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REVIEW

Effects of Dairy Protein and Fat on the Metabolic Syndrome and Type 2 Diabetes

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■ Abstract

The incidence of the metabolic syndrome (MetS) and type 2 diabetes (T2D) is increasing worldwide. Evidence supports a negative relationship between the consumption of dairy products and risk of MetS and T2D. Dairy proteins are known to have a directly beneficial effect on hypertension, dyslipidemia, and hyperglycemia, but a detailed understanding of the underlying mechanisms is missing. It has been confirmed by observations that the insulinotropic effect of dairy proteins is associated with the amino acid composition; in particular branched-chain amino acids (BCAA) seem to be of vital importance. Dairy protein-derived peptides may also contribute to the insulinotropic effect via dipeptidyl peptidase-4 (DPP-4) inhibitory activity, and may lower the blood pressure (BP). The lipid metabolism may be improved by whey protein (WP), which acts to reduce the postprandial triglyceride (TG) response. The effect of dairy fat is much more controversial because of the potentially harmful effect exerted by saturated fatty acid (SFA) on metabolic health. Recent observations suggest less adverse effects of SFA on metabolic health than previous assumed. However, little is known about dairy lipid fractions belonging to the groups of monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and phospholipids (PL). Dairy fat seems to act differently depending on the dairy product and the composition of macronutrients in the meal. Therefore, for a better understanding of the mechanisms behind the dairy protein and fat effect on MetS, we suggest that more human studies should be carried out to clarify the interactions of dairy protein and fat with macronutrients in the meal and other dairy components, such as micronutrients and microorganisms from fermented products.

Keywords: type 2 diabetes · metabolic syndrome · glucose metabolism · lipid metabolism · blood pressure · dairy fat

1. Introduction

The incidence of metabolic syndrome (MetS) and type 2 diabetes (T2D) is increasing world-wide. MetS is characterized by a combination of metabolic characteristics, including abdominal obesity, dyslipidemia, impaired glucose homeostasis (impaired glucose tolerance or impaired fasting glycemia), and hypertension [1]. Furthermore, individuals with MetS are often affected by low-grade inflammation. These risk factors contribute to a 2-fold increased risk of developing cardiovascular disease (CVD) [2] and a 5-fold risk of developing T2D [3].

Meta-analyses and systematic reviews show an inverse association between consumption of dairy products and risk of MetS [4, 5] and T2D [6-8], respectively. Individual risk markers such as low-grade inflammation [9] and hypertension are also inversely associated with dairy consumption [10]. Some findings refer to dairy products in general [5, 7], others to specific low-fat dairy products [6, 8, 11]. Consumption of cheese and a mixture of fermented dairy product are also inversely associated with diabetes incidence [12, 13]. Recently, a review concluded that even a small rise from 2-3 to 4-5 daily servings of dairy products adds to the beneficial effect [14]. This is in agreement with a study suggesting that dairy intake reduces the risk of T2D in a dose-dependent manner [7].

The main ingredient of dairy products is milk. Milk consists of 87% water, 3.5% protein, 3.4-4.0%

fat, and 4.9% lactose. Dairy protein and fat are complex constituents and can be fractionated at many levels. Results from studies to-date indicate that the beneficial effects of different fractions of dairy protein and fat on metabolic disturbances such as MetS and T2D remain inconclusive. It is well established that consumption of dairy products reduces the risk of developing MetS [4, 5] and T2D [6-8]. Therefore, it is important to clarify how single components in the complex dairy food matrix affect MetS and T2D risk factors. This would help us to evaluate the compounds' therapeutic potential in combating T2D and CVD. Therefore, the aim of the present review is to examine the existing evidence on the effects of dairy constituents on glucose metabolism, lipid metabolism, and blood pressure (BP), with specific focus on the following components:

1. Dairy proteins, including whey protein (WP) and casein
2. Fat including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and phospholipids (PL)

Furthermore, the review aims to clarify potential mechanisms of dairy protein and fat on the metabolic risk factors.

Abbreviations:
ACE - angiotensin-converting enzyme
BCAA - branched-chain amino acids
BP - blood pressure
C10:0 - 10 carbons atom length
CVD - cardiovascular disease
DBP - diastolic blood pressure
DPP-4 - dipeptidyl peptidase-4
FFA - free fatty acids
GIP - glucose-dependent insulinotropic polypeptide
GLP-1 - glucagon-like peptide 1
HDL - high-density lipoprotein
HOMA-IR - homeostasis model assessment of insulin resistance
iAUC - incremental area under the curve
Lactopeptide C12 - Phe-Phe-Val-Ala-Pro-Phe-Pro-Glu-Val-Phe-Gly-Lys
LDL - low-density lipoprotein
LPL - lipoprotein lipase
MAP - mean arterial pressure
MetS - metabolic syndrome
mTOR - mammalian target of rapamycin
MUFA - monounsaturated fatty acids
NO - nitric oxide
PL - phospholipids
PUFA - polyunsaturated fatty acids
SBP - systolic blood pressure
SFA - saturated fatty acids
T2D - type 2 diabetes
TG - triglyceride
VLDL - very low-density lipoprotein
WP - whey protein
WPH - whey protein hydrolysate
WPI - whey protein isolate

2. Consideration of the relevant literature

Studies appropriate for inclusion in this review were identified by searching nine electronic databases (bibliotek.dk, SveMed+, Medline, PubMed, Embase, The Cochrane Library, Cinahl, Web of Science, and Scopus) for relevant reviews and meta-analyses examining the effects of dairy protein and dairy fat on human individuals with MetS and T2D up to 21th of February 2014. Supplementary evidence from recent research publications, independent of species, were covered by including studies on dairy protein and fat, in relation to MetS and T2D, during the period 2004-2014. The following search terms were included: diabetes mellitus type 2, insulin, blood glucose, metabolic syndrome, hypertension, dyslipidemia, dairy protein, milk protein, whey protein, casein, dairy fat.

3. Milk and dairy proteins

Milk contains two protein fractions, namely casein and WP, representing 2.8% and 0.7% of the total weight of milk, respectively. Fractions are primarily separated by acid precipitation in the cheese-making process [15].

Over the past 20 years, investigation on dairy protein has increased. Today we know that dairy protein has a direct positive effect on hypertension, dyslipidemia, and hyperglycemia. Dairy protein may also improve metabolic health indirectly by increasing lean body mass and reducing obesity [16, 17, 18]. The beneficial effects are primarily linked to WP. There is no apparent difference between the effects of different dairy proteins on satiety and hunger, stimulating hormones, and body weight [19]. Furthermore, it is unknown whether dairy protein outperforms other dietary protein sources in relation to metabolic health.

4. Whey protein

The WP fraction is a heterogeneous, polymorphic group of proteins, dominated by β-lactoglobulin, α-lactalbumin, and glycomacropeptide [15]. Commercially, WP occurs in three different condensed forms: WP concentrate (WPC), WP

isolate (WPI), and WP hydrolysate (WPH). WPC has 30-85% protein, whereas WPI has >90% protein [20]. WPI contains little or no lactose or fat, which is not separated from WPC. WPH is a peptide product derived from WP through a process involving enzymatic hydrolysis or microbial fermentation[21]. The absorption rate of WP is dependent on the degree of hydrolysis and thereby the size of the available peptides. Consumption of WPH results in a more rapid increase of plasma amino acids compared with intact WP [22].

4.1 Glucose metabolism

The consumption of WP is known to acutely reduce the postprandial glucose response in both healthy [23-26] and T2D subjects [27]. WP also improves the insulin response in lean individuals [24, 28] and diabetics [27]. In healthy Japanese subjects, the consumption of WPH induces greater insulin stimulation than intact WP [22]. The consumption of WP 30 minutes prior to a carbohydrate-rich meal stimulates insulin and incretin secretion more than WP as part of a mixed meal in T2D subjects [29]. These results indicate that WP acutely improves glycemic control by stimulating insulin secretion, and reduces plasma glucose levels, an effect that may be enhanced by consumption of WP prior to meal. Thus, in healthy adults, WP contributes to an insulin-independent reduction of blood glucose concentration [26].

A couple of studies have investigated the impact of chronic consumption of WP on glucose and insulin homeostasis. In healthy animals, insulin sensitivity improved after consumption of WP within a period of 6 weeks, as indicated by reduced plasma insulin concentrations [30]. Continued consumption (12 weeks) of 55 g/day WP in obese individuals improved fasting insulin levels and homeostasis model assessment of insulin resistance (HOMA-IR) scores compared with control in overweight and obese subjects [31]. These results point to a beneficial effect of WP by way of continued ingestion, but further studies on MetS and T2D subjects are needed to confirm this. The results are summarized in **Table 1**.

The insulinotropic effect of WP is probably linked to the high content of specific amino acids. A mixture of leucine, isoleucine, valine, threonine, and lysine can mimic glycemic and insulinemic responses similar to those generated by WP [32]. The effect of amino acid-mediated insulin secretion from pancreatic β-cells is substantial, but depends on the kind and composition of amino acids included in the mixture. Liu *et al*. demonstrated that arginine, lysine, alanine, proline, and glutamine stimulate insulin secretion from isolated mouse islets and clonal INS-1E β-cells in a dose- and glucose-dependent manner [33]. Leucine, isoleucine, and valine belong to the group of branched-chain amino acids (BCAA). BCAA are metabolized by branched-chain aminotransferase and branched-chain α-keto acid dehydrogenase. The liver has little aminotransferase activity, and therefore BCAA are primarily delivered to the systemic circulation and metabolized in the skeletal muscle. Additionally, BCAA (especially leucine) activate both the mammalian rapamycin (mTOR) pathway and protein synthesis leading to an increased hormone (e.g. insulin, GIP) expression and secretion [34, 35]. mTOR might also potentially improve insulin sensitivity by activating IRS-1 through phosphorylation [36].

Another possible explanation for the insulinotropic effect of WP is through activation of the incretin system. The incretin system involves glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1). The hormones are released from the intestine in response to a meal, and subsequently further stimulate insulin secretion [37]. WP consumption increases postprandial GIP response in both healthy individuals [26, 38] and T2D patients [27] more than other dairy or protein sources or water. However, the GIP response cannot be mimicked by the supply of specific amino acids that are abundant in WP (BCAA, threonine and lysine) [32]. This indicates that the effect of GIP is not mediated via amino acids *per se*. Interestingly, in the initial postprandial period, the GIP response is lower after ingestion of WP in combination with a fat-rich diet than after ingestion of other dietary protein sources in both obese non-diabetic [39] and T2D subjects [40].

The effect of WP on GLP-1 is ambiguous. Two studies, comparing WP with soy and casein, report a rise in GLP-1 levels in healthy subjects [41, 42]. WP consumed as a pre-meal resulted also in a higher GLP-1 concentration in healthy subjects [26]. This may be related to dipeptidyl peptidase-4 (DDP-4) inhibitory activity of dairy protein, as shown in an animal study [43]. DPP-4 is an enzyme, which plays a major role in glucose metabolism by degrading GLP-1; hydrolytic products of α-lactalbumin and β-lactoglubulin possess DDP-4 inhibitory activity [44, 45]. Other studies that investigated the GLP-1-stimulating effect of WP in healthy [32, 38] and T2D subjects [27, 40, 46] did not detect any changes in the GLP-1 responses when expressed as incremental area under the

Table 1. Effects of milk proteins on glucose metabolism in human subjects

Milk protein (g)	Study design (duration)	Effect	Comparison group	Subjects (n)	Reference
WP (20 g)	Acute	↓ Glucose response	0 g WP	Healthy (10)	[23]
WP (50.8 g)	Acute	↓ Glucose response	Turkey, egg	Lean men (22)	[24]
WP as pre-meal (10-40 g)	Acute	↓ Glucose response	Water	Healthy (16/21)	[25]
WP as pre-meal (10-20 g)	Acute	↓ Glucose response	Water	Healthy (10)	[26]
WP (27.6 g)	Acute	↓ Glucose response	Ham (96 g) + lactose (5.3 g)	T2D (14)	[27]
Casein hydrolysate (12 g)	Acute	↓ Glucose response	Intact casein	T2D (36)	[63]
WP (50.8 g)	Acute	↑ Insulin response	Tuna, turkey, egg	Lean (22)	[24]
WPI (50 % of diet)	Acute	↑ Insulin response	Glucose, casein, soy	Lean (23)	[28]
WP (27.6 g)	Acute	↑ Insulin response	Ham (96 g) + lactose (5.3 g)	T2D (14)	[27]
WP (18.2 g)	Acute	↑ Insulin response	White-wheat bread, milk, cod, cheese, gluten-low, gluten-high	Healthy (12)	[38]
WPH	Acute	↑ Insulin response	WP, soy protein and soy hydrolysate	Healthy (10)	[22]
Casein hydrolysate (12 g)	Acute	↑ Postprandial insulin response	0 g casein hydrolysate	T2D (13)	[62]
WPI (2x27 g/d)	Chronic (12 weeks)	↑ Fasting insulin + HOMA-IR	Glucose	Overweight and obese (70)	[31]
WP or casein (35 g/d)	Chronic (12 weeks)	↑ C-peptide	Baseline	Overweight adolescents (203)	[61]
WP as pre-meal (55 g)	Acute	↑ Insulin and incretin response	WP in main meal	T2D (8)	[29]
WP as pre-meal (10-20 g)	Acute	↑ Postprandial GIP	Water	Healthy (10)	[26]
WP (18.2 g)	Acute	↑ Postprandial GIP	White-wheat bread, milk, cod, cheese	Healthy (11)	[38]
WP (27.6 g)	Acute	↑ Postprandial GIP	Ham (96 g) + lactose (5.3 g)	T2D (14)	[27]
Hydrolysed casein	Acute	↑ Postprandial GIP	Intact casein	Healthy (6)	[60]
WPI + fat-rich meal (45 g)	Acute	↓ Postprandial GIP	Casein and gluten protein	Obese non-diabetic (11)	[39]
WPI + fat-rich meal (45 g)	Acute	↓ Postprandial GIP	Gluten protein	T2D (12)	[40]
WP (48 g)	Acute	↑ GLP-1	Casein	Healthy (9)	[41]
WP (10-25 %)	Acute	↑ GLP-1	Casein, soy	Healthy (25)	[42]
WP as pre-meal (10-20 g)	Acute	↑ GLP-1	Glucose, water	Healthy (10)	[26]
WP (18 g)	Acute	→ GLP-1	Glucose, drinks supplemented with amino acids.	Healthy (12)	[32]
WP (18.2 g)	Acute	→ GLP-1	White-wheat bread, milk, cod, cheese, gluten-low, gluten-high	Healthy (11)	[38]
WP (27.6 g)	Acute	→ GLP-1	Ham (96 g) + lactose (5.3 g)	T2D (14)	[27]
WPI + fat-rich meal (45 g)	Acute	→ GLP-1	Casein, cod- and gluten protein	T2D (12)	[40]
WPI + fat-rich meal (45 g)	Acute	→ GLP-1	WP specific fractions	T2D (12)	[46]
WPI + fat-rich meal (45 g)	Acute	→ GLP-1	WP specific fractions	Obese non-diabetic (12)	[47]

Legend: ↑: significant increase. ↓: significant decrease. →: unchanged or insignificant effect compared with the comparison group. GIP: glucose-dependent insulinotropic polypeptide, GLP-1: glucagon-like peptide 1, HOMA-IR: homeostasis model assessment of insulin resistance, T2D: type 2 diabetes, WP: whey protein, WPH: whey protein hydrolysate, WPI: whey protein isolate.

curve (iAUC). Also, no difference was found in obese, non-diabetic subjects between WPI and WP-specific fractions (WPH and WP enhanced with α-lactalbumin and caseinoglycomacropeptide, respectively) [47]. The incretin results are summarized in **Table 1**.

4.2 Lipid metabolism

The effect of WP on lipid metabolism has been studied in a few acute and chronic setups. In acute studies, postprandial triglyceride (TG) responses measured as iAUC decreased significantly in both obese non-diabetic subjects [39] and T2D subjects [40] after consumption of WPI in comparison with other dietary protein sources (cod, casein or gluten) or glucose. In contrast, no difference in postprandial TG responses was observed when the acute effects of WPI and WP-specific fractions in obese, non-diabetics [47] and T2D subjects [46] were compared. Furthermore, studies comparing WP to other dietary protein sources found a sup-

pression of the response to free fatty acids (FFA) [39, 40]. Interestingly, this WPH caused less postprandial suppression of FFA than WPI and other WP-specific fractions [47]. Intervention with WPI (54 g/day) in overweight and obese individuals reduced fasting TG by 22% after 6 weeks and by further 22% after 12 weeks compared with glucose intervention over the same time frame. Furthermore, WPI reduced total cholesterol and LDL cholesterol more than casein and glucose [31]. However, 12-week intervention with WP (50 g/day) did not affect fasting lipid parameters in moderate obese subjects [48]. **Table 2** summarizes the effects of WP on lipid metabolism.

Contrary to the results from chronic studies, findings from six acute studies noted no changes in other parameters related to lipid metabolism such as total cholesterol, low-density lipoprotein (LDL) cholesterol, or high-density lipoprotein (HDL) cholesterol. This may indicate that WP has an outstanding acute postprandial effect on the TG response compared to other dietary protein sources and glucose, and that the effect can either be attributed to WPI or a single WP fraction alone. The reason for this difference is unknown. Postprandial TG concentration is affected by many factors, e.g., intestinal chylomicron secretion, hepatic very low-density lipoprotein (VLDL) secretion, conversion of TG-rich lipoproteins to TG-depleted lipoproteins, and tissue uptake of TG-depleted lipoproteins [49]. Thus, WP consumption may result in a lower production of chylomicrons, which is consistent with the more pronounced FFA suppression. Lipoprotein lipase (LPL) is a key enzyme in the hydrolysis of TG in chylomicrons and VLDL. The insulinotropic effect of WP may enhance LPL activity, and hence accelerate chylomicron clearance. In the chronic state, WP is also proposed to affect intestinal lipid absorption and/or excretion and/or *de novo* cholesterol biogenesis in the liver [31].

Amino acids with high availability such as BCAA and essential amino acids contained in WP may possibly influence lipid metabolism. Lysine is involved in the metabolism of long-chain fatty acids, which are essential for the endogenous synthesis of carnitine. Lysine deficiency reduces carnitine concentrations in the body and increases lipid accumulations. Supplementation of lysine reduces the level of TG in tissues [50]. However, a 12-week intervention with lysine did not change the lipid profile in men with hypertriglyceridemia [50]. Leucine and alanine have also been linked to beneficial metabolic effects, such as reduced body fat accumulation [36, 51]. However, some investigations have revealed that the beneficial effects might be due to the supply of nitrogen rather than leucine itself. Thus, to better understand the mechanism by which WP affects lipid metabolism in general and TG responses in particular requires more investigation into the effect of single amino acids.

4.3 Blood pressure

Recent studies investigating the antihypertensive effect of milk protein and peptides are inconclusive, despite growing evidence that supports the hypothesis that milk proteins and peptides have beneficial effects on vascular health [52]. Only two studies in humans have compared the effects of WP and casein on BP. They found that WPI (45 g/day) compared to casein and glucose did not acutely change systolic blood pressure (SBP) or diastolic blood pressure (DBP) in postmenopausal women [53]. However, consumption of WP and casein 2 x 27 g/day during a 12-week period was found to reduce DBP in non-hypertensive subjects compared to glucose [54].

BP is controlled and regulated by different biochemical mechanisms. The renin-angiotensin system is a central pathway causing vasodilation and consequently decreased BP by inhibition of the angiotensin-converting enzyme (ACE). Peptides derived from casein are known to have an antihypertensive effect via inhibition of the ACE system. However, consumption of milk drinks supplemented with peptides from WP did not reduce BP in hypertensive subjects [55]. Besides the effect on the ACE system, the vasodilator nitric oxide (NO) also plays a major role in BP regulation. A whey-derived peptide (NOP-47) has been found to improve the arterial vasodilation in healthy women and men after 2 weeks intervention (5 g/day) [56]. NOP-47 is suggested to increase NO synthesis in the endothelium. Nevertheless, Ballard *et al*. observed that NOP-47 acutely (within 2 hours) increased brachial artery flow-mediated dilation in overweight persons, without causing inhibition of ACE activity or detectable changes in circulating endothelium-derived factors including plasma NO, prostacyclin, and endothelium-1 [57].

Several studies have investigated the influence of intact WP or WPH on BP in young people with elevated SBP and DBP. Consumption of WP and WPH (28 g/day) during a 6-week period reduced SBP, DBP, and mean arterial pressure (MAP) compared with baseline, with no significant difference between the two groups of young individuals consuming 28 g per day of either hydrolyzed or non-hydrolyzed whey protein [58]. Likewise, SBP

Table 2. Effects of milk proteins on lipid metabolism in human subjects

Milk protein (g)	Study design (duration)	Effect	Comparison group	Subjects (n)	Reference
WPI or casein + fat-rich meal (45 g)	Acute	↓ TG response	Cod- and gluten protein	Obese non-diabetic (11)	[39]
WPI + fat-rich meal (45 g)	Acute	↓ TG response	Casein, cod- and gluten protein	T2D (12)	[40]
WPI (45 g)	Acute	↓ TG response	Casein, glucose	Postmenopausal women (20)	[53]
WPI or casein + fat-rich meal (45 g)	Acute	↓ TG concentration in chylomicron-rich fraction	Cod- and gluten protein	Obese non-diabetic (11)	[39]
Casein combined with carbohydrates and a fat-rich meal (45 g)	Acute	↓ TG concentration in chylomicron-rich fraction	Control meal, control meal + carbohydrates, control meal + casein	T2D (11)	[64]
WPI + fat-rich meal (45 g)	Acute	→ TG response	WP specific fractions	Obese non-diabetic (11)	[47]
WPI + fat-rich meal (45 g)	Acute	→ TG response	WP specific fractions	T2D (12)	[46]
WPI or casein + fat-rich meal (45 g)	Acute	↓ FFA	Cod- and gluten protein	Obese non-diabetic (11)	[39]
WPI + fat-rich meal (45 g)	Acute	↓ FFA	Casein, cod- and gluten protein	T2D (12)	[40]
WPI (2x27 g/d)	Chronic (12 weeks)	↓ Fasting TG, ↓ total cholesterol, ↓ LDL cholesterol	Glucose	Overweight and obese (70)	[31]
Casein (35 g/d)	Chronic (6 weeks)	↓ Total cholesterol	Baseline	*Hypercholesterolemic (43)*	[65]
WP (2x25 g/d)	Chronic (12 weeks)	→ Fasting lipids	Casein	Moderate obese (48)	[48]

Legend: ↑: significant increase. ↓: significant decrease. →: unchanged or insignificant effect compared with the comparison group. FFA: free fatty acids, T2D: type 2 diabetes, TG: triglycerides, WP: whey protein, WPI: whey protein isolate.

and DBP were reduced in hypertensive subjects after 6 weeks of ingestion of WP-derived peptides (20 g/day) [59]. Therefore, whey-derived peptides may have a potential to reduce both SBP and DBP, however more studies are needed to identify the activating peptides and their mechanism of action. The results are summarized in **Table 3**.

5. Casein

Casein is the milk protein fraction that precipitates upon acidification. Consequently, casein as opposed to WP coagulates in the acidic environment in the stomach. This coagulation delays the gastric emptying and reduces the postprandial increase in plasma amino acids. Casein is known to be more slowly digested than WP, and the different digestion rates of the proteins may affect the insulin response. According to Bendtsen *et al*. this may result in a more pronounced satiating effect of casein over time [19]. Consumption of hydrolyzed casein results in a more rapid increase in plasma amino acid concentrations compared with intact casein [60]. The main components of the casein fraction are α_{s1}-, α_{s2}-, β-, and κ-casein.

5.1 Glucose metabolism

The effect of casein on glucose metabolism is not as well studied as the effect of WP (**Table 1**). Casein is often used as a protein benchmark in studies on WP. Acutely both WP and casein show insulinotropic properties in healthy subjects, with the WP fraction being a more potent insulin secretagogue than casein [38]. Continued consumption of 35 g casein/day (over 12 weeks) increased the concentration of C-peptide in the plasma of overweight adolescents [61]. The slow absorption of casein can be regulated by hydrolysis of the casein fraction. This ensures faster digestion and thereby quicker availability of BCAA, essential amino acids, and total plasma amino acids in the circulation [60]. Hydrolyzed casein in doses higher than 12 g/day enhance the carbohydrate-induced insulin and glucose responses compared to lower doses [62] and to intact casein [63] in T2D subjects. Interestingly, the induction from hydrolyzed casein was independent of the leucine content. The incretin hormones GIP and GLP-1 are proposed only to play a minor role in the insulinotropic effect of casein in T2D subjects [64]. However, hydrolyzed casein stimulates the release of GIP more substan-

tially than intact casein, whereas the GLP-1 response is independent of protein fractionation [60].

5.2 Lipid metabolism

The consumption of casein in combination with carbohydrates and a fat-rich meal acutely suppresses the TG response in the chylomicron-rich fraction in T2D [64]. This is in agreement with observations in non-diabetic subjects, where casein and WP have similar suppressive effects on postprandial TG levels and TG concentrations in the chylomicron-rich fraction after a fat-rich meal [39]. An enhanced TG response is a common finding in MetS and T2D subjects as a part of postprandial dyslipidemia, which is due to insulin resistance. Therefore, the effect of casein is beneficial for the lipid metabolism.

In agreement with this finding, Holmer-Jensen *et al*. observed a positive correlation between FFA and plasma levels of TG after WP and casein consumption, primarily as a result of more pronounced FFA suppression [39]. This indicates that both WP and casein are able to reduce lipotoxicity. Other studies have found that WP is more efficient than casein in reducing the postprandial TG response in overweight post-menopausal women [53] and T2D subjects [40]. A six-week intake of casein reduces plasma total cholesterol in hypercholesterolemic adults compared with baseline, but the reduction was mainly caused by a decrease in HDL cholesterol [65]. The results are summarized in **Table 2**.

5.3 Blood pressure

Specific peptides derived from casein have antihypertensive effects by inhibiting the ACE system. Among those are the lactotripeptides Val-Pro-Pro and Ile-Pro-Pro. Two meta-analyses of randomized controlled trials report that the lactotripeptides reduce BP both in European and Asian subjects [66, 67]. Another lactopeptide C12 (Phe-Phe-Val-Ala-Pro-Phe-Pro-Glu-Val-Phe-Gly-Lys) is also able to reduce BP, by a mechanisms of ACE inhibition. Townsend *et al*. report that in hypertensive subjects lactopeptide C12 (100 mg) in combination with alginic acid (1754 mg) reduce both SBP (9.2 mmHg) and DBP (6.0 mmHg) 6 hours after oral administration [68]. Administration of lactopeptide C12 in a higher dose (3.8 g) reduces SBP and DBP in pre-hypertensive subjects [69]. The continued effect (12 weeks) of intact casein (54 g/day) was only tested in one study by Pal and Ellis [54], where casein and WPI demonstrate similar suppressive effects on DBP in overweight individuals. Therefore, more randomized, controlled intervention studies are needed to disentangle the potential benefits of both casein and WP on vascular health in general and BP in particular. **Table 3** summarizes the effect of milk proteins on blood pressure.

6. Dairy fat

The results from only a few randomized clinical trials are published on the effect of dairy fat as a specific macronutrient in relation to MetS and T2D. However, there is a considerable amount of literature dealing with observational evidence. In recent years, consumption of dairy fat and its role in MetS and T2D has been extensively discussed. Dairy fat has been proposed as unlikely to independently protect against diabetes due to the association between low-fat and reduced risk of diabetes [70]. Although previous data indicate that dairy fat and especially high-fat dairy food contribute to obesity and cardiometabolic risk, a recent review rejects this hypothesis [71]. Likewise, there is no convincing evidence between high-fat dairy consumption and risk of T2D [71]. Consequently, the present evidence concludes that dairy fat consumption is not typically associated with an increased risk of weight gain, CVD, and T2D. Furthermore, SFA may be less adverse than previously thought [72, 73]. SFA comprises a range of distinct SFAs, which may affect the metabolism differentially.

Milk fat occurs as globules emulsified in the aqueous phase of milk. The globule is a complex substance composed of different layers, where the outermost protect against coalescing and stabilizing of the emulsion. The large surface of the globule makes it sensitive to lipolytic enzymes, while passing through the gastrointestinal tract.

The composition of the lipid fraction in milk is dominated by TG (95-96%). The remaining percentage is composed of phospholipids (PL), cholesterol, 1,2-diacylglycerol, FFA, monoacylglycerol, cholesteryl ester, and hydrocarbons [74]. The fatty acid composition is dominated by SFA (70%) followed by MUFA (25%) and PUFA (2.3%) [75]. Milk is characterized by a relatively high content of low molecular weight fatty acids, with approximately 400 different fatty acids, where butyric acid (C4:0) is the primary constituent. About 15 of these fatty acids are present in >1% and the others are present only in trace amounts. Furthermore, milk contains odd numbered fatty acids, e.g. pentadecanoic acid (C15:0) and margaric acid (C17:0).

Table 3. Effects of milk proteins on blood pressure in human subjects

Milk protein (g)	Study design (duration)	Effect	Comparison group	Subjects (n)	Reference
WPI (45 g)	Acute	→ SBP, → DBP	Casein, glucose	Postmenopausal women (20)	[53]
NOP-47 (5 g)	Acute	↑ Artery flow-mediated dilation	Placebo	Overweight (21)	[57]
Casein hydrolysate (C12) (0,1 g) + alginic acid (1,754 g)	Acute	↓ SBP, ↓ DBP	Placebo, and C12 + alginic acid (0,877 g)	Hypertensive (10)	[68]
WP or casein (2x27 g/d)	Chronic (12 weeks)	↓ DBP	Glucose	Obese, non-hypertensive (70)	[54]
WP-derived peptides (2.6 g/d)	Chronic (12 weeks)	→ BP	Placebo	Hypertensive (54)	[55]
NOP-47 (5 g/d)	Chronic (2 weeks)	↑ Arterial vasodilation	Placebo	Healthy (20)	[56]
WP or WPH (28 g/d)	Chronic (6 weeks)	↓ SBP, ↓ DBP, ↓ MAP	Baseline	Young people with elevated SPB and DBP (71)	[58]
WP-derived peptides (20 g/d)	Chronic (6 weeks)	↓ SBP, ↓ DBP	WP	Hypertensive (30)	[59]
C12 (3.8 g/d)	Chronic (4 weeks)	↓ BP	Baseline	Pre-hypertensive (48)	[69]

Legend: ↑: significant increase. ↓: significant decrease. →: unchanged or insignificant effect compared with the comparison group. BP: blood pressure, C12: lactopeptide C12 (Phe-Phe-Val-Ala-Pro-Phe-Pro-Glu-Val-Phe-Gly-Lys), DBP: diastolic blood pressure, MAP: mean arterial pressure, NOP-47: whey-derived peptide, SBP: systolic blood pressure, T2D: type 2 diabetes, WP: whey protein, WPH: whey protein hydrolysate, WPI: whey protein isolate.

Fatty acids originate from two sources: directly from peripheral circulation or from *de novo* synthesis. Mammary *de novo* synthesis accounts for all C4:0-C12:0, most of myristic acid (C14:0), and typically about half of palmitic acid (C16:0). Microorganisms in the rumen are able to synthesize C15:0 and C17:0 plus hydrogenate linoleic acid (C18:2) to yield oleic acid (C18:1) and stearic acid (C18:0) together with the formation of conjugated linoleic acids (CLA) [76]. C18:0 or longer are derived from circulating plasma lipids. The composition of milk lipids varies during seasons, and depends on genetic and environmental factors [74].

6.1 Saturated fat

C14:0, C16:0, and C18:0 are the most abundant SFAs. The remaining SFAs appear in <5% of the total [74]. Reducing the intake of SFA is recommended to avoid the negative effects on LDL-cholesterol, inflammation, and insulin sensitivity [77, 78]. However, Kuipers *et al*. suggest that reduction of carbohydrates with a high glycemic index may be more efficient in the prevention of CVD induced by low-grade inflammation [79]. Furthermore, substitution of starch with SFA is found to improve glycemic control in T2D subjects [80]. Besides the interaction with other macronutrients, SFA may also modulate the susceptibility to impaired insulin sensitivity, and may cause MetS in genetically disposed individuals [81]. Toll-like receptor 4 (TLR-4) is likely to be involved in the effects of SFA. SFA may activate TLR-4 signaling, thus increasing inflammatory cytokine productions and insulin resistance [82].

As mentioned above, SFA is referred to as dietary SFA in general. Literature on dairy-derived SFA is limited. Butter is often referred to as a source of SFA due to its high SFA content. In mixed meals, butter contributes more to the incretin effect by increasing insulin secretion than protein represented by dried meat [83]. In spite of the capability to stimulate insulin secretion, dairy SFA may impair insulin signaling through stimulation of low-grade inflammation, thus initiating insulin resistance [84]. C16:0 inhibits the activation of insulin signaling molecules (IRS-1, Akt, PI3-K), and causes insulin resistance [85]. C16:0 may also inhibit insulin signaling through sphingolipid synthesis, whereby C16:0 may serve as a precursor [86]. Substitution of butter with sunflower oil, a rich source in PUFA, induces a significant reduction in inflammatory markers in overweight men [87]. This observation points to adverse effects of dairy SFA on low-grade inflammation.

Synthetic C6:0-C10:0 (medium-chain fatty acids) have beneficial effects on metabolic health and improve insulin sensitivity. Under certain *in vitro* conditions, they are proinflammatory, but *in vivo* they may reduce intestinal injury and protect from hepatotoxicity [88]. C6:0-C10:0 fatty acids from

dietary sources are found to be incorporated in adipose tissue TG and potentially affect adipose tissue and systemic functions. The SFAs, C12:0-C16:0, increase LDL and HDL cholesterol, and thereby have an unfavorable effect on the lipid profile [89]. On the other hand, specifically designed butter with 20% lower content of C12:0-C16:0 does not change inflammatory markers in healthy subjects [90]. Substituting butter for sunflower oil decreases the late phase of postprandial lipidemia in overweight men, which may be elicited via a slower absorption of SFA and a lower clearance rate of C18:0 and C16:0 [87].

The effect of SFA on the metabolism may also be affected by the remaining components in the given product. Hjerpsted *et al*. showed that cheese and butter with the same fat content and composition do not affect the lipid metabolism in the same way [91]. Cheese lowers LDL and HDL cholesterol concentrations after 6 weeks intervention. The explanation of this observation may be associated with other components than SFA, but the mechanism is not known. The authors propose potential effects of calcium, fermentation levels, or the protein content in cheese. Another explanation may be related to the mechanical handling of milk during processing which may cause a restructuring of elements contained in the milk fat globule.

6.2 Monounsaturated fat

Palmitoleic acid (*cis* and *trans* C16:1) and oleic acid (C18:1) are the MUFAs with quantitative significance in the dairy lipid fraction. They represent 1-2% and 20-30% of the total fatty acid composition in milk fat, respectively [74]. In the cohort of the Multi-Ethnic Study of Atherosclerosis, circulating levels of *trans* C16:1n-7 are associated with higher LDL cholesterol [92]. Conversely, *trans* C16:1n-7 is also associated with reduced TG, fasting insulin, and incidence of diabetes [92]. This is in agreement with previous results from the same group determined in the cohort of the Cardiovascular Health Study [93]. These results are in accordance with Kratz *et al*. who suggest *trans* C16:1 to be beneficial for obesity and metabolic health [71].

C18:1 is suggested as a dietary element reducing the incidence of metabolic diseases [78]. In mice, C18:1 augments the insulin response by 1.5-fold, without stimulating insulin secretion [94]. Milk supplemented with C18:1 and in combination with folic acid and vitamins A, B6, D, and E reduce total, LDL, and HDL cholesterol in subjects with MetS [95] and moderate CVD risk [96]. The three milk supplements applied were not isocaloric. Therefore it is hard to know the absolute effect of C18:1. There is possibly a synergistic effect between C18:1, milk, and the added supplements.

6.3 Polyunsaturated fat

CLA (C18:2) is one of the most studied fatty acids in a dietary context. It represents 1-3% of the total fatty acid composition in milk [74]. The primary CLA isomers in dairy fat is *cis*-9, *trans* 11 CLA (70-90%); the second most frequent CLA is *trans* 10, *cis*-12. Despite CLA unlikely affect the risk factors associated with MetS and T2D in humans [71], the consumption of *trans* 10, *cis*-12 CLA isomer exerts a negative effect on metabolic risk factors by aggravating insulin resistance and increasing plasma glucose concentration in obese men [97]. Consumption of a mixture (4.2 g/day) of *cis*-9, *trans* 11 CLA and *trans* 10, *cis*-12 CLA for three months increased the inflammatory marker C-reactive protein compared to control [98]. However, naturally enriched *cis*-9, *trans* 11 CLA cheese reduces IL-6, IL-8, and TNF-α in healthy subjects after 10 weeks intervention [99]. Taken together, the results from studies on CLA isomers are ambiguous.

α-linolenic acid is another major PUFA in milk; it comprises 0.5-2% of the total fatty acid composition in milk [74]. Djousse *et al*. report that subjects consuming ≥2 servings of dairy products per day together with a high intake of linolenic acid have the lowest prevalence of hypertension [10]. However, no interaction between dairy and linolenic acid consumption was observed.

6.4 Phospholipids

Polar lipids in milk, such as phosphorylated lipids, are concentrated in milk fat globule membranes surrounding the lipid droplets that are secreted by the mammary gland. The milk fat globule membrane has a complex structure. The key function is to prevent coalescence of fat globules in milk [100]. Phospholipids (PL) in milk represent approximately 0.25 to 0.96% of milk fat, with variability in the content attributed to numerous factors including the feeding regime, period of lactation, and differences in the methods of extraction and analysis [100].

Sphingomyelin, one of the PL contained in milk, accounts for about 1% of dairy fat [74]. Sphingomyelin is bioactive through its metabolites ceramide, spingosine, and sphingosine 1-sphosphate. Studies in animal models suggest an inhibitory effect of milk-derived sphingolipids on cholesterol absorption [101].

Ohlsson *et al*. have investigated whether buttermilk enriched with polar milk lipids, primarily sphingomyelin, acutely affect postprandial lipidemia after a fat-rich meal in healthy men [102]. They found no significant difference in plasma TG concentrations, but it seems to increase HDL cholesterol when compared with control who did not receive the sphingolipids within the milk formulation. Recently, Keller *et al*. assessed the effects of lipamin M20 on plasma lipids [103]. Lipamin M20 is a milk phospholipid concentrate prepared from butter concentrate. It contains 52% protein, 5% lactose, 38% total fat, and 18.4% total phospholipid. In this open-label, cross-over study of lipamin M20, healthy women consumed low and high doses of milk phospholipids (PL) and a combination of PL and plant sterol over a test period of ten days. The low dose comprised approximately 3 g of milk PL daily (corresponding to approximately 16.2 g lipamin M20), the high dose comprised approximately 6 g of milk PL (corresponding to approximately 32.4 g lipamin M20), and the combination dose comprised approximately 6 g of milk PL combined with 2 g plant sterols. Intake of 32.4 g lipamin M20 provided approximately 16.8 g protein (milk fat globule membrane fraction not specified) and 6 g PL to the body. Total plasma PL, phosphatidylcholine, sphingomyelin, and phosphatidyl inositol concentrations did not differ from baseline values or between groups after the interventions with lipamin M20 [103]. Following intake of 16.2 g lipamin 20, the plasma level of phosphatidyl ethanolamine was higher than at baseline. Total and HDL cholesterol levels in plasma were significantly lower after daily intake of 16.2 g lipamin 20 compared with levels at baseline, while total and LDL cholesterol levels following intake of 32.4 g lipamin 20 were significantly higher than levels after intake of 16.2 g lipamin 20. Stool weight was higher following daily intake of 6 g milk PL compared with baseline [103]. Fecal pH was significantly lower following the intervention than at baseline, and fecal weight of PL (mg/g dry matter) increased.

Conway and colleagues assessed the effects of daily intake of two servings of buttermilk (prepared from powder, 45 g/day) on plasma lipids, sterols, C-reactive protein, and BP in a randomized, controlled, crossover study [104, 105]. The total daily intake of PL from the buttermilk and control beverages was 188 mg and 34 mg, respectively. Thirty-four adults completed the study and consumed buttermilk or the control beverage for consecutive periods of four weeks. Total cholesterol, TG, and BP were significantly lower after consumption of buttermilk than after consumption of control beverage. There were no changes in HDL cholesterol or body weight.

Human studies indicate that milk PL may have a regulating impact on the lipid metabolism. However, more studies are needed to determine the mechanism of PL.

7. Conclusions and perspectives

Taken together, dairy proteins have a high potential to improve metabolic disturbances in relation to MetS and T2D due to the amino acid composition. To identify the mechanisms underlying the protein-specific insulinotropic effects more studies are needed investigating specific amino acids and dairy protein-derived peptides. Likewise, we need to gain understanding of the beneficial properties on lipid metabolism in general and plasma TG in particular by testing the effects of specific amino acids and dairy protein-derived peptides. Interestingly, dairy proteins have a DPP-4 inhibitory potential, which possibly can improve glucose metabolism. This should be tested in humans.

Little is known about the specific dairy lipid fractions, not least the potential differences between short- and medium-chain SFA versus long-chain SFA and their effects on metabolic risk factors. Human studies are needed on specific fatty acids contained in SFA, MUFA, PUFA, and PL to determine their potential effects and underlying mechanisms. Moreover, we need studies to clarify the effects of dairy products on metabolic risk factors and the mechanisms by which dairy lipids act and if they possibly interact synergistically with other dietary components. A large part of our knowledge derives from observational studies that do not allow the identification of cause-effect relationship. Therefore, long-term, randomized, controlled human clinical trials are needed to clarify whether dairy products with various SFA compositions influence the metabolism differentially in MetS and T2D subjects.

It will also be of interest to study the impact of dairy proteins and fat on body composition and fat distribution (gynoid versus android distribution) as these measures play a key role in the risk of CVD. An important question is how dairy proteins and fat impact diurnal BP and pulse (i.e. the influence over 24 h). It seems that dairy proteins counteract inflammatory factors, but we need a more accurate picture of the effects of dairy components on both fasting and postprandial levels of inflam-

mation, which are critical aspects in atherogenesis and the development of CVD. Apparently, low-fat-fermented dairy products have a positive influence on T2D risk [13]. However, we have little knowledge about the mechanisms of action. The beneficial effects of fermented dairy products on various risk factors may be related to probiotic bacteria, vitamin K_2, and energy density. This needs to be explored in more detail.

In conclusion, the present evidence confirms that dairy proteins have an insulinotropic effect, but the underlying mechanism is not yet clear. The amino acid composition and specific amino acids like BCAA seem to be of particular importance. Dairy protein-derived peptides may also contribute to the insulinotropic effect via DPP-4 inhibitory activity and improvement of BP. Data indicate that in particular dairy fat has different effects depending on the dairy product and composition of macronutrients in the meal. To better understand the biological mechanisms of dairy proteins and fat on metabolic risk factors more human studies are needed; they should focus on the interactions of dairy proteins and fat with macronutrients in the meal and other dairy components such as micronutrients and microorganisms from fermented products. Also, there appears to be an impact of manufacturing methods of dairy products on their potential to act insulinotropic or antiatherogenic. This should be determined. Although not easy to achieve, these findings will provide a more clear knowledge of the bioactive mechanisms of dairy proteins and fat.

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