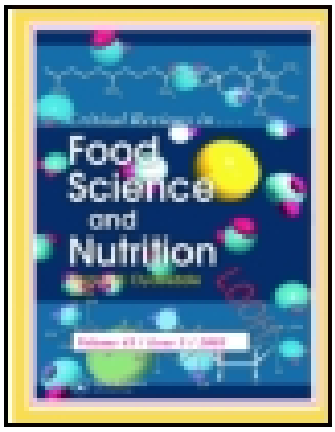

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A Role for Whey-derived Lactoferrin and Immunoglobulins in the Attenuation of Obesity-related Inflammation and Disease?

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A role for whey-derived lactoferrin and immunoglobulins in the attenuation of obesity-related inflammation and disease?

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ABSTRACT

Obesity is a strong predictive factor in the development of chronic disease and has now superseded under-nutrition as a major public health issue. Chronic inflammation is one mechanism thought to link excess body weight with disease. Increasingly, the gut and its extensive population of commensal microflora are recognized as playing an important role in the development of obesity-related chronic inflammation. Obesity and a high fat diet are associated with altered commensal microbial communities and increased intestinal permeability which

contributes to systemic inflammation as a result of the translocation of lipopolysaccharide into the circulation and metabolic endotoxemia. Various milk proteins are showing promise in the prevention and treatment of obesity and chronic low grade inflammation via reductions in visceral fat, neutralization of bacteria at the mucosa and reduced intestinal permeability. In this review, we focus on evidence supporting the potential anti-obesogenic and anti-inflammatory effects of bovine whey-derived lactoferrin and immunoglobulins.

Keywords

obesity, inflammation, microbiota, lactoferrin, immunoglobulins

1. INTRODUCTION

Overweight and obesity are defined by the World Health Organization (WHO) as abnormal or excessive fat accumulation that may impair health (WHO 2013). Excess body weight is now the leading risk factor contributing to the overall burden of disease worldwide (Murray et al., 2012). The incidence of obesity-related conditions has been declared an epidemic in both developed and developing countries (WHO 2003) and has now superseded under-nutrition as a major public health issue (Pal et al., 2013). Health problems associated with overweight and obesity, including insulin resistance, dyslipidemia and glucose intolerance, metabolic syndrome, type 2 diabetes mellitus and cardiovascular disease (Despres et al., 2006; Pal et al., 2013) impose significant economic and personal burden and are projected to increase as the rate of excess body weight continues to rise worldwide.

Evidence implicates chronic low grade inflammation as one mechanism linking excess body weight to metabolic dysregulation (Esposito et al., 2004; Pal et al., 2013). The interplay between adipose tissue, the immune system and metabolism occurs across multiple organ systems with widespread implications for physiology at the whole-body level. Excess adipose tissue directly activates effector mechanisms of the immune system, in particular macrophages, inflammatory cytokine production, hormone balance, patterns of gene expression and intracellular signaling (Fantuzzi 2005). For example, several studies highlight the role of tumor necrosis factor alpha (TNF-Ŭ) in the development of insulin resistance by influencing the phosphorylation state of the insulin receptor, with the nuclear factor(NF)-əB and c-Jun N-terminal kinase pathways linking inflammation and insulin resistance (Hotamisligil et al., 1994; Ozcan et al., 2004; Arkan et al.,

2005). The increasing recognition of the contribution of inflammation to disease risk in obesity has spawned research on immunologically-mediated processes as a strategy to overcome obesity-related disease.

Increasingly, the gut and its extensive population of commensal microflora are recognized as playing an important role in the development of obesity-related chronic inflammation. Altered composition of the commensal microbiota and changes in intestinal permeability, resulting in the translocation of lipopolysaccharide (LPS) into the systemic circulation (Frazier et al., 2011), are observed in obese cohorts (Ley et al., 2006; Turnbaugh et al., 2009; Schwiertz et al., 2010). These changes to the composition of the microbiota and to intestinal permeability may be mediated through high fat/high energy diets, setting up a positive feedback cycle that disrupts mucosal homeostasis (Jumpertz et al., 2011; Wu et al., 2011) and propagates a chronic low grade inflammatory response.

Modulation of the intestinal microbiota and reduction of intestinal permeability thus have the potential to counteract obesity-related inflammation. Various supplementation strategies have been investigated in this context, including functional foods. Bovine whey-derived lactoferrin and immunoglobulins are isolated milk proteins shown to have immunomodulating properties (Severin et al., 2005). Of further interest, findings from a recent study suggest that lactoferrin supplementation may have direct anti-obesogenic effects (Ono et al., 2010). This review considers the evidence to date from both animal and human studies for the role of intestinally-derived inflammation in obesity and the potential for bovine whey-derived lactoferrin and immunoglobulins to ameliorate inflammation and obesity-related pathologies.

2. MICROBIOME

2.1 The intestinal microbiome and obesity

Humans have co-evolved with a diverse and complex group of bacteria, viruses and fungi at mucosal surfaces known collectively as the commensal microbiota. The number of bacterial species at the mucosa is now estimated to exceed 10^{14} and outnumber human cells by a factor of 10 (Qin et al., 2010). The vast number and diversity of the bacterial species contained in the gut have led to it being termed the metagenome with the main phylogenic categories in the gut, bacteroidetes and firmicutes, constituting 90% of the bacterial species (Qin et al., 2010). Both human and animal studies indicate that the metagenome is essential to the ontogeny of the immune system, to homeostasis within the mucosal milieu and subsequently to health and disease. The evolution of the microbiome and its host have led to a mutual interdependence in which bacterial metabolites provide vitamins, enzymes and short chain fatty acids that the host relies upon for normal physiological functions, while the host, in turn, provides a stable ecosystem for survival (Carroll et al., 2009).

The relationship between the intestinal microbiota and host spans a continuum of symbiosis to dysbiosis that is associated with differential profiles in the composition of bacterial species (Madupu et al., 2013). Differences in the composition of fecal microbiota between obese and lean individuals have been observed, although it should be noted that overall, the evidence is equivocal (Ley et al., 2005; Turnbaugh et al., 2009; Jumpertz et al., 2011). Some studies have shown obese individuals to have a lower relative abundance of bacteroidetes and a higher abundance of firmicutes compared to lean individuals (Ley et al., 2005; Turnbaugh et al., 2009). However, a study in a sample of 20 subjects found no differences in the composition of intestinal microbiota between obese and lean individuals (Jumpertz et al., 2011). Animal knock-out studies

have shown that the gut microbiota may promote obesity. Germ free mice have been shown to have lower body fat than their wild type counterparts, which is reversed following transplantation of wild type microbiota (Turnbaugh et al., 2008). Furthermore, transplantation of the microbiota from genetically obese, leptin receptorï deficient *ob/ob* mice induced a greater increase in fat mass in germ free animals than did microbiota from lean *ob/+* and +/+ mice (Turnbaugh et al., 2006). Whether microbiota associations with body mass are species-specific or relate only to a particular combination of microbial species is yet to be determined.

The observed changes in the intestinal microbiota associated with obesity are most likely driven by dietary factors, with evidence suggesting that nutrient load and fat intake alter the microbial community structure (Frazier et al., 2011). In human studies, caloric load has been associated with variations in microbial composition. A crossover study comparing consumption of either 2400-kcal/day or 3400-kcal/day at similar macronutrient profiles (24% protein, 16% fat, and 60% carbohydrates) for three days found an increase in the number of firmicutes and a decrease in the number of bacteroidetes at the higher caloric intake (Jumpertz et al., 2011). This mirrors findings in animal studies. In murine models, high fat diets have been associated with lower bacteroidetes to firmicutes ratio (Hildebrandt et al., 2009; Ding et al., 2010), while in humans both high fat diets and high protein diets have been shown to reduce the relative abundance of bacteroidetes (Russell et al., 2011). While dietary intake is recognized to alter the composition of the microbiota, the role of specific macronutrients in shaping the relative abundance of specific bacterial groups remains poorly understood.

2.2 Microbiome-Immune Interactions

Changes in the abundance of microbial species in obesity may promote inflammation that contributes to disease. Cross-talk between the microbiota, host epithelial and immune cells alters signaling pathways and regulatory mechanisms within the mucosa. Research into probiotic supplements has shown the ability of microbes to alter inflammation in the intestinal mucosa. A comparison of four strains of lactobacilli found varying capacities of each to elicit secretion of the cytokines Interleukin(IL)-10, IL-12p70, interferon-כ (IFN-כ) and TNF-Ŭ from human epithelial cell cultures (Hsieh et al., 2013). Other probiotic research indicates that these microbes promote homeostasis by activating regulatory cells. Analysis of the T-cell receptor repertoires of regulatory T-cells and their reactivity to commensal microbes revealed the regulatory T-cell repertoire to be heavily influenced by the composition of the intestinal microbiota (Cebula et al., 2013). These research findings suggest that changes in the microbial composition may lead to a reduction in the frequency of intestinal regulatory cells and a breakdown in mucosal immune homeostasis. Metabolic byproducts of bacterial fermentation, in particular short chain fatty acids, also exert inflammatory influence in the intestine. Smith et al, using a mouse model, noted that colonic homeostasis and tolerance to the microbiota was regulated by bacterial metabolites that altered the size and function of the regulatory T-cell pool in the colon (Smith et al., 2013). These findings provide evidence that the microbiota play a role in determining the inflammatory tone of the mucosal milieu.

A breakdown in the balance of interactions between the intestinal microbiota and the immune system may lead to uncontrolled inflammation both locally and systemically. Increased permeability of the intestine allows the translocation of bacterial products into the circulation (Geurts et al., 2014). In a seminal murine study, Everard *et al*. reported that administration of

Akkermansia muciniphila, a species that typically resides in the mucin layer, resulted in a two-fold increase in the concentrations of endocannabinoids, which are mediators known to regulate intestinal barrier function (Everard et al., 2013). Similarly, the use of antibiotic regimens that alter intestinal bacteria have also been associated with reduced intestinal permeability in mice (Cani et al., 2008), further supporting the relationship between the intestinal microbial composition and permeability.

Microbiota-induced increases in intestinal permeability may be further exacerbated by a high fat/high sugar diet typical in obesity. A three month trial of a probiotic supplement reported greater intestinal permeability (assessed using a dual sugar absorption test), but no difference in blood-derived LPS in 28 overweight/obese adults diagnosed with metabolic syndrome compared to 10 healthy controls (Leber et al., 2012). This is in contrast to a study that examined serum lipopolysaccharide binding protein (LBP), as a surrogate for LPS, in 420 individuals; metabolic syndrome was associated with higher LBP concentrations (MetS: 8.02µg/mL v Control: 6.82 µg/mL) (Gonzalez-Quintela et al., 2013). Animal studies have also demonstrated that high fat/sugar diets increase intestinal permeability, increase circulating LPS concentrations, and induce local and systemic inflammation (de La Serre et al., 2010; Lam et al., 2012; Martinez-Medina et al., 2014).

Interestingly, LPS has also been implicated in inducing changes in cholesterol and triglyceride metabolism via induction of inflammatory mediators. An early murine model, demonstrated that administration of pro-inflammatory cytokines results in an increase hepatic lipid and cholesterol synthesis (Feingold et al., 1989). A subsequent study using C57Bl/6 LPS-sensitive mice, found LPS administration induced increases in the activity of hydroxy-3-methylglutaryl-coenzyme A

(HMG-CoA) reductase (the rate limiting enzyme for cholesterol synthesis) and both serum cholesterol and triglycerides. These effects were attenuated when endotoxin was administered following pre-treatment with anti-TNF-Ŭantibodies implicating TNF-Ŭas a potential mediator of alterations in lipid metabolism (Memon et al., 1993). Collectively these data support an interplay between microbial species, inflammation and metabolism and represent an important paradigm when considering the aetiology of obesity-related disease.

3. FUNCTIONAL FOODS

Functional foods demonstrate physiological benefits beyond that of simple nutritional value and are showing promise in the prevention and attenuation of chronic disease (Cencic et al., 2010). Over the last 20 years, immunonutrition has emerged as an important area of research in the context of chronic diseases (Szarc vel Szic et al., 2010; Satyaraj 2011). With 65% of the bodyôs immune cells located within the gastrointestinal tract, diet-derived compounds may actively influence immune function (Satyaraj 2011). Nutrition has been suggested as a primary environmental factor involved in the epigenetic mechanisms underpinning inflammation and complex diseases, such as obesity and type 2 diabetes mellitus (Szarc vel Szic et al., 2010). Functional foods may enhance immunocompetence and physiological functioning, although evidence thus far is largely conflicting (Lopez-Varela et al., 2002). Despite the lack of a firm regulatory definition, the food industry has begun to market certain foods with a ófunctional foodô label and interest in nutraceuticals has grown strongly (Alissa et al., 2012). Nutraceuticals, a term coined in 1989, describes a set of functional foods that either prevent or treat a disease and encompasses various bioactive components found in food (Cencic et al., 2010; Alissa et al., 2012).

3.1 Effect of Dairy on Obesity

Evidence from research involving dairy foods supports their consideration as a functional food in the context of obesity and metabolic disease. In the Coronary Artery Risk Development in Young Adults study of over 5000 individuals, there was a 21% reduction in the risk for insulin resistance syndrome for each daily occasion of dairy consumption, over a 10 year follow-up (Pereira et al., 2002). Inverse relationships between dairy food consumption and indices of body mass, adiposity and metabolism continue to be reported in subsequent studies including adult (Holmberg et al., 2013), predominately obese (Murphy et al., 2013) and adolescent cohorts (Abreu et al., 2012). However, meta-analyses do not always support significant weight reductions, suggesting that the effects may be short-term (<1 year) only or only observed with concomitant reduction in energy intake (Abargouei et al., 2012). As such, wide-spread prescription of increased dairy consumption as a tool to promote weight loss is generally not supported, although the roles of individual milk proteins are less clear.

Whey-protein, as one of the key constituents of milk-based products, has been assessed for its contribution to the potential beneficial effects of dairy consumption. Whey-protein isolate is produced by the filtering of whey to yield a highly concentrated protein supplement (>90% protein). Evidence from animal studies confirms the capability of whey-protein to modify a number of biological pathways associated with obesity-related pathologies. A study conducted by Pilvi *et al*. reported that C57Bl/6J mice fed a diet high in calcium and whey-protein demonstrated altered adipose tissue gene expression relating primarily to insulin signaling, adipocytokine signaling and the fatty acid metabolism pathways (Pilvi et al., 2008). Another study using C57Bl/6J mice fed whey-protein isolate, rich in lactoperoxidase, lactoferrin, growth

factors and immunoglobulins, reported that whey-protein isolate enhanced weight and body fat loss during energy restriction, limited hepatic fat accumulation and was associated with decreased phosphorylation of S6 Kinase 1 (Shi J 2011), a protein implicated in lipogensis/adipogenesis and insulin signaling pathways (Um et al., 2004). While detailed study of biological pathways can be complicated in human clinical trials, evidence suggests the potential for beneficial health effects in response to whey-protein supplementation. In a cohort of 70 overweight or obese subjects, 12 weeks of whey-protein supplementation was associated with significant reductions in blood pressure (3-4%) (Pal et al., 2010a), cholesterol (6-7%) and measures of insulin resistance (~10%) (Pal et al., 2010b). However, such findings have not been widely replicated, limiting full delineation of the biological pathways via which whey-protein may mediate such effects.

The potentially beneficial effects of whey-protein in ameliorating risk for obesity-associated disease may be attributable to the thermogenic costs of digestion, increased satiety or alterations in metabolic signaling. Whey-protein has been found to have a greater thermic effect than casein or soy (whey 14.4 ± 0.5%; casein 12.0 ± 0.6%; soy 11.6 ± 0.5%) in a study involving 23 lean healthy subjects; a trend toward significantly increased fat oxidation was also noted (Acheson et al., 2011). Other studies using pre-load with whey-protein supplements report increased short-term post-prandial satiety (Veldhorst et al., 2009; Solah et al., 2010; Poppitt et al., 2011), although these findings are not always replicated (Abou-Samra et al., 2011; Lorenzen et al., 2012). Finally, stimulation of the incretin signaling pathways has been observed in response to whey-protein intake. Signaling responses to whey supplementation include reports of 60-65% post-prandial increases in the incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent

insulintropic polypeptide (GIP) following a whey preload (Hall et al., 2003) and greater increases in GLP-1, insulin and ghrelin following a meal based on 25% caloric content from whey compared to soy and casein proteins (Veldhorst et al., 2009). Enhancement of incretin hormones may increase both insulin secretion and insulin stores in ϐ-cells, and promote differentiation of precursor cells into ϐ-cells resulting in increased ϐ-cell mass (Jakubowicz et al., 2013) - all important in the context of glucose homeostasis. Collectively these studies provide evidence supporting further consideration of whey-protein as a functional food for management of obesity and associated disease.

4. WHEY-DERIVED PROTEINS

Whey contains a number of biologically active constituents that may explain the responses associated with dairy consumption. Several proteins, in particular the antimicrobial peptide lactoferrin and immunoglobulins, have immunomodulating actions and are involved in maintaining immune homeostasis and/or enhancing immune cell function, proliferation and antibody synthesis (Meisel et al., 2003). Furthermore, whey-derived proteins are showing promise to ameliorate the chronic low-grade inflammation and metabolic disturbance associated with excess body mass (see reviews by Graf et al., 2011 and Severin et al., 2005). Whey-derived lactoferrin and immunoglobulins are two particular proteins of interest.

4.1 Lactoferrin

Lactoferrin is a component of the innate immune system expressed in bodily secretions, such as milk and the mucous secretions of the digestive tract, and is also contained within the secondary granules of neutrophils (Ward et al., 2005; Vogel 2012). Consistent with the innate immune system being evolutionarily conserved, lactoferrin is found in various mammalian species,

including cows, sheep, horses, dogs and other species, (Nara et al., 2011; Quintana et al., 2011; El-Fakharany et al., 2013). Structural similarities between species have been reported and include the two symmetrical lobes (N and C lobes) and the highly basic nature of the protein (Baker et al., 2009). Biological similarities also exist; lactoferrin plays similar physiological roles across species, in particular as a mediator of innate defences. As part of the transferrin family, lactoferrin possesses a range of microbicidal properties (reviewed by (Ward et al., 2005)) and through its function as an iron binding protein, can retard the growth of iron dependent bacteria and the formation of bacterial biofilms (Singh et al., 2002). Lactoferrin has also been recognized as possessing anti-viral capacity by binding to glycosaminoglycans present in the cell membrane of some viruses and inhibiting their entry into cells, as well as inhibiting specific DNA and RNA viruses (Andersen et al., 2001; Ward et al., 2005; Adlerova et al., 2008). Interestingly, pepsin digestion of lactoferrin in the gut cleaves the C-terminal lobe of lactoferrin to produce lactoferricin (Legrand et al., 2005; Adlerova et al., 2008; Marnila P 2009; Vogel 2012), which has been shown to possess greater potency than the parent molecule (Ward et al., 2005; Vogel 2012). Interestingly, the C-terminal lobe has been recently reported to be resistant to trypsin digestion and retains antimicrobial activity through the conservation of a defined iron-binding site (Rastogi et al., 2014a; Rastogi et al., 2014b), which may be important when considering use of oral lactoferrin supplements and transit through the gastrointestinal tract.

4.1.1 Immune-modulation

Lactoferrin has both regulatory and stimulatory effects on the immune system and can exert both pro-inflammatory and anti-inflammatory effects (Legrand et al., 2005; Tomita et al., 2009; Vogel 2012). As a constituent component of mucosal secretions, lactoferrin contributes to the

maintenance of immune homeostasis. Lactoferrin, due to its cationic nature, shows high affinity for anionic molecules and is capable of binding LPS, allowing for direct neutralization and limiting activation of macrophage CD14 and other pattern recognition receptors (Legrand et al., 2005) important for initiation of innate immune responses. The LPS-binding abilities of lactoferrin have been reported in various in vitro and animal models using both bovine (Dohler et al., 2002; Pecorini et al., 2010) and human lactoferrin (Zhang et al., 1999; Baveye et al., 2000; Li et al., 2012). In this context, the ability of lactoferrin to prevent LPS-induced inflammatory responses may also play a key role in preventing chronic inflammation mediated by bacterial translocation from the gut, as has been described in obesity (Frazier et al., 2011).

Lactoferrin may also stimulate the immune system by promoting the maturation of dendritic cells (DCs) and subsequent priming of naïve T cells, increasing migration of leukocytes, enhancing natural killer (NK) cell activity and inducing activation of macrophages resulting in increased TNFŭ, IL-8 and nitric oxide (Legrand et al., 2005; Tomita et al., 2009; Vogel 2012). A small number of lactoferrin binding sites on immune and epithelial cells have been identified, including intelectin and a low-density lipoprotein receptor-related protein. These receptors bind to lactoferrin to promote its cell entry (Legrand et al., 2005; Tomita et al., 2009) where it has been hypothesized that lactoferrin can affect gene transcription (Sang-Muk et al., 2001). Evidence suggests that nucleolin expressed by dividing cells may act as a carrier between the cell surface and the nucleus (Legrand et al., 2005). Other studies have identified a 105-kDa receptor present on activated immune cells, Jurkat lymphoblastic T cells, platelet and mammary gland cells that also binds lactoferrin (Mazurier et al., 1989; Leveugle et al., 1993; Damiens et al.,

1998). The binding of lactoferrin to receptors supports its potential to directly influence cellular responses, including the induction of inflammatory signaling.

Evidence from both animal models and human clinical studies supports the potential immunomodulatory effects of supplementation with bovine whey-derived lactoferrin (bLf). Potential mechanisms underpinning these effects are illustrated in Figure 1. In murine models, oral administration of bLf has been shown to: (i) augment circulating numbers of CD4+ T cells, CD8+ T cells and NK cells (Kuhara et al., 2000); (ii) increase numbers of CD4+ and CD8+ T cells in the intestinal mucosa (Wang et al., 2000); and (iii) induce increased production of regulatory cytokines IFN-ɔ and IL-10 from isolated intestinal intraepithelial lymphocytes (Takakura et al., 2006), all suggesting modulation of both local and systemic immunity. Similarly, in a clinical trial involving supplementation with 250mg/day bovine ribonuclease-enriched lactoferrin in conjunction with calcium (100% recommended daily allowance) in post-menopausal women over a six month period, decreased plasma concentrations of IL-6 (~44%) and TNF-Ŭ (~10%), and increased IL-10 secretion (140%) were noted along with moderate increases in IL-12, IL-1ɓ and IFN- ɔ (Bharadwaj et al., 2010). Collectively this evidence supports the need for the further consideration of any potential immunomodulatory effects of lactoferrin supplementation in the regulation of chronic inflammation in obesity-associated disease.

4.1.2 Health Benefits

In addition to potential immunomodulatory effects, bLf and recombinant human lactoferrin may offer additional health benefits. To date, antimicrobial properties, modulation of lipid metabolism, anti-cancer properties, and ability to increase hemoglobin and serum iron

concentrations have all been recognized (Brock 2002; Marnila P 2009). Animal studies have shown reduced hepatic cholesterol, plasma triglycerides and non-esterified fatty acids (Takeuchi et al., 2004); enhanced weight loss, suppressed weight regain, improved glucose tolerance and decreased adipose tissue inflammation (Shi J 2011); and improved glucose homeostasis (Li et al., 2014) in response to lactoferrin supplementation. Evidence from human clinical studies also supports an association between lactoferrin and indices of obesity-associated disease. Moreno-Navarrete et al previously reported significant inverse relationships between circulating lactoferrin concentrations and both body mass index and waist-hip ratio in their study assessing natural variation in circulating lactoferrin concentrations due to genetic polymorphism (Moreno-Navarrete et al., 2008). The same group has also observed a positive association between lactoferrin concentrations and measures of insulin sensitivity in an overweight cohort (Moreno-Navarrete et al., 2009), and an inverse relationship between baseline lactoferrin concentrations and inflammatory responses to acute fat feeding in obese individuals (Fernandez-Real et al., 2010).

Of further interest is the randomized double-blind placebo-controlled trial by Ono et al which examined the effect of daily supplementation with 300mg enteric coated bLf over an eight week intervention in 26 Japanese adults considered to be abdominally obese (visceral fat area >100cm^2). Supplementation with bLf resulted in a significant decrease in visceral fat accumulation by 14.6cm^2 (p=0.0089), as well as body mass index (-0.6kg/m2), hip circumference (-2.6cm) and body weight (-1.5kg) compared to placebo, in the absence of lifestyle change (Ono et al., 2010). This study suggests lactoferrin supplementation as a novel approach with the potential to modulate indices of obesity and associated disease.

4.2 Immunoglobulins

It has long been established that immunoglobulins (Igs) in colostrum and maternal milk are important for conveying passive immunity to infants and function to protect the host from viral and bacterial infections (Korhonen et al., 2010). Produced by B cell-derived plasma cells, Igs work by recognizing and binding pathogenic antigens, promoting clearance by phagocytosis and activating the complement system to induce cell destruction. Secretion of immunoglobulins onto mucosal surfaces is a key immune mechanism given the significant exposure of the mucosal surfaces to environmental antigens and the need to induce tolerance to food borne antigens and commensal micro-organisms. Traditionally mucosal antibody secretion has been thought to be restricted to secretory immunoglobulin A, with cross-talk between the intestinal luminal contents and intestinal epithelial cells contributing to the regulation of the polymeric immunoglobulin receptor and immunoglobulin secretion (Kaetzel 2005; Johansen et al., 2011). The presence of Igs at the mucosal surfaces is essential for effective immune exclusion (Woof et al., 2005) and as such may also play a role in regulating the activation state of the underlying mucosa-associated lymphoid tissue.

4.2.1 Immune-modulation

Supplementation with non-specific bovine immunoglobulins (bIgs) or specific bIgs fractions obtained from hyper-immunized cows have been shown to be efficacious in various models of infection in both animals and humans (see Reviews by (Korhonen et al., 2000; Mehraa R 2006)). Positive clinical outcomes are generally attributed to the known functions of immunoglobulins as described above. For example, bIgs may act to modify the intestinal microbiota and thus be useful in the treatment of individuals suffering gastrointestinal disturbances (Korhonen et al.,

2000). Despite these outcomes, possible immunomodulatory effects of bovine immunoglobulin supplementation have not been extensively documented and although a number of studies have utilized supplementation with bovine colostrum (Shing et al., 2009; Jenny et al., 2010; Jensen et al., 2012), these are generally a poor surrogate given the presence of other growth factors and bioactive compounds.

4.2.2 Health Benefits of Immunoglobulins

Immunoglobulin supplementation may provide additional health benefits beyond reducing risk for and/or attenuating the duration of infectious disease. An animal model utilizing leptin deficient *ob/ob* mice have documented the effects of eight weeks of supplementation with IgG-rich colostrum from cows immunized against enterotoxigenic *E.Coli* or the purified anti-LPS IgG alone on blood lipids, glucose tolerance and hepatic steatosis (Adar et al., 2012). Reductions in circulating triglycerides (but not total cholesterol) and hepatic fat were noted following supplementation with either the IgG-rich colostrum or the purified IgG, in addition to improved glucose tolerance following supplementation with the purified IgG, all suggesting the potential for bovine immunoglobulin supplementation to affect indices of metabolic disease.

Human clinical studies also allude to a potential metabolic benefit of immunoglobulin supplementation. An initial placebo-controlled cross-over trial in 11 patients with hypercholesterolemia reported 8% reductions in total cholesterol and 4% reductions in LDL cholesterol following eight weeks of supplementation with skim milk from hyperimmunized cows (90g per day) when compared to supplementation with regular skim milk and suggested the increased IgG content of the hyperimmunized skim milk as responsible for the differing responses (Golay et al., 1990). Subsequently, an independent group performed a similar study

utilizing a 10-week supplementation protocol involving 30 adults with moderately elevated blood cholesterol (Sharpe et al., 1994). Significant reductions in total (~5%) and LDL cholesterol (~7%) were again noted, along with significant reductions in both systolic (~4%) and diastolic blood pressure (~4.5%), further suggesting positive effects of bovine Ig supplementation on indices of cardiometabolic disease. An additional double-blind placebo controlled trial involving a six week supplementation with isolated bIgs (5g per day) in cohort of 52 adults with mild untreated hypercholesterolemia has replicated these outcomes (Earnest et al., 2005). Significant decreases total cholesterol (6.33 ± 0.1 mmol/L v 5.97 ± 0.7 mmol/L) and LDL cholesterol (4.12 ± 0.6 mmol/L v 3.84 ± 0.6 mmol/L) were noted post-supplementation for the treatment group (Earnest et al., 2005). The exact mechanisms by which bIgs mediate these effects is not known, however multiple mediators are suspected, including the direct binding of bIgs to cholesterol in the intestinal lumen and binding of bIgs to LPS preventing endotoxin-mediated lipogenesis (Earnest et al., 2005) and inflammation (Adar et al., 2012). These studies provide evidence that the use of immunoglobulins may have therapeutic efficacy in obesity-related metabolic dysfunction.

5. CONCLUSIONS

Broadly speaking, consumption of dairy foods has been shown to be inversely associated with indices of obesity and metabolic disease, and a number of potential mechanisms underpinning these outcomes have been suggested (Pereira et al., 2002; Pilvi TK et al., 2008; Pilvi et al., 2008; Jakubowicz et al., 2013). However, the health implications of specific whey-derived proteins remain ambiguous. The development of new chromatographic and membrane separation technologies has meant that specific milk proteins may be isolated on a large scale from whey,

thus allowing for commercial scale production at concentrations similar to that found in colostrum (Korhonen et al., 2010) and rapid growth in availability of products marketed as functional foods or nutraceuticals.

The development of isolated whey-derived milk proteins by the nutraceutical industry has allowed for further scientific investigation into clinical applications, including immune modulating effects and efficacy in the context of obesity and associated-disease. Bovine lactoferrin and immunoglobulin supplements are generally regarded as safe and, in addition to potential anti-obesogenic effects, both agents may act synergistically to modify immune activation and subsequent inflammation in obesity and associated disease (Figure 2). However, large-scale well-controlled human clinical studies assessing the benefits of whey-derived lactoferrin and immunoglobulins in the treatment and prevention of obesity remain limited. Future studies should include assessment of any impacts on the relative abundance of intestinal microbial species known to be either beneficial or harmful to the host, and the dosages required to achieve this effect. Furthermore, evidence that these immune milk proteins can reduce intestinal permeability or gene expression as key regulators of inflammation and metabolism would provide mechanistic evidence supporting their role in improving health outcomes in obese individuals.

CONFLICT OF INTEREST

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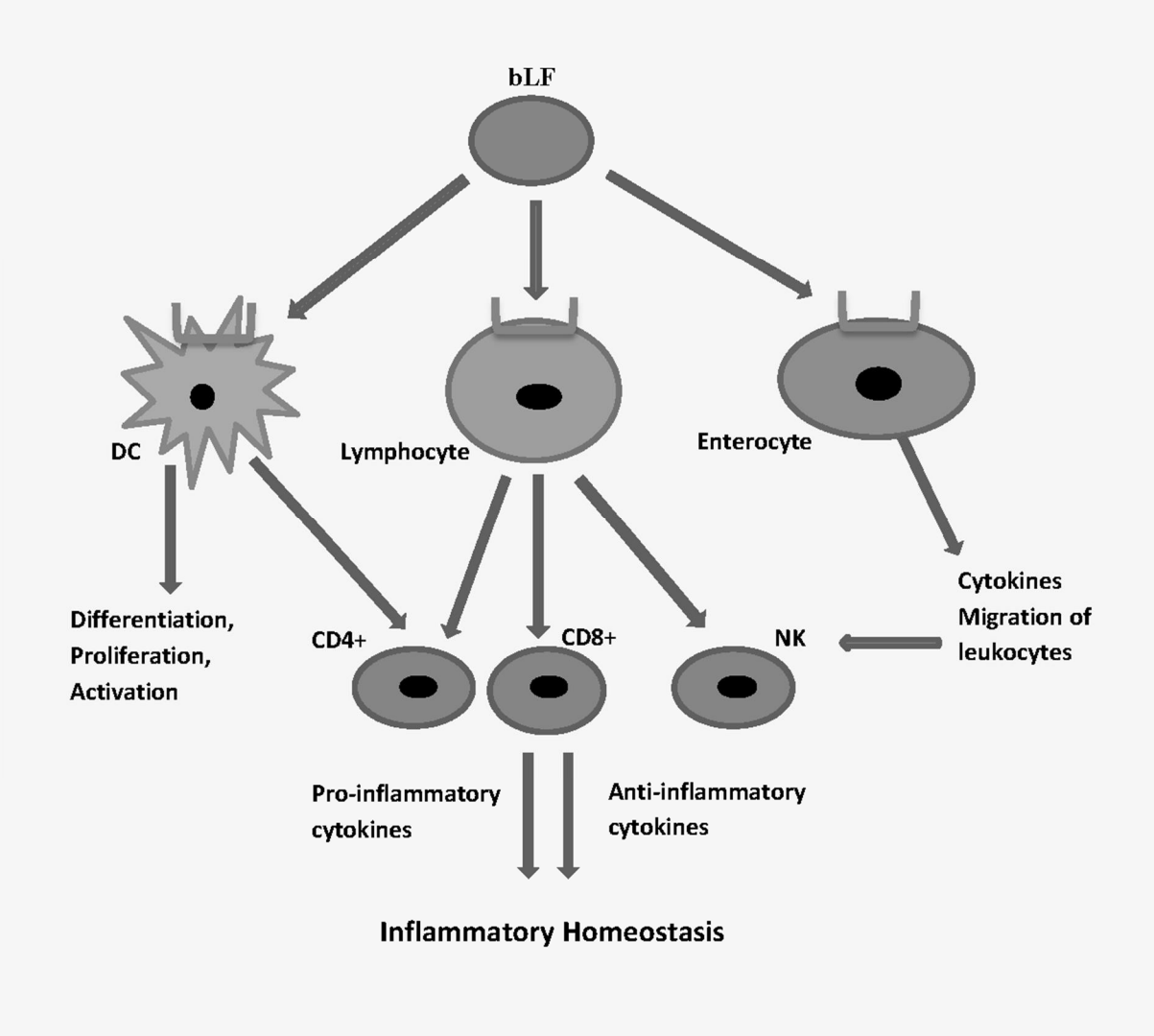

Figure 1. Proposed mechanism of action of orally administered bLF in immunomodulation bLF and digested peptides bind to receptors on dendritic cells, intraepithelial lymphocytes and enterocytes resulting in alterations of cytokine levels and effector mediators. Binding of bLF to DCs results in their maturation, proliferation and activation leading to an increased immune response and the priming of naïve T cells. The binding of bLf to intraepithelial lymphocytes results in increased migration of T cells and NK cells to the intestinal lumen and altered levels of

secreted pro- and anti-inflammatory cytokines. Binding of bLF to enterocytes also results in the secretion of cytokines, such as IL-18, and may increase the migration of immune cells.

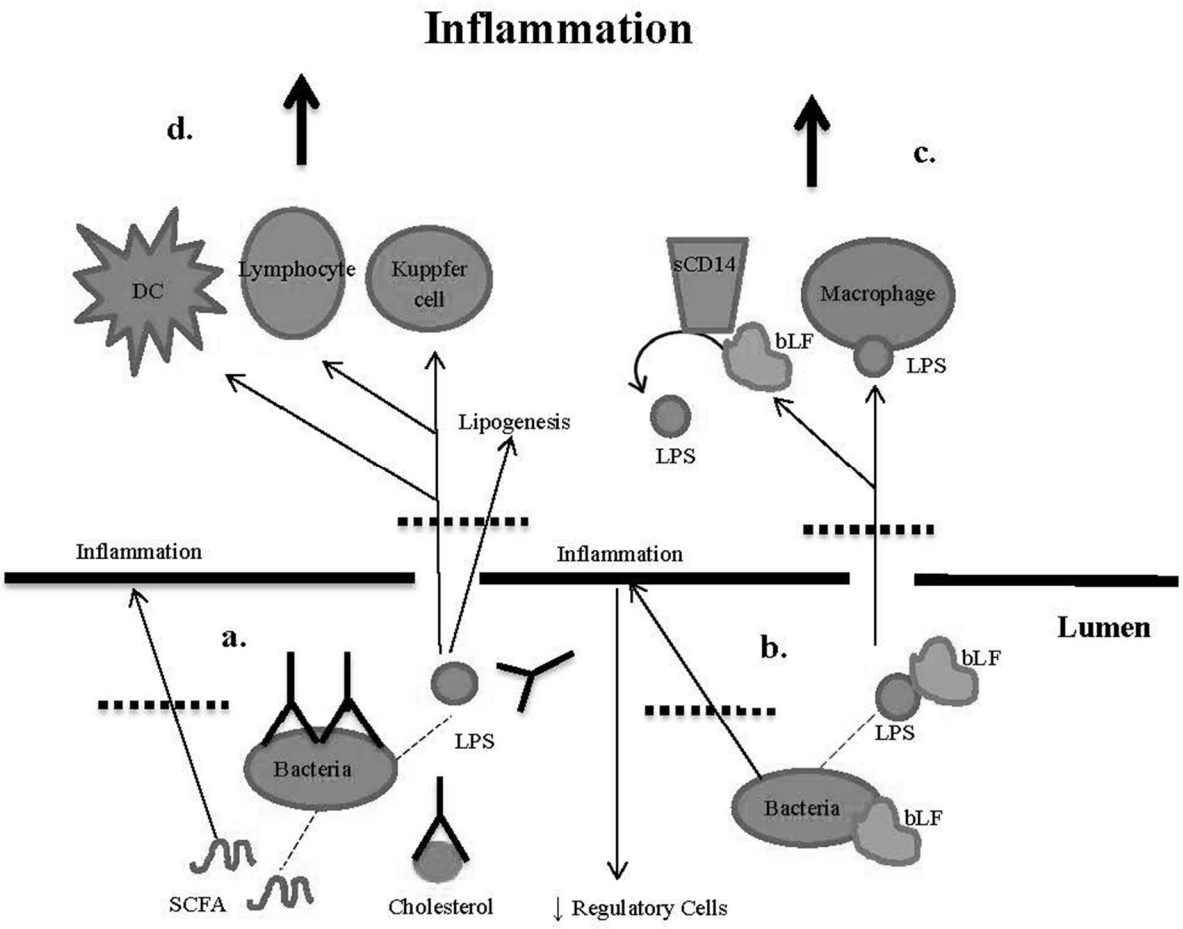

Figure 2. Proposed mechanism of action of orally administered bIgs and bLf in immunomodulation, inflammation and disease. a. bIgs bind pathogenic bacteria, preventing the release of short chain fatty acids (SCFAs) and lipopolysaccharide (LPS), as well as directly binding and neutralizing LPS. Elimination of both microbe and by-products prevents their interaction with the intestinal epithelium and subsequent inflammation. bIgs may also directly bind cholesterol in the lumen preventing absorption. b. bLf binds pathogenic bacteria aiding in elimination and preventing interaction with the intestinal epithelium and subsequent inflammation. Elimination of pathogenic microbial species may correct the reduced frequency of

intestinal regulatory cells observed in response to an altered microbial composition. bLf may also bind LPS in the lumen.c. Binding of LPS by bLf prevents transfer to macrophages. bLf also binds sCD14 preventing immune activation and inflammation d. Binding of bIgs to LPS prevents interaction of LPS and immune cells, limiting immune cell activation and proliferation. Elimination of endotoxin also attenuates endotoxin-mediated lipogenesis and associated increases in cholesterol and TG synthesis.

