CHAPTER

19

Milk Proteins and Human Health

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OUTLINE

INTRODUCTION

Milk provides a rich source of proteins that are not found in any other food source and that have a range of putative positive health outcomes in both adults and children. Epidemiology has pointed to a positive association between cow's milk and human health, the underpinning mechanisms of which are as yet not well understood (Elwood et al., 2008). Humans consume milk primarily from cows, but around the world communities consume milk from many other animals, including buffalo, yaks, goats, sheep, reindeer, and camels. Cow's milk protein consists of approximately 80% (w/w) casein and 20% (w/w) whey protein, both of which may elicit beneficial health outcomes, and which are the source of a wide range of peptides with potential bioactive properties. The processing of milk protein, which may be an important factor in digestion and absorption kinetics, may also have physiological effects and in turn potential health benefits (Morr and Ha, 1993; Dalgleish and Corredig, 2012).

Milk Proteins. DOI: 10.1016/B978-0-12-405171-3.00019-2

In clinical studies, whey protein concentrate (WPC), micellar casein, or sodium caseinate have most commonly been investigated, but trials have also used milk protein concentrate (MPC), calcium caseinate, casein hydrolysate, whey hydrolysates, and whey protein isolate (WPI) as sources of cow's milk protein. The production of these products is described elsewhere in this volume (see in particular Chapters 2 and 12).

The impact of milk proteins and their associated peptides on health outcomes across the life span is a growing area of research interest. Whey protein in particular has been a focus due to its properties of rapid absorption, serum AA profile, and insulinotropic effects among other properties of interest. In this chapter, we review the evidence (primarily from human clinical trials) that milk proteins may improve or prevent a range of age-related chronic health conditions, particularly those associated with metabolic health, including metabolic syndrome, type 2 diabetes (T2DM), atherosclerosis, and hypertension, as well as the role that they may play in the control of body weight and maintenance of lean body and/or skeletal muscle mass during aging and weight loss. We also review recent evidence in support of bone health and maternal and infant nutrition.

MILK PROTEINS, METABOLIC HEALTH, AND TYPE 2 DIABETES

Over the past two decades poor metabolic health has emerged as a major public health concern. It has been linked to a more sedentary lifestyle and poor diet, and is a forerunner of T2DM and adverse cardiovascular health. Metabolic syndrome has been defined to represent a cluster of commonly measured metabolic markers of adverse health, including abdominal obesity, hyperglycemia, dyslipidemia, and hypertension (Grundy et al., 2005; Alberti et al., 2009). These provide intermediary biomarkers of poor health that can be targeted for improvement. Individuals with metabolic syndrome are at higher risk of developing T2DM and also cardiovascular disease (CVD) (Alberti et al., 2009), and younger, lean individuals typically have a better metabolic profile than older, overweight men and women. This gradual impairment is commonly accompanied by physiological changes such as an excess accumulation of lipids in adipose tissue and subsequent overspill into liver, skeletal muscle, and other tissues. Adipose tissue provides a major lipid storage depot; however, excessive expansion of abdominal and possibly other adipose stores and consequent lipid overspill and infiltration into other tissues is linked to release of proinflammatory mediators (Despres and Lemieux, 2006). Blunting of CHO, fat and protein metabolism, insulin resistance, and impairment in endothelial function are other common consequences. Metabolically active lean body mass is important for good metabolic health, not least as a mediator for glucose regulation among many other roles. For example, skeletal muscle accounts for up to 75% of whole-body glucose uptake. In combination, progressive loss of skeletal muscle and excess accumulation of abdominal fat (recently termed 'sarcobesity' (Parr et al., 2013)) can severely impair metabolic health.

Evidence from large epidemiological studies has shown that the consumption of dairy products may be associated with a lower risk of metabolic disorders and CVD (Elwood et al. 2008; Rice et al., 2011), which in turn has been attributed to milk protein and in particular

to the whey protein component. Insulin is sensitive to both the composition and concentration of plasma AAs, and both whey protein and casein ingestion stimulate increased insulin secretion (Nilsson et al., 2004; 2007). Ingestion of whey protein leads to more rapid secretion of insulin than micellar casein (Boirie et al., 1997), a consequence of its rapid absorption kinetics, and hence underpins better glucose control in metabolically impaired individuals (Pal and Radavelli-Bagatini, 2012). Evidence predominantly generated from single-meal postprandial studies shows that milk proteins may increase tissue glucose uptake and suppress post-meal blood glucose fluctuations (Claessens et al., 2008; 2009b; Pal and Ellis, 2010a; Akhavan et al., 2010).

These effects have also been shown in patients with T2DM. In a postprandial study of T2DM patients, the addition of 18 g whey protein into a breakfast or lunch meal caused a marked increase in plasma insulin concentrations compared to an isoenergetic substitution of nondairy protein and carbohydrate (CHO). The higher insulin levels were associated with a greater suppression of postprandial blood glucose levels (Frid et al., 2005). Casein also has effects on blood glucose. In a group of overweight individuals with T2DM, casein hydrolysate (0.3 g/kg) with leucine (0.1 g/kg) decreased plasma glucose levels over 24 hours when consumed after breakfast, lunch, and dinner (Manders et al., 2006). Notably, however, the casein supplement had been enriched in this study with the branched-chain amino acid (BCAA) leucine. Conversely, ingestion of 40 g casein hydrolysate at breakfast, lunch, and dinner caused little improvement in 24-hour blood glucose levels in a group of patients with long-standing T2DM. The discrepancy in outcome has been attributed to impairment of the insulin-secreting pancreatic beta-cell in long-standing T2DM patients, impairing the response to the milk protein (Manders et al., 2009). This remains unconfirmed, with some preliminary evidence that free AAs may in fact reactivate the insulin secretory mechanism in this metabolically impaired T2DM patient group (van Loon et al., 2003). Obesity and metabolic syndrome are often accompanied by the development of nonalcoholic fatty liver disease (NAFLD), where excessive lipids accumulate in the liver, eventually leading to fibrosis and cirrhosis. Experimental evidence from rodents has shown whey protein to decrease lipid accumulation in the liver (Hamad et al., 2011), but there are as yet no clinical studies.

There have been few long-term clinical studies of milk proteins on hyperglycemia. In overweight and obese individuals with increased metabolic risk through impaired glucose tolerance (IGT), raised plasma triglyceride (TG) levels, low HDL-cholesterol levels, and/or abdominal obesity, daily ingestion of a high-dose WPI supplement (54 g/day) for 12 weeks successfully decreased fasting blood insulin levels and insulin resistance but unexpectedly did not in turn decrease fasting glucose concentrations (Pal et al., 2010a). Clearly, more long-term studies of insulin and glucose control are needed.

MILK PROTEINS, OBESITY, AND WEIGHT CONTROL

There is growing evidence that high-protein diets may be efficacious for weight loss and/or longer-term weight-loss maintenance (Larsen et al., 2010), but the issue as to whether different protein sources may be more or less successful is not well understood. The primary mechanisms that underpin high-protein diets or protein-based supplements for weight loss are enhanced appetite control and suppression of food intake (Poppitt et al., 1998; Anderson

and Moore, 2004; Paddon-Jones et al., 2008; Dove et al., 2009). Sparing of skeletal muscle in favor of adipose loss during weight reduction through use of a higher-protein (>20% of energy as protein), energy-restricted diet may also be important. This is of particular relevance to dairy protein, where the high BCAA content drives muscle protein synthesis, which will contribute to the maintenance of metabolically active lean body mass. High-protein diets suppressing hunger and food intake have been reported in many studies, although the majority of data have been from shorter-term, postprandial interventions. Gradually, evidence for longer-term suppression and weight loss is growing (Clifton et al., 2008; Larsen et al., 2010). Milk proteins are commonly used in meal replacement drinks, which provide a convenient way to undertake a low-energy, high-protein diet. Some preliminary data show that milk proteins may promote greater satiety than other protein sources, but it is not well substantiated (Anderson et al., 2004; Veldhorst et al., 2009; Baer et al., 2011). Whether whey protein or casein may have differential effects is also not well understood. In a recent study, ingestion of an isoenergetic bolus of skimmed milk containing both casein and whey protein was reported to decrease energy intake more than protein alone (Lorenzen et al., 2012). In contrast, other studies have reported whey protein to be more anorectic than casein (Hall et al., 2003), with *a*-lactalbumin proposed as the whey protein fraction responsible for the greatest suppression of intake in a study comparing whey, whey protein plus glycomacropeptide (GMP), casein, and soy (Veldhorst et al., 2009). In a recent study on overweight women, there was no difference in energy intake between four whey protein fractions comprising WPC, colostrum WPC, GMP, and *b*-lactoglobulin, despite promising rodent studies, although *b*-lactoglobulin induced greater fullness (Poppitt et al., 2013). GMP has long been purported to differentially suppress appetite, but there is little evidence to support this hypothesis (Veldhorst et al., 2009; Keogh et al., 2010).

To date, there have been few long-term studies investigating the role of milk proteins in weight loss, in the absence of other lifestyle interventions. A 6-month study of overweight and obese individuals reported that 56 g/day WPC resulted in significant loss of body weight, fat mass, and waist circumference compared to a CHO control (Baer et al., 2011), while a shorter 12-week study found no effect of 54 g/day of WPI (Pal et al., 2010a). Interestingly, fasting blood lipids and insulin levels improved in this study, supporting the hypothesis that whey protein may improve metabolic health even in the absence of weight loss (Pal et al., 2010a). Energy-restricted diets are a common way to successfully decrease body weight, at least in the short term, but characteristic of this is loss of both fat mass and lean mass. Loss of lean mass results in concomitant reduction in basal metabolic requirements, and a return to habitual dietary habits may then result in rapid weight regain. Middle-aged or older individuals with the age-related sarcopenia and obesity known as 'sarcobesity' (Parr et al., 2013) who lose weight through traditional energy-restricted diets may be even more susceptible to rapid weight regain due to already low muscle mass. Ingestion of whey protein as part of an energy-restricted diet has been proposed as a strategy to decrease fat mass while preserving lean mass. There is some data to support this proposal. In a study of obese individuals undertaking a severely energy-restricted diet of ~2 MJ/day for 12 weeks, supplementation twice daily with a milk protein successfully led to greater weight loss, fat loss, and maintenance of lean muscle mass (Frestedt et al., 2008). Another similar study supplementing with a high-protein meal replacement comprising whey, soy, and free AAs led to greater loss of body fat compared to a low-protein meal replacement (Treyzon et al., 2008). Milk proteins may also

be beneficial for maintaining a lower body weight after energy restriction and fat loss. In a study of whey protein and casein supplementation, there was significantly better weight loss maintenance over a 12-week period compared with a CHO control (Claessens et al., 2009a).

Whether whey protein or casein supplements may be more effective for preservation of lean muscle mass during periods of weight loss induced by an energy-restricted diet was addressed in a recent trial (Adechian et al., 2012) where obese individuals underwent a 6-week energy-restricted diet with whey protein or casein supplementation. While all individuals showed weight loss after 6 weeks, there were no differential effects of whey protein or casein for weight loss, fat loss, or preservation of lean muscle mass. Intriguingly, assessment of whole body protein synthesis and whole body protein breakdown showed casein to cause greater inhibition of protein breakdown, while whey protein increased protein synthesis (Adechian et al., 2012). The implications are that casein supplementation may be optimal for preservation of skeletal muscle mass during energy restriction. Notably, however, no long-term trials have as yet compared milk proteins with other protein sources for long-term weight loss and maintenance.

Milk Proteins, Muscle Wasting, and Sarcopenia

Advancing age and a sedentary lifestyle is associated with a gradual decline in skeletal muscle mass, function, and strength, which in the extreme form is termed sarcopenia or muscle wasting. Loss of skeletal muscle mass or function of the muscle has major implications for quality of life since activities of daily living such as walking upstairs become difficult or are no longer possible. At the extreme end, patients with chronic or end-stage diseases, including cancer, heart failure, AIDS, and chronic obstructive lung disease and sepsis, are also often susceptible to muscle wasting (Tan and Fearon, 2008; Fearon et al., 2011).

In addition to these mobility issues, skeletal muscle also has a significant impact on metabolic health. As one of the major organs responsible for insulin-stimulated glucose uptake, loss of skeletal muscle mass is often associated with insulin resistance (Evans, 2010). In addition to the insulin stimulatory effect of milk protein, it is the high level of BCAAs in whey protein and casein that prevents loss of lean body mass through increased skeletal muscle protein synthesis and/or decrease in breakdown (Adechian et al., 2012). There is evidence that the anabolic effect of milk protein is decreased during aging, which has been termed anabolic resistance (Volpi et al., 2000). Whether this is an anabolic resistance to dietary intake of BCAAs or simply a reflection of underutilization of the major muscle groups in older people as exercise levels decline is a matter of considerable debate. Whether milk proteins can prevent the development of anabolic resistance or overcome established anabolic resistance in older individuals is of great interest.

Both circulating insulin and AAs are important for the activation of muscle protein synthesis. Whey protein and casein are high-quality proteins based on both the protein digestibility corrected AA score (PDCAAS) (Boye et al., 2012) and the recently developed digestible indispensable AA score (DIAAS) (FAO, 2013), both of which take into account human AA requirements and protein digestibility. However, whey protein contains a greater amount of the BCAAs leucine, isoleucine, and valine than does casein. Of the BCAAs, leucine is thought to be the most potent activator of protein synthesis (Katsanos et al., 2006; van Loon, 2012), although a recent study has shown that high levels of nonleucine BCAAs can induce equivalent protein

synthesis when given with a whey protein supplement (Churchward-Venne et al., 2012). Casein in turn contains several essential amino acids (EAAs), including histine, methionine, and phenylalanine in a greater amount than whey protein, and also contains a greater amount of the non-EAAs arginine, glutamic acid, proline, serine, and tyrosine (Hall et al., 2003). The EAAs have been demonstrated to be primarily responsible for the activation of muscle protein synthesis (Volpi et al., 2003), although they are not necessarily efficacious for inhibition of protein breakdown. As noted above, studies have demonstrated greater whole body protein synthesis following ingestion of whey protein versus casein, while protein catabolism was greater following casein supplementation (Boirie et al., 1997; Adechian et al., 2012). The faster digestion rate of whey protein compared with casein, which due to its micellar structure tends to clot in the stomach, has been commonly believed to lead to more rapid delivery of AAs into plasma following whey protein, and longer, more sustained delivery following casein ingestion. There is certainly a body of data showing greater muscle protein accretion over the 6 hours following whey protein supplementation compared to casein or casein hydrolysate (Pennings et al., 2011).

Conversely, a recent study of intrinsically labeled whey protein and casein, co-ingested as milk, showed the absorption and retention of AAs from whey protein and casein to be similar after 2 hours, with AAs derived from casein showing greater absorption and retention rates beyond 3 hours (Soop et al., 2012). Some discrepancies in outcome might be due to the differential effects of aging on the response to these two dairy proteins. It may be worthwhile to increase the typically low whey protein content of milk, which then theoretically would provide both an early (whey) and sustained (casein) stimulation of muscle protein synthesis and an inhibition of muscle protein breakdown (Reitelseder et al., 2011). However, it remains to be seen whether milk protein-enhanced muscle protein synthesis is effective for prevention of muscle wasting and promotion of muscle protein accretion in healthy aging or elderly populations, or in patients with chronic end-stage disease. Additional evidence is also required to confirm that this in turn results in functional improvements in strength and/or mobility.

Regular resistance-type exercise transiently activates muscle protein synthesis and over time can lead to increases in skeletal muscle mass. Milk protein supplements are popular among recreational gym users seeking to increase muscle mass, but there is also research interest in whether milk protein ingestion in conjunction with resistance exercise may be beneficial for individuals with sarcopenia. Whey protein and casein ingestion after resistance exercise have been shown to both cause comparable increases in net protein balance (Tipton et al., 2004) and myofibrillar protein synthesis rate (Reitelseder et al., 2011), while other data show that whey protein ingestion causes greater increase in the muscle protein synthesis rate during the early 3-h period (Tang et al., 2009). Longer-term studies to date have produced mixed findings. In obese postmenopausal women, WPI in combination with an energy-restricted diet plus exercise over 6 months led to 4% greater weight loss than in the control group, and notably greater loss of subcutaneous adipose tissue and greater increase in leg muscle mass (Mojtahedi et al., 2011). Conversely, in elderly men who undertook resistance training for 12 weeks, protein supplementation did not improve muscle hypertrophy (Verdijk et al., 2009). Although the effects on muscle anabolism and catabolism are clear, the evidence underpinning clinically significant gains in lean body remain equivocal (Cermak et al., 2013).

Milk Proteins and Heart Health

Atherosclerosis

Atherosclerosis is a common cause of myocardial infarction, stroke, and peripheral vascular disease. It represents the progressive damage to the vascular endothelium due to build-up of lipids, immune cell infiltration, and plaque formation, leading to impaired endothelial function and reduced blood flow. It has been suggested that milk protein may improve adverse circulating lipid levels, one of the primary metabolic risk factors for CVD. Most of these studies have focused on the effects on postprandial lipemia, based on the premise that rapid clearance of blood lipids after a meal decreases arterial exposure to these circulating lipids. One study in overweight postmenopausal women given a high-fat meal found WPI and sodium caseinate to both decrease circulating TG and TG:ApoB48 ratio compared with glucose (Pal et al., 2010a, 2010b).

Another study of obesity compared the effect of different milk protein fractions, including WPI, whey protein hydrolysate, α-lactalbumin, and GMP, on postprandial lipemia after a high-fat meal, but found no significant differences (Holmer-Jensen et al., 2012). Whey protein has also been shown to suppress postprandial circulating TG, free fatty acids (FFA), and chylomicron-rich lipoprotein appearance in diabetic patients following a high-fat meal, compared to controls of casein, fish, or plant protein sources (Mortensen et al., 2009). Casein alone, on the other hand, failed to improve postprandial TG in T2DM patients (Brader et al., 2010). There have been few long-term clinical studies. A 3-month trial of a fermented whey product in individuals with metabolic syndrome found some improvements in metabolic markers, although confounding effects on body weight make this trial difficult to interpret (Gouni-Berthold et al., 2012).

Blood Pressure and Vascular Reactivity

Observational and clinical studies have shown that the consumption of dairy products is associated with decreased risk of hypertension (Soedamah-Muthu et al., 2012). Much work has focused on identifying and isolating the bioactive peptides that may be responsible. The discovery that milk-derived peptides inhibit angiotensin converting enzyme (ACE) activity, and hence alter vasoconstriction, vasodilation, and blood pressure (BP) *in vitro*, led to a plethora of animal and subsequently human trials. Despite promising evidence that lactokinins or caseinkinins can reduce BP in spontaneously hypertensive animals, these findings are yet to be confirmed.

The most well-studied milk protein-derived peptides are isoleucine-proline-proline (IPP) and valine-proline-proline (VPP), which are generated by the fermentation of milk (Saito, 2008; Boelsma and Kloek, 2009). IPP and VPP have been shown to be weak ACE in- hibitors based on *in vitro* experiments (FitzGerald and Meisel, 2000). Several meta-analyses of clinical trials of IPP and VPP on BP have been published which suggest these milk-derived peptides may have antihypertensive effects in humans (Xu et al., 2008; Turpeinen et al., 2013; Cicero et al., 2013). For example, a recent meta-analysis of 19 clinical trials of a daily dose of <10 mg milk tripeptides on BP over the last 15 years reported that overall systolic BP was decreased –4.0 mmHg, and diastolic BP was decreased –1.9 mmHg (Turpeinen et al., 2013). Previous meta-analysis reports also reported that VPP and IPP supplementation resulted in

significant decreases in systolic and diastolic BP, although suppression was greater in hypertensive individuals (Xu et al., 2008).

Not all studies have been positive, however. A European trial of VPP and IPP supplementation in a workplace environment found no effect on BP (Engberink et al., 2008). In the few human trials that assessed changes in ACE activity, neither VPP nor IPP appears to result in detectable inhibition of *in vivo* ACE activity. Hence, the mechanism behind the BP lowering effects observed in many studies is not yet clear. A recent report from the European Food Safety Authority (EFSA), which assesses the scientific basis for health claims of nutraceutical food products, concluded there was currently insufficient evidence to substantiate the claim that consumption of the milk-derived peptides IPP or VPP help maintain normal BP (European Food Safety Authority, 2012). Efforts are ongoing to identify further milk-derived peptides with potential to improve cardiovascular health.

There is evidence that supplementation with intact milk proteins may also potentially exert beneficial effects on endothelial function and BP control. An acute trial showed that ingestion of 5 g whey protein extract increased brachial artery flow mediated dilation in older, overweight individuals (Ballard et al., 2012). This was not associated with an inhibition of ACE activity. Hence regulation of vascular reactivity/endothelial function takes place by some other as yet unknown mechanism. In overweight individuals, whey protein hydrolysate or WPC (28 g/day) decreased systolic and diastolic BP compared to baseline, but only in subjects with hypertension at the start of the trial, and was again not associated with any detectable changes in ACE activity. A more recent study found 12-week supplementation with WPI or sodium caseinate (54 g/day) decreased BP compared to glucose supplementation (Pal and Ellis, 2010b). In summary, human intervention studies suggest that milk peptides or milk protein supplementation may improve hypertension, but this is primarily in individuals with increased risk. Moreover, the mechanism underlying these improvements is not well understood.

MILK PROTEINS AND BONE HEALTH

Bone remodeling occurs across the life span in response to environmental changes such as diet, physical activity, and external loading. Nutrition during childhood and adolescence is one of the major factors that influences the development of bone mass and strength, alongside physical activity, endocrine status, and exposure to risk factors (Caroli et al., 2011). Calcium, protein, and vitamin D are essential nutrients for bone development and bone health maintenance (Pampaloni et al., 2011), and dairy foods such as milk and cheese are a rich source of both Ca and protein, arguably providing an optimal source of essential nutrients for bone health (Caroli et al., 2011). Aging is associated with decreased bone mineral density (BMD), which leads to increased susceptibility to fracture in the elderly.

Although Ca derived from cow's milk has long been shown to ameliorate bone loss (Tang et al., 2007) and provides a more conservative and arguably safer route by which to increase dietary Ca than through supplementation with Ca salts per se (Reid et al., 2006; Bolland et al., 2008; 2010), recent studies show that cow's milk protein may influence bone remodeling (Tsuji-Naito and Jack, 2012). In these studies, whey proteins have been shown to promote growth of bone cells through a number of mechanisms, including stimulation of

osteoblast differentiation, activation of intracellular signaling molecules, increased alkaline phosphatase activity, and mineralization (Tsuji-Naito and Jack, 2012). It has long been known that milk protein supplementation increases serum insulin-like growth factor 1 (IGF-1). In turn it has been hypothesized that this may be a mechanism for protection of BMD. Supplementation with MPC over a period of 6 months successfully increased serum IGF-1 in elderly individuals with recent osteoporotic hip fracture, decreased mean rehabilitation ward stay to 33 days compared with 54 days in control patients, and 6 months after the intervention was completed continued to show attenuation of proximal femur BMD loss (Schürch et al., 1998). Conversely, in a longer 2-year study of elderly women, while a high-protein whey drink increased IGF-1 levels when measured after 1 and 2 years of supplementation, there were no effects on BMD outcomes (Zhu et al., 2011). Whether whey protein may enhance the effects of multinutrient supplementation for improving recovery from fractures in the elderly is of interest, but is yet to be established.

The effects of overweight, obesity, and weight loss have long been of concern to the field of bone remodeling and fracture risk. Energy-restricted diets for weight loss have long been reported to increase the risk of bone loss, possibly due to inadequate energy and nutrients for maintenance of BMD (Villareal et al., 2006). Interestingly, there is some evidence that bone health may be preserved when the diet is rich in dairy products (Josse et al., 2012). Which component of dairy may be responsible for this protective effect, however, is not clear.

Milk Proteins and Infant Health

Appropriate early nutrition is essential for the growth and development of infants. The World Health Organization and other health bodies recommend breastfeeding infants up to 6 months of age and continuing breastfeeding with complementary foods up to 2 years of age. However, commercial infant formulas (IF) have been widely developed to provide appropriate nutrition should breastfeeding not be possible. Cow's milk proteins are often used as the main protein source in IF, the composition of which is governed by strict regulatory guidelines. Milk protein, in particular whey-modified protein, is used as a primary protein source. Cow's milk requires modification in order for it to be the basis of IF since it contains two to three times the level of total protein compared with human breast milk, and has a different protein composition. Whether high-protein IFs may exceed infant requirements is widely debated, with discussion focused on later development of obesity and associated noncommunicable diseases (NCDs) (Michaelsen et al., 2012), although systematic reviews examining associations between early feeding and later-life obesity or BMI are not conclusive (Owen et al., 2005a,b).

Certainly there may be differences in infant body composition driven by feeding practices (Gale et al., 2012). In light of these issues, IFs with low protein levels close to that of human milk have been developed, and these lower protein content IFs remain an area of great interest and a current 'hot topic.' Differences in protein composition between human and cow's milk include the whey protein:casein ratio and the level of free AAs, which are high in human milk. Mature human milk contains ~60% whey protein, of which *a*-lactalbumin is the major component, and ~40% casein. It also has high levels of EAAs. Conversely, the ratio of whey protein:casein in cow's milk is 20:80, and it contains a major whey protein *b*-lactoglobulin that is absent in human breast milk. The primary objective in protein-modified IFs

has been to achieve a low-protein composition with sufficient indispensable and conditionally indispensable AAs but with decreased nonessential AAs. Adequate protein nutrition in infants given low-protein IF (as demonstrated by comparable growth curves to infants fed standard IF) has been demonstrated in formulas enriched with components such as *a*-lactalbumin (Lien et al., 2004).

In low-birth-weight (LBW) infants, dairy protein provides a useful way to increase intake in order to improve growth and development. A systematic review of IF feeding showed that 3–4 g/kg body weight per day of protein may successfully accelerate weight gain (Premji et al., 2006). Again there has been debate as to whether this accelerated growth may increase susceptibility to weight and adipose gain in later life, with data showing that it may be associated with school age obesity (Koletzko et al., 2009). Studies are ongoing to establish whether the benefits of increased protein intake in infancy may be offset in later adolescence and adulthood, or whether IF-fed infants have better long-term health in adult life. Outcomes remain equivocal, with prospective studies showing both IF and breast milk to be more beneficial in areas such as bone health in later adult life (Fewtrell et al., 2009; Mølgaard et al., 2011; Pirilä et al., 2011).

An area of protein composition that has been reviewed in detail is immune function. Breast milk contains a range of fractions, including immunoglobulins, lactoferrins, antibodies, macrophages, neutrophils, lymphocytes, and cytokines among others, which may aid immune development (Field, 2005). Because of the importance of this fact, IF commonly also contains factors that may help support immunity. In addition, the use of hydrolyzed proteins for suppression of allergy is of great interest. Hydrolyzed whey protein in IF has been shown to decrease the risk of atopic dermatitis, an inflammatory, chronic form of childhood eczema, after birth and up to 3 years of age. This beneficial effect has been attributed to the presence of immune components such as *b*-lactoglobulin, GMP, and lactoferrin (Osborn and Sinn, 2006). Also of possible relevance with respect to allergy is recent evidence from a probiotic supplementation study. Having initially supplemented mothers during pregnancy with two different probiotic strains, these were then given to their infants using a range of delivery formats, including cow's milk IF. This study showed *Lactobacillus rhamnosus* to decrease the risk of childhood eczema (Wickens et al., 2012). Whether the mother or the infant is the critical window for supplementation, and whether there may be synergies between specific strains of probiotics and the protein component of dairy, is not known.

CONCLUSIONS

There is an evolving body of evidence showing that milk proteins or milk-derived peptides may have significant functional properties for the prevention and/or treatment of important chronic health conditions, although much of the science is as yet at an early stage. Clearly, there remains considerable scope for optimizing the processing of whey protein or casein in order to maximize positive health outcomes. The role that dairy protein may play in the prevention of adverse metabolic health and T2DM, atherosclerosis, hypertension and CVD risk, muscle wasting and sarcopenia, as well as osteoporosis, is gradually being elucidated, but research to identify specific protein components, optimum protein dose, synergies with other nutrients, and also the underpinning mechanisms of these positive effects on health

are needed. Bovine milk proteins are also an important component of infant formulas and provide an essential alternative when breastfeeding cannot be achieved or maintained. However, further clinical intervention studies, particularly long-term well-controlled studies, are required to build the evidence base necessary to fully support the development of functional foods based on bovine milk proteins for the maintenance and improvement of human health.

DISCLOSURES

SDP holds the Fonterra Chair in Human Nutrition at the University of Auckland. RAM receives financial support from the New Zealand Primary Growth Partnership (PGP) program, funded by Fonterra Co-operative Group and the New Zealand Ministry for Primary Industries (MPI).

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