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THE HEALTH ASPECTS OF BUTTERMILK COMPONENTS. A REVIEW.

Technical-scientific information

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Contents

1 Abstract

Buttermilk has been underestimated for many years. For a long time it has been regarded as an unwanted and useless by-product which accumulates during the production of butter. However, the high-value components of this food which are found to be beneficial to human health, are slowly changing these opinions. Currently specific interest in the milk fat globule membrane (MFGM), which is destroyed because of the churning process, is increasing. The main function of MGFM is to protect the milk fat from coalescing. The single constituents that are released from the membrane after churning are the focus of current nutritional research projects all over the world and many scientists are trying to identify them as functional molecules. Other typical milk components such as caseins and whey proteins are also present in buttermilk and continue to be the subjects of research in the near future.

This review outlines some health aspects of buttermilk components and focuses on MFGM, caseins and whey proteins.

Keywords: buttermilk, MFGM, casein, whey protein, diabetes mellitus, coronary heart disease

Table 1: Selected constituents in 100 g buttermilk and 100g skimmed milk

constituent	/100 g buttermilk	/100g skimmed milk
water	90.4 g	90.7 g
protein	3.43 g	3.43 g
fat	0.51 g	0.07 g
cholesterol	4.0 mg	3.0 mg
carbohydrates	4.01 g	4.80 g
minerals	0.75 g	0.75 g
vitamin B12	200 ng	300 ng
α-tocopherol	20 µg	traces
folic acid	5 µg	5 µg

2 Introduction

Buttermilk originates from churning butter out of cream. The per capita consumption of fresh buttermilk varies within the European countries but is in general quite low (1) - for this reason this dairy product mainly finds application as powder in the food industry or is used for feeding animals. Concerning its impacts on human health, buttermilk has been considered as worthless for many years. Even to this very day its full potential with regard to health has not yet been completely exploited (2). Indeed, buttermilk consists of high-value components which are listed in Table 1. This table also shows how similar the composition of buttermilk and skimmed milk is.

In comparison to any other dairy product, buttermilk is characterized by the existence of high amounts of residual milk fat globule membrane (MFGM). Intact MFGM is responsible for the stability, integrity and protection of milk fat in the aqueous phase of whole milk (3). Due to the churning process this membrane is finally destroyed and its compounds, such as phospholipids and membrane specific proteins, are released. Some of these MFGM compounds are considered to be beneficial with regard to human health (4;5).

How exactly humans can benefit from buttermilk as a whole food is not yet clear since research is mostly focusing on the functions of single compounds of this dairy product, such as phospholipids, MFGM, caseins and whey proteins. Accordingly, the primary objective of current dairy research is to investigate mechanisms and effects of the buttermilk -components on the human metabolism. Studies, which concentrate on the influence of buttermilk as a whole food on the metabolism are not existent, therefore, this review has to focus on the existing data of the literature, which mainly describes the positive and negative impacts of the single buttermilk components (especially proteins) on human health. Minerals and vitamins are disregarded due to space limitations of this publication but of course they also have to be considered in order to successfully evaluate the full health potential of buttermilk.

3 Milk fat globule membrane (MFGM)

Regarding caseins and whey proteins, buttermilk is not a better provider than skimmed milk or whey. But buttermilk specificity is to be rich in destroyed MFGM, whose compactly bound constituents such as proteins, phospholipids, minerals etc. are released during the process of churning. This technological step finally results in an increased availability of the MFGM-constituents to the human organism (2).

In general, the MFGM encloses a triglyceride core in order to prevent the coalescence of the fat globules in the aqueous solution milk. That means in the first instance, MFGM acts as an emulsifier. However, at second glance, the specific proteins, unique polar lipids and minor molecules (minerals, vitamin E, carotenoids) of this membrane seem to possess additional functional properties, especially in terms of health (5). Some of the most interesting outcomes of research concerning MFGM compounds and human health will be presented in the following sections.

3.1 Composition of MFGM

The composition of MFGM which can be found in the literature is highly variable as a consequence of differences in MFGM-isolation, purification or analysing methods. Table 2 *summarizes* the composition of MFGM according to Walstra *et al.*, 2006 (6).

Polar lipids and membrane-specific proteins are important parameters for determining the functional and nutritional value of MFGM and therefore also of buttermilk. The schematic figure representing the structure of MFGM and the arrangement of membrane-specific proteins and lipids is located at the end of this chapter (Fig.I).

Table 2: Composition of MFGM according to Walstra *et al.* 2006 (6)

component	mg/100g fat globules
protein	1800
phospholipids	650
cerebrosides	80
cholesterol	40
monoglycerides	present, but quantity unknown
water	present, but quantity unknown
carotenoids C + vit.	0.04
iron	0.3
copper	0.01
total	> 2570

3.2 Polar lipids

MFGM contains both: polar lipids (e.g. phospholipids (PL) & sphingolipids) and, in much lower concentrations, neutral lipids such as tri-, di- and monoglycerides, cholesterol and its esters (4). Relating to the beneficial effects on human health, the polar lipids especially come to the fore and should be focused on. They consist of a hydrophilic head and a hydrophobic tail and they are often esterified with two fatty acids via glycerol. The third hydroxyl-group of glycerol is linked to a phosphate residue which, in turn, is able to bind several organic groups such as serine, choline or ethanolamine. After binding, these molecules are characterized as **(glycerol)phospholipids** (7;8).

Sphingolipids represent the second group of polar lipids which are of great interest to nutritional research. The feature of these polar lipids is the sphingoid-base, which is a long-chain aliphatic amine with two or three hydroxyl-groups. The amino group is often linked with a fatty acid leading to the so-called ceramides-molecules (9).

The mainly occurring and most important polar lipids of MFGM are: phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SM), phosphatidylinositol (PI) and phosphatidylserine (PS) (Table 3). Other molecules such as glucosylceramide, lactosylceramide and gangliosides exist in trace amounts (3).

Table 3: Profile of glycerophospholipids and sphingolipids in milk fractions

polar lipid	Fauquat ***et al.*** (10)	Lopez ***et al.*** (11)	Keenan ***et al. (12)***
PE	6.4±3.6*	23.2±2.2*	35.7‡
PI	7.6±1.8*	8.1±0.8*	5.7‡
PS	6.5±1.9*	16.1±1.0*	4.9‡
PC	32.1±5.0*	26.6±1.3*	26.8‡
SM	17.3±2.3*	26.0±1.0*	21.4‡

*in g/100g phospholipids; means ± standard deviation

‡in g/100g phospholipids

PS=phosphatidylserine

PC=phosphatidylcholine

PE=phosphatidylethonalamine

PI=phosphatidylinositol

SM=sphingomyline

3.2.1 Impact of (glycerol)phospholipids on the human organism

Buttermilk is an excellent source of glycerophospholipids whose effects on human health are very similar to those of sphingolipids: they play an important role in cell signalling and have a strong impact on the development of brain function. Especially, cholin-containing glycerophospholipids can positively affect cognition by ensuring ion permeability, fluidity and the suitable environment of the dynamic membrane of the adult brain (13-15). These functions are crucial for children, ageing persons and probably for Alzheimer patients as well.

Furthermore, there are effects of glycerophospholipids on the cholesterol metabolism but they still remain controversial. Animal studies showed a decrease in total cholesterol, non-HDL and triglycerides (TG) in the serum as well as smaller aortic fatty streaks in the blood vessels after the consumption of diets high in phospholipids (16-19).

Intervention studies with egg- and soybean-glycerophospholipids with humans generated different results depending on the concentration and source of the phospholipid as well as the study design, the duration of intervention and the composition of the fatty acids (16;20;21). Nishimukai and other researchers investigated the impact of endogenous and dietary phosphatidylcholine (PC) on intestinal lipid absorption. The results of these studies are not consistent – on the one hand lymphatic transport of triglycerides in bile-diverted rats was shown to be increased (22;23) whereas other works has not clearly shown a rise of the lymphatic transport of TG (especially at low ratios of TG to PC from 16:1-7:1) (16;24). Higher administered ratios of TG to PC of 3:1, in turn, promoted absorption of TG and enhanced plasma TG concentrations in rats. Such a ratio is considered as unrealistic in terms of being consumed via regular nutrition. However, it is an important option for people suffering from impaired digestive function (25;26). The potential of milk PL extracts containing different species of PL on inhibiting intestinal cholesterol absorption in mice which were fed a high-fat diet as well as their ability to decrease cholesterol and triacylglyceride levels in mice, was subject of the work by Kamili *et al.* (27). This research group provided evidence that milk PL extracts have the potential to reduce intestinal cholesterol uptake in mice fed an obesogenic and atherogenic diet. The relevance for humans has to be determined in near future (28).

3.2.2 Impact of sphingolipids on the human organism

Although there is no evidence for the necessity of sphingolipids for normal physiological processes yet, researchers believe that these polar lipids are indispensable to human nutrition. Scientists assume that they have the potential to inhibit colon cancer (29-31) and might play an important role in regulating the cholesterol metabolism, as well (16;32;33). In this context, several studies were conducted in the past. Nyberg *et al.* administered cholesterol in combination with sphingomyelin to normal rats and detected an inhibitory effect on cholesterol absorption (32). Same observations have been found by others in mice and Caco2 cells (33). Another study with ApoE Leyden mice explored that sphingolipids decreased the levels of plasma cholesterol and triacylglycerides after feeding the animals with a high-cholesterol and high-fat diet. Furthermore, the accumulation of fat in the livers of the mice was reduced (34). Whether this effect is transferable to humans has been studied by Ohlsson *et al.* (35) who investigated the effect of a sphingolipid-enriched dairy formulation on postprandial lipid levels and lipoprotein profile of healthy male volunteers. In summary, they found no significant impact on plasma lipid parameters. However, research in this topic continues for a lack of further evidence and data.

Besides, sphingolipids are important compounds of the myelin-covering which encloses and protects the axons of the nerve cells (36). In addition, they seem to regulate the irritability and transmitter release in the nervous system (37).

Sphingomyelin (SM), as the most important and abundant representative of the group of sphingolipids, as well as metabolites from SM have highly bioactive potential by being involved in trans-membrane signalling and cell -regulation, -proliferation, -differentiation as well as programmed cell death. These properties, in turn, influence human health by having impacts on cancer, cardiovascular diseases, the cholesterol and lipid metabolism (29;30;38).

In summary, sphingolipids are molecules of high potential in association with health promoting effects on the human organism. But once again: because of several methodological limitations in the animal and human studies conducted, all these impacts have to be verified with further studies in the near future.

3.3 MFGM-specific proteins

MFGM-specific proteins are special proteins which do not occur in this form in other milk phases. 25-70% of the MFGM is composed of these proteins whereas they constitute only 1-4% of the total milk protein. However, these proteins seem to fulfil important biological functions (39).

About 120 different proteins were determined in the bovine MFGM (40) and of these, eight major glycoproteins have been identified, purified and characterized up to now: mucin 1 (MUC1), xanthine dehydrogenase/oxidase (XDH/XO), cluster of differentiation 36 (CD36), periodic acid Schiff 6/7 (PAS 6/7, also known as lactadherin), adidophilin (ADPH) and butyrophilin (BTN) (41). They are mainly responsible for cell signalling (23%), membrane/protein trafficking (23%), fat transport or metabolism (11%), protein metabolism (7%), general transport (9%) and immune functions (4%). Other smaller proteins, loosely linked to the membrane, are enzymes, immunoglobins, proteins from the cytoplasm of the secretory-epithelial cells. All these biomolecules fulfil essential functions in the mammal cells of the cows. However their properties in the human organism (after digestion, potential structural alterations, changes in conformations) are mainly unknown (42).

3.3.1 MFGM proteins and anticancer effects

Several proteins of MFGM are supposed to reduce the risk of different cancers in the human organism: 1) FABP, which was able to inhibit the growth of breast cancer cells in vitro (43), 2) the proteins BRCA1 and BRCA2 were both identified in MFGM and are involved in DNA-repair. In addition, the onco-supressor BRCA2 regulates the cell division – an important function relating to the prevention of cancer (44). A third component of MFGM helping to prevent the occurrence of cancer is an inhibitor of the enzyme glucuronidase. This enzyme is present in some of the intestinal bacteria and facilitates the degradation of glucuronides. Normally these molecules are synthesized in the liver in order to neutralize toxic substances. The degradation of glucuronides finally leads to the release of potential toxins and, as a consequence, to an impaired risk of the formation of cancer (5). Accordingly, the consumption of MFGM-specific proteins could help prevent the occurrence of cancers, but again, further research is necessary.

3.3.2 MFGM proteins and antibacterial effects

XDH/XO were shown to be effective antimicrobial compounds. These proteins are not only existent in MFGM but also expressed in different cells of the gastrointestinal tract (4). Their functionality is based on the formation of reactive oxygen species, superoxide and hydrogen peroxide in order to inactivate hazardous bacteria. Furthermore, this MFGM and gut protein reduces inorganic nitrite to nitric oxide and peroxynitrite which both have antibacterial effects. Besides that, XDH/XO inhibits the growth of bacteria such as *Staphylococcus aureus, Escherichia coli* and *Salmonella enteritidis* by activating the lactoperoxidase system in milk or the formation of hydrogen peroxide (45-47).

Instead of binding to the protein-receptors located on the inner surface of the gut, pathological bacteria can also attach to structurally similar proteins of MFGM and thus, can easily be eliminated without developing their toxic potential (4). According to the literature, MUC1 and other bovine milk glycoproteins especially from MFGM were found to inhibit gastric colonization with *Helicobacter pylori* and *Escherichia coli* in animal models as well as in vitro (48;49).

3.3.3 MFGM proteins and coronary heart disease (CHD)

In 2003, Moss and Freed published data based on epidemiological observations, which reveals positive correlations between death rates from CHD and milk consumption, depending on the country. They propose the hypothesis that the occurrence of the CHD is related to the non-fat fraction of the milk, namely MFGM. Consequently, antibodies against bovine, but not human MFGM-proteins are circulating in the human blood and finally bind to these proteins, probably to CD36 (5;50). These antibodies are suspected to be responsible for biochemical and immunological coronary atherogenic effects (51). Several scientists doubt this hypothesis and further research is important in order to prove this assumption.

3.3.4 MFGM proteins and Multiple Sclerosis (MS)

Multiple Sclerosis is an autoimmune disease affecting the central nervous system (CNS). Chronic inflammation in the CNS finally leads to demyelination of neurons, axonal degeneration and a loss of oligodendrocytes. Both genetic and environmental causes are held responsible for the development of MS (4). Scientists have also suggested that milk and dairy products are involved in the development and progression of MS (52-54). This assumption is based on the existence of BTN which was found to be structurally similar to a putative auto-antigen "myelin oligodendrocyte glycoprotein" (MOG) in human MS and which may finally lead to the mentioned disease pattern (55). In order to investigate this assumption, researchers induced the so-called experimental autoimmune encephalomyelitis (EAE) in animal models. EAE is a disease which exhibits similar clinical characteristics to MS. Eventually, Mana *et al.* were able to show that the treatment of mice with BTN before and after immunization with the auto-antigen MOG has a positive impact on the suppression and prevention of the clinical manifestation of EAE and thus, probably also of MS (56). In conclusion, MFGM, in particular BTN, from dairy products do not evoke the development and suppression of this neurodegenerative disease but can even positively influence the aetiopathology of EAE and probably MS, too (57).

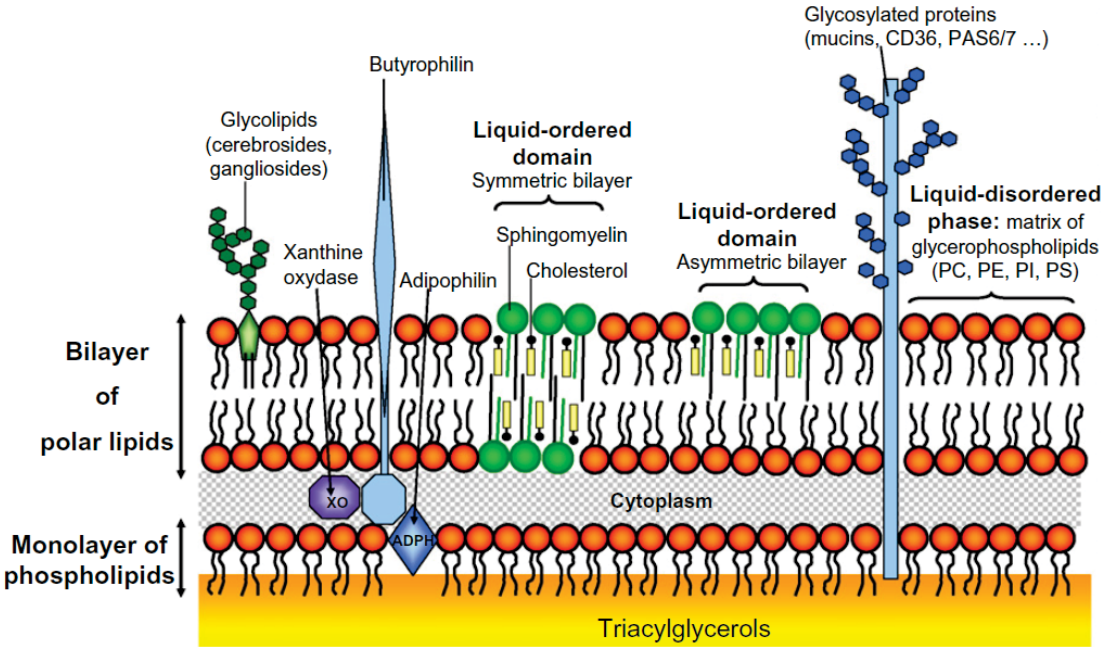

Figure 1: Structure of MFGM (figure extracted from Lopez *et al.* [2011]). MFGM encloses a triglyceride core. The trilayer of polar lipids is the backbone of the membrane. MFGM-specific proteins such as butyrophilin or mucin but also cholesterol, sphingomyelin and glycolipids are incorporated in the triplelayer-matrix of glycerophospholipids.
Abbreviations: PC=phosphatidylcholine, PE=phosphatidylethanolamine, SM=sphingomyelin, PI=phosphatidylinositol and PS=phosphatidylserine (58).

4 Caseins in buttermilk

The protein content of buttermilk, with a percentage of 3.2% corresponds approximately to the content of skim milk (59). Caseins cover 77-81% of all the existing proteins in dairy products, whereas whey proteins represent circa 20% of the proteins (60).

Caseins can be divided into several subclasses: α-casein (50-55%), β-casein (30-35%), κ-casein (15%) and γ-casein (5%) (61). These subgroups significantly differ in their content of phosphate per casein molecule (62). In solution, bovine caseins are arranged in micelles which can also bind calcium and magnesium, their salts as well as fat (63;64). This micelle-structure causes reduced digestibility and a slow release of the protein molecules from this compound in the duodenum in comparison to whey proteins because of agglutinating in the stomach (65). Since α-casein and γ-casein are not present in human milk as well as the different homology of bovine and human β-casein and κ-casein, these proteins are often held responsible for milk allergy in infants and adults (66). But due to a lack of reliable data in literature, no clear evidence can confirm this assumption.

Several other metabolic consequences after casein ingestion in humans and animals are described in the scientific literature, some of which become an object of the following sections.

4.1 Antioxidant activity of caseins

Oxidations are essential processes in human organisms especially relating to the production of energy. Though an excess of oxidation reactions leads to the augmented appearance of reactive oxygen species (ROS) and the damage of biological molecules. ROS have been identified as one of the initiators in the aetiology of cardiovascular diseases, cancer, diabetes and ageing and also as an important parameter in physical exercise, stress, trauma, stroke and infection (67-69). Healthy individuals possess several physiologic mechanisms and substances such as enzymes, gluthatione, ubiquinal and uric acid in order to reduce oxidative stress (70;71).

However, special antioxidant compounds of food may also reduce the oxidant damage in humans – as do caseins (49;72). The phosphates located in these protein molecules seem to be responsible for that function (73). Accordingly, casein micelles can bind to non-heme iron (a producer of ROS) by interactions of phosphoserine residues of the casein micelle or free inorganic phosphate which was released out of the micelles. This phenomenon was confirmed by Demott *et al.* in 1976, who stated that 72% of non-heme iron added to skim bovine milk was recovered from α-casein and 21% from β-casein (74).

Furthermore, several studies investigated the inhibitory effects of caseins and casein-derived peptides on enzymatic and non-enzymatic lipid peroxidations in foods (75). It is suggested that these phosphorylated proteins and peptides seem to be the most preferred targets of free fatty acid radicals (76;77). In doing so, the primary structure of the casein-derived peptides is of high importance in determining their antioxidant activity (78). Besides the amino acids sequences, the presence of certain peptidic bonds, the structural conformation arrangement (79) and the hydrophobicity (80) of the peptides are also influencing factors concerning the antioxidant activity.

Interestingly, caseinates are used in the food industry to avoid oxidative browning of fruits and vegetables (81).

4.2 Caseins and cardiovascular diseases (CVD)

4.2.1 A1 β-casein

Multi-factorial pathological processes and the long-term exposure of the human organism to several risk factors entail to ischemic heart disease (IHD) and stroke. A lot of risk factors in association with CVD are well studied, such as high blood pressure, low density lipoproteins (LDL), obesity and smoking. In the last decades, RB Elliott and CNS McLachlan hypothesized that β-casein of some cow races is also one of those risk factors for IHD. For analyzing the effect of β-casein on human health it is necessary to observe each genetic variant of this protein. A1, A2 and B β-casein are the most common variants and especially A1 and B are mentioned in the context with dramatic impacts on human health. Enzymatic cleavage of the protein-variants A1 and B β-casein releases a molecule built of 7 amino acids which is called "β-casomorphin 7" (CM-7) (82). This molecule is known for its opioid properties and potential immunosuppressive activity on the human body (83). Ecological data from McLachlan shall indicate a correlation of the mortality from CVD in 16 countries with a high national A1 β-casein consumption (84). Laugesen and Elliott published similar correlations in 2003 (85) and a rabbit study released the same year seemed to confirm this hypothesis: after feeding the animals for 6 weeks with 10% A1 β-casein or A2 β-casein, they were sacrificed. The areas of aortic fatty steaks in the vascular system of the rabbits were larger in the A1 β-casein group than in the A2 group. Furthermore, serum cholesterols increased in the A1 group (86).

AS Truswell, author of a critical review concerning this topic, argued against this hypothesis by listing several lacks and limitations of the experiments and calculations of these works. Regarding the rabbit study, Truswell and other scientists point out that the study design was crude; the animal model used in the trial, the shortness of the casein intervention, the amount of animals per group and the highly artificial diets are only some of the reasons why these results cannot be transferred to humans (83;87).

With regard to the hypothesis and the calculation of the risk from A1 β-casein he argued that Laugesen, Elliott and McLachlan did not include other risk factors for CVD such as smoking or high blood pressure. Furthermore, in Truswell's opinion the observation time between food consumption and mortality of patients was too short (5 years). Several other inconsistencies are referred to. Finally, the scientist concluded: "There is thus no convincing or probable evidence that the A1 β-casein in cow's milk is a factor causing CVD" (83) and the European Food Safety Authority also came to the conclusion that this hypothesis does not meet the reality (88).

4.2.2 Caseins and its influence on lipid profile

Besides an unhealthy lifestyle and insufficient physical exercise, abnormal serum lipid levels also contribute significantly to the development of CVD. Especially a high ratio of LDL:HDL (high density lipoprotein) is a strong predictor of cardiac events in humans (89). High concentrations of lipoprotein a (Lp(a)) and triglycerides in the blood serum are further important parameters in terms of cardiac events (90;91). A decrease in LDL cholesterol and an increase in HDL are known to reduce the risk of the CVD development (89).

Scientific animal studies and a few human trials particularly investigated the influence of soy-based proteins on some of these parameters. Casein often served as a reference protein. In summary, soy protein interventions lasting for several weeks decreased total cholesterol and LDL more in comparison to casein (92). Lp(a) was significantly decreased after a casein treatment but increased after soy protein consumption (93). A recently published review of Blachier *et al.* states that soy based proteins might be the better source in order to decrease the occurrence of these CVD risk factors. But the authors of this publication also indicate that the data of the studies is extremely heterogenic due to the existence of secondary plant compounds in soy-based diets, differing amounts of used proteins and the existence of bioactive peptides from soy which might also have an influence on the lipid profile. Furthermore, the results from animal studies described in the literature should not be easily transferred to humans. Understanding the underlying mechanisms of the different proteins on parameters of the lipid profile would be one step forward in evaluating the effect of caseins on CVD particularly (92).

4.2.3 Caseins and blood pressure

A number of animal and human studies demonstrated impressively that caseins have great potential to lower blood pressure. The angiotensin-converting enzyme (ACE) inhibiting casein-derived peptides called casokinins seem to be responsible for that effect. The ACE belongs to the renin-angiotensin system and plays a major role in regulating blood pressure. Its inhibition is one of the most important targets in terms of high blood pressure therapy (94). The first study conducted with caseins that reported this positive effect was published in 1992 by Sekiya *et al.* 20g of hydrolysated caseins per day, which is, however, a large amount of proteins, reduced the diastolic and systolic blood pressure significantly in hypertensive and normotensive humans (95). This observation was verified by several other research groups (96-98) and demonstrates the potential of natural food sources such as proteins and in particular caseins to get the CVD-risk factor "high blood pressure" under control.

4.3 Caseins and type 1 diabetes mellitus

Diabetes mellitus is a disease which is characterised by a loss of function of the insulin producing pancreatic β–cells. In short, the body loses its ability to distinguish between foreign and self-proteins, resulting in the destruction of pancreatic islet cells. This modification leads to the inability of the pancreas to release the hormone insulin. Lifelong supplementations of this hormone accompanied by extensive outcomes on the quality of life are dramatic consequences for the often young individuals (99). Type 1 diabetes is a disease which already occurs in children and young adults (100). Hereditory transmission is responsible for its development, but non-genetic determinants also cause this disease. In this context, pathogens and inappropriate dietary intake are especially named in scientific literature. Finnish researchers assumed that cow's milk exposure in early life could trigger this autoimmune disease. This hypothesis was supported by several other scientists such as Cavallo *et al.* who discovered increased amounts of antibodies against β-casein in diabetes type 1 patients (101). In particular, the variation A1 β–casein is once again the focus of research in connection with type 1 diabetes (84;85). The hypothesis of a positive correlation between the risk of occurrence of this disease and the casein variant was established by Elliot and colleagues, who assumed that βCM-7 negatively affects the immune system of children resulting in the formation of auto-antibodies against the insulin-producing β-cells of the pancreas (82;84;85;102).

The argumentation of this hypothesis is based on the estimation of Elliott, which reveals that there is a correlation between the incidence of type 1 diabetes in 0-14-year-old children and the average national consumption of A1 β-casein in several selected countries. This method of estimation has been proven unreliable according to some scientists, and in addition, animal studies with diabetes-prone mice and rats demonstrated no difference in the occurrence of diabetes between A1 β-casein and the A2 variant (83;87).

Several numbers of meta-analyses and case-control studies were discordant concerning the impact of caseins on type 1 diabetes: on the one hand, Gerstein found positive correlations (103) whereas on the other hand, Vitranen *et al.* and others made contradictory observations and pointed out that the risk of developing type 1 diabetes is not increased after consuming cow's milk in early childhood (104-107). Crawford *et al.* even showed, in 2003, that although the milk consumption has remained the same in Switzerland since 1990 the number of type 1 diabetes patients has increased three-fold (108).

All these discrepancies finally lead to the conclusion that further research is necessary. In particular, non-hazardous clinical intervention trials with children are promising approaches to clarify open questions and, in particular, the role and physiological properties of βCM-7 on diabetes.

4.4 Other effects of caseins on human health

The following table, Table 4, shows further supposed effects of caseins on the human organism. But caution is advised since most of the studies have limitations with regard to their study design, the amount of proteins used or the animal model which was used for the intervention.

Table 4: Effects of caseins on human health

protein fraction	reference	properties
whole caseins and casein-derived peptides	(109)	- increase of amino acid oxidation & protein synthesis; strongly inhibit proteolysis
	(93)	- decrease Lp(a) concentration; inhibit lipoxygenase-mediated lipid autoxidation
	(110)	- appetite suppression
	(111)	- tripeptides IPP, VPP: anti-hypertensive; immunomodulatory activities; increasing osteoblast-proliferation, differentiation and signalling - radical scavenging activity; cytomodulatory effects
	(112)	- enhanced re-mineralization of the enamel on tooth; antimicrobial effects
κ -casein and κ -casein-derived peptides	(113)	- antithrombotic
	(114)	- appetite suppression
	(111)	- ACE-inhibitory activity
	(112)	- opioid antagonist
caseinoglycomacropeptides	(115)	- antithrombotic, opioid agonist
	(116)	- antiobese
		- binding of toxins
	(111)	- binding of toxins
casomorphines (peptide from α- or β-casein)	(114)	- opioid agonist
peptides of β-casein	(111)	- immunostimulation; opioid; ACE-inhibitory activities

5 Whey proteins in buttermilk

As already mentioned, buttermilk contains about 3.2g protein/100ml (59) whereas approximately 20% are allotted to whey proteins. Whey proteins include β-lactoglobulin (48%), α-lactalbumin (18%), immunoglobulins (11%), proteose-peptones (11%), serum albumin (6%), lactoferrin (1%) and others (4%). In the scientific literature they are described as a component of milk and dairy products whose primary function is not the nourishment of the offspring but the provision of functional properties. The biological value of whey proteins is therefore much higher than that of caseins (104% versus 87%, respectively) (117).

Unlike caseins, whey proteins are not arranged in micelles but characterized by their molecular-disperse structure (118). This arrangement causes a completely different physical, chemical and physiological behaviour of the whey proteins in comparison to caseins: they were discovered to be more effective in satiation according to observed ratings of fullness and hunger stated by study volunteers as well as measurements of postprandial gastrointestinal hormone releases and changes of the metabolism (119). Furthermore, the fast absorption rate of whey proteins and high contents of special amino acids like leucine lead to physiological protein synthesis (especially muscles) (120). In addition, this protein fraction contributes to a reduced risk of osteoporosis by strengthening the bone structure (121;122), increases the bioavailability of vitamins, minerals and lipids as it provides a lot of free binding sites for these substances (123) and serves, directly and indirectly, as a modulator of the immune system (124;125). Many other studies have been conducted, focusing on the physiological properties of whey proteins and whey protein derived peptides. Some of the results found are mentioned in the following text, others have already been excellently reviewed in other publications (118;126;127).

5.1 Antioxidative activity of whey proteins

The antioxidant properties of whey proteins are based on the existence of the amino acids cysteine and glutamic acid (128). Both are compounds of the constituent glutathione which may be the most important water-soluble, self-built antioxidant in humans. Glutathione defends the human organism from oxidative stress and an excess of ROS (127). The amino acid tyrosine is also able to scavenge free radicals (129). Furthermore, α-lactalbumin binds heavy metals which protects the body from radicals (123).

5.2 Whey proteins and cardiovascular diseases

5.2.1 Whey proteins and their influence on lipid profile

Whey proteins and peptides derived from whey proteins were shown to have a positive impact on the decrease in the concentrations of triglycerides and total cholesterol in blood serum leading to beneficial alterations of the plasma lipid profile from atherogenic to cardioprotective (130-132). This effect is most likely caused by a special whey protein fraction called lactostatin. Lactostatin increased the HDL concentrations in rats and significantly lowered the total cholesterol in their serum (133). Furthermore, Morikawa *et al.* observed that lactostatin induces the expression of an enzyme called human cholesterol 7α-hydroxylase which, in turn, metabolizes cholesterol and finally leads to hypocholesterolemic consequences. An amino acid mixture that was equivalent to lactostatin did not induce the production of this hydroxylase (134).
Most of the existing studies which examined the effects of dietary proteins on the blood lipid concentrations were performed with soy proteins and whey proteins or caseins as a control. It was noticed that soy-based proteins have the highest potential to reduce triglycerides, LDL and total cholesterol. However, research demonstrated impressively that whey proteins are analogous to soy proteins with regard to their impact on the lipid profile (135).

5.2.2 Whey proteins and blood pressure

Only recently, Pal and Ellis (136) published results concerning the chronic effects of whey proteins on blood pressure in 70 overweight men and women. A supplementation of whey proteins for 12 weeks resulted in a reduced systolic and diastolic blood pressure in comparison to the baseline levels and control group (glucose). The authors of this study did not identify the origin of the beneficial effect - whether it is due to the whey protein as a whole, special peptide fractions, amino acids or even synergistic actions among these compounds. Furthermore, whey proteins were found to ameliorate the arterial stiffening in patients which is considered to be another benefit in combating high blood pressure and thus, cardiac events (137).
As with caseins, whey proteins also release bioactive peptides with possible antihypertensive properties. They are called lactokinins and seem to act similary to casokinins (138).

However, previous studies generated data with differing observations which is due to strongly inconsistent study designs (95;98;139-141). Further research on this topic is necessary in order to evaluate the effect of whey proteins on blood pressure.

5.3 Whey proteins and diabetes mellitus type 1

In the case of bovine milk proteins, not only caseins are under suspicion of causing diabetes - in the last two decades whey protein fractions were also made responsible for the development of diabetes.

For example, "bovine serum albumin" (BSA) was suspected of contributing to the development of type 1 diabetes by triggering the human auto immune response (142;143). BSA and human blood serum albumin are classified as identical regarding their homology (144) and BSA was suggested to cause type 1 diabetes due to increased levels of antibodies against BSA found in diabetic patients (142). One special amino acid section of BSA called ABBOS is made responsible for the immunogenic reactions in humans of diabetogenic genotypes (145;146). The partial digestion of BSA facilitates the accessibility and absorption of ABBOS in the immature gastrointestinal tract of the newborns where specific epitopes of this fraction are recognized by T-cells and B-cells of the immune system. Several immuno-reactions such as the activation of macrophages, the secretion of lymphokines from T-cells and the production and release of antibodies from transformed B-cells follow after the recognition process, finally leading to diabetes (146;147). Researchers assume that the problem is a cell-surface-protein (p69) of the β-cells of the pancreas, which is actually targeted against viral infections and which probably has a similar homology to ABBOS. Finally, p69 is activated by all of the immuno-reactions mentioned above after BSA consumption and its presence on the β-cell-surface may lead to an immune response against the insulin-producing pancreatic cells yielding to their destruction. Every exposure to cow's milk in the first three months of life would evoke such an immune reaction in the diabetes-predestined newborns. After this period, the risk of developing type 1 diabetes due to bovine milk seems to be averted in proned individuals (100). Nevertheless, the evidence of this theory is lacking and many questions and mechanisms need to be clarified to confirm this hypothesis.

Another hypothesis concerning this topic is mentioned by several scientists who assume that the loss of function of β-cells is due to their sensitivity against too much oxidative stress (148;149) but clarification is urgently necessary as this knowledge has a tremendous impact on human health, the health care system and, of course, the dairy industry.

5.4 Other effects of whey proteins on human health

Further effects of whey proteins on human health are summarized in Table 5. However, it is important to consider that many of these studies have methodological limitations such as lacking study designs, unphysiological amounts of protein used or experiments on cells or animals instead of using human volunteers.

Table 5: Effects of whey proteins on human health

protein fraction	reference	properties
whole whey proteins and	(109)	- strongly stimulate amino acid oxidation & protein synthesis
whey protein-derived peptides	(117)	- stimulate insulin secretion; improve lean body mass
	(150)	- decrease arterial stiffening
	(151;152)	- antiobese
	(153)	- facilitate growth of some bifidobacteria & lactobacilli
	(112)	- provide protection against intestinal, mammary and colon cancers; opioid agonists
branched chain amino acids – esp. leucine	(154)	- important substrate for protein synthesis and muscle formation
	(154)	- modulator of insulin signalling
cysteine, glutamic acid	(117)	- used as a component of important glutathione against ROS
β-lactoglobulin	(123)	- elicitor of milk allergy in childhood
	(123)	- increases absorption of minerals, liposoluble vitamins and lipids
immunoglobulins	(124;155)	- beneficial effects against infections
	(112)	- immune protection
whey glycomacropeptide	(156)	- antimicrobial; antiviral
	(112)	- appetite-inhibiting properties; bifidogenic
lactoferrin	(112;127)	- antimicrobial; anti-oxidative; immunomodulatory - antimicrobial; anti-oxidative; immunomodulatory

6 Conclusion

In the past, buttermilk was regarded as a worthless by-product of the butter churning process and only small amounts found application in animal feeding and the food industry. Unlike whey, which is the fall-out during cheese production, buttermilk is not used as a novel functional ingredient, a protein supplement or an antioxidant. However, buttermilk has the potential to be all of this – especially because of the presence of MFGM but also because of its high-quality proteins, including caseins and whey proteins.

The studies published in literature cannot clearly determine the health effects of buttermilk components on human health, mainly due to inconsistencies in study designs. On the one hand, many properties of these compounds were observed in animal studies. Either the type of animal model or an inappropriate amount of animals, as well as unphysiological amounts of the investigated compound and the duration of the intervention were limiting factors in those trials, though. On the other hand, researchers of human intervention studies with buttermilk components also failed to generate exact data by ameliorating the designs of the human trials.

Furthermore, it has to be noticed that the effects of single components or compounds cannot easily be transferred to buttermilk as a whole food. The interactions of the buttermilk macro -and micronutrients with the human organism have not yet been explored.

This review shows the tendency and potential that buttermilk components can positively influence human health and its contribution to a balanced daily diet is without doubt (although its per capita consumption is still quite low in many European countries). In the future, much more research is necessary in order to investigate the full bioactive potential of buttermilk components on the one hand and buttermilk as a whole food on the other hand.

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