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EFFECTS OF A DAIRY-RICH DIET ON MODULATING BIOMARKERS OF INFLAMMATION IN WEIGHT-STABLE OVERWEIGHT AND OBESE ADULTS

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ABSTRACT

Excess adiposity is associated with persistent low-grade inflammation, which may contribute to the development of obesity comorbidities. Overweight and obesity alter the pattern of inflammatory adipokine and cytokine expression. Dairy has been proposed to help reduce inflammation in overweight and obese adults with low habitual dairy intake, although the mechanisms of this potential effect are unclear. Without weight loss, adequate dairy consumption of 3 servings/d may modulate inflammatory stress in the context of excess adiposity. The current study examined the effects of a dairy-rich diet (DRD) on modifying concentrations of biomarkers of inflammation in overweight and obese adults. This randomized, controlled, crossover design study added 3 nonfat dairy or non-dairy control (soy) smoothies per day for 30 days to the diets of weight-stable overweight and obese adults (n= 23), with a 30-day washout period before crossing over to the other diet treatment. Measurements and blood samples were collected at baseline, day 10, and day 30 of each treatment period. Body weight, body mass index, fat mass, and body fat % remained constant throughout the study. Blood concentrations of CRP, TNF-α, IL-6, adiponectin, leptin, and resistin did not vary with the dairy-rich diet treatment over time. However, treatment order effects were present for TNF-α, IL-6, adiponectin, and resistin. Meeting dairy intake recommendations did not impact measures of body weight and body fatness or biomarkers of inflammation, suggesting that dairy can be incorporated into a weight-maintaining diet for overweight and obese adults with a neutral effect on metabolic health.

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Chapter 1

Introduction

Overweight and obesity plague over 68% of adults in the United States, so modulating metabolic health in the context of excess adiposity to reduce the risk of obesity comorbidities represents a pressing public health concern (1). Chronic, low-grade systemic inflammation is characteristic of obesity, and inflammation is associated with cardiovascular disease, diabetes, and some cancers (2). In addition, people with the clustering of cardiometabolic risk factors including abdominal obesity, high triglycerides, low high-density lipoprotein-cholesterol (HDL-C), hypertension, and elevated fasting blood glucose, collectively referred to as metabolic syndrome (MetS), experience a pro-inflammatory state and increased morbidity and mortality (3). The persistent activation of the acute-phase immune response is a putative mechanistic link between obesity and comorbid diseases, so attenuating this state is crucial to preserving health (4). Due to the pervasiveness of overweight and obesity and the limited long-term success rates in reducing body weight and body fatness, minimizing inflammation in overweight and obese conditions could promote health and limit the development of obesity comorbidities.

With increased body fatness, adipose tissue morphology changes with greater incidence of macrophage infiltration and alterations in adipokine secretion (5). In obesity, macrophages infiltrate white adipose tissue, scavenge dead adipocytes, and assemble into multinucleate giant cells (6). Activated macrophages accumulate in the adipose tissue of individuals with obesity proportional to adiposity (7). These multinucleate giant cells represent persistent areas of macrophage activation, and they secrete greater amounts of the pro-inflammatory cytokines and chemokines tumor necrosis factor (TNF)-α, interleukin (IL)-6, monocyte chemotactic protein (MCP)-1, and nitric oxide, among other factors. The morphological alterations of adipose tissue

associated with increasing adiposity coincide with changes in the expression of inflammatory cytokines.

Specifically, obesity is associated with increased secretion of TNF-α, IL-6, and C-reactive protein (CRP) and decreased release of adiponectin. MCP-1 recruits macrophages to enable white adipose tissue infiltration, and TNF-α activates these macrophages. TNF-α affects metabolism at the organismal level by decreasing insulin sensitivity and signaling (8). Both TNF- α and IL-6 promote lipolysis, and IL-6 also stimulates the liver to increase secretion of triglycerides (9; 10). IL-6 increases hepatic CRP secretion, and circulating levels of CRP are positively correlated with adiposity (11). In contrast, adiponectin expression decreases during obesity as a response to increased levels of TNF-α and IL-6. Adiponectin promotes insulin sensitization, exerts protective effects against atherogenesis, and reduces macrophage secretion of TNF-α (12). Expression of the adipose-derived protein leptin increases with adipocyte expansion, but obese people may be resistant to the appetite-lowering effects of leptin (13). Resistin, a presumed connection between obesity and the progression of insulin resistance, is expressed by mononuclear cells in the blood and adipocytes, and *in vitro*, mononuclear expression of resistin has increased with the application of IL-1β, TNF-α, and IL-6 (14; 15). Shifts in inflammatory cytokines due to excess adiposity may precipitate the development of conditions such as insulin resistance and cardiovascular disease, so maintaining a protective inflammatory status in the context of excess adiposity may be advantageous to health.

Although only 18% of men and 10% of women achieve the suggested intake of 3 servings/d of dairy products put forth by the *Dietary Guidelines for Americans, 2010* (16), adequate dairy intake is associated with favorable body weight status and beneficial levels of indicators of metabolic health (17). Recent meta-analyses have concluded that without energy restriction, however, dairy product consumption at the recommended level of 3 servings/d does not lower body weight or body fatness (18; 19), although dairy intake is thought to help maintain

lean body mass during weight loss (20). Meeting adequate dairy intake levels is associated with lower blood pressure (21), an improved serum lipid profile (22), and reduced risk of cardiovascular disease (23; 24). Furthermore, in a 10-year prospective cohort study, the incidence of individual MetS components (obesity, abnormal glucose homeostasis, hypertension, and dyslipidemia) were reduced with increasing levels of dairy intake, and the odds of developing MetS were 72% lower (OR 0.28, 95% CI: 0.14-0.58) for those consuming the greatest amount of dairy (≥35 servings/wk) compared to those consuming the least amount of dairy (<10 servings/wk) (25). Therefore, dairy products can benefit metabolic health and contribute to a healthy weight-maintaining diet that reduces the risk of chronic disease.

An emerging body of evidence associates adequate levels of dairy intake with a more favorable inflammatory profile (26; 27; 28; 29). A randomized, controlled, crossover study conducted by Zemel and colleagues compared plasma concentrations of inflammatory biomarkers in free-living, weight-stable overweight and obese adults on 28-day treatments with eucaloric nonfat dairy supplemented (3 servings/d) and non-dairy supplemented (3 servings/d of soy) diets. Compared to plasma concentrations of inflammatory biomarkers on the non-dairy diet, reductions in plasma TNF-α (15%, $P< 0.001$), MCP-1 (20%, $P< 0.004$), IL-6 (13%, $P< 0.001$), and CRP (57%, $P< 0.05$) and an increase in plasma adiponectin (20%, $P< 0.002$) were apparent at day 7 of the dairy-supplemented diet and became more pronounced throughout the dairy diet treatment (29). Significant reductions in CRP and increases in adiponectin have been reported by Zemel and Sun (26) with the implementation of eucaloric and hypocaloric diets designed to meet adequate dairy intake. However, other studies have not found significant alterations in biomarkers of inflammation with eucaloric (30; 31) or energy-restricted dairy interventions (32). The extent to which dairy foods can temper inflammatory stress remains unclear.

To examine the role of dairy products in modulating biomarkers of inflammation, the current study utilized a randomized, crossover design to administer 3 servings/d of nonfat dairy

smoothies, a dairy-rich diet (DRD) treatment, for 30 days compared to 3 servings/d of non-dairy (soy) smoothies, a control (CON) diet treatment, with a 30-day washout between treatments. Most Americans do not meet the recommended 3 servings/d of dairy products, so the dairy treatment enabled participants to meet adequate dairy intake levels. To minimize confounding by changes in adipose tissue, weight-stable overweight participants maintained constant body fatness throughout the study. Based on the limited, but suggestive evidence in previous literature (Scoular KM, Nickols-Richardson SM. Systematic review of emerging evidence regarding the influence of dairy intake on inflammation and oxidative stress in overweight and obesity. J Obes. 2013;In review.), it was predicted that compared to the CON diet, the DRD would reduce or maintain blood levels of the pro-inflammatory biomarkers CRP, TNF-α, and IL-6 and increase or maintain plasma concentrations of the anti-inflammatory biomarker adiponectin within individuals. Due to their associations with inflammatory cytokines, plasma concentrations of leptin and resistin were not predicted to differ between diet treatments. Overall, it was hypothesized that increased dairy intake would benefit inflammatory status, indicating a more favorable, chronic disease preventing metabolic profile.

Chapter 2

LITERATURE REVIEW

Systematic review of emerging evidence regarding the influence of dairy intake on inflammation and oxidative stress in overweight and obesity

Submitted to the *Journal of Obesity*

ABSTRACT

Inflammation and oxidative stress are exacerbated in the overweight and obese conditions and may contribute to the progression of obesity comorbidities. Dairy consumption has been associated with more favorable inflammatory status and reduced oxidative stress. A systematic search of the literature was conducted to identify experimental and observational studies in overweight and obese adults, some of whom had metabolic syndrome (MetS) that assessed the effects of dairy intake on modulating biomarkers of inflammation and oxidative stress. Eight randomized controlled trials (RCTs) and 1 cross-sectional study were found and reviewed. Among habitual low-dairy consumers, clinical trials in overweight and obese adults suggest a modest reduction in inflammation with an adequate dairy diet. Participants with MetS did not respond consistently to an increase in dairy consumption. The cross-sectional study supported the notion that adequate dairy intake is associated with lower concentrations of inflammatory

biomarkers. In the context of excess adiposity, increasing dairy intake to the recommended 3 servings/d may modify inflammation and oxidative stress. Methodological consistency and manipulations using various dairy foods will enable a better, more mechanistic understanding of how dairy affects inflammation and oxidative stress.

INTRODUCTION

The state of persistent, low-grade inflammation that occurs with obesity drives the progression of comorbidities such as insulin resistance and cardiovascular disease (CVD) [1]. In addition, obesity comorbidities may stem from the increased oxidative stress characteristic of obesity [2]. Adipose tissue functions not only as an endocrine organ but also as a body of cells that responds to increased energy consumption. Surplus energy inputs exceed metabolic demands and the body's reduction-oxidation (redox) capacity for dealing with energy substrates, and this oxidative stress increases susceptibility to cellular damage [3]. Changes in adipocytes, macrophages, and inflammatory signaling pathways causally link obesity to other metabolic disorders, highlighting the need to understand metabolic consequences of excess adiposity [4], [5]. Overweight and obesity continue to affect millions of people worldwide, and obesity rates exceed 30% in most sex-age groups of American adults examined in the 2009-2010 National Health and Nutrition Examination Survey (NHANES) [6]. The concomitant inflammation and oxidative stress associated with excess adiposity warrant attention as mechanisms of disease progression and underscore the importance of modulating inflammation and oxidative stress in the context of overweight and obesity [7].

Numerous investigations have examined the impact of dairy intake on weight status and adiposity, and results have been mixed. A recent meta-analysis concluded that dairy products exert a neutral effect on change in body weight (-0.14 kg) and fat mass (-0.45 kg) in parallel arm

or crossover studies comparing dairy and control groups without energy reduction or interventions ≥1 year and modest but significant beneficial effects in reduced-energy interventions (-0.79 kg for body weight; -0.94 kg for fat mass) and studies <1 year (-0.47 kg for body weight; -0.91 kg for body fat) [8]. Similarly, a meta-analysis conducted by Abargouei and colleagues [9] found that in the absence of energy restriction, increasing dairy consumption to the recommended, adequate level (3 servings/d) did not significantly affect body weight (0.33 kg), fat mass (-0.16 kg), lean body mass (0.35 kg), or waist circumference (-2.68 cm). Changes in fat mass alter adipokine secretion and inflammatory signaling [4], so interventions that reduce adiposity must be considered in this context. A review of prospective cohort studies on the effect of dairy intake on body weight found that in adults, evidence was inconclusive but suggested a beneficial, though small in magnitude, effect of dairy food consumption [10]. Five of 9 studies in adults observed a beneficial protective effect of dairy in preventing inappropriate weight gain, as defined within each study [10]. Because the evidence for a protective effect of dairy in modulating body weight and adiposity is not clearly apparent at the organismal and body composition level, perhaps benefits of adequate dairy consumption occur at a cellular or subcellular scale.

Current recommendations from the *Dietary Guidelines for Americans, 2010* suggest including 3 servings/d of dairy as part of a healthy diet [11]. Dairy products provide protein and lipids as well as a variety of vitamins, minerals, and bioactive compounds. Specifically, fat-free and reduced-fat fluid milk and milk products provide vitamin D, calcium (Ca), and potassium, all nutrients of concern, in the form of low energy-density, nutrient-rich foods [11]. While consumption of dairy foods has been linked to an eating pattern that better meets micronutrient requirements [12] and a lower glycemic index diet [13], individual components of dairy products may contribute to improved health. Milk is voluntarily fortified with vitamin D in the United States, and adequate blood concentrations of vitamin D confer bone health [14]. The mineral

content of milk, specifically the balance of magnesium, potassium, and Ca, may be linked to lower blood pressure [15]. Casein and whey proteins present in dairy include angiotensin-I-converting enzyme (ACE) inhibitory proteins, which are bioactive peptides that reduce peripheral blood pressure by preventing the formation of angiotensin II (for review see [16], [17]). Whey protein is also recognized as a valuable source of branched chain amino acids (BCAAs) including leucine, which stimulates muscle protein synthesis, and whey protein has been linked to maintenance of lean body mass in weight loss trials (for review see [18]). Furthermore, the fatty acid profile of dairy is comprised primarily of the saturated fatty acids myristic (14:0), palmitic (16:0), and stearic (18:0) acid, and milkfats increase serum concentrations of high-density lipoprotein-cholesterol (HDL-C) and improve the HDL-C:total cholesterol (TC) ratio [19].

Consumption of low-fat dairy foods is recommended as part of the Dietary Approaches to Stop Hypertension (DASH) combination diet to reduce blood pressure. In general, compliance with the DASH diet may lower systolic blood pressure by 5.5 mm Hg and diastolic blood pressure by 3.0 mm Hg compared to a control diet typical of many Americans in as little as 2 weeks [20]. This effect may rely mechanistically on the actions of Ca suppression of circulating 1,25-dihydroxyvitamin D (1,25-OH_2-D) [21] or on the ACE peptides in casein and whey protein. While the antihypertensive effects of dairy are well established (for review see [22]), other metabolic benefits of dairy consumption, including non-blood pressure and non-lipid risk factors for CVD, warrant further investigation [17]. Dairy consumption benefits the serum lipid profile by raising serum HDL-C and improving the HDL-C:TC ratio, which has implications for CVD as well as other conditions associated with dyslipidemia (for review see [18], [23]). Inflammation has been implicated in the progression of a number of diseases [5]. A small but emerging body of evidence suggests that dairy may modulate the inflammatory burden [24-28], which has implication for reducing chronic disease risk with dietary interventions.

Several reviews have examined the inverse relationship between dairy intake and the clustering of cardiometabolic risk factors within individuals diagnosed with metabolic syndrome (MetS) [16], [29], [30]. An individual is clinically diagnosed with MetS if he/she meets at least 3 criteria for MetS as defined by the Adult Treatment Panel III: 1) waist circumference ≥102 cm in men or ≥88 cm in women; 2) serum HDL-C <40 mg/dL in men or <50 mg/dL in women; 3) serum triacylglycerol concentration ≥150 mg/dL; 4) blood pressure ≥130/85 mm Hg; 5) fasting plasma glucose concentration ≥110 mg/dL [31]. However, well-defined conclusions about dairy and the development of MetS cannot be drawn due to lack of randomized controlled/clinical trials (RCTs) manipulating reduced-fat dairy intake over long periods of time [30]. A prospective cohort study conducted by Pereira and colleagues [32] found that in individuals who were overweight or obese at baseline, the odds of developing MetS 10 years later were reduced by 21% (OR 0.79, 95% CI: 0.72-0.88) with an increase in 1 serving/d of dairy. Emerging evidence suggests that in specific populations, such as women ≥45 y, total dairy intake (OR 0.66 for highest intake quintile) and total Ca intake (OR 0.64 for highest intake quintile) were significantly inversely associated with MetS prevalence [33]. Similarly, Tehranian adults with the highest quartile of dairy intake had lower odds of meeting at least 3 of the criteria of MetS (OR 0.69, 95% CI 0.59-0.71) [34]. An association between dairy consumption and modulation of the individual metabolic abnormalities of MetS has not been consistently detected [35], [36] or has varied by sex [37]. Individuals with MetS have excess adiposity, so the role of dairy in altering inflammation and oxidative stress is relevant to MetS as both a condition of excess body fatness and a disease with metabolic disturbances thought to be modulated by dairy intake. The purpose of this systematic review was to assess current knowledge of how dairy intake influences inflammation and oxidative stress in overweight and obese conditions, as well as in MetS.

MATERIALS AND METHODS

A literature search was conducted to gather articles published from 1980 through March 2013, using PubMed/MEDLINE, AGRICOLA Articles, Cochrane Controlled Trial Register, and CAB Abstracts to identify studies examining dairy intake and inflammation in overweight and obese subjects. The search was limited to articles in English that involved adults and included the key search terms "dairy" OR "dairy product" OR "dairy-rich" OR "high dairy" OR "adequate dairy" OR "total dairy" AND "obese" OR "obesity" OR "overweight" OR "adiposity" AND "inflammation" OR "inflammatory stress" OR "inflammation status" OR "inflammatory status" OR "oxidative stress" OR "oxidative status" OR "metabolic."

Papers were identified through database searching and a manual search of references cited by included articles (Figure 1). Abstracts were screened for evaluation in humans, observational or experimental study design, relevant inflammatory and oxidative stress endpoints, and RCTs conducted for ≥4 weeks. Experimental designs <4 weeks did not intend to assess changes in dietary patterns concerning dairy, and outcome measures represented changes after a single meal challenge. Only papers evaluating dairy products consumed as foods in the context of the diet (i.e., milk, yogurt, cheese) were included. Excluding studies that assessed only specific components of dairy foods allowed any effects noted to be ascribed to dairy products in the way they are typically consumed. Therefore, articles had to include a quantitative measure of dairy intake over a daily or weekly timescale. For the purpose of this review, overweight was defined as body mass index (BMI) ≥25 kg/m^2 rather than the criteria used in each individual study. Since people with excess adiposity may also suffer from MetS, studies examining relevant metabolic outcomes in individuals with MetS were included. Studies that evaluated dairy consumption in overweight and obese people on both eucaloric and energy-restricted diets were considered. Evidence from 8 RCTs was graded using a quality checklist (Supplemental Figure).

RESULTS

This systematic review identified 9 papers, including 8 RCTs and 1 cross-sectional analysis. A summary of papers on the relationships between dairy product intake and inflammation and oxidative stress in overweight and obese adults is given in Table 1. Studies were conducted in the United States [25-27], [38]; the Netherlands [24], [39]; Australia [40]; Greece [28]; and Finland, Norway, and Sweden [41]. In RCTs where baseline dairy intake was reported [24], [38-41], habitual dairy consumption was below the *Dietary Guidelines for Americans, 2010*, recommendation of 3 servings/d [11], which is representative of American adults [42]. Because international literature was included in the current review, participants also were considered low dairy consumers by the standards of their country of origin. Eight RCTs examined dairy intake, overweight or obesity, and inflammation or oxidative stress, with 5 focusing on hypocaloric diet treatments, 2 not using dietary energy content manipulations, and 2 examining overweight or obese participants with MetS in eucaloric diet interventions (with data from multiple RCTs included in a single publication [26] and 2 papers resulting from a single RCT [24], [39]). The cross-sectional analysis included participants with an average BMI >25 kg/m^2 consuming their typical diets.

When van Meijl and Mensink [39] supplemented the diets of Dutch participants with 2 servings/d of dairy (500 mL low-fat milk and 150 g low-fat yogurt) for 8 weeks, no overall changes in metabolic risk factors, except for a decrease in systolic blood pressure, were observed; however, blood sample analysis demonstrated a significant decrease in the tumor necrosis factor (TNF)-α index [24], an indicator of bioavailable TNF-α. A separate study that used a randomized crossover design of a 6-month high-dairy diet (4 servings/d) followed immediately by a 6-month low-dairy diet (≤1 serving/d) did not find any relationship between dairy intake level and measures of cardiometabolic health [40]. Conversely, archival blood sample analyses from a

parallel group intervention that compared a high-dairy diet (3 dairy servings/d, 1200 mg Ca/d) to a low-Ca/low-dairy diet (<1 dairy serving/d, 500 mg Ca/d) reported significant increases in plasma adiponectin and significant decreases in plasma C-reactive protein (CRP) after 24 weeks [26]. Zemel and colleagues [27] used a randomized crossover study design to examine how dairy influences inflammatory and oxidative stress in overweight and obesity without changes in adiposity. Similar to their other experiments, these authors detected increases in plasma adiponectin and decreases in plasma CRP after 28 days on a high-dairy diet of 3 servings/d, in addition to increases in plasma malondialdehyde, 8-isoprostane-$F_{2\alpha}$, TNF-α, interleukin (IL)-6, and monocyte chemotactic protein (MCP)-1 (with an effect of treatment order) [27]. Results of the effects of increased dairy consumption on biomarkers of inflammation and oxidative stress are mixed, but it appears that there is emerging evidence from a small body of studies to suggest that inflammatory and oxidative stress in overweight and obese populations of adults can be reduced by altering this single dietary variable.

Energy restriction studies (500 kcal/d deficit) that assigned participants to an adequate-dairy (3-4 servings/d) or low-dairy control (≤1 serving/d) group noted a significant decrease in post-intervention plasma concentrations of CRP relative to controls [26] and a reduction in individual plasma concentrations of high sensitivity (hs)-CRP (1.0 mg/L) associated with weight loss (-6.3 ± 2.9 kg) [38]. A 12-week intervention induced significant increases in plasma concentrations of adiponectin in the adequate-dairy treatment compared to the low-dairy treatment [26], but there were no significant within-subjects changes in plasma adiponectin in another intervention of the same length [38]. The study by Van Loan and colleagues [38] did not find any significant alterations in subcutaneous adipose tissue transcript abundances or plasma concentrations of inflammatory markers or cytokines measured, but plasma concentrations of all pro-inflammatory biomarkers [plasminogen activator inhibitor-1, IL-1β, IL-6, IL-8, TNF-α, hs-CRP] decreased in participants assigned to the dairy treatment by 3% for TNF-α to 29% for hs-

CRP. The low-dairy control group also had decreases in endocrine hormones and inflammatory cytokines, which may have been related to the significant weight loss (-6.0 ± 3.1 kg) and changes in body fat (-5.1 ± 3.0 kg) associated with the energy-deficit diet. Reductions in fat mass alter the amount of tissue available to secrete adipokines and the pattern of cytokine production; hence, energy-restricted diets that induce weight loss and reduce fat mass can induce modest reductions in biomarkers of inflammation as observed in both the low-dairy and adequate-dairy groups in the study by Van Loan and colleagues [38].

Wennersberg and colleagues [41] compared an adequate-dairy diet (3-5 servings/d) to a low-dairy diet (≤2 dairy servings/d) in a 6-month parallel arm study of adults with MetS and habitually low dairy intake (≤2 servings/d). Stancliffe and colleagues [25] evaluated weight-maintaining diets with adequate dairy (>3.5 servings/d) relative to low dairy (<0.5 servings/d) in a randomized, parallel group, 84-day intervention in overweight and obese adults with MetS. Both studies included individuals with MetS, and both examined changes in body composition measurements and biomarkers of inflammation and oxidative stress. While adults in both studies maintained constant body weight, unlike the Wennersberg and colleagues [41] intervention, the adequate-dairy treatment in the study by Stancliffe and colleagues [25] significantly reduced waist circumference (-2.8 ± 0.8 cm), fat mass (-1.3 ± 0.9 kg), and trunk fat mass (-1.4 ± 0.7 kg) compared to the low-dairy treatment. However, Wennersberg and colleagues [41] found a treatment effect for reductions in waist circumference (-2.8 cm) and sagittal abdominal diameter (-0.8 cm) in participants with low habitual Ca intake (<700 mg/d) in the adequate-dairy group. Tumor necrosis factor-α, MCP-1, IL-6, and CRP were significantly reduced in an adequate-dairy condition [25], but these pro-inflammatory cytokines, as well as complement factors C3 and C4 showed no relationship with increased dairy consumption in a separate study [41]. In addition to reductions in biomarkers of inflammation, participants had significant reductions in malondialdehyde (-1.39 ± 0.89 nmol/L) and oxidized low-density lipoprotein (-88 ± 36 ng/mL) in

the adequate-dairy treatment compared to the low-dairy control (25), but no effect of adequate dairy on change in plasma 8-isoprostane-$F_{2\alpha}$ concentration was detected in the Wennersberg and colleagues [41] study. Moreover, Wennersberg and colleagues [41] observed a significant increase in serum TC in the adequate-dairy group relative to controls (82 mg/dL) that was related to the proportion of the milk fatty acid 15:0 (pentadecanoic acid) to TC esters in serum ($r=0.24$, $P=0.011$), while Stancliffe and colleagues [25] found the opposite effect with a significant decrease in plasma TC with increased dairy consumption ($P<0.02$) in obese adults. While adequate levels of dairy consumption can alter anthropometrics and body composition of adults with MetS, effects of dairy on biomarkers of inflammation and oxidative stress are inconsistent.

The single cross-sectional survey was conducted in the ATTICA study of Greek adults and assessed dairy intake (full-fat and low/non-fat products) with a valid food frequency questionnaire [28]. Those with moderate dairy consumption (11-14 servings/wk) and high dairy consumption (>14 servings/wk) exhibited lower concentrations of TC and triglycerides and had reduced rates of hypercholesterolemia compared to those with low dairy intake (<8 servings/wk). A single blood draw was used to measure plasma inflammatory markers. Compared to the lowest consumption group, those with moderate and high dairy intake had significantly lower plasma concentrations of CRP, IL-6, and TNF-α, with a more pronounced effect at the highest dairy consumption level. Even with adjustments for weight status and other potential confounders, these findings persisted. Overall, this cross-sectional analysis [28] supports an inverse relationship between dairy consumption and inflammation.

DISCUSSION

This systematic review identified and evaluated the evidence from 8 RCTs and 1 cross-sectional study for the relationship between dairy intake and biomarkers of inflammation and oxidative stress. Clinical trials conducted in overweight and obese adults support a modest beneficial effect of adequate dairy intake in reducing inflammation in habitual low-dairy consumers. Mixed results from 2 RCTs in participants with MetS do not identify a clear association between dairy and inflammatory and oxidative stress. A cross-sectional study echoed the trends noted in RCTs of reduced inflammation in adults consuming high or adequate levels of dairy.

Future research directions

Suggestive, but inconclusive evidence linking dairy to an improved metabolic profile highlights the need for replication of studies, consistent methodology, consensus on meaningful outcome measurements, identification of key baseline characteristics that influence effects of dairy consumption, and exploration of possible mechanisms. Emerging evidence in the field will be most useful for drawing associations across studies with consistent methodology. Scientific replication is necessary for the development of theory driven science. Only with duplication of results across multiple laboratories and sample study populations will a cohesive framework for the influences of dairy consumption on metabolic health emerge. A meta-analysis of 29 RCTs on dairy and changes in body weight and adiposity found significant effects of study duration and energy restriction protocols, so the regression analysis was stratified to account for this heterogeneity [8]. Study design elements can substantially influence outcome variables, and the covariates of adiposity, baseline dairy intake, initial concentrations of inflammatory and oxidative

stress biomarkers, and metabolic risk factors also may play a role. Consistent methodology is required to identify the independent and synergistic contributions of multiple variables as well the importance of baseline participant characteristics.

Furthermore, a consensus in the field as to which outcome variables are most reflective of the underlying processes will move investigations forward. The 9 papers addressed in this review included 11 different pro-inflammatory biomarkers, 2 anti-inflammatory biomarkers, and 4 biomarkers of oxidative stress. If inflammation and oxidative stress represent mechanisms in the development of obesity comorbidities, then agreement upon which biomarkers best represent disease progression will enable more meaningful comparisons across studies. Reductions in plasma TNF-α and IL-6 were apparent at day 7 in the adequate-dairy treatment of the study by Zemel and colleagues [27]. These biomarkers continued to fall throughout the duration of the study, suggesting that beneficial effects of dairy can occur in a short interval and are sustainable with consistent dairy consumption. In addition, it should be noted that serum concentrations of biomarkers of inflammation may respond to dairy interventions differently than subcutaneous adipose tissue transcript amounts of these molecules, as demonstrated by Van Loan and colleagues [38]. Different results across studies likely arise from variations in study design, but determining the most clinically meaningful measures of remediating effects of dairy foods and the amount of time necessary for such changes to occur will help inform future interventions and disease prevention efforts. Future studies examining the relationship between dairy foods and MetS should adopt the same criteria for defining MetS to strengthen homogeneity of included participants. For example, the inclusion criteria in the study by Wennersberg and colleagues [41] stipulated that people meet at least 2 of 5 risk determinants for MetS, while participants in the study by Stancliffe and colleagues [25] met at least 3 of the MetS criteria. Consistent methodology will enable more accurate and meaningful comparisons across studies and study populations.

With refinement of techniques to quantify dairy ingestion with biochemical markers, better assessments of habitual dairy consumption will be possible. Controlled feeding trials are not always feasible, so replacing self-report measures of dairy intake with biochemical measurements can help minimize the limitations associated with self-reported data, enabling better quantification of dairy intake across populations and enhanced assessment of compliance in interventions that manipulate dairy. For example, serum 15:0 and 17:0 fatty acids, derived primarily from dairy products, have been used as measures of dairy intake [43]. Widespread adoption of such measures would facilitate more meaningful comparisons across studies.

Distinguishing how different populations may benefit from dairy consumption presents an opportunity for the development of targeted interventions. Studies included in this review recruited overweight and obese low-dairy consumers, some of whom also met the criteria for MetS. Although dairy does not appear to exert a strong effect on body weight in long-term intervention trials [8], the relationship between dairy intake and body fatness and fat distribution is less clear. A high-dairy diet (3 servings/d, 1200-1300 mg/d Ca) induced greater body weight and fat loss in a 6-month trial of obese adults compared to a low-dairy diet (≤1 serving/d) and a high-Ca diet (control diet with supplemental 800 mg/d Ca), specifically with favorable reductions in trunk fat [44]. If dairy influences fat distribution patterns, then initial adiposity allocation may modulate any beneficial effects of dairy in relation to central and visceral fat stores. Similar to the approach taken by Zemel and colleagues [27], holding body weight and body fatness constant throughout the course of the study enables examination of metabolic changes independent of changes in adiposity. In continuing to explore MetS, the specific MetS criteria of individuals may highlight the inflammatory pathways and physiological mechanisms most affected and potentially most susceptible to remediation with dairy. Analyses of NHANES data from 1999-2004 found that while some dairy foods and dairy-related nutrients were inversely related to MetS prevalence (1 serving/d yogurt increase, OR 0.40, 95% CI: 0.18-0.89; 100 mg magnesium intake,

OR 0.83, 95% CI: 0.72-0.96), others were not (1 serving/d cheese increase, OR 1.16, 95% CI: 1.04-1.29; 100 g low-fat milk increase, OR 1.02, 95% CI: 0.97-1.06; 100 g phosphorous increase, OR 1.05, 95% CI: 1.01-1.10) [42]. Due to heterogeneity in the effects of different dairy products, the type of dairy foods as well the fat and mineral content of those products must be considered. Regional variation in fortification of milk and milk products should also be noted as, for example, most milk in the United States is fortified with vitamin D, but only some milk and milk products are fortified with vitamin D in Australia [45]. Baseline dairy intake is highly variable, and much attention has been directed to low-dairy consumers. While a threshold effect for dairy and Ca consumption to alter body weight and adiposity has not been supported across clinical trials [46], habitual dairy intake levels may be important in the ability of increased dairy consumption to temper inflammation and oxidative stress. Meeting the recommendation of 3 servings/d of dairy can help meet nutrient requirements, but for adults to achieve the nutrient concentrations for optimal bone health, 4 servings/d may be required [47]. Therefore, understanding the benefits of various levels of increased dairy consumption warrants further investigation.

The possible mechanisms underlying the role of dairy in regulating body weight, body fatness, and inflammatory and oxidative stress encompass theories concentrating on specific dairy components and dairy as a whole product. Zemel [48] proposed that increased dietary Ca suppresses 1,25-OH_2-D and parathyroid hormone to reduce lipogenesis and stimulate lipolysis through increased intracellular concentrations of Ca and inhibition of uncoupling protein 2. Acute, but not habitual Ca intake is associated with 24-h fat oxidation ($r=0.38$, $P=0.03$), suggesting a role of dairy Ca in modulating fat oxidation in the short term [49]. In addition, the Ca in dairy products may combine with fatty acids to form insoluble soaps that are excreted in feces, thereby increasing fecal energy loss [50]. However, mechanisms focusing on Ca alone do not account for the bioactive components of dairy products and differential reductions in body weight and body fatness seen in studies that compare energy restriction diets with Ca

supplementation and Ca consumed in dairy products [44]. Branched-chain amino acids (valine, leucine, isoleucine) in dairy peptides have been implicated in sparing muscle with weight loss to favor lipid metabolism. It has been proposed that BCAAs in the whey fraction of dairy exert anabolic effects [51]. Conjugated linoleic acid (CLA) has been purported to regulate adipogenesis, inflammation, and lipid metabolism, but the amount of CLA consumed in dairy products and their effects is probably minimal [18]. Focused research on the identification of the most crucial components of dairy may allow insight into the mechanisms of how dairy impacts metabolic health both as a whole food and through its various constituents.

CONCLUSION

Overall, this systematic review suggests that based on a limited body of available evidence, consumption of dairy products may have a modest beneficial effect on concentrations of inflammatory and oxidative stress biomarkers in overweight and obese conditions. This area warrants further investigation to determine the potential benefits of dairy products in ameliorating inflammation and oxidative stress in populations with excess adiposity and other metabolic concerns. Consistency in methodology and exploration of the effects different dairy products will better inform recommendations about the potential therapeutic effects of dairy foods in modulating inflammation and oxidative stress in overweight and obesity.

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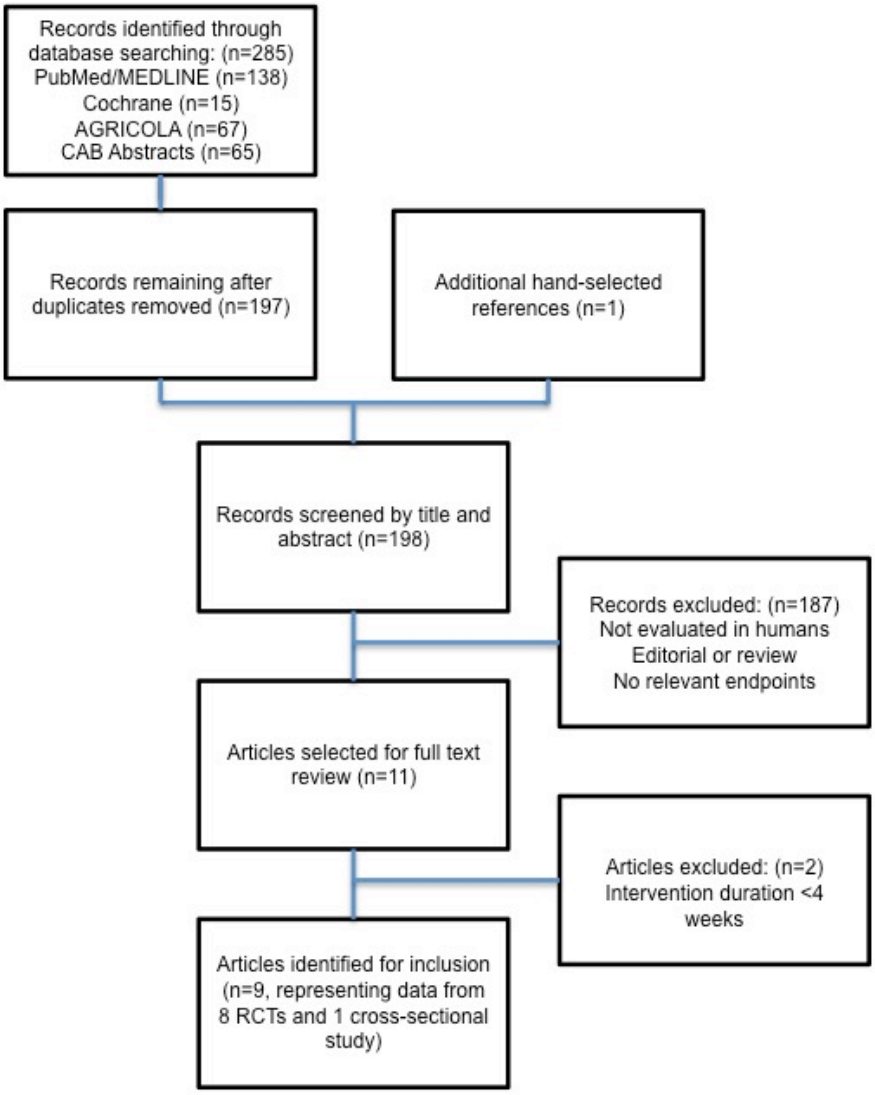

Figure **2-1**. Flowchart of systematic review search and select process.

Table **2-1**: Summary of randomized controlled/clinical trials evaluating dairy intake and biomarkers of inflammation and oxidative stress.

Study reference	Subjects	Design	Primary outcome	Dairy intervention	Control	Effects on inflammation and oxidative stress
Crichton et al., 2012	61 OW/OB 29.5% male BMI 31.5±0.9	Randomized; crossover; weight maintenance; 2 x 6-mo	Waist circumference	4 servings/d reduced-fat dairy	≤1 dairy serving/d	(neutral) hs-CRP
van Meijl and Mensink, 2011	35 OW/OB 28.6% male BMI 32.0±3.8	Randomized; crossover; weight maintenance; 8-wk, 2-wk washout, 8-wk	Metabolic risk factors	500 mL milk (1.5% fat) and 150 g yogurt (1.5% fat)	600 mL fruit juice and 43 g fruit biscuits	(neutral) CRP, PAI-1
van Meijl and Mensink, 2010						(neutral) IL-6, TNF-α, s-TNFR-1, MCP-1, ICAM-1, VCAM-1 (+) s-TNFR-2 (-) TNF-α index
Zemel and Sun, 2008	34 OB African Americans High dairy: 17.6% male BMI 42.5±2.6 Low dairy control: 47.1% male BMI 34.9±0.8	Randomized; parallel; weight maintenance; 2-wk lead-in, 24-wk	Body weight; body fat	3 servings/d with 1200 mg Ca/d	<1 serving/d with 500 mg Ca/d	(+) adiponectin (-) CRP
Zemel et al., 2010	20 OW/OB 70% male OW: BMI 28.0±1.01 OB: BMI 32.5±1.10	Randomized; crossover; weight maintenance; 28-d, 28-d washout, 28-d	Biomarkers of oxidative and inflammatory stress	3 servings/d with 1200-1400 mg Ca/d	Soy-based placebo with 500-600 mg Ca/d	(neutral) IL-15 (+) adiponectin (-) malondialdehyde, 8-isoprostane-$F_{2\alpha}$, TNF-α, IL-6, CRP, MCP-1 (treatment order effect)
Van Loan et al., 2011	71 OW/OB % Body fat 43.1±6.9	Randomized; parallel; energy-restricted; 3-wk run-in, 12-wk	Body weight; body fat; intra-abdominal adipose tissue	3-4 servings/d (low- or reduced-fat to full fat) with ~1339 mg Ca/d	≤1 serving/d with ≤600 mg Ca/d	(neutral) transcript abundance: TNF-α, IL-6, MCP-1
Zemel and Sun, 2008	34 OB Yogurt: 27.8% male BMI 32.1±0.4 Control: 12.5% male BMI 33.2±0.9	Randomized; parallel; energy-restricted; 2-wk lead-in, 12-wk	Body weight; body fat	3 servings/d of fat-free yogurt (6 oz) with 500-1100 mg Ca/d	≤1 serving/d with 400-500 mg Ca/d	(+) adiponectin (-) CRP
Stancliffe et al., 2011	40 OW/OB with MetS 47.5% male BMI 30.1±4.4	Randomized; parallel; weight maintenance; 84-d	Biomarkers of oxidative and inflammatory stress	≥3.5 servings/d with ≥1000 mg Ca/d	≤0.5 servings/d with ≤600 mg Ca/d	(+) adiponectin (-) malondialdehyde, oxidized LDL, TNF-α, MCP-1, IL-6, CRP
Wennersberg et al., 2009	113 OW with MetS 32.7% male Milk: BMI 30.1±3.6 Control: BMI 30.0±3.3	Randomized; parallel; 6-mo	Waist circumference; body mass; body fat	3-5 servings/d	≤2 servings/d	(neutral) IL-6, hs-CRP, TNF-α, C3, C4, PAI-1, 8-isoprostane-$F_{2\alpha}$, adiponectin (+) leptin (females)

OW: overweight; OB: obese; BMI: body mass index; hs-CRP: high-sensitivity C-reactive protein; PAI-1: plasminogen activator inhibitor-1; IL: interleukin; TNF-α: tumor necrosis factor-α; s-TNFR-1: soluble TNF receptor; MCP-1: monocyte chemotactic protein-1; ICAM-1: intercellular adhesion molecule-1; VCAM-1: vascular cell adhesion molecule-1; Ca: calcium; MetS: metabolic syndrome; LDL: low-density lipoprotein; C3: C3 complement; C4: C4 complement.

Quality Criteria Questions	Crichton 2012	van Meijl 2010	van Meijl 2011	Stancliffe 2011	Van Loan 2011	Wennersberg 2009	Zemel 2008	Zemel 2010
1. Was the research question clearly stated?	Y	N	N	Y	Y	Y	Y	N
2. Was the selection of study subjects/patients free from bias?	Y	Y	Y	Y	Y	Y	N	Y
2.1 Were inclusion/exclusion criteria specified?	Y	Y	Y	Y	Y	Y	N	Y
2.2 Were criteria applied equally to all study groups?	Y	Y	Y	Y	Y	Y	Y	Y
2.3 Were health, demographics, and other characteristics of subjects described?	N	N	N	Y	Y	N	N	Y
2.4 Were the subjects/participants a representative sample of the relevant population?	Y	Y	Y	Y	Y	Y	Y	Y
3. Were study groups comparable?	Y	Y	Y	Y	Y	Y	Y	Y
4. Was method of handling withdrawals described?	Y	Y	Y	N/A	N	Y	N	N/A
4.1 Were follow up methods described and the same for all groups?	Y	Y	Y	Y	Y	Y	N	Y
4.3 Were all enrolled subjects/patients (in the original sample) accounted for?	Y	Y	Y	Y	Y	Y	U	Y
4.4 Were reasons for withdrawals similar across groups?	U	U	U	N/A	U	U	U	N/A
5. Was blinding used to prevent introduction of bias?	N	N	N	N	N	N	N	N
5.1 In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	N	N	N	N	N	N	N	N
5.2 Were data collectors blinded for outcomes assessment?	N	N	N	N	N	N	N	N
6. Were interventions/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were intervention factors described?	Y	Y	Y	Y	Y	Y	Y	Y
6.1 In RCT or other intervention trial, were protocols described for all regimens studied?	Y	Y	Y	Y	Y	Y	Y	Y
6.3 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Y	Y	Y	Y	Y	Y	Y	Y
6.4 Was the amount of exposure and, if relevant, subject/patient compliance measured?	Y	Y	Y	Y	Y	Y	Y	Y
7. Were outcomes clearly defined and the	Y	Y	Y	Y	Y	Y	Y	Y

measurements valid and reliable?								
7.1 Were primary and secondary endpoints described and relevant?	Y	Y	N	Y	Y	Y	N	Y
7.2 Were nutrition measures appropriate to question and outcomes of concern?	Y	Y	Y	Y	Y	Y	Y	Y
7.3 Was the period of follow-up long enough for important outcome(s) to occur?	Y	Y	Y	Y	Y	Y	Y	Y
7.4 Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Y	Y	Y	Y	Y	Y	Y	Y
7.5 Was the measurement of effect at an appropriate level of precision?	N	Y	Y	Y	Y	Y	Y	Y
7.6 Were other factors accounted for (measured) that could affect outcomes?	Y	Y	Y	Y	Y	Y	N	Y
7.7 Were the measurements conducted consistently across groups?	Y	Y	Y	Y	Y	Y	Y	Y
8. Was the statistical analysis appropriate for the study design and type of outcome indicators?	Y	Y	Y	Y	Y	Y	Y	Y
8.1 Were statistical analyses adequately described, results reported appropriately?	Y	Y	Y	Y	Y	Y	Y	Y
8.2 Were correct statistical tests used and assumptions of test not violated?	Y	Y	Y	Y	Y	Y	Y	Y
8.3 Were statistics reported with levels of significance and/or confidence intervals?	Y	Y	Y	Y	Y	Y	Y	Y
8.5 Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Y	Y	Y	Y	Y	Y	N	Y
8.6 Was clinical significance as well as statistical significance reported?	N	N	N	N	N	N	N	N
8.7 If negative findings, was a power calculation reported to address type 2 error?	Y	Y	N	Y	Y	Y	N	N
9. Are conclusions supported by results with biases and limitations taken into consideration?	Y	Y	Y	Y	Y	Y	Y	Y
10. Is bias due to study's funding or sponsorship unlikely?	Y	Y	Y	Y	Y	Y	Y	N
NEGATIVE = If>6 "No" NEUTRAL = If 6 "No" PLUS = Most "Yes" including criteria 2, 3, 6, 7, and at least one additional "Yes" Y = Yes N = No U = Unknown	+	+	+	+	+	+	0	+

Figure **2-1S**. Quality control checklist for RCTs examining how dairy intake influences inflammatory and oxidative stress in overweight and obese participants.

Chapter 3

METHODS

Participants

Twenty-three overweight or obese [body mass index (BMI) ≥ 27 to ≤ 33 kg/m^2], but otherwise healthy weight-stable males (18-55 years) and females (18-45 years) completed the study. Data were collected from November 2011, to April 2013, at the main campus of The Pennsylvania State University (University Park, PA). Participants were recruited by word-of-mouth, in-class announcements, newspaper advertisements, and posted flyers. Initial inclusion criteria included appropriate age and self-reported BMI; self-reported weight stability [i.e., weight change ≤ 5% of body weight (BW)] over the past 6 months; moderate physical activity level (i.e., ≤ 150 minutes of planned physical activity/wk); absence of dairy or soy aversion, intolerance, or allergy or lactose-intolerance; lack of habitually high soy consumption; limited consumption of caffeinated beverages (i.e., ≤ 16 ounces/d); daily alcoholic beverage intake of less than 2 drinks in males and 1 drink in females (i.e., 1 alcoholic beverage= 12 fluid ounces regular beer, or 5 fluid ounces wine, or 1.5 fluid ounces 80-proof distilled liquor); absence of use of steroids, bisphosphonates, anticonvulsants, and glucocorticoids; and in females, eumenorrhea (21- to 35-d menstrual cycle).

Interested participants telephoned or emailed study personnel for a general synopsis of the study (n= 431). Study personnel verbally conveyed the objective, requirements, expectations, risks, and benefits of the study. Individuals expressing continued interest (n= 43) were provided with an Informed Consent Form for informational purposes and a Health Care Provider Clearance Form that was completed by a health care provider to determine if the potential participant were of adequate health to participate in the study (Fig. 3-1). Eight participants did not return the health care provider form and withdrew due to lack of medical clearance, and ten people returned the health care provider form but declined continued

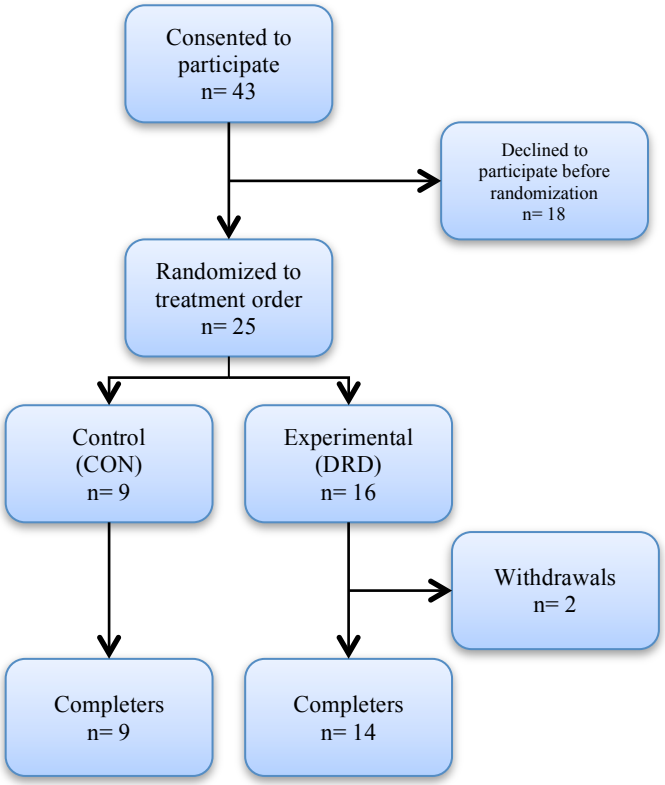

Figure **3-1**. Consolidated standards of reporting trials for consenting participants.

CON= control; DRD= dairy-rich diet.

participation in the study. Study personnel reviewed returned and completed screening forms to determine eligibility (criteria listed above) for randomization (n= 25). Exclusion criteria included a BMI or < 27 or > 33 kg/m^2; weight instability during the last 6 months; unwillingness to maintain weight; gastric bypass surgery; use of weight loss of medications or supplements in past 6 months; metabolic disorders including type 1 or type 2 diabetes, metabolic syndrome, thyroid disorders or thyroid hormones, gastrointestinal disorders including Crohn's disease or irritable bowel syndrome; impaired renal function; diagnosed osteopenia and osteoporosis; periodontal disease; eating disorders; current cigarette smoking or use of tobacco products; hypertension or hypercholesterolemia; and in women, hysterectomy or ovariectomy without hormone replacement; lactating, pregnant, or attempting to become pregnant, and initiation or change in use of oral contraceptive or hormone replacement therapy within the past 2 years.

There were no differences in BW or BMI among randomized participants who withdrew from or completed the study (n= 2; n= 23). Since there were not any withdrawals among participants randomly assigned to complete the CON diet first, comparisons of non-completers by treatment order were not possible. Time constraints, missed appointments, and unwillingness to comply with BW and BMI maintenance and/or DRD and CON diet treatments were the primary reasons for the individuals who began a diet treatment to drop out (n= 2). A total of 23 participants completed the study. This study was approved by the Institutional Review Board for Research Involving Human Subjects at The Pennsylvania State University. Each participant provided written informed consent before completion of any study procedure.

Design

The study utilized a randomized crossover design with repeated measures within participants to deliver dairy-rich (DRD) and non-dairy control (CON) treatments. Enrolled participants were assigned a code number, stratified by sex, age, BMI, and randomized to treatment order. Randomized participants (n= 26) were scheduled for obtainment of smoothies (3-5 pickup dates/wk) and data collection dates (baseline, day 10, day 30 of each diet phase). For the weight-maintaining diet treatments, participants consumed either 3 dairy smoothies (DRD, total calcium intake= ~1,300 mg/d) or 3 soy-based smoothies (CON, total calcium intake= ~550 mg/d) per day for 30 d. Then, after a 30-d washout period, individuals crossed over to the other treatment arm. Testing sessions at baseline, day 10, and day 30 of each dietary period (total of 6 testing dates) included measurement of anthropometric and body composition indices and a fasting blood draw.

Diets

Two diet treatments in the form of smoothies (dairy or non-dairy) were consumed 3 times per day throughout each 30-d treatment period. Smoothies were incorporated into the total daily caloric allotment necessary for each individual to maintain his/her body weight. Each smoothie provided approximately 170 kcal, 30 g of carbohydrate, 10 g of protein, and 1 g of dietary fat such that total daily intake from smoothies was 510 kcal, 90 g of carbohydrate, 30 g of protein, and 3 g of dietary fat. In the DRD treatment, each dairy smoothie included 28 g of nonfat dry milk powder and provided 350 mg of calcium (total calcium intake from dairy smoothies= 1,050 mg/d). Each soy smoothie in the CON treatment included 11.5 g of soy protein isolate powder and provided 50 mg of calcium (total calcium intake from soy-based smoothies= 150 mg/d). DRD and CON smoothies provided ~8.3 mmol of antioxidants (total antioxidant intake from smoothies= ~25 mmol/d). All smoothies were prepared from different flavored (e.g., blueberry banana, strawberry, etc.) recipes. Both dairy-based and soy-based smoothies included fruit and/or juice blend, water when indicated, Splenda (non-nutritive sweetener), and flavoring when indicated to produce 16 ounces of beverage when blended on high speed for 3-5 minutes or until the desired consistency was achieved. Smoothies were packaged in plastic 20-ounce bottles. Participants collected smoothies from study personnel daily or every other day throughout the week and picked up weekend allotments on Fridays. Three smoothies per day were consumed throughout the day at breakfast, lunch, and dinner or as a snack.

Each participant also consumed a weight-maintaining diet with macronutrient composition and fiber intake consistent with pre-study habits. BW was measured daily or every other day on weekdays to monitor for energy balance. The Mifflin-St. Jeor equation was used to determine energy needs for weight maintenance. Dietary compliance was monitored by daily weekday measurements of BW, return of empty smoothie bottles, and completion of all questionnaires. Study personnel counseled participants to adjust the background diet as necessary to maintain BW consistent with pre-study measurements.

Anthropometric and body composition measurements

Body height (cm) was measured at the beginning of the study with a calibrated scale-mounted stadiometer (Seca 700, Hanover, MD) to the nearest 0.1 cm. At each of 6 intervals (baseline, day 10, day 30) throughout the study, BW (kg) was measured using a calibrated balance-beam scale (Tanita, 410GS, Arlington Heights, IL) to the nearest 0.1 kg and BMI was calculated as BW/height (kg/m^2). Fat mass (FM) (kg, to the nearest 0.1 kg) and body fat percentage (BF%) (to the nearest 0.1%) were measured with a foot-to-foot bioelectrical impedance analyzer (TBF-410GS, Arlington Heights, IL). Participants wore lightweight clothing and were shoeless during these readings, and they were instructed to empty the bladder, avoid strenuous exercise and food, caffeine, and alcohol intake, and maintain normal hydration before each of the Tanita scale measurements. Waist circumference (WC) was measured (cm) standing erect using a retractable measuring tape. Two measurements were taken and averaged if the measurements were within 1 cm of another (33).

Blood draw and biochemical markers

On the morning of each testing date (between 0700 and 0900) after a fast of at least 10 hours but not exceeding 12 hours, fasting venous blood samples were collected from participants. A registered nurse drew approximately 40 mL of whole blood into serum tubes and plasma tubes treated with EDTA. Serum samples were allowed to clot at room temperature for 30 minutes before they were spun in a 4°C centrifuge at 839 *x g* for 12 minutes. Plasma samples were immediately handled and spun in a 22°C centrifuge at 1207 *x g* for 10 minutes. Samples were then aliquotted into cryovials and stored at -80°C until bioassays were completed.

Enzyme-linked immunosorbent assays (ELISA) were used to measure concentrations of serum CRP (ng/mL) (ALPCO Diagnostics, Salem, NH) and plasma TNF-α (pg/mL) and IL-6 (pg/mL) (R&D

Systems Inc., Minneapolis, MN). ELISA were used to measure serum adiponectin (ng/mL), resistin (ng/mL), and leptin (ng/mL) (R&D Systems Inc., Minneapolis, MN). Samples were run in duplicate, and duplicates with a coefficient of variation greater than 25% were run again. Intra-assay variation ranged from 2.4% for leptin to 14.6% for IL-6.

Statistical analyses

Descriptive statistics were applied to characterize the total sample population. Paired t-tests were used to verify lack of differences in measures of BW, WC, BMI, FM, and BF% between baseline DRD and baseline CON; baseline DRD and day 30 DRD; and baseline CON and day 30 CON. The Shapiro-Wilkes test statistic was computed to assess for normality of distributions of biomarkers (DRD and CON) at baseline. Homogeneity of variance in outcome measures was assessed with Levene's test. Data were log-transformed for skewed distributions, and outliers >3 standard deviations from the mean were removed from distributions of biomarkers at baseline (34). Multivariate normality was checked using Mahalanobis distances (35). A multivariate (treatment order, diet treatment, baseline values for biomarker) analysis of covariance with repeated measures on the time axis (baseline, day 10, day 30) was used to test for differences in concentrations of the 6 inflammatory biomarkers between diet treatments (DRD, CON). For each analysis, Box's test of equality of covariance matrices was used to verify that the covariance matrices of the dependent variable were equal across groups (36). A probability of $p < 0.05$ was considered significant. Statistical analyses were conducted using IBM SPSS Statistics for Windows, version 19.0 (IBM Corp., Armonk, NY, USA).

Chapter 4

RESULTS

A total of 23 participants (11 males, 12 females) completed the study. Of those who completed the study, 60.9% (n= 14) were randomly assigned to start with the DRD treatment arm and cross over to the CON diet treatment. Participants were overweight or obese (BMI= 29.3 ± 1.8 kg/m^2) and weighed an average of 85.1 ± 10.5 kg (Table 4-1). Participant anthropometric and body composition measurements and other characteristics are summarized in Table 4-2.

Maintenance of participant BW, WC, BMI, FM, and BF% were included as study design elements to minimize the influence of changes in adiposity on the effects of dairy in modulating inflammation. Paired t-tests were used to verify consistency in BW, WC, BMI, FM, and BF% between baseline DRD and baseline CON, baseline DRD and day 30 DRD, and baseline CON and day 30 CON (Table 4-3). BW, WC, BMI, FM, and BF% did not change throughout the study (baseline DRD and baseline CON), during the DRD treatment (baseline DRD and day 30 DRD), and during the non-dairy control treatment (baseline CON and day 30 control).

After log transformation and removal of outliers, distributions of biomarker concentrations at baseline for the DRD and CON treatments were normally distributed and had homogeneity of variance (Table 4-4). Multivariate normality was verified by a maximum Mahalanobis distance less than the critical value (df= 6, critical value= 22.46) (35).

Table **4-1**. Summary characteristics of study completers at study start date.

Variable	Mean ± st dev (n= 23)	Range (minimum – maximum)
Male (%)	48%	
Age (y)	26.3 ± 7.3	20 – 45
Height (cm)	170.3 ± 8.6	146.8 – 188.0
BW (kg)	85.14 ± 10.54	60.7 – 104.2
BMI (kg/m^2)	29.27 ± 1.76	26.3 – 32.7

BW= body weight; BMI= body mass index.

Table **4-2**. Mean ± st dev anthropometric and body composition measurements of study completers at baseline of DRD and CON diet treatments.

Measurement	Baseline dairy-rich diet (DRD) (n= 23)	Baseline non-dairy control diet (CON) (n= 23)
BW (kg)	85.1 ± 10.7	85.2 ± 10.0
WC (cm)	95.8 ± 6.9	95.9 ± 5.4
BMI (kg/m^2)	29.3 ± 1.9	29.3 ± 1.6
FM (kg)	26.2 ± 6.7	26.5 ± 6.8
BF (%)	31.1 ± 7.6	31.4 ± 8.0

BW= body weight; WC= waist circumference; BMI= body mass index; FM= fat mass; BF= body fat.

Table **4-3**. Results of paired t-tests for measurements of adiposity.

	Baseline DRD and Baseline CON		Baseline DRD and Day 30 DRD		Baseline CON and Day 30 CON	
Measurement	t	*p*-value	t	*p*-value	t	*p*-value
BW (kg)	-0.204	0.841	0.506	0.618	0.198	0.845
WC (cm)	-0.229	0.821	-1.529	0.141	-0.858	0.400
BMI (kg/m^2)	-0.252	0.803	0.649	0.523	0.077	0.939
FM (kg)	-0.756	0.458	-0.102	0.919	-0.048	0.962
BF (%)	-0.783	0.442	-0.323	0.750	-0.151	0.881

DRD= dairy-rich diet; CON= non-dairy control diet; BW= body weight; WC= waist circumference; BMI= body mass index; FM= fat mass; BF= body fat; n= 23; df= 22; results not significant at $p< 0.05$.

Concentrations of baseline biomarkers did not differ between the DRD and CON diet treatments, except for a higher concentration of plasma IL-6 at the start of the DRD (t= 2.282, df= 21, p= 0.033) Table 4-5. Biomarker concentrations at baseline DRD and baseline CON were included in the final model. The effects of diet treatments on inflammatory biomarkers on each testing date are summarized in Table 4-6.

Percent change in biomarker concentration throughout each diet treatment overall and separated by treatment order is listed in Table 4-7. For all biomarkers, change in biomarker concentration over time was not explained by diet treatment.

Variations in serum concentration of CRP were not explained by diet treatment $F(1, 20)= 0.084$, $p= 0.775$ or by the interaction of diet treatment*time $F(2,19)= 1.491$, $p= 0.250$. There were no treatment order effects for CRP outcomes.

Table **4-4**. Distribution characteristics of biomarker values at baseline of DRD and CON diet treatments.

		Shapiro-Wilkes			Mahalanobis maximum distance	Levene's test		
Biomarker	Diet	W	df	*p*-value		*F*	df	*p*-value
CRP	DRD	0.979	21	0.915	3.790	3.530	(1, 20)	0.075
	CON	0.977	21	0.878	4.492	3.471	(1, 20)	0.077
TNF-α	DRD	0.959	21	0.488	5.128	1.698	(1, 20)	0.207
	CON	0.930	21	0.139	6.204	0.385	(1, 20)	0.542
IL-6	DRD	0.965	21	0.631	6.533	2.150	(1, 21)	0.157
	CON	0.941	21	0.229	4.303	0.923	(1, 21)	0.348
Adiponectin	DRD	0.937	21	0.192	7.930	0.996	(1, 21)	0.330
	CON	0.935	21	0.177	5.799	0.000	(1, 21)	0.999
Leptin	DRD	0.937	21	0.194	7.723	0.284	(1, 21)	0.600
	CON	0.949	21	0.328	8.068	0.002	(1, 21)	0.967
Resistin	DRD	0.934	21	0.162	3.205	0.101	(1, 21)	0.754
	CON	0.937	21	0.186	5.435	1.068	(1, 21)	0.313

DRD= dairy-rich diet; CON= non-dairy control diet; CRP= C-reactive protein; TNF-α= tumor necrosis factor-α; IL-6= interleukin-6; results not significant at $p < 0.05$.

The DRD and CON treatments did not affect plasma levels of TNF-α $F(1, 20) = 0.283$, $p = 0.601$, and plasma concentrations of TNF-α did not change over time based on diet treatment $F(2, 19) = 0.126$, $p = 0.882$. The interaction of diet treatment and treatment order explained significant

Table **4-5**. Results of paired t-tests for concentrations of baseline biomarkers at the start of the DRD and CON diet treatments.

Biomarker	Concentration at baseline DRD	Concentration at baseline CON	t	*p*-value
CRP	889.6 ± 4.1 (ng/mL)	906.8 ± 4.4 (ng/mL)	-0.082	0.935
TNF-α	1.15 ± 1.58 (pg/mL)	1.18 ± 1.48 (pg/mL)	-0.449	0.658
IL-6	1.95 ± 1.49 (pg/mL)	1.60 ± 1.66 (pg/mL)	2.282	0.033*
Adiponectin	4020.6 ± 2.3 (ng/mL)	4589.1 ± 2.0 (ng/mL)	-1.452	0.161
Leptin	13.82 ± 3.11 (ng/mL)	13.34 ± 2.45 (ng/mL)	0.328	0.746
Resistin	7.70 ± 1.56 (ng/mL)	7.05 ± 1.44 (ng/mL)	1.323	0.199

DRD= dairy-rich diet; CON= non-dairy control diet; values reported as mean ± st dev; df= 21; *$p< 0.05$ is significant.

variation in concentrations of TNF-α [$F(2, 20)= 13.117$, $p= 0.002$], as did the interaction of diet treatment and treatment order and time [$F(2, 19)= 8.889$, $p= 0.002$] (Fig. 4-1). Starting with the DRD treatment and crossing over to CON resulted in increasing concentrations of TNF-α through the course of the DRD and CON diets, whereas beginning with the CON treatment and crossing over to the DRD diet yielded decreasing concentrations of TNF-α through the DRD diet and increasing concentrations of TNF-α through the CON diet.

IL-6 did not vary by treatment $F(1, 21)= 0.005$, $p= 0.946$ or by treatment over time $F(2, 20)= 2.921$, $p= 0.077$. However, there were significant interactions of diet treatment*treatment order [$F(1, 21)= 10.585$, $p= 0.004$] and diet treatment*treatment order*time [$F(2, 20)= 10.732$, $p= 0.001$] (Fig. 4-2). Similar to the pattern seen with TNF-α, starting with the CON diet treatment yielded decreasing concentrations of IL-6 over the course of the DRD treatment and

Table **4-6**. Concentrations of inflammatory biomarkers in overweight and obese participants at baseline, day 10, and day 30 in a crossover study examining changes in the inflammatory profile with weight-maintaining dairy-rich (DRD) or control (CON) diets.

Biomarker	Diet	Baseline	Day 10	Day 30	*p*-value
C-reactive protein (ng/mL)	DRD	889.6 ± 4.1	648.0 ± 8.2	1497.0 ± 4.5	Diet= 0.775
	CON	906.8 ± 4.4	1129.0 ± 4.6	678.6 ± 30.0	Time= 0.885
					D x T= 0.250
					D x O= 0.932
					D x O x T= 0.965
Tumor necrosis factor-α (pg/mL)	DRD	1.15 ± 1.58	1.26 ± 1.63	1.28 ± 1.68	Diet= 0.601
	CON	1.18 ± 1.48	1.27 ± 1.61	1.23 ± 1.54	Time= 0.093
					D x T= 0.882
					D x O= 0.002*
					D x O x T= 0.002*
Interleukin-6 (pg/mL)	DRD	1.95 ± 1.49	1.62 ± 1.78	1.66 ± 2.56	Diet= 0.946
	CON	1.60 ± 1.66	1.74 ± 2.06	1.59 ± 2.24	Time= 0.677
					D x T= 0.077
					D x O= 0.004*
					D x O x T= 0.001*
Adiponectin (ng/mL)	DRD	4020.6 ± 2.3	4040.2 ± 2.4	4339.8 ± 2.4	Diet= 0.496
	CON	4589.1 ± 2.0	4414.0 ± 2.0	4648.9 ± 2.0	Time= 0.129
					D x T= 0.748
					D x O< 0.001*
					D x O x T= 0.012*
Leptin (ng/mL)	DRD	13.82 ± 3.11	12.86 ± 3.08	15.19 ± 2.53	Diet= 0.138
	CON	13.34 ± 2.42	11.33 ± 2.92	13.78 ± 3.38	**Time= 0.027***
					D x T= 0.677
					D x O= 0.750
					D x O x T= 0.668
Resistin (ng/mL)	DRD	7.70 ± 1.56	7.19 ± 1.46	7.52 ± 1.40	Diet= 0.442
	CON	7.05 ± 1.44	6.43 ± 1.82	7.00 ± 1.93	Time= 0.203
					D x T= 0.985
					D x O< 0.001*
					D x O x T= 0.792

D= diet treatment; T= time; O= treatment order; D x T= diet treatment*time interaction; D x O= diet treatment*treatment order interaction; D x O x T= diet treatment*treatment order*time interaction. For each biomarker at each timepoint, n= 23 except for CRP (n= 22) and TNF-α (n= 22); **p*< 0.05 is significant.

Table **4-7**. Summary of changes in concentrations of inflammatory biomarkers throughout the DRD and CON diet treatments (baseline to day 30, n= 23) split by treatment order (n= 14 Phase 1 DRD, n= 9 Phase 1 CON).

Biomarker	Total sample (n= 23[a])		Treatment order (n= 14[a])		Treatment order (n= 9)	
	DRD	CON	Phase 1: DRD	Phase 2: CON	Phase 2: DRD	Phase 1: CON
CRP	↑ 68.3%	↓ 25.2%	↑27.5%	↓ 39.2%	↑ 115.8%	↓ 15.8%
TNF-α	↑ 10.9%	↑ 4.3%	↑ 31.0%	↑ 9.2%	↓ 12.1%	↑ 16.9%
IL-6	↓ 15.2%	↓ 1.0%	↑ 12.1%	↓ 27.1%	↓ 45.0%	↑ 59.5%
Adiponectin	↑ 7.9%	↑ 1.3%	↑ 15.8%	↓ 5.5%	↓ 3.2%	↑ 12.9%
Leptin	↑ 9.9%	↑ 3.3%	↑ 2.3%	↓ 0.6%	↑ 23.0%	↑ 9.6%
Resistin	↓ 2.3%	↓ 1.0%	↓ 9.3%	↓ 4.4%	↑ 9.8%	↑ 5.4%

DRD= dairy-rich diet; CON= non-dairy control diet; CRP= C-reactive protein; TNF-α= tumor necrosis factor-α; IL-6= interleukin-6; [a]For TNF-α and CRP total sample n= 22, n= 13 Phase 1 DRD, n= 9 Phase 1 CON; results not significant at $p< 0.05$.

increasing concentrations over the course of the CON treatment. However with IL-6, participants who began with the DRD diet treatment had increases in this pro-inflammatory biomarker during the DRD treatment and decreases during the CON treatment.

Serum concentration of adiponectin did not differ by diet treatment [$F(1, 21)= 0.480$, $p= 0.496$], and there was not a significant interaction of diet treatment*time [$F(2, 20)= 0.294$, $p= 0.748$]. Treatment order interactions were significant for diet treatment*treatment order [$F(1, 21)= 19.375$, $p< 0.001$] and diet treatment*time*treatment order [$F(2, 20)= 5.566$, $p= 0.012$] (Fig. 4-3). When participants were randomly assigned to begin with the DRD diet treatment, serum concentrations of adiponectin decreased throughout the DRD treatment and increased during the

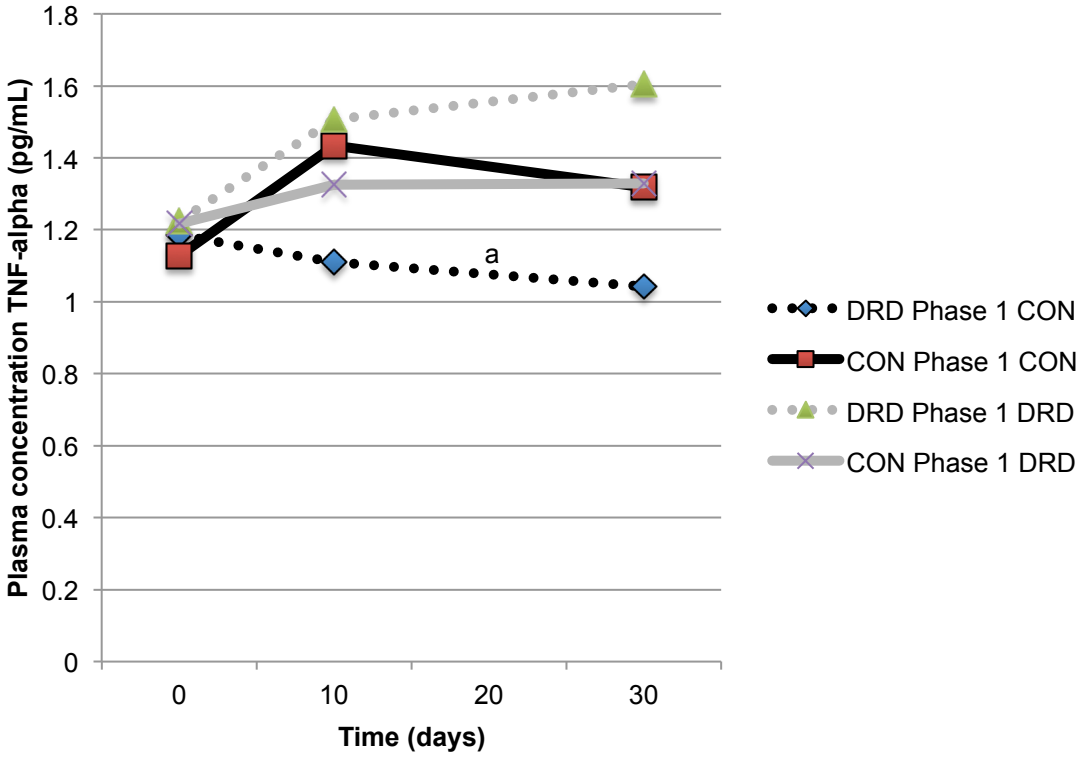

Figure **4-1**. Plasma concentrations of TNF-α (pg/mL) over time throughout the 30-day DRD and CON diet treatments split by treatment order (Phase 1 CON, Phase 1 DRD).

[a]different from DRD Phase 1 DRD and DRD Phase 1 DRD over time; [b]different from CON Phase 1 DRD and CON Phase 1 DRD over time, using $p < 0.05$ for statistical significance; *p*-values from multivariate analysis of covariance with repeated measures on the time factor. Diet treatment x treatment order $p = 0.002$. Diet treatment x treatment order x time $p = 0.002$. At all Phase 1 CON timepoints n= 9, baseline CON Phase 1 DRD n= 13, all other Phase 1 DRD timepoints n= 14.

course of the CON treatment. Those that started with the CON diet treatment had increases in serum adiponectin through the DRD treatment and decreases in serum adiponectin over the course of the CON treatment.

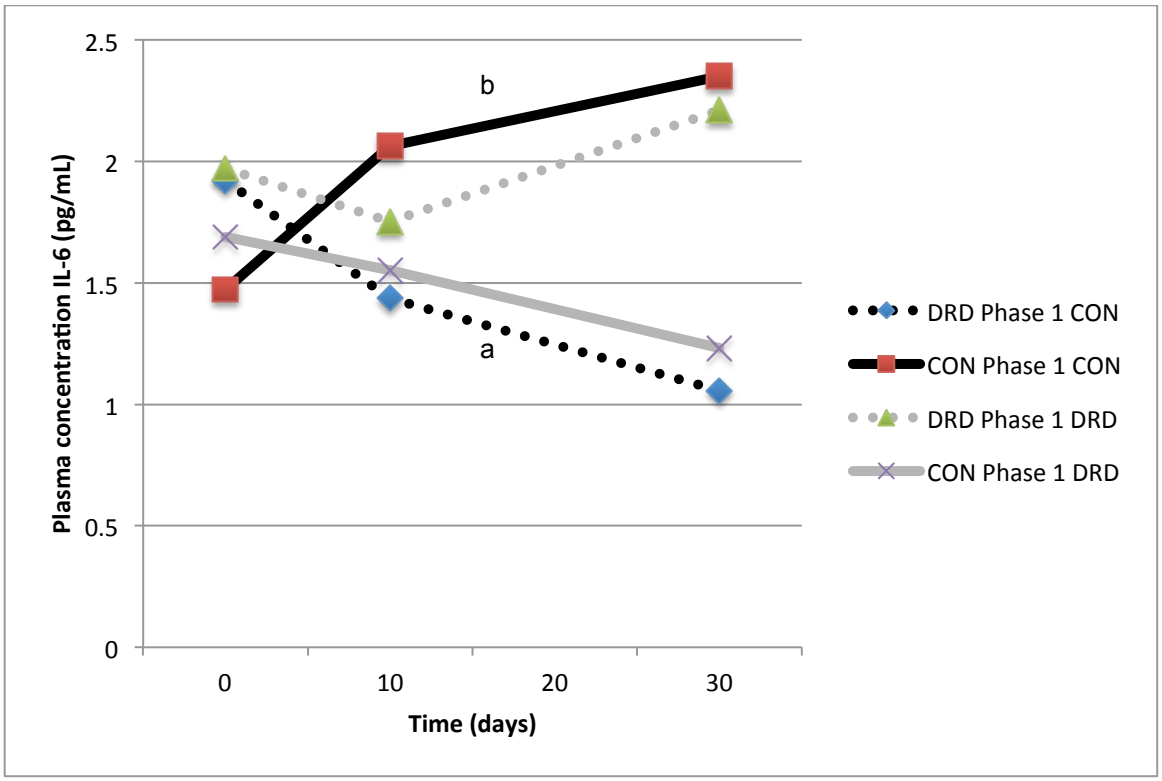

Figure **4-2**. Plasma concentrations of IL-6 (pg/mL) over time throughout the 30-day DRD and CON diet treatments split by treatment order (Phase 1 CON, Phase 1 DRD).

[a]different from DRD Phase 1 DRD and DRD Phase 1 DRD over time; [b]different from CON Phase 1 DRD and CON Phase 1 DRD over time, using $p < 0.05$ for statistical significance; *p*-values from multivariate analysis of covariance with repeated measures on the time factor. Diet treatment x treatment order $p = 0.02$. Diet treatment x treatment order x time $p = 0.001$. At all timepoints, n= 9 Phase 1 CON, n= 14 Phase 1 DRD.

There was a significant effect of time on serum concentrations of leptin $F(2, 20) = 4.338$, $p = 0.027$. Polynomial contrasts were performed comparing all time points, and there was a quadratic relationship of leptin outcomes over time ($F = 6.791$, $p = 0.016$). Diet treatment [$F(1,$

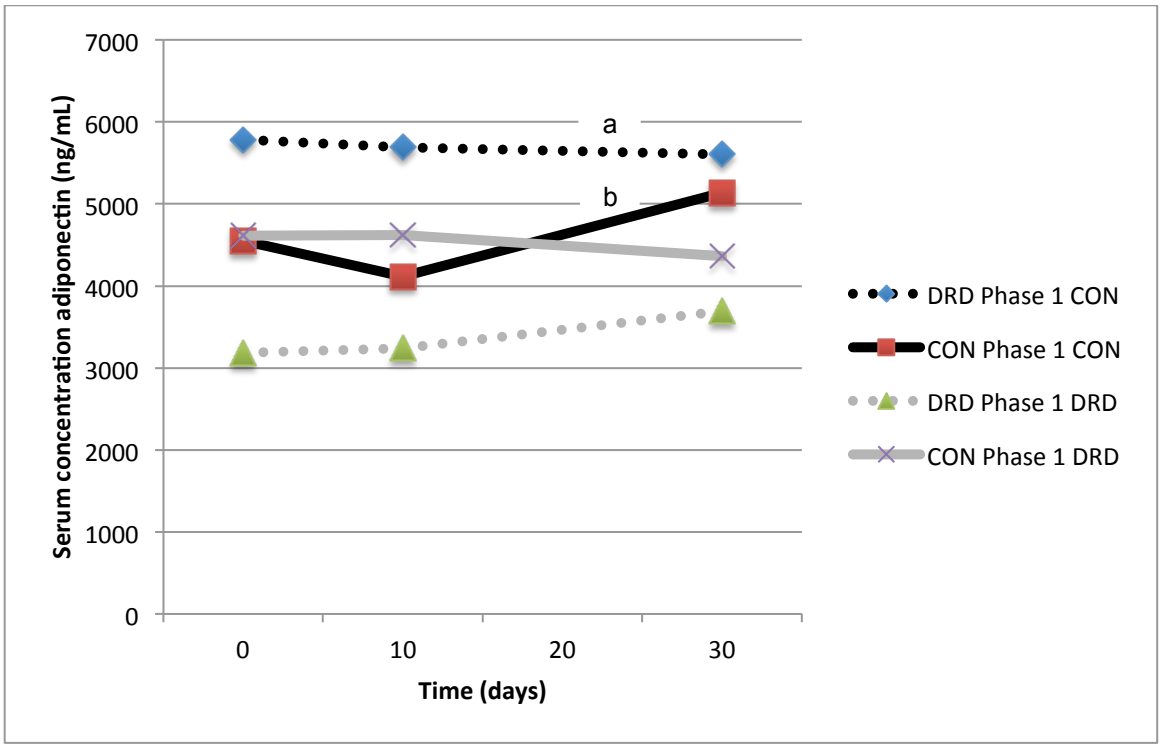

Figure **4-3**. Plasma concentrations of adiponectin (ng/mL) over time throughout the 30-day DRD and CON diet treatments split by treatment order (Phase 1 CON, Phase 1 DRD). [a]different from DRD Phase 1 DRD and DRD Phase 1 DRD over time; [b]different from CON Phase 1 DRD and CON Phase 1 DRD over time, using $p< 0.05$ for statistical significance; *p*-values from multivariate analysis of covariance with repeated measures on the time factor. Diet treatment x treatment order $p< 0.001$. Diet treatment x treatment order x time $p= 0.012$. At all timepoints, n= 9 Phase 1 CON, n= 14 Phase 1 DRD.

21)= 2.378, $p= 0.138$] or diet treatment by time [$F(2, 20)= 0.398$, $p= 0.677$] did not explain variation in serum concentrations of leptin (Fig. 4-4).

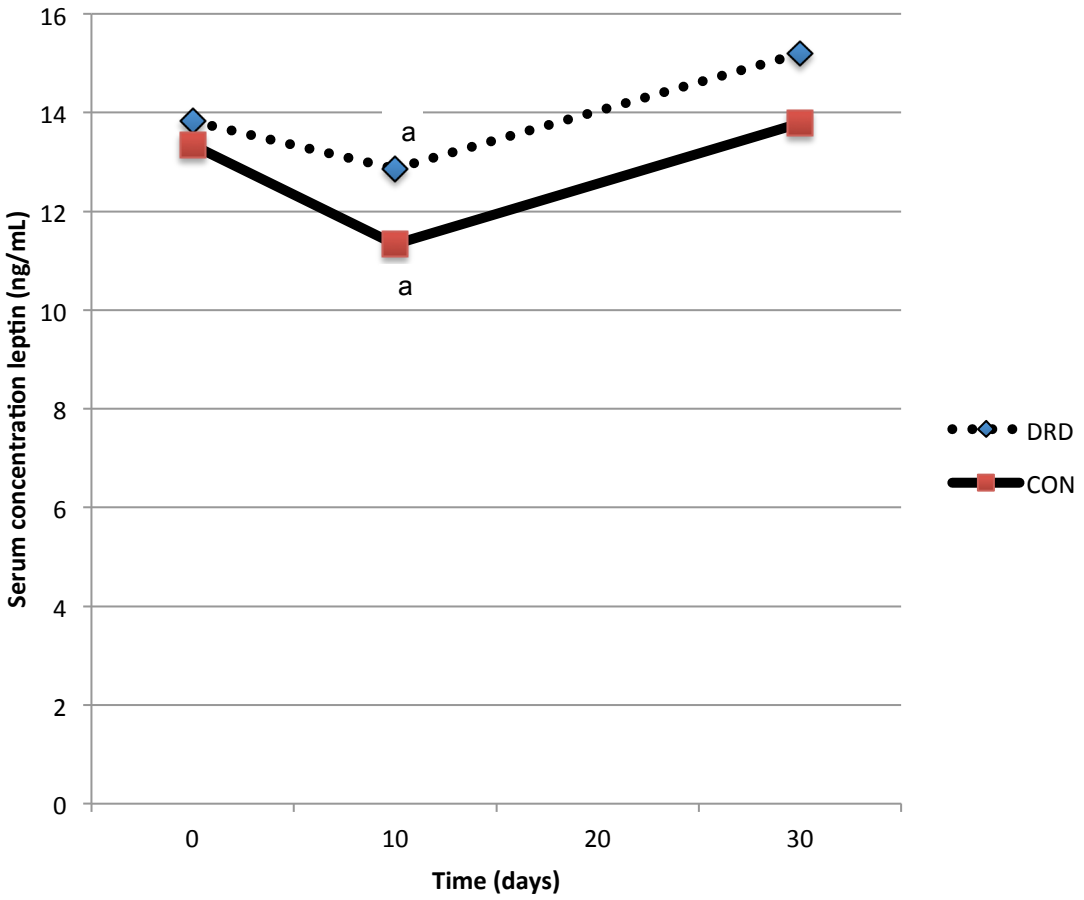

Figure **4-4**. Plasma concentrations of leptin (ng/mL) over time throughout the 30-day DRD and CON diet treatments.

[a]different from day 30, using $p < 0.05$ for statistical significance; p-values from multivariate analysis of covariance with repeated measures on the time factor. Main effect of time $p = 0.027$. Diet treatment x time $p = 0.677$. At all timepoints, n= 23.

Resistin concentrations in serum did not vary by diet treatment $F(1, 21) = 0.613$, $p = 0.442$ or by the interaction term diet treatment*time $F(2, 20) = 0.015$, $p = 0.985$. There was a significant interaction of diet treatment by treatment order [$F(1, 21) = 18.011$, $p < 0.001$] (Fig. 4-5).

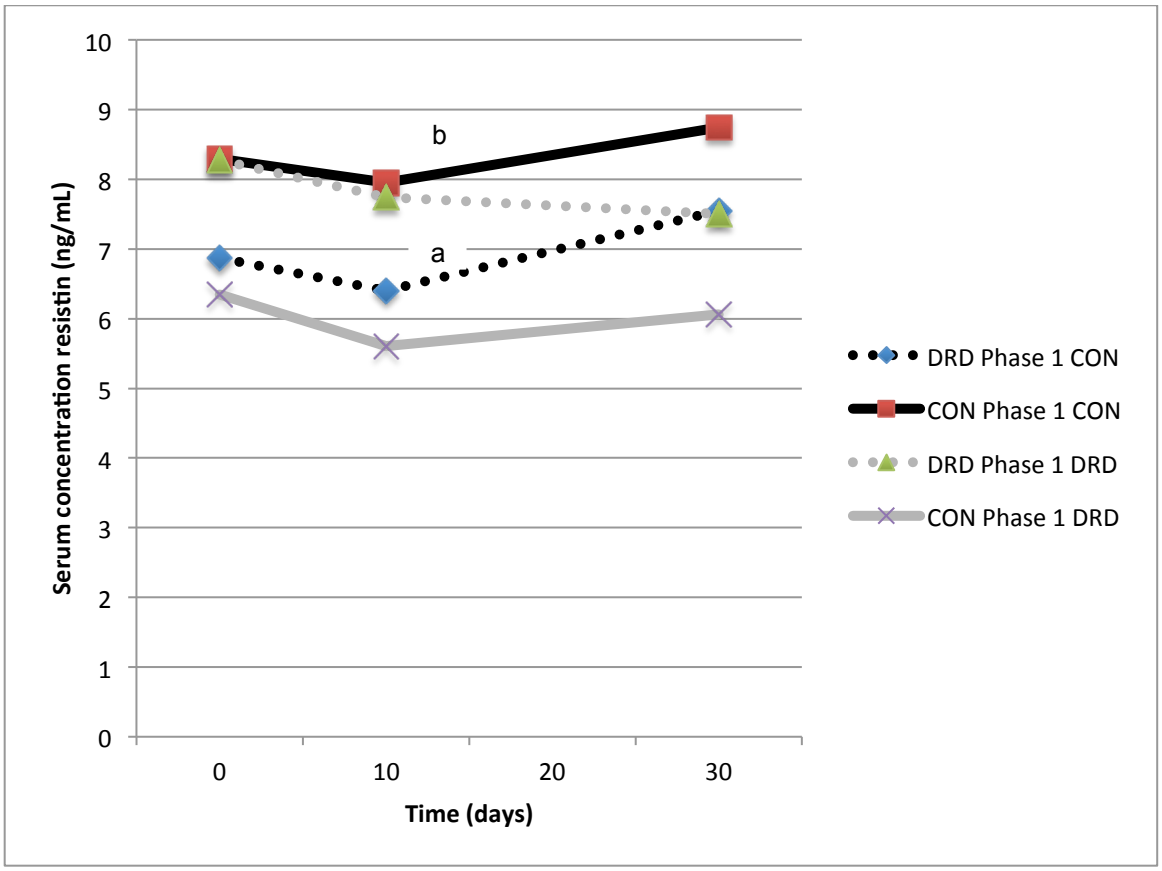

Figure **4-5**. Plasma concentrations of resistin (ng/mL) over time throughout the 30-day DRD and CON diet treatments split by treatment order (Phase 1 CON, Phase 1 DRD).

[a]different from DRD Phase 1 DRD; [b]different from CON Phase 1 DRD, using $p < 0.05$ for statistical significance; *p*-values from multivariate analysis of covariance with repeated measures on the time factor. Diet treatment x treatment order $p < 0.001$. Diet treatment x treatment order x time $p = 0.792$. At all timepoints, n= 9 Phase 1 CON, n= 14 Phase 1 DRD.

Chapter 5

DISCUSSION

Data from this randomized, controlled, crossover study indicate that consumption of 3 servings/d of nonfat dairy for 30 days may alter biomarkers of inflammatory stress in overweight and obese participants. Although the DRD did not induce differential changes in markers of inflammation over time compared to the CON diet, treatment order effects by diet treatment and by diet treatment by time ($p< 0.05$) were present for TNF-α, IL-6, and adiponectin. Additionally, treatment order effects by diet treatment altered resistin concentrations, and leptin concentrations varied over time. The DRD treatment did not have consistent effects on pro-inflammatory biomarkers (CRP, TNF-α, IL-6), anti-inflammatory biomarkers (adiponectin) or adipokines (leptin, resistin). There were no differences in biomarker concentrations by diet treatment over time, so the null hypotheses was not rejected that the DRD would maintain blood concentrations of all inflammatory biomarkers compared to the CON diet treatment. In support of the null hypotheses, comparisons of the DRD and CON diet treatments found that CRP, TNF-α, and IL-6 levels were maintained or increased over time, serum adiponection concentrations were maintained or reduced over time, and serum leptin and resistin concentrations were maintained over time.

Throughout the study, participants maintained BW, WC, BMI, FM, and BF%, indicating that adiposity was held constant. Consistent with recent meta-analyses, it was possible to incorporate 3 servings/d of dairy into the diet without negatively affecting BW or measures of adiposity (18; 19). Therefore, changes in concentrations of inflammatory biomarkers were probably not due to alterations in adipose tissue morphology, but rather more likely attributable to the dairy-rich diet manipulation. For example, circulating levels of the anti-inflammatory

adipokine adiponectin increase with weight loss (37) and plasma concentrations of the pro-inflammatory cytokine CRP increase with weight gain (11). Adipose tissue maintenance could have contributed to the observed lack of variation in concentrations of CRP, TNF-α, IL-6, adiponectin, and resistin over time and across diet treatments.

Although time was a significant predictor of serum leptin concentration, this effect was not explained by the dairy manipulation. During both the DRD and CON diet treatments, leptin concentrations at day 10 were significantly lower than at day 30. Expression of leptin and resistin is stimulated by increasing adipose tissue mass (14; 38). BW, BMI, FM, and BF% were maintained throughout the present study, so changes in adiposity are not likely explanations for changes in leptin concentration. Similarly, relative constancy in adipose tissue supports the lack of differences in resistin concentration between diet treatments. Resistin concentrations decreased during DRD and CON diet treatments or increased DRD and CON diet treatments according to treatment order randomization, so dairy consumption fails to explain this pattern.

Concentrations of the pro-inflammatory biomarkers CRP, TNF-α, and IL-6 were not altered by increased dairy consumption in the present study. In the randomized, crossover design study by Zemel and colleagues where overweight and obese participants consumed 28-day weight-maintaining high dairy (3 nonfat dairy smoothies/d) or control (3 soy smoothies/d) diets, however, plasma concentrations of TNF-α, MCP-1, IL-6, CRP were reduced while plasma concentrations of adiponectin increased with the dairy treatment (29). Furthermore, in a randomized, controlled, crossover study by van Meijl and Mensink, 8 weeks of a low-fat dairy supplemented diet (500 mL low-fat milk and 150 g low-fat yogurt/d) compared to 8 weeks of a non-dairy supplemented control diet (600 mL fruit juice and 43 g fruit biscuits/d) increased plasma concentrations of s-TNFR-2 and decreased the TNF-α index in overweight and obese participants, suggesting a possible effect on the actions and bioavailability of TNF-α (27). However, IL-6 and *hs*-CRP were not affected in this intervention (31), similar to the findings of

the present study. A 12-mo crossover study in overweight adults with 6-mo high dairy (4 servings/d) and low dairy (≤1 serving/d) treatment arms did not detect a difference in plasma concentrations of *hs*-CRP with increased dairy consumption (30). But, analogous to the results of the study conducted by Zemel and colleagues (29), a 24-week high-dairy (3 servings/d) weight maintenance diet significantly lowered plasma concentrations of CRP in obese participants (26). The response of pro-inflammatory biomarker concentrations to adequate dairy diets is not consistent across studies.

In contrast to other weight maintaining diets including 3 servings/d of dairy administered to overweight and obese participants (26; 29), the DRD diet treatment did not alter serum concentrations of adiponectin. In the study conducted by Zemel and colleagues, significant increases in adiponectin (20%, $p< 0.002$) occurred after 28 days on a diet supplemented with smoothies containing nonfat milk (3 servings/d) (29). Similarly, obese participants in the 24-week adequate dairy intervention led by Zemel and Sun experienced increases in circulating levels of adiponectin (26). The present study detected nonsignificant increases in serum adiponectin after the 30-day DRD treatment (7.9%), and perhaps a longer dairy-supplemented diet treatment would yield significant differences in circulating adiponectin.

Various components of dairy products have been mechanistically implicated in the potential inflammation-reducing effects of dairy. For example, BCAAs such as leucine in the whey portion of dairy may be involved in preferential oxidation of lipids with weight loss to maintain muscle mass (20). This would reduce adipose tissue mass and lessen the inflammatory effects associated with excess body fat. Dietary Ca may influence body weight and body composition by suppression of 1,25-OH_2-D and parathyroid hormone (39). The present study delivered smoothies containing 1050 mg Ca/d in the DRD treatment and 150 mg Ca/d in the CON treatment, which is comparable to the dietary Ca supplementation administered in the high dairy and low dairy treatments used in the study by Zemel and colleagues (29). Calcium may also

influence the short-term rate of lipid oxidation (40) and contribute to insoluble soaps in the gut that elevate fecal energy excretion (41). The current study does not help elucidate the role of Ca as well as other components of dairy in modulating inflammation, as the potential inflammation-reducing effects of dairy remain unclear.

Treatment order effects for concentrations of TNF-α, IL-6, adiponectin, and resistin do not present a clear pattern to explain any carryover effects induced by timing of the DRD and CON diet treatments. For example, circulating levels of the pro-inflammatory cytokine IL-6 and the anti-inflammatory cytokine adiponectin increased during the DRD when it was administered first, decreased during the DRD when it was administered second, increased when the CON diet treatment was presented first, and decreased when the CON treatment was presented second. These parallel alterations in mean concentrations of IL-6 and adiponectin over time by treatment order do not represent a significant effect of diet treatment over time. Therefore, the treatment order effects do not clarify the potential role of dairy in modulating inflammatory stress.

A key strength of this study included its crossover design, which allowed for comparisons of the diet treatments within individuals. Each participant served as his/her own control to reduce study population differences between the DRD and CON treatments. This study adds to the limited literature on whether dairy consumption can attenuate inflammation in overweight and obese participants. A defining feature of the present study was the maintenance of adiposity to minimize changes in adipose tissue as a potential confounder. Weight loss and changes in adipose tissue mass alter adipokine secretion, so weight maintenance enabled minimization of this effect (5). The relatively short duration of the treatment phases (30 days each) was sufficient to observe changes in concentrations of inflammatory biomarkers, although a longer intervention would provide more information about the maximum possible magnitude of changes induced by dairy consumption and how any changes might be sustained over a longer period of time.

The current study was limited by the small sample size and baseline characteristics of the participants. Low habitual dairy intake was not specified in the inclusion criteria, and low dairy consumers may preferentially benefit from increasing dairy intake to adequate levels. Additionally, habitual Ca intake should be accounted for, as the study by Wennersberg and colleagues detected reductions in WC and sagittal abdominal diameter in the adequate-dairy treatment group of participants with MetS and habitually Ca intake below 700 mg/d (42). In addition, while the present study utilized a single nonfat dairy product (powdered milk) to supplement the diets of participants, this limited the ability of the investigation to detect any changes in inflammatory biomarkers that could be altered by the consumption of a variety of dairy products to meet 3 servings/d. The current study used a free-living population to examine how inflammatory outcomes changed with dairy supplementation. Although the dairy component of the diet was controlled through the treatment manipulations, this was not a total feeding trial, so the remainder of the participants' diet was subject to variability.

In conclusion, the present study tested the ability of dairy to reduce inflammation in weight-stable overweight and obese participants using a 30-day DRD treatment compared to a non-dairy CON diet with a randomized, controlled, crossover study design. Reduction of the chronic, low-grade inflammation concomitant with excess adiposity could improve metabolic health and help prevent the development of obesity comorbidities in overweight and obese populations (43). Therefore, the current study was designed to examine the effects of meeting adequate dairy intake recommendations on the inflammatory profile. Adiposity remained constant throughout the duration of the study, indicating that dairy can be incorporated into the diet without increasing BW or body fatness. The dairy-supplemented diet did not appreciably alter biomarkers of inflammation in comparison to the control diet, although treatment order effects were observed for circulating concentrations of TNF-α, IL-6, adiponectin, and resistin. Overall, this study did not detect an effect of dairy on attenuation of indicators of inflammation in

overweight and obese participants. The limited and inconsistent evidence on the relationship between dairy consumption and inflammation in adults with excess adiposity warrants further research. While the current study suggests that meeting the dairy intake recommendation of 3 servings of dairy per day does not alter the inflammatory burden of overweight and obesity, the potential role of dairy in modulating inflammation remains unclear (16).

Chapter 6

FUTURE DIRECTIONS

The current study did not find differences in concentrations of biomarkers of inflammation with a 30-day DRD treatment compared to a 30-day non-dairy CON diet treatment in weight-stable, adiposity-stable overweight and obese adults. Treatment order effects were detected for TNF-α, IL-6, adiponectin, and resistin, and leptin concentrations varied over time independent of diet treatment. Achieving adequate dairy intake did not impact the inflammatory profile of participants. The inconclusive results of this study and the limited evidence available on the relationship between dairy intake and inflammation suggest that more research is necessary to examine the potential inflammation-reducing effects of dairy products. With the pervasiveness of obesity, there is a need to understand how specific dietary components contribute to and can potentially improve metabolic health.

The striking improvements in inflammatory stress reported in the studies conducted by Zemel and colleagues (29) and Zemel and Sun (26) warrant further attempts at replication. Habitual dairy and Ca consumption as well as dairy and Ca intake throughout the study represent study design elements that require rigorous examination. If Ca is mechanistically involved in dairy modulation of inflammation, then this dairy component merits attention in the framework of a dairy-supplemented diet intervention. A future study to probe how dairy can affect the inflammatory profile independent of changes in adiposity should recruit overweight and obese participants with minimal (≤0.5 servings/d), moderate (1-2.5 servings/d), and adequate (≥3 servings/d) habitual dairy consumption. Habitual Ca consumption should be calculated, and while this will most likely be reflective of habitual dairy intake, participants should be stratified by Ca intake. Weight-maintenance diets could be administered in a crossover fashion with adequate dairy/adequate Ca (3 servings/d of dairy delivering 1,000 mg Ca/d) and low dairy/no

supplemental Ca (≤0.5 servings/d, no Ca supplementation) treatment arms to maximize the disparity in dairy intake across treatments. A dairy supplementation intervention in participants with MetS reported a treatment effect for WC and sagittal abdominal diameter those with Ca intake <700 mg/d at baseline (41). Detection of a possible threshold effect for benefits of dairy due to baseline Ca intake and/or habitual dairy intake could be achieved using the suggested diet treatments across the spectrum of typical dairy and Ca intake. Ideally, the intervention would be conducted in a metabolic ward where all dietary components could be precisely controlled. If consistently low dairy and/or low Ca consumers experience attenuation of inflammation by meeting dairy and Ca intake recommendations, then these populations should be targeted for dietary interventions to reduce inflammation.

Elucidation of the effects of specific types of dairy on the inflammatory profile is necessary for a more complete understanding of how dairy potentially modulates inflammation. The few eucaloric studies on dairy supplementation and inflammatory outcomes in overweight and obese populations have used nonfat dry milk (29; present study); low-fat milk and yogurt (27; 31); and reduced fat milk, yogurt, and custard (30). A future randomized, controlled investigation among overweight participants could compare changes in biomarkers of inflammation with eucaloric diets that include 3 servings/d of low-fat fluid milk, low-fat yogurt, low-fat cheese, or a control food. The type of dairy consumed may be important for overall metabolic health, as yogurt consumption was negatively associated with the prevalence of MetS in NHANES data from 1999-2004, but cheese and low-fat milk were positively related to MetS incidence (17). Therefore, identification of any divergences in how specific dairy products influence inflammation is crucial.

An additional future study could assess how an adequate dairy diet encompassing an assortment of dairy products influences the inflammatory burden. In a cross-sectional survey of Greek adults, those that consumed ≥11 servings/wk of milk, yogurt, and cheese had significantly

lower CRP, TNF-α, and IL-6 than those that ate <8 servings/wk (28). Incorporation of a variety of dairy products into diet manipulations would present the opportunity to explore how dairy as a food group could influence inflammation. While the current study utilized 3 servings/d of a single dairy product for consistency, incorporation of various dairy products into the diet would be beneficial to more closely mimic consumption patterns of typical Americans. The flexibility of increasing the variety of dairy consumed in an intervention could more easily transition to sustainable lifestyle changes aimed at following dietary guidelines beyond the duration of the study. Overall, future research should examine how meeting dairy intake recommendations with various dairy products could benefit overweight and obese populations across a range of habitual dairy and Ca intakes.

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