced an inhibitory effect of FA oxidation on glucose metabolism that is independent of triglycerides [130]. *Trans* fatty acids were recently reported to affect insulin release from mouse islets in contrast to the corresponding *cis*-isomers [131].

Islet cells, however, do not only respond to fatty acids by alterations in insulin secretion, but also by proliferation [132]. Oleic and  $\alpha$ -linoleic acid and even more palmitic acid (unpublished data) induced proliferation of neonatal rat islets, whereas others were without effect. It may be speculated that hyperinsulinemia found in the early stage of the metabolic syndrome might be favored by induction of increased islet cellularity in early infancy.

## CONCLUSION

A relation between bottle feeding (milk consumption) in early infancy and the development of type 2 diabetes has only been found in one study in Pima Indians. Since diabetes prevalence among these people is very high (also in breast-fed children) and problems of satiety and of hygiene accompanying bottlefeeding could contribute to subsequent diabetes development, the association is rather questionable.

Concerning milk intake beyond infancy, consumption of whole milk and high fat dairy products in higher amounts substantially contributes to intake of (saturated) fat, which is associated with the development of the metabolic syndrome (including obesity, hypertension, hyperlipidemia and atherosclerosis). In contrast, addition of low fat dairy milk products to a vegetarian diet has been shown to have a positive influence on blood pressure, and milk and cheese consumption showed no association to diabetes-related mortality. However, in the latter study, no information about the fat content is given. Among the different components of milk fat, cholesterol, SFA (except from stearic acid) and TFA have to be regarded as unfavourable, whereas oleic acid and—keeping in mind, that there are only a few studies at present—CLA and ACE inhibiting peptides from milk proteins can potentially exert protective effects with respect to the development of the metabolic syndrome or atherosclerosis.

Concerning direct effects on insulin release, SFA, TFA but also oleic acid have been shown to negatively affect islet function *in vitro*, whereas CLA has been shown to increase insulin sensitivity.

However, potential unfavorable or beneficial effects of milk can not be attributed to the mechanism of action of a single fatty acid or other components observed *in vitro*, since a mixture of positive and negative compounds is always ingested. Elimination of disadvantageous components and enrichment of beneficial ones could be a solution. So far, the consumption of low fat dairy products in combination with plant food as a lactovegetable diet is recommended.

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