9-10-2015

Reduction of Obesity-associated Chronic Inflammation by Low-fat Yogurt

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University of Connecticut, 2015

ABSTRACT

The objective of this study was to conduct an intervention study to test how low-fat yogurt consumption affects postprandial metabolism, chronic inflammation, and intestinal barrier function. Apparently healthy premenopausal women with body mass index (BMI) of 18.5 to 27 (lean) or 30 to 40 kg/m^2 (obese) participated in a 9-week controlled intervention study. Participants in each BMI range were randomly assigned to either a low-fat yogurt-supplemented group (Y, yogurt lean; YO, yogurt obese) or a soy pudding-supplemented control group (C, control lean; CO, control obese), with n = 30/group. Participants consumed 12 oz. of yogurt or the control food daily for 9 weeks while maintaining usual caloric intake. At wk 0, 3, 6 and 9, participants provided fasting blood samples for determination of inflammation and endotoxemia, and for weight and blood pressure measurement. At wk 0 and 9, participants consumed 8 oz. of yogurt or the control food, followed by a high-fat, high-carbohydrate challenge meal. Blood samples were taken at baseline, 1, 2, 3, and 4 h after the meal to assess postprandial metabolism. At baseline, obese participants had increased plasma glucose, triglyceride, insulin, interleukin-6 (IL-6), high-sensitivity C-reactive protein (hsCRP), tumor necrosis factor alpha (TNF-α), lipopolysaccharide-binding protein (LBP), LBP/soluble CD14 (sCD14) ratio, but less immunoglobulin M endotoxin-core antibody (IgM EndoCAb) than lean participants. The

challenge meal induced postprandial increases in IL-6, glucose, and LBP/sCD14 ratio in CO. However these postprandial responses were suppressed in YO. Yogurt consumption also reduced postprandial hypoglycemia in Y, possibly by preventing insulin over-secretion. Both challenge meals had similar responses, except 15% decreased IL-6 area under the curve in the second test. After 9 weeks, YO had decreased fasting plasma IL-6, hsCRP, TNF-α, TNF-α/soluble tumor necrosis factor receptor II (sTNF-RII) ratio, but increased IgM EndoCAb. These improvements occurred despite ~ 0.9 kg weight gain in YO and CO. YO diastolic blood pressure decreased at wk 3, but was not maintained at wk 6 and 9. In conclusion, consuming low-fat yogurt improved postprandial metabolism and reduced biomarkers of chronic inflammation in healthy obese premenopausal women, in part because of improved intestinal barrier function.

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B.E., China Agricultural University, **2006**

M.S., China Agricultural University, **2009**

A Dissertation

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

at the

University of Connecticut

2015

APPROVAL PAGE

Doctor of Philosophy Dissertation

Reduction of Obesity-associated Chronic Inflammation by Low-fat Yogurt

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ACKNOWLEDGEMENTS

This the dissertation is the collaborative work of many people to whom I would like to express my sincerest gratitude. First, thank you, my major advisor Dr. Bradley Bolling, for providing me with all the support, resources and techniques that are necessary to complete this dissertation. Thanks you for giving me your constant trust and guidance that made me a better scientist. You are a wonderful person to work with.

Dr. Bruno, Dr. Fernandez, Dr. Freake, and Dr. Volek, thank you for serving on my doctoral committee, for providing sound criticism that improves the quality of this dissertation work, and for challenging me to reach my potential. It has been a great honor working with every one of you.

To professors at NUSC, Dr. Koo, Dr. Rodriguez, Dr. Lee, Dr. Blesso, Dr. Park and Dr. Mobley, thank you for offering your knowledge and expertise to me. To other professors, Dr. White, Dr. Parkin, Dr. Steele, Dr. Venkitanarayanan and Dr. van Pijkeren, thanks for offering your support to my study.

I would like to thank my current and past lab mates, Diana, Kelley, Derek, Liyang, Rod, Dr. Noh, Eunice, Chris, Yi, Lei, Minyu, Allyson, and Kevin for your help in my study life. Special thanks to Diana and Kelley for your great help with the study recruitment. Thank my friends Sanggil, Jianping, Terry, Lai, Ying, Rui, Xi, Sarah, Katherine, Yuan, Xueqi, Abhinav, Leelyn, Chai Siah, Yue, Tho, Jeff, Mo, Fei, Fang, Qinlei, Yiming, Shigang, Hong, Bang, Leslie and Lei for your friendship and help. You guys brightened my life every day.

Thank all the study participants for their time and support that made this study possible, and thank the funding agency Diary Research Institute for the financial support.

Most importantly, my mum and dad, Yingying, Ruixi, my grandpa and grandma, and my other family members, I love you and miss you every day.

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List of Abbreviations

ANCOVA: analysis of covariance

ANOVA: Analysis of variance

AUC: Area under the curve

BMI: Body mass index

BP: Blood pressure

C: Control lean

CI: Confidence interval

CO: Control obese

CVD: Cardiovascular disease

DiaBP: Diastolic blood pressure

EDTA: Ethylenediaminetetraacetic acid

GIP: Gastric inhibitory peptide

GLP: Glucagon-like peptide

HOMA-IR: Homeostatic model assessment of insulin resistance

hsCRP: High-sensitivity C-reactive protein

IgM EndoCAb: Immunoglobulin M endotoxin-core antibody

IκB-α: Nuclear factor kappa B inhibitor alpha

IL-6: Interleukin-6

JNK: c-Jun N-terminal kinases

LAB: Lactic acid bacteria

LBP: Lipopolysaccharide-binding protein

LPS: Lipopolysaccharide

MAPK: Mitogen-activated protein kinases

MUFA: Monounsaturated fatty acids

NDSR: Nutrition data systems for research

NF-κB: Nuclear factor kappa B

PBS: Phosphate-buffered saline

PUFA: Polyunsaturated fatty acid

RDA: Recommended Dietary Allowance

RR: Relative risk

sCD14: Soluble CD14

SFA: Saturated fatty acids

sTNF-RII: Soluble tumor necrosis factor receptor II

SysBP: Systolic blood pressure

TG: Triglyceride

TGFβ1: Transforming growth factor beta 1

TLR4: Toll-like receptor 4

TNF-α: Tumor necrosis factor alpha

WC: Waist circumference

Y: Yogurt lean

YO: Yogurt obese

Chapter 1: Introduction

1.1 Overview

Obesity is an abnormal or excessive accumulation of fat that poses a risk to health. A person with a body mass index (BMI) between 25 and 30 is classified as overweight and a BMI greater than 30 is obese. In the US, nearly 70% of adults are classified as overweight or obese (Flegal, Carroll et al. 2010). Obesity is associated with cardiovascular disease, Type II diabetes, and certain cancers (Kastarinen, Nissinen et al. 2000, Grundy 2007, Hossain, Kawar et al. 2007). Obesity decreases quality of life, life expectancy, and its prevalence causes a sizable economic burden (Peeters, Barendregt et al. 2003, Wang, McPherson et al. 2011, Warkentin, Das et al. 2014).

1.2 Central Hypothesis and Specific Aims

Inflammation links obesity to various chronic diseases (Lumeng and Saltiel 2011). In obese humans and other animals, proinflammatory markers at both protein and the transcriptional level are increased in systemic circulation and various tissues (Kern, Ranganathan et al. 2001, De Souza, Araujo et al. 2005, Brake, Smith et al. 2006, Kim, Park et al. 2006, Ehses, Perren et al. 2007). Several inflammatory mechanisms in obesity have been proposed. Obesity-associated inflammation is considered to originate from the interplay between immune cells and metabolic tissues in response to excessive nutrients intake, resulting in increased secretion of inflammatory cytokines (Gregor and Hotamisligil 2011). In addition, persistent postprandial dysmetabolism-induced inflammation, which is exacerbated in obese population, also leads to chronic inflammatory status (Burdge and Calder 2005, Esposito, Ciotola et al. 2007, Calder, Ahluwalia et al. 2011). Furthermore, obesity is associated with impaired intestinal barrier function, leading to increased endotoxin which effectively induces inflammation in humans (Kemna, Pickkers et al. 2005, Andreasen, Larsen et al. 2010, Pei, Martin et al. 2015).

Intervention studies report the anti-inflammatory effects of yogurt in children and the elderly (Sakamoto, Igarashi et al. 2001, Schiffrin, Parlesak et al. 2009, Yang and Sheu 2012). Animal and human studies suggest that yogurt consumption is beneficial for intestinal barrier function, thus reducing the risk of endotoxin exposure (Isolauri, Kaila et al. 1993, Matsumoto, Aranami et al. 2007, Schiffrin, Parlesak et al. 2009). Yogurt consumption might reduce body weight and blood pressure, and weight loss is associated with reduced chronic inflammation (Ziccardi, Nappo et al. 2002, Zemel, Richards et al. 2005, Thomas, Wideman et al. 2011, Drouin-Chartier, Gigleux et al. 2014). However, evidence from randomized, controlled studies on the anti-inflammatory effects of low-fat yogurt in healthy premenopausal women is still lacking. Most yogurt intervention studies are relatively small in sample size and have lacked proper dietary controls for yogurt. In addition, yogurt intervention studies in obese population usually do not include the non-obese control, and some of these studies incorporate yogurt in calorie-restricted diets which may obscure the effect of yogurt consumption in a free-living population. Furthermore, the possible dietary change brought by yogurt intervention is not considered.

Therefore, the *objective of this dissertation research* is to better define how low-fat yogurt consumption affects body weight, blood pressure, postprandial inflammation, intestinal barrier function, and chronic inflammation in apparently healthy premenopausal women using a randomized, controlled, isocaloric study design. We selected a non-dairy control food with similar macronutrient and micronutrient content, total calories and texture, and also included a group of non-obese control participants. The *central hypothesis* is that low-fat yogurt consumption reduces postprandial and chronic inflammation by decreasing metabolic endotoxemia. The central hypothesis was assessed by evaluating the following specific aims:

Aim 1: Define the effects of low-fat yogurt consumption on weight, blood pressure and dietary pattern. The *working hypothesis* is that isocaloric low-fat yogurt intervention will improve weight status and reduce blood pressure without changing dietary pattern. This hypothesis was tested by evaluating the changes in body weight, waist circumference, blood pressure, and dietary pattern.

Aim 2: Determine how pre-meal consumption of low-fat yogurt affects postprandial metabolism after a high-fat, high-carbohydrate meal. The *working hypothesis* is that pre-meal consumption of low-fat yogurt will reduce postprandial inflammation by improving postprandial metabolism and suppressing metabolic endotoxemia. This hypothesis was tested by measuring the postprandial changes in glucose, triglyceride (TG), insulin, interleukin-6 (IL-6), lipopolysaccharide-binding protein (LBP) and soluble CD14 (sCD14) after consuming low-fat yogurt followed by a high-fat, high-carbohydrate meal.

Aim 3: Define the effects of low-fat yogurt consumption on chronic inflammation and endotoxemia. Building on the findings under Aim 2, the *working hypothesis* is that low-fat yogurt consumption will reduce chronic inflammation and metabolic endotoxemia. This hypothesis was tested by determining the changes in markers for inflammation and endotoxemia before and after yogurt intervention.

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Chapter 2: Literature Review

This chapter is reprinted from “Pei, R., D. A. Martin, D. M. DiMarco and B. W. Bolling (2015). "Evidence for the Effects of Yogurt on Gut Health and Obesity." Critical Reviews in Food Science and Nutrition: DOI: 10.1080/10408398.2014.883356” with permission from Taylor & Francis Group.

2.1 Introduction

Yogurt has been consumed for centuries. As early as 1908, Metchnikoff ascribed the prolonged life of the Bulgarians to consumption of sour milk fermented by lactic acid bacteria (O'Sullivan, Thornton et al. 1992). Yogurt is a milk product fermented by *L. bulgaricus, S. thermophilus* and *L. acidophilus* (CODEX STAN 243-2003). In addition to these Lactic acid bacteria (LAB), other strains of *Lactobacillus* and *Bifidobacterium* are commonly used as yogurt starter cultures (Desobry-Banon, Vetier et al. 1999). Yogurts may also be enriched in other probiotic strains that convey additional health benefits beyond those of traditional yogurt cultures (Shah 2007).

The global rise in obesity is an increasing health concern. The causes of obesity and approaches needed to reduce obesity are multifactorial in nature (Holes-Lewis, Malcolm et al. 2013). Effective social, behavioral, and dietary interventions are needed to mitigate the adverse effects of obesity on personal health outcomes (Wadden, Webb et al. 2012). Obesity impairs gut health, which may be a potential target for therapeutic dietary interventions (Tilg and Kaser 2011). Yogurt is rich with potential bioactive components and emerging evidence points toward the efficacy of yogurt and its components to improve gut health in obesity.

2.2 Yogurt Bioactives

2.2.1 Nutrients in yogurt

Dairy products are rich in high-quality proteins, calcium, potassium, phosphorus, magnesium, zinc and B vitamins (**Table 2.1**) (Buttriss 1997). Fermentation can improve the nutrient content of dairy products. For example, some bacteria synthesize B vitamins. *S. thermophilus* can produce folate during yogurt fermentation, and certain inoculations can

increase folate levels 6-fold (Crittenden, Martinez et al. 2003). Yogurt also contains conjugated linoleic acid (CLA), a derivative of linoleic acid (Shahani and Chandan 1979, Aneja and Murthi 1990). CLA may improve body composition by increasing lean body mass while decreasing fat mass, and has immunostimulatory and anticarcinogenic effects (Park, Albright et al. 1997, Whigham, Cook et al. 2000).

Table 2.1 Representative nutrient data bank values for plain yogurts in the U.S.[1]

Nutrient (per 6 oz.)	**Whole**	**Low-fat**	**Fat-free**	**Fat-free** (Greek)
calories	104	107	95	100
total fat (g)	5.9	2.64	0.31	0.66
saturated fat (g)	3.56	1.7	0.12	0.20
MUFA (g)	1.52	0.72	0.05	0.09
PUFA (g)	0.16	0.08	0.01	0.02
cholesterol (mg)	22	10	2	8
carbohydrates (g)	7.92	11.97	13.06	6.12
sugar (g)[2]	7.92	11.97	13.06	5.51
dietary fiber (g)	0.0	0.0	0.0	0.0
protein (g)	5.9	6.77	5.73	17.32
thiamin (mg)	0.05	0.08	0.08	0.04
riboflavin (mg)	0.24	0.36	0.40	0.47
niacin (mg)	0.13	0.19	0.21	0.35
vitamin B6 (mg)	0.05	0.08	0.09	0.11
folate (mcg)	12	19	20	12
vitamin B12 (μg)	0.63	0.95	1.04	1.28
vitamin A (RAE[3])	46	24	3	2

vitamin C (mg)	0.8	1.4	1.50	0.0
vitamin D (μg)	3.0	2.0	0.0	0.0
vitamin E (mg)	0.1	0.05	0.0	0.02
vitamin K (μg)	0.3	0.3	0.30	0.0
calcium (mg)	206	311	338	187
phosphorus (mg)	162	245	267	230
magnesium (mg)	20	29	32	19
sodium (mg)	78	119	131	61
potassium (mg)	264	398	434	240
iron (mg)	0.08	0.14	0.15	0.12
zinc (mg)	1	1.51	1.65	0.88

[1] Derived from USDA National Nutrient Database for Standard Reference, Release 26.

[2]Sweetened or fruit yogurts typically have an additional 20 g sugars per 6 oz.

[3]Retinol activity equivalents

Fermentation also improves the digestibility of milk proteins. LAB proteolytic enzymes and peptidases increase free amino acids in yogurt (Gorbach 1990). Upon digestion, yogurt had smaller clots of curd than milk, which facilitated digestive enzyme activity (Breslaw and Kleyn 1973). In addition, the viscous texture of yogurt might decrease the gastric emptying rate, which increases duration of the enzymatic hydrolysis (Shahani and Chandan 1979, Gaudichon, Roos et al. 1994).

Yogurt is also considered to be a good source of minerals. Dairy products are a good source of calcium, not just because of the abundance of calcium but also because of the high

absorbability of calcium from yogurt. The presence of lactose, phosphopeptides, and amino acids derived from casein in dairy products facilitates the absorption of calcium by promoting its active transport or passive diffusion (Gueguen and Pointillart 2000). Although intervention studies have not demonstrated greater bioavailability of dairy calcium than supplemental calcium, dairy calcium was more effective than supplementary calcium in reducing weight and fat in energy-restricted adults (Sheikh, Santa Ana et al. 1987, Recker, Bammi et al. 1988, Zemel, Shi et al. 2000, Zemel, Thompson et al. 2004, Zhao, Martin et al. 2005). Although there is no evidence showing that yogurt serves as a better source of calcium than milk or other dairy products, yogurt has the advantage of being well tolerated by lactase-deficient individuals (Smith, Kolars et al. 1985).

2.2.2 Other bioactives

Dairy products contain bioactive proteins, such as immunoglobulins, α-lactoglobulin, β-lactoglobulin, lactoferrin, and phosphopeptides, which may regulate immune response, modulate blood pressure, and facilitate mineral absorption (Ebringer, Ferenčík et al. 2008). Bacterial hydrolysis of milk protein can yield oligopeptides with additional biological activities. For instance, some peptides (e.g. Val-Pro-Pro and Ile-Pro-Pro) have hypotensive effects via inhibiting angiotensin-converting-enzyme (Nakamura, Mizutani et al. 2009). A pentapeptide hydrolyzed from casein, Ile-Ile-Ala-Glu-Lys, has hypocholesterolemic effects *in vitro* (Morikawa, Kondo et al. 2007). Other effects of bioactive peptides such as antithrombotic, antioxidant, antimicrobial and antifungal activities have also been reported (Ebringer, Ferenčík et al. 2008).

Dairy products also contain various bioactive lipids and oligosaccharides. Phospho- and sphingolipids may reduce blood cholesterol, enhance brain function, and inhibit colon cancer (Rombaut, Camp et al. 2005, Ebringer, Ferenčík et al. 2008). Some short chain fatty acids in dairy products such as butyric acid (C4:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0) have anticarcinogenic, antiviral and antibacterial activities (Ebringer, Ferenčík et al. 2008). In addition, dairy products contain some oligosaccharides such as lactulose, which could serve as prebiotics to support the growth of commensal bacteria (Marconi, Messia et al. 2004).

2.2.3 Microorganisms

S. thermophilus and *L. bulgaricus* are the most frequent microorganisms used to produce yogurt. In the United States, some yogurts have additional *L. acidophilus, B. bifidum, B. lactis, L. casei,* and/or *L. rhamnosus* content, among others, and are branded as 'probiotic yogurts.' The most basic definition of probiotics is, "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Pineiro and Embarek 2002). However, others have proposed that probiotics must originate from humans, be viable through the gastrointestinal tract, adhere to the intestinal wall to facilitate colonization, produce antimicrobials, and provide a demonstrable health effect (Guarner, Perdigon et al. 2005).

It is commonly thought that 10^5 - 10^7 CFU/mL living probiotic bacteria are needed to confer a health benefit to the host (Schillinger 1999, Vélez, Hermans et al. 2007). Yogurt culture content ranges from 10^4 to 10^8 CFU/g/strain (Dunlap, Yu et al. 2009). In the US, yogurt can be certified with a "live and active culture" seal from the National Yogurt Association if it contains 10^8 CFU/g at the time of manufacture (National-Yogurt-Association 2008). While the viability of yogurt microorganisms may be enumerated at manufacture, viability declines throughout the

shelf-life of products. For example, *L. acidophilus*, a culture commonly added to yogurt post-fermentation, is relatively unstable in yogurt. This is likely due to hydrogen peroxide produced by *L. bulgaricus* during yogurt production (Gilliland and Speck 1977). A survey of yogurts in Columbia found poor survival and inconsistent labeling of strains (Vélez, Hermans et al. 2007). In a study of yogurts of European origin, bacterial counts in some products were as low as 10^4 CFU per gram per strain by the sell-by date (Schillinger 1999). Temperature fluctuations may also reduce viability of yogurt probiotics. After 6 h at room temperature, reductions of 9-46.2% were seen in the CFU count for *L. GG, L. johnsonii*, and *L. acidophilus* (Scharl, Geisel et al. 2010). Thus, it is expected that the amount of traditional and probiotic strains present in yogurt varies considerably by manufacturer, storage conditions, and time of consumption. Despite this, yogurt cultures may not need to be viable to confer a health benefit. For example, a preparation of mixed DNA from various probiotic strains inhibited colitis in IL-$10^{-/-}$ mice (Jijon, Backer et al. 2004). Conventional yogurt LAB improve lactose digestion, despite poor viability and the inability to survive the digestive process (Martini, Lerebours et al. 1991).

2.3 Obesity, Yogurt and Chronic Disease Risk

Obesity is an abnormal or excessive accumulation of fat that poses a risk to health. A person with a body mass index (BMI) between 25 and 30 is classified as overweight and a BMI greater than 30 is obese. The International Obesity Task Force estimates that at least 1.1 billion adults are overweight, with 312 million of those obese (Haslam and James 2005). In the US, nearly 70% of adults are classified as overweight or obese (Flegal, Carroll et al. 2010). Obesity is a major risk factor for a number of chronic diseases such as diabetes, cardiovascular disease (CVD) and certain cancers. Furthermore, obese adults are projected to lose 7 years of life expectancy (Peeters, Barendregt et al. 2003). The morbidity associated with obesity accounts for

2-7% of health care costs in the developed world (Hossain, Kawar et al. 2007). Morbidities attributed to obesity include CVD, type 2 diabetes, hypertension, cancer, chronic inflammation, and compromised gut health. A limited number of yogurt intervention studies relevant to obesity and chronic disease risk have demonstrated positive outcomes on lipid profiles and chronic inflammation (**Table 2.2**).

2.3.1 Cardiovascular diseases

CVD is one of the leading causes of death and premature mortality. Ischemic heart diseases and stroke account for nearly one in four deaths worldwide (Lozano, Naghavi et al. 2012). Visceral obesity has a critical role in the development of CVD (Grundy 2007). Mathieu et al. reviewed how inflammation linked obesity and CVD (Mathieu, Lemieux et al. 2010). Briefly, excessive accumulation of fat in the adipose tissue leads to macrophage infiltration and elevated production of proinflammatory cytokines, which contribute to the development of atherosclerosis. In addition, obesity is related to atherogenic dyslipidemia characterized by increased levels of triglyceride (TG), small dense low-density lipoprotein (sdLDL) particles, as well as decreased level of high-density lipoprotein cholesterol (HDL-C) (Tenenbaum and Fisman 2012). Obesity can also directly affect the structure and functions of the cardiovascular system. Obese individuals have increased cardiac output which can lead to left ventricular hypertrophy and other structural abnormalities (Lavie, Milani et al. 2009). Obesity also causes left atrial enlargement due to increased circulating blood volume and abnormal left ventricular diastolic filling (Lavie, Milani et al. 2009). These abnormalities compromise cardiovascular function and increase CVD risk for obese individuals (Lavie, Milani et al. 2009).

Table 2.2 Clinical studies of yogurt on biomarkers relevant to obesity and chronic disease risk.

Category	Reference	Population	Treatment	Outcome
Lipid profiles	(Schaafsma, Meuling et al. 1998)	n = 30 healthy men	375 mL/d for 3 wk, 0.5% fat, + *L. acidophilus*	↓ serum total cholesterol, ↓ LDL, and ↓ LDL/HLD-ratio.
	(Anderson and Gilliland 1999)	n = 40 hypercholesterolemic individuals	200 mL/d for 4 wk, + *L. acidophilus*, unspecified fat content	↓ serum cholesterol by 3.2%
	(Kießling, Schneider et al. 2002)	n = 29 women (14 hypercholesterolemic)	300 g/day for 6 mo, 3.5% fat, + *L. acidophilus* and *B. longum*	↑ HDL
	(Fabian and Elmadfa 2006)	n = 33 lean women	100 g/d for 2 wk and then 200 g/d for another 2 wk, 3.6% fat	↓ LDL/HLD ratio
	(Sadrzadeh-Yeganeh, Elmadfa et al. 2010)	n = 90 lean women	300 g/d for 6 wk, 2.5% fat	↓ total cholesterol and ↓ total:HDL cholesterol ratio
Inflammation	(Matsumoto, Ohishi et al. 2001)	n = 6 elderly (3 M, 3 F)	100 g/day for 2 wk, + *L. acidophilus* and *B. lactis*, unspecified fat content	↓ haptoglobin in feces
	(Sakamoto, Igarashi et al. 2001)	n = 31 elderly (29 M, 2 F)	180 g/day for 8 wk, + *L. gasseri*, unspecified fat content	↓ *H. pylori*-induced gastric mucosal inflammation
	(Schiffrin, Parlesak et al. 2009)	n = 36 elderly (9 M, 27 F)	300 g/day for 4 wk, + *L. johnsonii*, unspecified fat content	↓ plasma LBP, sCD14 and surrogate markers of LPS permeability

	(Yang and Sheu 2012)	n = 38 children	400 mL/day for 4 wk, + *L. acidophilus* and *B. lactis*, unspecified fat content	↓ serum IL-6
Appetite	(Tsuchiya, Almiron-Roig et al. 2006)	n = 32 healthy men and women	acute yogurt intake (200 Kcal)	↓ hunger, ↑ fullness, ↔ subsequent food intake
	(Chapelot and Payen 2010)	n = 18 lean men	acute yogurt intake (287 Kcal)	↑ satiety, ↔ subsequent food intake
	(Dougkas, Minihane et al. 2012)	n = 40 overweight men	acute yogurt intake (201 Kcal)	↓ appetite, ↓ subsequent energy intake
	(Douglas, Ortinau et al. 2013)	n = 15 women	acute yogurt intake (160 Kcal)	↓ hunger, ↑ fullness, and delayed subsequent eating

Several recent expert reviews have summarized the potential benefits of dairy consumption on CVD risk. Van Meijl et al. reviewed the physiological effects of dairy consumption on metabolic syndrome and concluded that dairy calcium and protein had important roles in reducing metabolic syndrome risk (Van Meijl, Vrolix et al. 2008). In a prospective, matched case-control study using serum milk fat biomarkers, it was found that biomarkers of milk fat were inversely associated with the first myocardial infarction in Swedish women after multivariable adjustment for confounders (OR 0.74, 95% CI: 0.58, 0.94); moreover, reported intake of fermented milk products were inversely related to the first myocardial infarction ($P <$ 0.05 for trend) (Warensjö, Jansson et al. 2010). German et al. reviewed the effects of dairy foods and dairy fats on CVD risk (German, Gibson et al. 2009). They suggested that although dairy products contributed to saturated fat intake, there was no consistent association between dairy consumption and risk of CVD (German, Gibson et al. 2009). Similarly, another group examined the influence of milk fat containing dairy foods and CVD health and concluded that dairy consumption did not increase the risk of CVD, coronary heart disease or stroke, regardless of milk fat levels (Huth and Park 2012). Most research on dairy and CVD risk have not evaluated yogurt specifically. Given the differences in nutrient and bioactive content between yogurt and other dairy products, more attention is needed on this specific product category.

Conventional yogurt consumption may improve lipid profiles in healthy and hypercholesterolemic adults. The effects of conventional yogurt, yogurt with *L. acidophilus* and *B. lactis,* or no yogurt on blood lipids were evaluated in healthy Iranian women (n = 90) (Sadrzadeh-Yeganeh, Elmadfa et al. 2010). Consumption of 300 g/d of conventional and probiotic yogurt for 6 wk reduced the total cholesterol and total:HDL cholesterol ratio relative to the control group (Sadrzadeh-Yeganeh, Elmadfa et al. 2010). The probiotic yogurt-consuming

group also experienced an 8.8% increase in HDL cholesterol (Sadrzadeh-Yeganeh, Elmadfa et al. 2010). Probiotic or prebiotic containing yogurt may also further improve lipid profiles in adults. A randomized cross-over study of 40 hypercholesterolemic US adults consuming 200 g yogurt with *L. acidophilus* L1 for 4 wk reduced serum cholesterol by 3.2% relative to a yogurt prepared with an *L. acidophilus* strain with poor viability and low *in vitro* cholesterol-lowering activity (Anderson and Gilliland 1999). In a cross-over study of 29 healthy women, which included hypercholesterolemic individuals, 300 g of yogurt with *L. acidophilus* and *B. longum* for 7 weeks increased serum HDL cholesterol by 0.3 mmol/L relative to a control yogurt without these strains (Kießling, Schneider et al. 2002). In a parallel study of 33 normocholesterolemic women, consumption of 100 g/d yogurt for 2 wk and 200 g/d for another 2 wk reduced the LDL/HDL cholesterol ratio in healthy women, with no differences from the probiotic culture *L. casei* containing yogurt (Fabian and Elmadfa 2006). Consumption of 375 mL of yogurt containing *L. acidophilus* and fructo-oligosaccharides for 3 wk lowered serum total cholesterol, LDL-cholesterol, and the LDL/HDL-ratio in 30 healthy men relative to conventional yogurt (Fabian and Elmadfa 2006).

2.3.2 Hypertension

Hypertension is associated with vascular disease mortality, CVD, and renal diseases (Lewington, Clarke et al. 2002, Chobanian, Bakris et al. 2003). Hypertension in US adults increased from 23.9% in 1988-1994 to 29% in 2007-2008 based on data from the National Health and Nutrition Examination Survey (NHANES) (Egan, Zhao et al. 2010). Previous studies have indicated a strong relationship between obesity and hypertension. Cross-sectional studies indicate that more than 85% of hypertensive individuals have a BMI of over 25 kg/m^2 (Kastarinen, Nissinen et al. 2000). Several mechanisms are involved in the pathogenesis of

obesity-related hypertension. The sympathetic nervous system, the renin-angiotensin system (RAS), and aldosterone contribute to the development of hypertension in obesity (Rahmouni, Correia et al. 2005). Long-term over-activation of sympathetic nervous system which is found in obesity could raise arterial pressure by inducing peripheral vasoconstriction and increasing sodium reabsorption in the renal tubules (Rahmouni, Correia et al. 2005). Adipose RAS is activated in obesity; animal models of visceral obesity suggest that adipose RAS contributes to obesity-associated hypertension (Massiéra, Bloch-Faure et al. 2001). Plasma aldosterone levels were elevated in obese hypertensive patients (Goodfriend and Calhoun 2004); on the other hand, an aldosterone antagonist was found to inhibit the development of high blood pressure in dietary-induced obese dog models (De Paula, Da Silva et al. 2004).

Therefore, given the need for dietary strategies to mitigate hypertension, the antihypertensive effects of dairy consumption have been investigated. The Dietary Approaches to Stop Hypertension (DASH) trial showed that a diet rich in fruits and vegetables lowered blood pressure and that additional inclusion of low-fat dairy products with reduced saturated and total fat further augmented these blood pressure-lowering effects (Appel, Moore et al. 1997). A recent review and meta-analysis of five cohort studies involving nearly 45,000 subjects revealed an inverse association between dairy consumption and development of elevated blood pressure, defined as ≥ 130 mm Hg systolic and/or ≥84 mm Hg diastolic blood pressure (RR 0.87, 0.81-0.94 95% CI) (Ralston, Lee et al. 2012). Another meta-analysis of prospective cohort studies similarly reported that increased consumption of 200 g/d of low-fat dairy products reduced the risk of hypertension (RR 0.96, 95% CI, 0.93-0.99) (Soedamah-Muthu, Verberne et al. 2012). Based on these data, low-fat dairy consumption appears protective against hypertension in adults,

but well-designed randomized, controlled trials (RCTs) are needed to confirm if yogurt is also antihypertensive.

2.3.3 Cancer

In 2010, 8 million people died from cancer globally, accounting for 15.1% of all deaths worldwide (Lozano, Naghavi et al. 2012). Overweight and obesity are estimated to contribute to 14% of all cancer deaths in men and 20% of deaths in women (Calle, Rodriguez et al. 2003). Meta-analyses indicate that higher BMI is associated with an increased incidence of endometrial, colorectal, and postmenopausal breast cancer (Larsson and Wolk 2007, Moghaddam, Woodward et al. 2007, Reeves, Pirie et al. 2007). It is hypothesized that obesity disturbs the physiological function of adipose tissue, which leads to insulin resistance, chronic inflammation, and dysregulation of adipokine secretion, factors contributing to the promotion and progression of cancer (Van Kruijsdijk, Van Der Wall et al. 2009).

Accumulating evidence indicates potential beneficial effects of yogurt consumption on cancers. A prospective study involving 82,220 Swedish individuals found that the risk for bladder cancer was lowest in individuals consuming the highest levels of sour milk and yogurt (RR 0.62, 95% CI, 0.46-0.85; $\geq$ 2 servings/d vs. 0 serving/d) (Larsson, Andersson et al. 2008). In another prospective study in an Italian cohort involving 45,241 volunteers, after adjusting for energy, simple sugar, calcium, fiber, animal fat, alcohol and red meat intake, body mass index, smoking, education, and physical activity, the hazard ratio for colorectal cancer in the highest versus lowest tertile of yogurt intake was 0.65 (95% CI, 0.48-0.89) (Pala, Sieri et al. 2011). Animal studies support the beneficial effects of yogurt. For example, LABs from yogurt were shown to effectively inhibit the genotoxic effects of heterocyclic aromatic amines on rats

(Zsivkovits, Fekadu et al. 2003). *L. acidophilus* isolated from yogurt reduced tumor growth rate and increased lymphocyte proliferation in a mouse model of breast cancer (Maroof, Hassan et al. 2012). A potential mechanism for reduced cancer risk is lower fecal mutagenicity, as demonstrated by consumption of yogurt with *B. lactis* by elderly individuals (Matsumoto, Ohishi et al. 2001). Given these promising results for yogurt intake and reduced risk for bladder and colon cancers, further work is warranted to evaluate if yogurt is similarly protective against other cancers.

2.3.4 Diabetes

The worldwide prevalence of diabetes in adults was estimated at 6.4% in 2010, and is projected to increase to 7.7% by 2030 (Shaw, Sicree et al. 2010). Excess weight may contribute to 90% of type 2 diabetes cases (Hossain, Kawar et al. 2007). More than 197 million people worldwide have impaired glucose tolerance attributed to obesity or metabolic syndrome (Hossain, Kawar et al. 2007). Many studies have illustrated the mechanisms linking obesity and type 2 diabetes. Adipose tissue has a pivotal role in type 2 diabetes by releasing non-esterified fatty acids (NEFAs), hormones, and various proinflammatory cytokines (Shoelson, Lee et al. 2006). Overabundant intracellular NEFAs inhibit key enzymes involved in glucose metabolism (Kahn, Hull et al. 2006). Furthermore, the corresponding intracellular fatty acid metabolites activate the serine/threonine kinase cascade which disturbs the insulin signaling pathway (Shulman 2000). To compensate for insulin resistance, the pancreatic β-cells secrete more insulin, eventually causing endoplasmic reticulum stress and protein misfolding which lead to β-cell apoptosis (Muoio and Newgard 2008).

Dairy consumption may reduce risk of type 2 diabetes. For example, an 8 yr prospective cohort study of 82,076 postmenopausal women demonstrated that low-fat dairy products were inversely associated with the risk of type 2 diabetes (RR 0.65; 95% CI: 0.44-0.96 for the highest quintile of intake) (Margolis, Wei et al. 2011). A recent meta-analysis of cohort studies showed that the adjusted relative risk of type 2 diabetes for highest versus lowest quartiles of dairy intake was 0.86 (95% CI, 0.79-0.92) (Tong, Dong et al. 2011). A subgroup analysis revealed a relative risk of 0.83 (95% CI, 0.74-0.93) for the intake of yogurt (Tong, Dong et al. 2011). A newer prospective study including 340,234 subjects did not find an association between total dairy products and diabetes. However, in the dairy subtype analysis, a higher combined intake of fermented dairy products (cheese, yogurt and thick fermented milk) was inversely associated with diabetes (HR, 0.88; 95% CI, 0.79-0.99) (Sluijs, Forouhi et al. 2012). In another smaller-sized prospective study, fermented dairy intake was inversely associated with fasting plasma glucose and HbA_{1c}, although no significant association between intake and incidence of diabetes was found (Struijk, Heraclides et al. 2013). Although epidemiological studies support the beneficial effects of yogurt consumption on reduced type 2 diabetes risk, RCTs are needed to confirm the causal effects of dairy consumption on improved diabetes outcomes.

2.4 Obesity, Yogurt and Chronic Inflammation

2.4.1 Obesity is associated with elevated chronic inflammation

The anti-inflammatory effects of low-fat dairy products have been well documented (Sakamoto, Igarashi et al. 2001, Schiffrin, Parlesak et al. 2009, Yang and Sheu 2012). The classic signs of inflammation are characterized by redness, swelling, heat, and pain and the inflammation is typically resolved shortly after the insult or stimuli are removed (Hotamisligil 2006). In contrast, obesity-associated chronic inflammation is unresolved, low-grade

inflammation that originates from metabolic cells (e.g. adipocytes) in response to excessive nutrient intake (Gregor and Hotamisligil 2011). Overactive metabolic signals induce the activation of proinflammatory pathways, which cause low-level induction of cytokines in metabolic tissues; these inflammatory signals recruit immune cells into metabolic tissues and disrupt the normal metabolic cell functions (Gregor and Hotamisligil 2011).

Obesity leads to increased levels of inflammatory biomarkers in a variety of tissues (**Table 2.3**). For example, protein kinases such as c-Jun N-terminal kinases (JNK) and inhibitor of κ kinase (IKK) induce the expression of proinflammatory cytokines (Solinas and Karin 2010). Obese women had a significantly higher amount of phosphorylated (active form) JNK in omental fat compared with lean women (Bashan, Dorfman et al. 2007). In rodents, Hirosumi et al. observed significant increases in total JNK activity in liver, muscle and adipose tissues of both dietary and genetic (ob/ob) obesity models (Hirosumi, Tuncman et al. 2002). Increased activation of JNK and nuclear factor kappa B (NF-κB) pathways were also detected in the hypothalamus of high-fat-fed mice, accompanied by increased secretion of proinflammatory cytokines (De Souza, Araujo et al. 2005). Elevated NF-κB and IKK activities were found in the livers of both genetic and diet-induced obese mice (Cai, Yuan et al. 2005). In high-fat-fed mice, increased IKK activity and downstream products of NF-κB pathway were observed in lysates of the thoracic aorta (Kim, Pham et al. 2007).

Table 2.3 Obesity-related changes in biomarkers of inflammation.

Reference	Samples	Population	Markers
(Hotamisligil, Arner et al. 1995)	adipose tissue	premenopausal women, n = 18 lean/n = 19 obese	↑ TNF-α mRNA; body weight reduction ↓ TNF-α mRNA
(Kern, Ranganathan et al. 2001)	adipose tissue	n = 50 lean/n = 50 obese	↑ TNF-α secretion
	plasma		↑ IL-6
(Panagiotakos, Pitsavos et al. 2005)	serum	3042 adults	↑ CRP, ↑ TNF-α, ↑ amyloid A, ↑ IL-6 in subjects with central adiposity
(Kim, Park et al. 2006)	serum	50 obese and 50 lean adults	↑ MCP-1, ↑ IL-8 and ↑ CRP
(Herder, Schneitler et al. 2007)	serum	519 adolescents	IL-6, IL-18 and interferon-γ-inducible protein-10 positively associated with BMI and waist circumference
(Mauras, DelGiorno et al. 2010)	plasma	203 children	↑ hsCRP, ↑ fibrinogen, ↑ IL-6 and ↑ plasminogen activator inhibitor-1
(Brake, Smith et al. 2006)	adipose tissue	High-fat-fed male mice	↑ ICAM-I, ↑ IL-6 and ↑ MCP-1 mRNA
(Ehses, Perren et al. 2007)	pancreatic islets	High-fat-fed mice	↑ IL-6, ↑ IL-8 and ↑ macrophage inflammatory protein 1α
(De Souza, Araujo et al. 2005)	hypothalamus	High-fat-fed rats	↑ TNF-α, ↑ IL-1β, and ↑ IL-6

Obesity increases the infiltration of immune cells into various metabolic tissues. Macrophages infiltrate adipose tissue in obese individuals and are responsible for nearly all adipose-derived TNF-α expression (Weisberg, McCann et al. 2003). Similarly, obesity leads to increased inflammatory macrophages in visceral adipose tissue (Curat, Wegner et al. 2006). Macrophage-derived proinflammatory cytokines can subsequently initiate insulin resistance and compromise β-cells (Solinas and Karin 2010).

Animal models of obesity corroborate the infiltration of macrophages and other immunocytes. Macrophages and microphages were increased in white adipose tissue in both genetic and high-fat diet-induced models of obese mice (Xu, Barnes et al. 2003). Ehses et al. observed increased islet-associated macrophages in high-fat-fed mice and *db/db* obese mice (Ehses, Perren et al. 2007). Diet-induced obese mice had increased accumulation of T cells in adipose tissue relative to lean mice (Wu, Ghosh et al. 2007). Likewise, natural killer T (NKT) cells infiltrated visceral adipose tissue in high-fat-fed mice (Ohmura, Ishimori et al. 2010). In the same model, depletion of NKT cells ameliorated visceral adipose tissue inflammation (Ohmura, Ishimori et al. 2010). Therefore, the metabolic and inflammatory consequences of obesity affect a wide variety of tissues. Animal and a limited number of human studies indicate a potential role for dairy or yogurt consumption to mitigate chronic inflammation associated with obesity, as detailed below.

2.4.2 Increased infiltration of immune cells into metabolic tissues

Yogurt and LAB can modulate the immune response through cytokine production. However, studies have not focused on the role of yogurt or dairy on obesity-associated immunocyte dysregulation. In diet-induced obese mice, compared to the high calcium diet, a

nonfat dry milk-supplemented diet reduced weight gain and associated adipose tissue inflammation as shown by decreased mRNA abundance of (monocyte chemoattractant protein) MCP-1, TNF-α, and IL-6; this suggested that some active components in dairy other than calcium could modulate the immune response (Thomas, Dunn et al. 2012).

Yogurt and its associated cultures also have immunostimulatory effects in healthy individuals. Consumption of yogurt containing *L. bulgaricus* and *S. thermophilus* increased production of IFN-γ by T cells in young adults (Halpern, Vruwink et al. 1991). IFN-γ regulates the induction of pro-inflammatory cytokines and the activation of macrophages and natural killer cells. LAB directly stimulates human lymphocyte IFN-γ *in vitro* (De Simone, Bianchi Salvadori et al. 1986). An observational retrospective study showed that supplementation with yogurt containing *L. rhamnosus* increased the CD4 count in a group of people living with HIV (Irvine, Hummelen et al. 2010). Consumption of fermented milk containing *L. acidophilus* significantly increased the phagocytosis of *E. coli* in adults (Schiffrin, Brassart et al. 1997). Likewise, fermented milk with *L. casei*, *L. acidophilus*, or a mixture of both increased phagocytic lymphocytic activities in Swiss mice (Perdigon, Alvarez et al. 1995). Oral administration of *L. acidophilus* alone improved immunoreactivity of peripheral blood leukocytes and peritoneal phagocytes and enhanced serum antibody response to orally and systemically administrated antigens in mice (Gill, Rutherfurd et al. 2000). Since yogurt consumption in obese individuals does not produce pro-inflammatory effects (Labonté, Couture et al. 2013), further work is needed to identify how yogurt modulates immune cells in obesity, and whether these effects are localized to the gut or have broader activities at metabolic tissues.

2.5 Obesity, Yogurt and Intestinal Barrier Function

The chronic inflammation associated with obesity may be exacerbated by impaired intestinal barrier function. Leptin-deficient and hyperleptinemic obese mice have increased intestinal permeability, modified distribution of junction proteins in the intestinal mucosa, as well as increased circulating levels of inflammatory cytokines compared with lean control mice (Brun, Castagliuolo et al. 2007). Diet-induced obese mice fed high-fat diets had increased intestinal permeability assessed by gavage of fluorescent-dextran, increased plasma LPS levels, and reduced expression of genes for tight junction proteins (Cani, Bibiloni et al. 2008). Obese women had increased paracellular permeability measured by lactulose excretion relative to lean women (Teixeira, Souza et al. 2012). Intestinal paracellular permeability was correlated with waist circumference and homeostatic model assessment (HOMA) values (Teixeira, Souza et al. 2012). Likewise, intestinal barrier function was more strongly correlated with central adiposity than BMI in overweight adults (Gummesson, Carlsson et al. 2011). Dysregulation of intestinal barrier function may be attributed to dysregulation of gut microbiota, endotoxin exposure, the mucus bilayer, secretory immunoglobulin A (sIgA), antimicrobial peptides, and tight junction proteins. Emerging evidence supports the ability of yogurt consumption to modulate these functions, as discussed below.

2.5.1 Dysregulation of gut microbiota

The intestine is essential for nutrient absorption and host defense. Gut microbiota facilitate these functions by fermenting non-digestible nutrients, vitamin synthesis, and participating in host defense (Salzman, Underwood et al. 2007). Favorable gut microbiota may compete with pathogens for space and nutrients and produce anti-microbial compounds such as bacteriocins and lactic acids (O'Hara and Shanahan 2006). Gut microbiota also contribute to

energy homeostasis and fat storage. Interestingly, germ-free mice were protected against diet induced obesity (Bäckhed, Manchester et al. 2007). On the other hand, conventionalization of germ-free mice with a normal microbiota harvested from the cecum of conventionally raised mice caused a 60% increase in body fat within 14 d despite reduced food consumption (Bäckhed, Ding et al. 2004). The authors proposed that the gut microbiota helped to absorb monosaccharides from the lumen which further induced de novo hepatic lipogenesis (Bäckhed, Ding et al. 2004). Colonization of germ-free mice with microbiota from obese mice induced a more significant increase in total body fat than colonization with microbiota from lean mice (Turnbaugh, Ley et al. 2006). This suggested that the composition of gut microbiota affects the development of obesity. In both mice and humans, *Bacteroidetes* and *Firmicutes* are the major species comprising the microbiota (Bäckhed 2009). Obese adults have a lower proportion of *Bacteroidetes* to *Firmicutes* than lean, although this ratio can be improved with weight loss from energy restriction (Ley, Turnbaugh et al. 2006).

Conventional yogurt cultures have limited viability in the gut and a limited ability to influence the composition of the gut microbiota. Adults consuming yogurt with *S. thermophilus* and *L. bulgaricus* had less than 10^3 CFU/g of these cultures in feces (Del Campo, Bravo et al. 2005). In another study, participants consumed 125 g of a commercial yogurt twice per day for one week, providing 10^8 CFU of *S. thermophilus* and *L. bulgaricus* (Elli, Callegari et al. 2006). *S. thermophilus* was not present in feces, although *L. bulgaricus* was present in about 70% of the fecal samples provided on days 2 and 7 of the yogurt-consumption period. However, the levels of *L. bulgaricus* detected on average did not exceed the 10^5 CFU/g minimum deemed necessary to exert beneficial effects (Elli, Callegari et al. 2006). Another study providing a higher dose of yogurt cultures (375 g yogurt, 10^8 CFU/g) for two weeks, reported a median value of

approximately 10^4 CFU each of *S. thermophilus* and *L. bulgaricus* per gram of feces (Mater, Bretigny et al. 2005). Although yogurt cultures have apparently low viability through the entire gastrointestinal tract, more information is needed about their small intestine viability.

Certain probiotic strains may have improved viability in the gut relative to *S. thermophilus* and *L. bulgaricus*. Healthy adults that consumed 230 mL yogurt with additional *L. acidophilus* and *B. bifidum* at 10^7 CFU/g daily for 10 days had decreased aerobic bacteria and increased anaerobic bacteria in fecal samples (Chen, Wu et al. 1999). Additionally, the bifidus to coliform ratio favorably increased and *B. bifidum* was measurable for up to 8 days after consumption (Chen, Wu et al. 1999). In contrast, *L. acidophilus, S. thermophilus,* and *L. bulgaricus,* were not detectable in feces (Chen, Wu et al. 1999).

McNulty et al (2011) investigated the effect of *B. animalis* subsp. *lactis* on the gut microbiota of mice and humans. Healthy pairs of monozygotic twins consumed a fermented milk product (FMP) with *L. bulgaricus* and *B. animalis* subsp. *lactis* or no product daily for seven weeks. Fecal samples analyzed before, during, and after the intervention did not show a statistically significant change in the microbiota composition (McNulty, Yatsunenko et al. 2011). Additionally, the FMP cultures did not persist in the microbiota longer than two weeks after ceasing its consumption. In the same study, human-gut-derived bacterial strains and FMP strains were transplanted into germ-free mice. Similar to humans, the humanized intestinal microbiota was not drastically altered by FMP, but genes related to carbohydrate metabolism were up-regulated by FMP consumption (McNulty, Yatsunenko et al. 2011).

Animal models suggest that yogurt-induced improvements in intestinal permeability are associated with changes to gut microbiota. In Wistar rat pups, consumption of yogurt with *L.*

casei counteracted acute gastroenteritis-induced barrier dysfunction (Isolauri, Kaila et al. 1993). In atopic dermatitis (AD) patients with increased intestinal permeability, 4 wk consumption of yogurt with *B. lactis*, *L. bulgaricus*, and *S. thermophilus* increased polyamine-producing bacterial species which was associated with improved intestinal barrier function (Matsumoto, Aranami et al. 2007).

Cultures used in yogurt may also be modified to improve their viability in the gut by protecting cultures from stomach acid. For example, yogurt with encapsulated or free *L. acidophilus* ATCC 4356 was subjected to a simulated human digestive system (Ortakci and Sert 2012). Encapsulated *L. acidophilus* had improved viability up to 2 h of incubation in artificial human gastric juice.

Thus, conventional yogurt cultures have low to no viability in the gut. Probiotic or encapsulated strains may have greater viability, and their metabolic effects or competition with coliforms in the intestine are apparent. These studies suggest that strains may not need to adhere to the intestinal epithelium and proliferate in order to exert the desired health effects. If this is the case, it suggests that consistent and prolonged probiotic consumption may be needed to achieve measurable health benefits from these strains.

2.5.2 Contribution of bacterial endotoxin to chronic inflammation

Gut microbiota contribute to systemic low-grade inflammation by increasing the exposure to proinflammatory bacterial products, especially the Gram-negative-derived LPS among others (Okamura, Watari et al. 2001, Rallabhandi, Bell et al. 2006). LPS typically consists of a hydrophobic domain known as lipid A, a non-repeating core oligosaccharide, and a distal polysaccharide (Raetz and Whitfield 2002). LPS initiates inflammatory signaling through

lipopolysaccharide binding protein (LBP), CD14, Toll-like receptor 4 (TLR4) and MD2. LBP is thought to extract LPS and subsequently deliver it to CD14 or lipoprotein; the former may lead to the activation of target cells while the latter may result in the clearance by liver (Van Bossuyt, De Zanger et al. 1988). CD14 serves as a pattern-recognition receptor in proinflammatory signaling which can be stimulated by various ligands (Pugin, Heumann et al. 1994, Park, Song et al. 2009). MD2 physically associates with TLR4 on the cell surface and acts as co-receptor with TLR4 for the detection of LPS (Manco, Putignani et al. 2010). Once activated by LPS, TLR4 undergoes oligomerization and recruits its two adaptor protein pairs, TRAM-TRIF and MAL-MyD88, ultimately activating the NF-κB pathway (Manco, Putignani et al. 2010). Human and animal studies have shown LPS as a strong inducer of proinflammatory cytokines such as IL-6 and TNF in most tissues including adipocytes (Stoll, Denning et al. 2004, Kemna, Pickkers et al. 2005, Cani, Amar et al. 2007, Creely, McTernan et al. 2007, Andreasen, Larsen et al. 2010).

Overweight and obese adults have increased endotoxin exposure (Sun, Yu et al. 2010). Acute and chronic fat consumption is associated with increased exposure to endotoxin. A cross-sectional study of 201 healthy French men reported that total energy and fat, but not carbohydrate or protein were correlated with plasma LPS (Amar, Burcelin et al. 2008). These observations were confirmed in mice and indicated fat was more efficient in facilitating translocation of LPS into circulation than carbohydrate (Amar, Burcelin et al. 2008). In addition, a single high-fat meal can induce postprandial endotoxemia and inflammation (Erridge, Attina et al. 2007, Ghanim, Sia et al. 2010, Laugerette, Vors et al. 2011). Obesity-associated dysregulation of gut microbiota may also increase endotoxin exposure (Cani, Amar et al. 2007, Cani, Bibiloni et al. 2008).

Preliminary studies in elderly individuals have demonstrated that yogurt consumption inhibits markers of endotoxin exposure in elderly individuals (Schiffrin, Parlesak et al. 2009). Elderly individuals (n = 23) with small-intestinal bacterial overgrowth consumed 300 g/d of yogurt with 10^9 CFU *L. johnsonii La1* for 4 wk. By the end of the trial, yogurt consumption decreased plasma levels of LBP and sCD14, LPS pattern recognition receptors in elderly with small-intestinal bacterial overgrowth (Schiffrin, Parlesak et al. 2009). Furthermore, yogurt consumption also reduced plasma endotoxin in healthy elderly participants (Schiffrin, Parlesak et al. 2009). Small intestinal bacterial overgrowth may affect 41% of obese individuals (Jouet, Coffin et al. 2011). Therefore, further studies are warranted to evaluate the ability of yogurt to reduce endotoxin exposure in obese individuals.

2.5.3 Mucus bilayer

The mucus bilayer is produced by goblet cells and separates gut microbiota from endothelial cells. This bilayer is formed by a mesh-like structure of mucins, high molecular weight glycoproteins with increased hydration capacity due to negative surface charges (Dharmani, Srivastava et al. 2009). Mucins lubricate and maintain the hydrated layer of the epithelium, as well as create a permeable unstirred, gel-like layer that facilitates nutrient exchange (Dharmani, Srivastava et al. 2009). The mucus bilayer is essential to the innate host defense. The outer mucus layer provides space and nutrients for the residence of commensal microflora which might inhibit the growth and invasion of pathogens; the inner layer is impervious to bacteria and acts like a protective barrier for the epithelium (Turner 2009, Kim and Ho 2010). The goblet cells also produce intestinal trefoil factor and resistin-like molecule-β, proteins that strengthen the barrier by stabilizing the mucin polymers or regulating mucin

secretions (Dharmani, Srivastava et al. 2009). Additionally, the mucus layer contains other defensive components such as secretory IgA and antimicrobial peptides.

Probiotics associated with yogurt may stimulate the production of intestinal mucins and improve host defense. For example, *L. plantarum* 299 v incubated with HT-29 intestinal epithelial cells increased mucin mRNA expression and inhibited the adherence of an attaching and effecting pathogenic *E. coli. in vitro* (Mack, Michail et al. 1999). Similarly, in Wistar rats, 7 d consumption of *Lactobacilli, Bifidobacteria,* and *Streptococci* increased basal luminal mucin content by 60% (Caballero-Franco, Keller et al. 2007). Whey peptides derived from α- and β-caseins also increased mucin secretion in HT29-MTX cells (Martínez-Maqueda, Miralles et al. 2012). Thus, this emerging evidence suggests that dairy products or their associated probiotics could be of benefit to the mucus bilayer. A recent diet-induced obese mice model showed that obesity was associated with decreased mucus layer thickness due to the decreased level of *Akkermansia muciniphila*, a mucus layer resident that played an essential role in mucus turnover (Everard, Belzer et al. 2013). Thus, it appears worthwhile to further investigate the effects of yogurt on the obese-compromised mucus layer.

2.5.4 Secretory IgA (sIgA)

sIgA is the major effector of the mucosa-associated lymphoid tissue (MALT) and protects against commensal bacterial penetration of the lumen (Brandtzaeg, Baekkevold et al. 1999). MALT consists of lymphocytes such as T cells and B cells, as well as plasma cells and macrophages, which are stimulated by antigens. sIgA, dimeric or polymeric IgA, is produced by plasma cells in the intestinal mucosa and is the predominant antibody class in the intestinal lumen (Macpherson and Uhr 2004, Woof and Ken 2006). Interstitial sIgA inhibits pathogens and

toxins by 1) preventing the adhesion and entry of pathogens and toxins by interfering with epithelial receptor recognition, 2) binding pathogens and promoting their clearance, or 3) inhibiting virus production (Corthésy 2007). Low serum IgA may indicate compromised immune function, while high serum IgA is associated with chronic inflammation, central adiposity, and advanced age (Gonzalez-Quintela, Alende et al. 2008).

Yogurt consumption appears to modulate the gut immune function by increasing sIgA. For example, consumption of yogurt with *L. acidophilus* by 30 healthy adults increased total serum IgA and the production of specific serum IgA against an attenuated strain of *S. typhimurium* (Link-Amster, Rochat et al. 1994). Consuming 400 mL yogurt with *L. acidophilus* daily for 4 wk reduced *H. pylori* and increased the serum IgA level in 38 infected children (Yang and Sheu 2012). Rodent studies also support the IgA-promoting effects of yogurt. In a mouse model, orally administrated LAB alone and in yogurt increased the intestinal IgA producing cells and IgA (Perdigon, Alvarez et al. 1995). Furthermore, a 7 d yogurt treatment partially prevented the infection of *S. typhimurium* and inhibited intestinal carcinomas induced by 1-2-dimethylhydrazine (Perdigon, Alvarez et al. 1995). Similarly, mice fed yogurt for 4 wk had increased serum IgA after a *S. typhimurium* challenge, relative to the milk-treated control group (Puri, Rattan et al. 1996). Thus, both animal and human studies have demonstrated induction of sIgA defenses following yogurt consumption, which may improve immunity.

2.5.5 Antimicrobial peptides

Defensins are antimicrobial peptides secreted by Paneth cells located in the crypts of the small intestinal mucosa (Porter, Bevins et al. 2002). Defensins have bactericidal activity against various Gram-positive and Gram-negative bacteria (Salzman, Underwood et al. 2007). These

peptides facilitate bacterial membrane collapse through electrostatic and hydrophobic interactions (Zasloff 2002). Paneth cell function and defensin levels are compromised in obese individuals, which may be explained by activated unfolded protein response in the intestine (Hodin, Verdam et al. 2011). In healthy women, consumption of 200 mL yogurt with or without *B. lactis* Bb12 for 3 wk did not alter fecal β-defensin-2, although both treatments increased fecal sIgA from baseline (Kabeerdoss, Shobana Devi et al. 2011). However, the probiotic *Lactobacillus* and *E.coli* Nissle 1917 increased β-defensin 2 expression in Caco-2 cells (Schlee, Wehkamp et al. 2007, Schlee, Harder et al. 2008). Further work is needed to determine if dietary approaches are feasible to overcome obesity-compromised defensin production.

2.5.6 Mucosal cells and tight junctions

The innermost layer of the intestine is a monolayer of enterocytes, endocrine cells, microfold cells, G cells, and Paneth cells (Scaldaferri, Pizzoferrato et al. 2012). Enterocytes are the most abundant cells and are connected by apical junctions, which are mainly adherent or tight junctions (Hartsock and Nelson 2008). Adherent junctions consist of the transmembrane protein E-cadherin and the catenin family members, including p120-catenin, β-catenin, and α-catenin (Hartsock and Nelson 2008). Adherent junctions initiate and stabilize cell-cell adhesion, regulate the actin cytoskeleton, and contribute to intracellular signaling (Hartsock and Nelson 2008). Tight junctions are composed of occludins, claudins, and junction adhesion molecules (JAM), transmembrane proteins that are linked to the cytoskeleton through zonula occludens (ZO) scaffolding proteins (Hartsock and Nelson 2008). Tight junctions are the primary barrier to intestinal intercellular space, but are not impermeable. The paracellular pathway is selective to ions and other small molecules, and depends on the cell type (Tsukita, Furuse et al. 2001).

Increased plasma endotoxin levels suggest that obese individuals have compromised intestinal barrier function (Sun, Yu et al. 2010). Compromised intestinal barrier function is proposed to contribute to chronic inflammation in obesity by initiating inflammation through endotoxin exposure (Cani, Amar et al. 2007). The perturbation of proinflammatory cytokines, gastrointestinal peptides, and endocannabinoids associated with obesity can compromise tight junctions (Blüher, Engeli et al. 2006, Côté, Matias et al. 2007, Cluny, Reimer et al. 2012). In rodents, glucagon-like peptide-2 (GLP-2) protects barrier function, while melatonin can increase permeability (Cameron, Yang et al. 2003, Cameron and Perdue 2005, Sommansson, Nylander et al. 2013). IFN-γ (Youakim and Ahdieh 1999, Yang, Kiristioglu et al. 2002, Bruewer, Utech et al. 2005, Clark, Hoare et al. 2005), TNF-α (Schmitz, Fromm et al. 1999, Mankertz, Tavalali et al. 2000, Li, Zhang et al. 2008, Al-Sadi, Boivin et al. 2009), and IL-6 (Yang, Han et al. 2003, Al-Sadi and Ma 2007) can disrupt barrier function. In contrast, IL-10 (Madsen, Lewis et al. 1997, Oshima, Laroux et al. 2001), transforming growth factor beta (TGF-β) (Howe, Reardon et al. 2005), and IL-17 (Kinugasa, Sakaguchi et al. 2000) improve barrier function in human T84 colonic epithelial cells. Obese *ob/ob* mice had improved barrier function and lower plasma LPS when treated with a CB receptor 1 antagonist (Muccioli, Naslain et al. 2010).

Yogurt and its associated probiotics may improve intestinal barrier function by maintaining the expression of tight junction proteins. Yogurt with *B. lactis* prevented the increase in intestinal permeability induced by partial restraint stress in rats, and restored occludin and JAM-A expression (Agostini, Goubern et al. 2012). In addition, calcium, which is rich in yogurt, plays a critical role in tight junction biogenesis and supplementation of calcium was shown to be able to inhibit alteration in tight junction function in a diabetic rat model (Stuart, Sun et al. 1994, Leal,

Martins et al. 2010). Therefore, these rodent studies suggest that dairy calcium or probiotic yogurt could be beneficial for maintaining function of tight junctions.

2.6 Other Potential Benefits of Yogurt Consumption on Gut Health

Increased yogurt consumption has the potential to improve intestinal health, ameliorate lactose intolerance, prevent constipation and diarrheal diseases, decrease allergies in vulnerable populations, and reduce the risks of colon cancer and inflammatory bowel diseases (Adolfsson, Meydani et al. 2004, Parvez, Malik et al. 2006). The mechanisms for these actions are not fully described, but may include modulating gut pH, inhibiting the proliferation and adhesion of pathogenic bacteria, secreting antibacterial substances, and regulating immune function.

2.6.1 Lactose intolerance

In lactase-deficient individuals, lactose enters the colon and is fermented by colonic bacteria. The colonic metabolites of lactose include short-chain fatty acids which, together with electrolytes, introduce an osmotic load that can cause diarrhea and discomfort (Lomer, Parkes et al. 2008). In a cross-sectional study, subjects with self-perceived lactose intolerance had a significantly lower calcium intake from dairy foods and reported higher rate of physician-diagnosed diabetes and hypertension (Nicklas, Qu et al. 2011). Early studies indicated that subjects with lactase deficiency had better digestion and absorption of lactose from yogurt than the lactose in milk (Kolars, Levitt et al. 1984). After ingestion of around 18 g of lactose in water, milk, or yogurt, subjects receiving yogurt had only one third of the hydrogen excretion, an indicator of undigested lactose, compared with those receiving lactose in water or milk (Kolars, Levitt et al. 1984). Furthermore, the consumption of yogurt led to fewer symptoms of diarrhea and flatulence relative to milk (Kolars, Levitt et al. 1984).

2.6.2 Diarrhea

Diarrhea is the leading cause of morbidity and death of children in developing countries (Boschi-Pinto, Velebit et al. 2008). Emerging evidence suggests that consumption of yogurt and its related probiotic cultures prevent or treat diarrhea. In a double-blind, placebo-controlled trial, infants that received formula with *B. bifidum* and *S. thermophilus* reduced the incidence of acute diarrhea and rotavirus shedding (Saavedra, Bauman et al. 1994). A meta-analysis of RCTs published from 1966 to 2000 suggested that *Lactobacillus* supplementation (10^8 to 10^{11} CFU daily) safely reduced the frequency and duration of acute infectious diarrhea in children (Van Niel, Feudtner et al. 2002). Moreover, a more recent meta-analysis of RCTs showed that administration of *Lactobacillus* through capsules or fermented milk during antibiotic treatment significantly reduced the risk of developing antibiotic-associated diarrhea (RR 0.35, 0.19-0.67 95% CI) (Kale-Pradhan, Jassal et al. 2010). However, the risk reduction was only significant in adults after subgroup analysis (Kale-Pradhan, Jassal et al. 2010).

2.6.3 H. pylori infection

Consumption of yogurt with *L. gasseri* for 8 wk significantly suppressed *H. pylori*-induced gastric mucosal inflammation in the elderly (Sakamoto, Igarashi et al. 2001). In children affected by *H. pylori*, yogurt consumption decreased serum IL-6 level after 4 wk (Yang and Sheu 2012).

2.6.4 Inhibition of Colitis

The prevalence of the inflammatory bowel diseases (IBD) Crohn's disease (CD) and ulcerative colitis (UC) is increasing in industrialized nations, and although the cause(s) are unknown, they likely result from an aberrant immune response to intestinal microbiota (Chaves,

Perdigon et al. 2011). Probiotics administered to murine models of IBD improve disease outcomes; this has been reviewed elsewhere (Claes, De Keersmaecker et al. 2011). Yogurt consumption also inhibits experimental IBD in mice. Consumption of yogurt with eight *L. bulgaricus* strains and two *S. thermophilus* strains decreased mortality rate and prevented intestinal inflammation and tissue damage in mice with trinitrobenzene sulfonic acid (TNBS)-induced intestinal inflammation (Chaves, Perdigon et al. 2011). Yogurt consumption prevented an increase in colonic $CD4^+$ and $CD8^+$ T cell numbers, decreased TLR4 positive cells at 14 days, but not 3 or 7 days post TNBS administration (Chaves, Perdigon et al. 2011). Yogurt without added probiotic strains inhibited TNBS-induced colitis in mice, increased the number of IgA producing cells, and decreased $CD8^+$ T cells two wk after TNBS administration (Gobbato, Rachid et al. 2008).

Clinical studies have mixed outcomes for the probiotic treatment of IBD and are strain-dependent (Kato, Mizuno et al. 2004, Hedin, Whelan et al. 2007, Lorea Baroja, Kirjavainen et al. 2007, Miele, Pascarella et al. 2009, Sood, Midha et al. 2009, Jonkers, Penders et al. 2012). Clinical studies have not used conventional yogurt as an intervention for IBD, despite self-reported benefits of yogurt reported by IBD patients (Cohen, Lee et al. 2013). Consumption of yogurt with *L rhamnosus* GR-1 and *L. reuteri* RC-14 improved markers of inflammation in monocytes from 20 patients with IBD, including increasing $CD4^+CD25^{high}$ T cells (Lorea Baroja, Kirjavainen et al. 2007). Yogurt could be an effective delivery vehicle for probiotic strains for treatment of IBD. However, more work is needed to identify clinically-significant probiotic strains for inhibiting colonic inflammation.

2.6.5 Appetite control

Obesity is a result of positive energy balance. Some studies have demonstrated that yogurt might help reduce energy intake by suppressing appetite. For example, consumption of yogurt either in semisolid or liquid form led to lower hunger and higher fullness feeling, compared with a fruit drink or dairy fruit drink (Tsuchiya, Almiron-Roig et al. 2006). Similarly, subjects felt higher satiety after consumption of yogurt as evidenced by rating of hunger, appetite, desire to eat, and fullness, compared with ingestion of chocolate bars (Chapelot and Payen 2010). Yogurt consumption also suppressed appetite rating and reduced subsequent food intake or delayed subsequent eating, compared with isovolumetric water (Dougkas, Minihane et al. 2012, Douglas, Ortinau et al. 2013). Therefore, yogurt consumption may provide a further benefit of appetite-suppression, although the molecular mechanisms for this effect remain uncharacterized.

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Chapter 3: Effects of Low-fat Yogurt Consumption on Weight, Blood Pressure and Dietary Pattern

3.1 Abstract

Background: Increasing dairy consumption may help obese individuals lose weight and lower blood pressure. However, the effect of increased yogurt consumption on weight, blood pressure, and diet quality without weight reduction is poorly understood.

Objective: Our objective was to determine how increased low-fat yogurt consumption affects body weight, body mass index (BMI), waist circumference (WC), blood pressure (BP), and diet in obese and non-obese women maintaining normal dietary patterns.

Design: Apparently healthy premenopausal women with BMI of 18.5 to 27 (lean) or 30 to 40 kg/m^2 (obese) participated in a 9-week randomized, controlled intervention study. Participants in each BMI range were randomly assigned to either a low-fat yogurt-supplemented group (Y, yogurt lean; YO, yogurt obese) or a soy pudding-supplemented control group (C, control lean; CO, control obese), with n = 30/group. Participants were advised to consume 12 oz. of yogurt or the control food daily for 9 weeks while maintaining their usual caloric intake. Weight, WC, and BP of participants were determined at wk 0, 3, 6 and 9, and 3-day food records were completed at baseline and the end of intervention.

Results: All groups maintained isocaloric intake with addition of the intervention foods, based on dietary records. Y and C maintained initial weights, but YO and CO had increased body weight by wk 9 (YO: 0.91 ± 0.31 kg, $P = 0.0023$; CO: 0.94 ± 0.42 kg, $P = 0.0215$). In YO, diastolic BP was lowest at wk 3 (-2.82 ± 0.90 mm Hg, $P = 0.0062$) with a reduced effect at wk 6 (-2.1 ± 0.83 mm Hg, $P = 0.1078$) and wk 9 (-1.90 ± 0.95 mm Hg, $P = 0.1833$) relative to baseline. In contrast, C and Y did not have consistent BP changes. Calcium intake increased 69% in Y and

57% in YO, and was higher than that in the control groups. Sugar and added sugar consumption increased by 48 - 317% in all groups by the end of the intervention.

Conclusion: Consumption of low-fat yogurt lowered blood pressure in obese premenopausal women within 3 weeks, but this effect decreased by wk 9, and coincided with ~ 0.9 kg increased body mass. Yogurt consumption induced a slight decrease in waist circumference but had no effect on blood pressure in lean premenopausal women. This trial was registered at clinicaltrials.gov as NCT01686204.

3.2 Introduction

Obesity is associated with increased risk of cardiovascular disease, type 2 diabetes, and certain cancers (Kastarinen, Nissinen et al. 2000, Grundy 2007, Hossain, Kawar et al. 2007). Hypertension is also linked to increased obesity, as more than 85% of hypertensive individuals have a BMI > 25 kg/m^2 (Kastarinen, Nissinen et al. 2000). Obesity decreases quality of life, life expectancy, and its prevalence causes a sizable economic burden (Peeters, Barendregt et al. 2003, Wang, McPherson et al. 2011, Warkentin, Das et al. 2014). Dietary and lifestyle changes that lead to weight loss are necessary approaches to reduce obesity-associated chronic disease risk.

Epidemiological studies and meta-analyses suggest that increased dairy consumption might benefit obese individuals by increasing weight loss and reducing blood pressure (Mozaffarian, Hao et al. 2011, Ralston, Lee et al. 2012, Soedamah-Muthu, Verberne et al. 2012). Furthermore, yogurt consumption is associated with lower BMI (28.0 ± 5.4 kg/m^2 in yogurt non-consumer vs. 26.9 ± 5.5 kg/m^2 in yogurt consumer, $P < 0.001$), and better diet quality and metabolic profile in US adults (Wang, Livingston et al. 2013).

Intervention studies also suggest that yogurt consumption aids weight loss while maintaining lean tissue and reducing diastolic BP in overweight and obese individuals when consumed with energy-restricted diets (Zemel, Richards et al. 2005, Thomas, Wideman et al. 2011). The design of these studies did not isolate the effect of weight loss and yogurt consumption on metabolic effects. Without energy restriction, intervention studies with dairy or fermented dairy consumption for BP reduction are equivocal (Maki, Rains et al. 2013, Drouin-Chartier, Gigleux et al. 2014, Hove, Brøns et al. 2015).

These prior intervention studies did not utilize conventional yogurt directly in a healthy, obese population, or include a non-obese control population. Therefore, we characterized the effect of yogurt consumption on body weight, WC, and BP as part of a larger randomized control trial investigating the anti-inflammatory mechanisms of yogurt consumption. To account for other potential dietary changes given the intervention, we further examined the nutrient intakes and diet composition of study participants.

3.3 Materials and Methods

3.3.1 Study participants

The study protocol was approved by the Institutional Review Boards at the University of Connecticut (#H12-168) and University of Wisconsin-Madison (#2014-0669) and participants were provided written consent before participating in study procedures. The study was also registered at ClinicalTrials.gov as NCT01686204.

Apparently healthy premenopausal women aged 21 to 55 years were recruited from the Storrs, CT and Madison, WI area by mass emails, flyers, announcement in newspapers. After consent, participants completed a questionnaire that included demographic health information. Study personnel then measured participants' height, weight, WC and BP. The study inclusion criteria included BMI from 18.5 to 27 or from 30 to 40 kg/m^2, an age of 21 to 55 years, stable body weight for the previous 2 months, willing to avoid yogurt and probiotic-containing foods and consume the provided 1.5 servings of yogurt or soy pudding (control treatment) for the duration of the study, no previous diagnosis of CVD, diabetes or arthritis, not being currently treated for cancer, not taking any anti-inflammatory drugs, not under prescribed estrogen replacement therapy, not on sliming, vegetarian or vegan diets, not currently taking dietary

supplements or smoking, not allergic to soy, egg or milk, not pregnant, lactating, or seeking to become pregnant.

3.3.2 Dietary treatment

Commercially available low-fat yogurts (Yoplait, General Mills, Inc. Minneapolis, MN) and soy puddings (ZenSoy, South Hackensack, NJ) packaged in 4 oz. containers were purchased from local suppliers. The soy pudding served as a non-dairy control food with macronutrient and micronutrient content, total calories and texture matched to yogurt. The yogurt had strawberry, strawberry banana, raspberry, and peach flavors, which was representative of most commercial low-fat yogurt. The detailed nutrient content of yogurt and control are listed in **Table 3.1**. Soy pudding contained 2.00 ± 0.09 mg/100g of daidzein, 1.64 ± 0.01 mg/100g of glycitein, and 5.34 ± 0.16 mg/100g of genistein (**Appendix A.1.1**). Therefore, 12 oz. soy pudding provided a small amount of isoflavones that were equivalent to that in ~ 5.0 g of soy bean (Franke, Custer et al. 1994). In addition, 12 oz. soy pudding provided much less than the 25 g of soy protein estimated to lower coronary heart disease risk (FDA 21 CFR 101.82). Further, 4 wk consumption of 10 g/day of soy protein, delivered as a soy-based smoothie, did not alter biomarkers of oxidative stress or inflammation in obese and overweight subjects (Zemel, Sun et al. 2010).

3.3.3 Experimental design

A randomized, controlled study was performed to test the impact of yogurt consumption on intestinal barrier function and chronic inflammation relative to consumption of a non-dairy control food. The sample size calculation was based on detecting an 8.2% difference in soluble CD14 (sCD14), the primary outcome (Laugerette, Vors et al. 2011). Measurement of weight, BP, and nutrient intake were nested in the study protocol as depicted in **Figure 3.1**.

Table 3.1 Nutrient comparison of low-fat yogurts and the soy pudding control food.

Nutrients/serving	Low-fat yogurts (Yoplait) [1]	Control food (ZenSoy)
Serving size (oz.)	4	3.8
Calories (kcal)	110	110
Total Fat (g)	1	1
Carbohydrate (g)	22	22
Protein (g)	3	2-3 [2]
Cholesterol (mg)	5	0
Sodium (mg)	60	55-70 [3]
Calcium (mg)	150	60-150 [3]
Vitamin A (IU)	500	200
Vitamin D (μg)	2.25	2.25

[1] Contained the active cultures *L. Bulgaricus*, *S. Thermophilus*, and *L. acidophilus*.

[2] Different flavors of pudding contained either 2 or 3 g of protein per cup.

[3] Due to the reformulation by the manufacture during the intervention, the sodium content decreased from 70 to 66 mg per cup; the calcium decreased from 150 to 60 mg per cup (n = 24, on the old formulation; n = 36, on the new formulation).

To avoid confounding by probiotic or dairy consumption, the participants restricted consumption of dietary supplements, fermented foods and limited their dairy consumption to ≤ 4 servings/day for 2 weeks before the intervention (washout period, wk -2 to wk 0) and throughout the intervention. From the beginning of wk 0 to the end of wk 9 (intervention period), the subjects consumed 12 oz. of yogurt or the control food daily. The lids of consumed yogurt and

pudding cups were collected weekly for assessment of compliance. Participants visited the study center at wk 0, 3, 6 and 9 for anthropometric and BP measurements.

After the initial consent and screening visit, 128 subjects were enrolled and randomly assigned to either the yogurt group or the soy pudding control group, in blocks of 6 (**Figure 3.2**). Participants were randomized upon enrollment by assigning random numbers generated by Minitab 17.0 (Minitab Inc., State College, PA) (Campbell and Swinscow 2009). After enrollment, 8 subjects were excluded or dropped from the study because of the following reasons: not able to obtain blood samples (n = 1), not able to eat pudding (n = 2), not compliant (n = 1), prescribed anti-inflammatory drug (n = 1), family emergency (n = 1), not specified (n = 2). At the end of the study, 60 obese (BMI 30-40 kg/m^2) and 60 lean (BMI 18.5-27 kg/m^2) participants completed the study, with n = 30/group (YO, yogurt obese; CO, control obese; Y, yogurt lean; C, control lean).

3.3.4 Anthropometric measurements

Participants were asked to remove shoes, jacket, phones, keys, or other extraneous mass before measurements. Body weight and height were measured on a digital physician scale (Rice Lake H150-10-5, Rice Lake, WI). WC was determined by placing a measuring tape evenly around a bare abdomen at hip bone level. BP was measured using an Omron HEM-780 with ComFit cuff for participants in Y and C, or an Omron BP710 with Omron H003D large adult cuff for YO and CO (Omron Healthcare, Inc. Lake Forest, IL). Before BP readings, participants were instructed to sit upright in a phlebotomy chair for a minimum of 5 min with their back supported, feet on the floor, legs uncrossed, bladder empty, and their upper arm supported at heart level (Pickering, Hall et al. 2005). BP was measured twice, at least 3 min apart. If BP readings were more than 5 mm Hg apart, a third reading was taken.

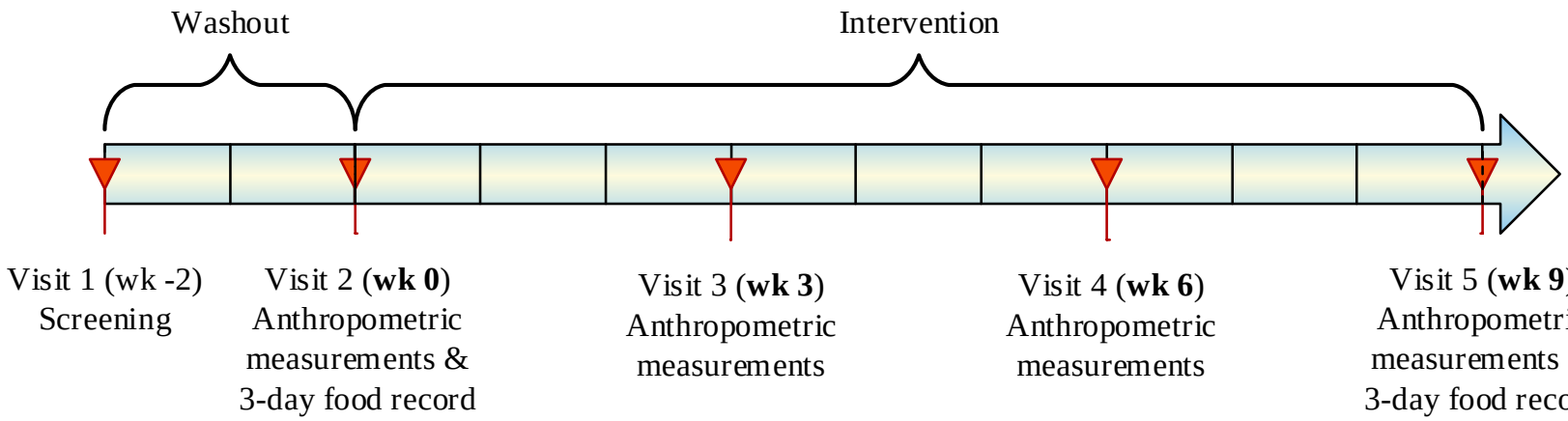

Figure 3.1 Experimental design.

After 2 weeks of washout, lean and obese participants were randomly assigned to either yogurt or the control food for 9 weeks. Starting from baseline (wk 0), subjects came to the study center to have their height, weight, waist circumference, blood pressure measured. The week before the start and end of intervention, participants completed a 3-day food record.

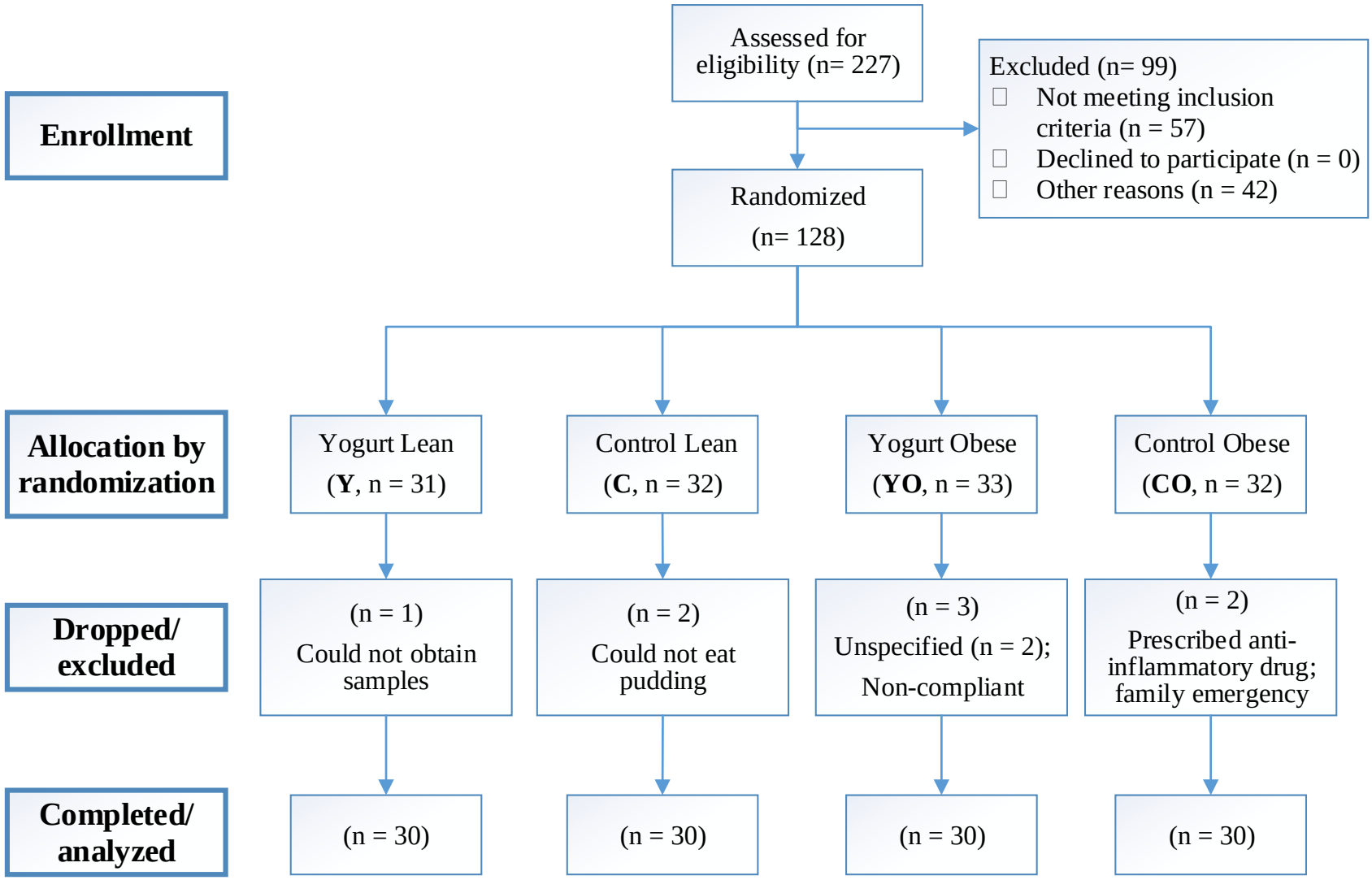

Figure 3.2 Enrollment and follow-up of participants.

3.3.5 Dietary analysis

Participants were instructed to maintain their usually dietary pattern and caloric intake, accounting for the additional ~ 300 calories provided by the intervention foods. Dietary records were used to examine if the intervention changed participants' nutrients intakes or dietary patterns and to determine their compliance to the requested dietary restrictions. Participants were instructed to complete two separate 3-day food records on two nonconsecutive weekdays and one weekend day during the washout period and near the end of the intervention. Participants were given detailed instructions on how to complete the record by study staff. The food records were reviewed by study staff upon submission and any incomplete or inaccurate information was corrected with participant input. The self-reported dietary intakes were analyzed by 2013 Nutrition Data System for Research (NDSR) (NCC, Minneapolis, MN). Because the NDSR did not include the exactly matched food item for yogurt and soy pudding, a similar alternative was used with nutrients corrected according to the nutrition facts label. The detailed NDSR nutrient content of the alternative is listed at **Appendix A.1.2**. The NDSR program output daily nutrient intakes and food group servings.

3.3.6 Statistical analysis

All data are expressed as mean ± standard error unless otherwise indicated. Statistical analysis was conducted on SAS 9.4 software (Cary, NC). The significance level was set at $\alpha = 0.05$ for all tests. At baseline, the difference of Y vs. C, and YO vs. CO were analyzed by independent T-test for numerical variables and Chi-square test for categorical variables, respectively. The effects of treatment (yogurt vs. control), time (wk 0, 3, 6 and 9) and treatment × time on weight, BMI and BP in the obese and lean groups were determined by repeated-measures ANOVA. The means of baseline and intervention dietary intakes within each group

were assessed by paired T-test. The significance of differences between group means was determined by analysis of covariance (ANCOVA) with baseline values as the covariate.

3.4 Results

Participants complied with consuming the study intervention foods. The compliance rates of returned cup lids were > 95% for all groups (data not shown). However, it should be noted that two participants could not comply with the control snack consumption, and dropped from the study. In addition, all participants reported adhering to the study dietary restrictions on food records.

3.4.1 Baseline characteristics of the study population

The baseline characteristics of lean and obese participants differed (**Table 3.2**). The mean age of obese participants was nearly 9 years older than the lean participants at 34.3 ± 1.3 and 25.1 ± 0.7 years, respectively ($P < 0.0001$). Compared to lean participants, obese participants had 49% greater weight, 51% higher BMI, and 33% greater WC. Systolic and diastolic BP were 12% and 10% higher in the obese than lean group, but the mean obese BP was less than prehypertension. Within each BMI group, the yogurt and control groups had no differences in weight, BMI, or BP. However, the WC of Y was 4.2 cm more than that of C ($P = 0.0424$).

Participant demographics were also different between lean and obese groups (**Table 3.3**). Obese participants were married at a greater rate than lean (22/60 vs. 11/60, $P = 0.0053$) and less likely to be at the lower income category (30/60 vs. 10/60 in the under $20,000 category, $P = 0.0001$) than the lean individuals. In addition, more obese participants reported more frequently reported non-disqualifying medical conditions than the lean (23/60 vs. 9/60, $P = 0.0039$). Although YO had more participants in the highest income category than the CO group ($P =$

0.0457), no differences in ethnicity, education, supplement use, smoking status, or medication use were found between yogurt and control treatment groups within each BMI group.

3.4.2 Changes in body weight, BMI and WC during dietary intervention

In Y and C, treatment, time or treatment × time did not affect weight, BMI or WC (**Table 3.4**). However, Y had a slight but statistically significant decrease in WC of -0.77 ± 0.33 cm at wk 6 (P = 0.0226) that was not maintained at wk 9 (-0.70 ± 0.32 cm, P =0.0647). In contrast to Y and C, both YO and CO gained BMI and body weight by wk 9, increasing 0.91 ± 0.31 kg (P = 0.0023) and 0.94 ± 0.42 kg (P = 0.0215) respectively. Despite weight gain, YO and CO WC did not increase significantly.

3.4.3 Changes in participants' BP during dietary intervention

Y and C BP were not significantly affected by treatment, time or treatment × time (**Table 3.5**). Within YO and CO, yogurt consumption significantly reduced the diastolic BP (P = 0.0023) and tended to lower the systolic BP (P = 0.0910) in YO, in comparison with CO. Within YO, diastolic BP decreased 2.82 ± 0.90 mm Hg at wk 3 (P = 0.0062). At wk 6, YO diastolic BP also decreased 2.10 ± 0.83 mm Hg from baseline, although this change did not achieve statistical significance (P = 0.1078). In addition, the changes in diastolic and systolic BP in YO were correlated (r = 0.6828, P < 0.001 at wk 3; r = 0.7452, P < 0.001 at wk 6; r = 0.6534, P = 0.001 at wk 9).

3.4.4 Baseline diet composition and nutrient intake in study participants

Prior to the intervention, the obese participants consumed 13% more total energy, 18% more total fat, 12% more total carbohydrate, 9% more total protein, 21% more total saturated fatty acids (SFA), 13% more sodium, 17% more sugar, and 41% more added sugar than the lean

participants (**Table 3.6**). YO had 18% greater intake of riboflavin than CO. No differences in other nutrients between the yogurt and control treatment groups within each BMI group achieved statistical significance.

3.4.5 Changes in diet and nutrient intake near the end of intervention

The average percentage of calories from fat decreased 7% - 15% from baseline among all groups, while the percentage of calories from carbohydrate increased 9% - 18% in C, CO, and YO (**Table 3.7**). Y also had 6.1 ± 3.1% increased percentage of calories from carbohydrate, although this change did not achieve statistical significance ($P = 0.0916$). The intakes of vitamin D, riboflavin, and pantothenic acid also increased 182% - 279%, 22% - 45%, and 22% - 36%, respectively in all groups. The calcium intake increased 21% - 69% in C, Y, and YO. CO also had 13.4 ± 8.3% calcium increase, but this was statistically insignificant ($P = 0.2736$). In all groups, total sugar intake increased 48% - 86% and added sugar intakes increased 102% - 317%. Within Y and C, Y had 16% higher intake of calcium but 22% lower intake of iron than C, when adjusting for baseline. Relative to CO nutrient intake YO had 37% more vitamin A, 94% more vitamin K, 24% more calcium, and 16% more potassium.

3.4.6 Food group consumption at baseline

Obese and lean participants had similar proportional food group consumption. At baseline, YO and CO consumed 32% more servings of dietary fat and 22% more beverages than Y and C (**Table 3.8)**. The proportional food group consumption between yogurt and control treatment groups within each BMI group did not differ.

3.4.7 Changes in food group consumption near the end of intervention

Each group substituted the yogurt and control food into their diets differently **(Table 3.9)**. Y, YO, and CO daily fruit consumption decreased by 0.50 - 0.78 servings/d. Y and C daily vegetable consumption decreased by 0.73 and 1.05 servings, respectively. YO and CO consumption of meat, eggs and nuts decreased by 1.20 - 1.56 servings/d, respectively. Y and YO had increased daily dairy consumption (1.15 - 1.33 servings) but decreased daily sweet consumption (0.38 - 0.79 servings). Daily dairy serving were < 4 in all groups, indicating compliance with the study's dietary restrictions.

After adjusting for baseline, Y consumed 1.22 servings of dairy products more than C daily. YO consumed more vegetable (+ 0.74 servings/day) and dairy (+ 1.45 servings/day) than CO.

Table 3.2 Baseline anthropometric and blood pressure values of enrolled participants (mean ± standard error, n = 30/group).

Characteristic (unit)	**Group**				***Significance** (P)*[1]		
	C	CO	Y	YO	*C vs. Y*	*CO vs. YO*	*Obese vs. Lean*
Age (year)	25.3 ± 1.1	31.9 ± 1.6	24.8 ± 0.8	36.7 ± 2.0	*0.7603*	*0.0606*	*<.0001*
Height (m)	1.66 ± 0.01	1.65 ± 0.01	1.67 ± 0.01	1.65 ± 0.01	*0.7534*	*0.6489*	*0.2883*
Weight (kg)	62.0 ± 1.5	93.4 ± 2.2	64.3 ± 1.5	94.2 ± 1.8	*0.3020*	*0.7894*	*<.0001*
BMI (kg/m^2)	22.4 ± 0.4	34.4 ± 0.5	23.1 ± 0.4	34.4 ± 0.6	*0.2546*	*0.9476*	*<.0001*
Waist circumference (cm)	69.5 ± 1.4	96.3 ± 1.6	73.7 ± 1.4	94.6 ± 1.4	*0.0424*	*0.4206*	*<.0001*
Systolic blood pressure (mm Hg)	105.5 ± 2.1	116.9 ± 2.2	103.5 ± 2.0	116.8 ± 2.1	*0.4985*	*0.9609*	*<.0001*
Diastolic blood pressure (mm Hg)	73.3 ± 1.7	79.0 ± 1.5	71.3 ± 1.5	79.3 ± 1.3	*0.3843*	*0.8918*	*<.0001*

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese; BMI, body mass index.

[1] Differences between yogurt and control treatment groups in non-obese [*P (C vs. Y)*] and obese individuals [*P (CO vs. YO)*], and the differences between obese and non-obese subjects [*P (Obese vs. Lean)*] were determined by independent T-test (PROC TTEST).

Table 3.3 Baseline demographics of study participants (n = 30/group).

Characteristic (unit)		**Group**				***Significance (P)***[1]		
		C	CO	Y	YO	*C vs. Y*	*CO vs. YO*	*Obese vs. Lean*
Ethnicity [n(%)]	Caucasian	22 (18.3)	24 (20.0)	23 (19.2)	26 (21.7)	*0.7656*	*0.4884*	*0.2611*
	Others[2]	8 (6.7)	6 (5.0)	7 (5.8)	4 (3.3)			
Marital status [n(%)]	Married	6 (5.0)	11 (9.2)	5 (427)	11 (9.2)	*0.7386*	*0.9162*	*0.0053*
	Single	24 (20.0)	16 (13.3)	25 (20.8)	15 (12.5)			
	Others[3]	0 (0.0)	3 (2.5)	0 (0.0)	4 (3.3)			
Annual Income [n(%)]	under $20000	12 (10.0)	8 (6.67)	18 (15.0)	2 (1.7)	*0.2886*	*0.0457*	*0.0001*
	$20000-$65000	10 (8.3)	18 (15.0)	6 (5.0)	18 (15.0)			
	$65000+	8 (6.7)	4 (3.3)	6 (5.0)	10 (8.3)			
Education [n(%)]	Undergraduate	10 (8.3)	12 (10.0)	12 (10.0)	14 (11.7)	*0.5464*	*0.4986*	*0.0761*
	Graduate	19 (15.8)	16 (13.3)	18 (15.0)	12 (10.0)			
	Others[4]	1 (0.8)	2 (1.7)	0 (0.0)	4 (3.3)			
Supplement use [n(%)]	Never used	23 (19.2)	23 (19.2)	19 (15.8)	18 (15.0)	*0.2598*	*0.1652*	*0.8433*
	Will stop	7 (5.8)	7 (5.8)	11 (9.2)	12 (10.0)			

Smoking status [n(%)]	Never smoked	29 (24.7)	26 (21.7)	27 (22.5)	27 (22.5)	*0.3006*	*0.6876*	*0.3426*
	Already quit	1 (0.8)	4 (3.3)	3 (2.5)	3 (2.5)			
Medication [n(%)]	None	16 (13.3)	13 (10.8)	14 (11.7)	14 (11.7)	*0.6056*	*0.7952*	*0.5834*
	Yes[5]	14 (11.7)	17 (14.2)	16 (13.3)	16 (13.3)			
Medical condition [n(%)]	None	25 (20.8)	16 (13.3)	26 (21.7)	21 (17.5)	*0.7177*	*0.1843*	*0.0039*
	Yes[5]	5 (4.2)	14 (11.7)	4 (3.3)	9 (7.5)			

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese.

[1] Differences between yogurt and control treatment groups in non-obese [*P (C vs. Y)*] and obese individuals [*P (CO vs. YO)*], and the differences between obese and lean subjects [*P (Obese vs. Lean)*] were determined by Chi-square test (PROC FREQ).

[2] Others included African American (n = 5), Asian (n = 9), Hispanic (n = 3), mixed (n = 8).

[3] Others included divorced (n = 4), engaged (n = 2), domestic partner (n = 1).

[4] Others included high school (n = 1), attended college (n = 5), some grad work (n = 1).

[5] Not include any inflammatory medication or medical condition.

Table 3.4 Changes from baseline in body weight, BMI and waist circumference (WC) of participants during the 9-wk consumption of 12 oz. yogurt or control snack (mean ± standard error, n = 30/group).

		Group	**Time** (wk)				***Significance** (P)*[1]		
			0	3	6	9	*Treatment*	*Time*	*Treatment × Time*
Lean (n=60)	Δ Weight (kg)	C	0 ± 0	-0.07 ± 0.22	0.19 ± 0.22	0.05 ± 0.26	*0.6323*	*0.4862*	*0.1400*
		Y	0 ± 0	-0.17 ± 0.16	-0.29 ± 0.29	0.22 ± 0.29			
	Δ BMI (kg/m^2)	C	0 ± 0	0.00 ± 0.08	0.10 ± 0.09	0.02 ± 0.10	*0.8400*	*0.9365*	*0.7614*
		Y	0 ± 0	-0.07 ± 0.06	-0.04 ± 0.09	0.08 ± 0.11			
	Δ WC (cm)	C	0 ± 0	0.08 ± 0.31	-0.06 ± 0.30	-0.22 ± 0.33	*0.2088*	*0.0679*	*0.3173*
		Y	0 ± 0	-0.12 ± 0.13	-0.77 ± 0.33 *	-0.70 ± 0.32			
Obese (n=60)	Δ Weight (kg)	CO	0 ± 0	0.37 ± 0.22	0.55 ± 0.32	0.94 ± 0.42 *	*0.8240*	*0.0015*	*0.8504*
		YO	0 ± 0	0.37 ± 0.21	0.51 ± 0.24	0.91 ± 0.31 *			
	Δ BMI (kg/m^2)	CO	0 ± 0	0.14 ± 0.08	0.30 ± 0.13	0.37 ± 0.16 *	*0.7451*	*0.0008*	*0.8054*
		YO	0 ± 0	0.15 ± 0.08	0.19 ± 0.09	0.33 ± 0.11 *			
	Δ WC (cm)	CO	0 ± 0	-0.32 ± 0.20	-0.22 ± 0.17	-0.55 ± 0.27	*0.4029*	*0.2816*	*0.7815*
		YO	0 ± 0	0.03 ± 0.16	-0.03 ± 0.36	-0.27 ± 0.43			

Abbreviations: C, control & lean; CO, control & obese; Y, yogurt & lean; YO, yogurt & obese; BMI, body mass index; WC, waist circumference.

[*] Changes in each group were determined by repeated-measure ANOVA (PROC MIXED) with time as independent variable.

[1] The effects of treatment, time and treatment × time were determined by repeated-measure ANOVA (PROC MIXED).

Table 3.5 Change from baseline in blood pressure of participants during 9-wk consumption of 12 oz. yogurt or control snack (mean ± standard error, n = 30/group).

		Group	**Time** (wk)				***Significance** (P)*[1]		
			0	3	6	9	*Treatment*	*Time*	*Treatment × Time*
Lean (n=60)	Δ SysBP (mm Hg)	C	0 ± 0	0.23 ± 1.48	-1.73 ± 1.04	0.33 ± 1.57			
		Y	0 ± 0	0.13 ± 1.29	-0.72 ± 1.46	-2.45 ± 1.64	*0.7011*	*0.4607*	*0.2104*
	Δ DiaBP (mm Hg)	C	0 ± 0	1.57 ± 1.22	-1.48 ± 1.05	0.15 ± 1.14			
		Y	0 ± 0	0.02 ± 0.77	0.50 ± 1.09	-0.75 ± 1.11	*0.8907*	*0.3449*	*0.0574*
Obese (n=60)	Δ SysBP (mm Hg)	CO	0 ± 0	2.57 ± 1.22	1.71 ± 1.39	1.12 ± 1.70			
		YO	0 ± 0	-1.13 ± 1.48	-0.68 ± 1.29	-0.25 ± 1.17	*0.0910*	*0.9057*	*0.2816*
	Δ DiaBP (mm Hg)	CO	0 ± 0	0.78 ± 0.91	1.02 ± 0.91	0.77 ± 1.22			
		YO	0 ± 0	-2.82 ± 0.90 *	-2.1 ± 0.83	-1.90 ± 0.95	*0.0023*	*0.5319*	*0.0710*

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese; SysBP, systolic blood pressure; DiaBP, diastolic blood pressure.

* Changes in each group were determined by repeated-measure ANOVA (PROC MIXED) with time as independent variable.

[1] The effects of treatment, time and treatment × time were determined by repeated-measure ANOVA (PROC MIXED).

Table 3.6 Self-reported dietary intakes at the start of intervention, determined by 3-day food record (mean ± standard error).

Intakes (unit)	**Group**				***Significance*** *(P)*[1]		
	C (n = 29)	CO (n = 30)	Y (n = 30)	YO (n = 30)	*C vs. Y*	*CO vs. YO*	*Obese vs. Lean*
Energy (kcal)	1657 ± 54	1869 ± 61	1666 ± 60	1893 ± 69	*0.9996*	*0.9921*	*0.0004*
Total Fat (g)	67.4 ± 3.1	75.2 ± 3.6	62.8 ± 3.7	77.5 ± 3.8	*0.7935*	*0.9684*	*0.0016*
Total Carbohydrate (g)	197.1 ± 7.8	226.4 ± 8.4	205.7 ± 6.4	223.7 ± 9.6	*0.8781*	*0.9954*	*0.0039*
Total Protein (g)	71 ± 2.8	78 ± 3.5	72.8 ± 3.4	78.6 ± 3.1	*0.9797*	*0.9992*	*0.0457*
% Calories from Fat	35.7 ± 1.1	34.8 ± 1.0	31.8 ± 0.9	35.4 ± 1.0	*0.0328*	*0.9757*	*0.1732*
% Calories from Carbohydrate	46.0 ± 1.2	48.0 ± 1.1	49.8 ± 1.1	46.0 ± 1.1	*0.0874*	*0.5950*	*0.3977*
% Calories from Protein	17.3 ± 0.7	16.7 ± 0.5	17.7 ± 0.7	17.3 ± 0.6	*0.9779*	*0.9157*	*0.4048*
Cholesterol (mg)	268 ± 23	249 ± 22	258 ± 21	332 ± 25	*0.9895*	*0.0481*	*0.2288*
SFA (g)	22.5 ± 1.2	25.4 ± 1.7	20.9 ± 1.5	27.2 ± 1.4	*0.8588*	*0.8289*	*0.0019*
Dietary Fiber (g)	22.0 ± 1.1	19.6 ± 1.1	19.3 ± 0.9	21.1 ± 1.3	*0.3406*	*0.7767*	*0.8264*
Vitamin A (IU)	11955 ± 1442	8545 ± 1974	10573 ± 1306	8373 ± 955	*0.9107*	*0.9998*	*0.0570*
Vitamin D (μg)	5.4 ± 0.6	4.5 ± 0.5	4.2 ± 0.5	4.4 ± 0.4	*0.3036*	*0.9992*	*0.4747*

Vitamin E (mg AT[2])	11 ± 0.8	12.4 ± 1.1	9.8 ± 0.7	9.4 ± 0.5	0.7202	0.0457	0.5390
Vitamin K	211.7 ± 27.4	165.5 ± 32	182.7 ± 30.2	151 ± 21.4	0.8861	0.9830	0.1672
Vitamin C (mg)	93.6 ± 9.0	88.7 ± 7.8	99.6 ± 7.2	76.2 ± 8.8	*0.9550*	*0.6995*	*0.0839*
Thiamin (mg)	1.6 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	*0.8362*	*0.9946*	*0.4096*
Riboflavin (mg)	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	*0.9948*	*0.0467*	*0.4668*
Niacin (mg)	21.5 ± 0.9	23.2 ± 1.3	22.8 ± 1.2	21.9 ± 1.1	*0.8473*	*0.8511*	*0.7199*
Pantothenic Acid (mg)	4.9 ± 0.2	4.9 ± 0.3	4.7 ± 0.2	5.0 ± 0.2	*0.9563*	*0.9998*	*0.4057*
Vitamin B-6 (mg)	1.9 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.7 ± 0.1	*0.9963*	*0.6839*	*0.0803*
Total Folate (μg)	434 ± 24	369 ± 20	397 ± 16	404 ± 22	*0.5804*	*0.6192*	*0.1555*
Vitamin B-12 (μg)	4.0 ± 0.3	3.6 ± 0.3	3.8 ± 0.4	4.4 ± 0.5	*0.9630*	*0.4781*	*0.7447*
Calcium (mg)	819 ± 36	811 ± 45	713 ± 35	835 ± 47	*0.2999*	*0.9764*	*0.1423*
Phosphorus (mg)	1119 ± 37	1130 ± 44	1072 ± 40	1218 ± 50	*0.8665*	*0.4616*	*0.0660*
Sodium (mg)	2751 ± 135	3350 ± 185	2989 ± 187	3116 ± 175	*0.7647*	*0.7707*	*0.0366*
Potassium (mg)	2492 ± 101	2476 ± 140	2433 ± 85	2473 ± 107	*0.9817*	*1.0000*	*0.9102*
Magnesium (mg)	290 ± 11	279 ± 13	283 ± 11	293 ± 14	*0.9732*	*0.8572*	*0.9573*
Iron (mg)	13.9 ± 0.7	14.3 ± 0.7	13.6 ± 0.6	15.3 ± 0.8	*0.9920*	*0.7539*	*0.1394*
Zinc (mg)	9.1 ± 0.4	9.6 ± 0.5	9.3 ± 0.5	10.1 ± 0.5	*0.9930*	*0.9136*	*0.1860*
Copper (mg)	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	*0.9982*	*0.9032*	*0.9155*

Selenium (μg)	106.0 ± 5.2	112.1 ± 6.3	104.7 ± 4.9	112.7 ± 5.4	*0.9986*	*0.9998*	*0.1989*
Manganese (mg)	4.0 ± 0.2	3.4 ± 0.2	4.7 ± 1.1	3.8 ± 0.2	*0.8097*	*0.9754*	*0.1685*
Alcohol (g)	2.9 ± 0.9	1.4 ± 0.5	2.7 ± 1.3	3.8 ± 1.2	*0.9993*	*0.3794*	*0.8617*
Total Sugars (g)	71.7 ± 3.7	87.2 ± 4.4	77.7 ± 3.7	87.7 ± 5.0	*0.7561*	*0.9997*	*0.0029*
Added Sugars (g)	37.2 ± 3.3	54.5 ± 4.1	38.8 ± 3.2	52.8 ± 5.2	*0.9919*	*0.9901*	*0.0001*

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese; SFA, total saturated fatty acids.

[1] Differences between yogurt and control treatment groups in non-obese [*P (C vs. Y)*] and obese individuals [*P (CO vs. YO)*], and the differences between obese and lean subjects [*P (Obese vs. Lean)*] were determined by independent T-test (PROC TTEST).

[2] alpha-tocopherol.

Table 3.7 Self-reported dietary intakes near the end of 9-wk daily consumption of 12 oz. yogurt or control snack (mean ± standard error).

Intakes (unit)	**Group**				***Significance** (P)*[1]	
	C (n = 29)	CO (n = 30)	Y (n = 30)	YO (n = 30)	*C vs. Y*	*CO vs. YO*
Energy (kcal)	1733 ± 48	1900 ± 67	1727 ± 60	1962 ± 63	*0.9135*	*0.5634*
Total Fat (g)	58.8 ± 2.7 ↓	67.6 ± 3.7	59.7 ± 3.5	71.8 ± 3.8	*0.5588*	*0.5067*
Total Carbohydrate (g)	234.2 ± 7.5 ↑	251.6 ± 9.2	226.7 ± 6.7	255.1 ± 8.1 ↑	*0.3965*	*0.7003*
Total Protein (g)	70.2 ± 2.5	72.9 ± 3.0	70.9 ± 3.3	76.4 ± 3.0	*0.9518*	*0.4045*
% Calories from Fat	29.3 ± 0.9 ↓	30.4 ± 0.9 ↓	28.8 ± 0.9 ↓	31.3 ± 0.9 ↓	*0.8026*	*0.5401*
% Calories from Carbohydrate	52.2 ± 1.0 ↑	51.7 ± 1.1 ↑	52.3 ± 1.1	50.6 ± 0.9 ↑	*0.6085*	*0.6162*
% Calories from Protein	18.1 ± 0.6	17.1 ± 0.6	17.8 ± 0.5	17.1 ± 0.5	*0.6227*	*0.9250*
Cholesterol (mg)	223 ± 22	240 ± 23	256 ± 22	255 ± 20 ↓	*0.2640*	*0.8294*
SFA (g)	20.5 ± 1.1	23.4 ± 1.7	19.7 ± 1.4	24.0 ± 1.3	*0.9842*	*0.9653*
Dietary Fiber (g)	19.1 ± 0.9	19.7 ± 1.0	15.7 ± 0.8 ↓	20.4 ± 1.4	*0.1056*	*0.9763*
Vitamin A (IU)	8613 ± 968 ↓	6806 ± 928	8272 ± 825	10841 ± 1367	*0.9738*	*0.0366*
Vitamin D (μg)	10.9 ± 0.5 ↑	9.3 ± 0.3 ↑	9.9 ± 0.4 ↑	10.1 ± 0.3 ↑	*0.5067*	*0.1400*
Vitamin E (mg AT^2)	8.2 ± 0.6 ↓	10.3 ± 1.3	8.3 ± 0.6	9.9 ± 0.6	0.7608	0.9085

Vitamin K (µg)	127.2 ± 14.8 ↓	88.5 ± 10 ↓	132.2 ± 16.3	171.9 ± 22	0.7737	0.0019
Vitamin C (mg)	72.8 ± 5.9	75.1 ± 7.3	64.9 ± 6.1 ↓	75.5 ± 8.3	*0.4246*	*0.7941*
Thiamin (mg)	1.5 ± 0.1	1.6 ± 0.1	1.4 ± 0.1	1.6 ± 0.1	*0.4310*	*0.6851*
Riboflavin (mg)	2.3 ± 0.1 ↑	2.4 ± 0.1 ↑	2.1 ± 0.1 ↑	2.3 ± 0.1 ↑	*0.1087*	*0.2302*
Niacin (mg)	19.6 ± 0.9	20.5 ± 1.6	18.9 ± 1.0 ↓	19.3 ± 1.0	*0.5099*	*0.8813*
Pantothenic Acid (mg)	5.7 ± 0.2 ↑	5.8 ± 0.2 ↑	5.9 ± 0.3 ↑	6.2 ± 0.2 ↑	*0.4979*	*0.3208*
Vitamin B-6 (mg)	1.7 ± 0.1	1.8 ± 0.2	1.6 ± 0.1 ↓	1.6 ± 0.1	*0.5198*	*0.8110*
Total Folate (µg)	380 ± 21	381 ± 23	364 ± 17	437 ± 30	*0.8956*	*0.4583*
Vitamin B-12 (µg)	4.3 ± 0.3	4.5 ± 0.5	4.8 ± 0.4 ↑	4.6 ± 0.2	*0.2023*	*0.7645*
Calcium (mg)	926 ± 38 ↑	887 ± 49	1058 ± 38 ↑	1166 ± 38 ↑	*0.0179*	*0.0012*
Phosphorus (mg)	1209 ± 37	1207 ± 41	1203 ± 44 ↑	1332 ± 46 ↑	*0.7880*	*0.2263*
Sodium (mg)	2492 ± 96	2823 ± 131	2573 ± 140 ↓	2954 ± 175	*0.9322*	*0.3572*
Potassium (mg)	2186 ± 80 ↓	2264 ± 96	2425 ± 89	2695 ± 101 ↑	0.0839	0.0113
Magnesium (mg)	274 ± 11	275 ± 12	280 ± 12	309 ± 12	*0.6105*	*0.1798*
Iron (mg)	14.7 ± 0.8	14.3 ± 0.7	12.1 ± 0.6	13.3 ± 0.7 ↓	*0.0426*	*0.2313*
Zinc (mg)	10.0 ± 0.4	11.2 ± 0.5 ↑	10.7 ± 0.5 ↑	11.7 ± 0.5 ↑	*0.3535*	*0.6024*
Copper (mg)	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.5 ± 0.1 ↑	*0.7283*	*0.1868*
Selenium (µg)	102.1 ± 4.0	107.0 ± 5.0	106.5 ± 5.6	111.3 ± 5.7	*0.5457*	*0.5310*

Manganese (mg)	3.3 ± 0.2 ↓	3.1 ± 0.2	3.7 ± 0.5	3.6 ± 0.2	*0.7679*	*0.2668*
Alcohol (g)	1.1 ± 0.4 ↓	2.2 ± 0.7	2.8 ± 1.3	2.8 ± 0.8	*0.1833*	*0.3267*
Total Sugars (g)	111.3 ± 3.3 ↑	116.6 ± 4.4 ↑	104.6 ± 3.5 ↑	115.1 ± 4.1 ↑	*0.1317*	*0.7887*
Added Sugars (g)	70.3 ± 2.5 ↑	83.4 ± 4.3 ↑	71.2 ± 3.2 ↑	81.6 ± 3.6 ↑	*0.9538*	*0.8408*

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese; SFA, total saturated fatty acids.

↓ Significant decrease from baseline determined by paired T-test (PROC TTEST).

↑ Significant increase from baseline determined by paired T-test (PROC TTEST).

[1] Dietary intakes at the end of intervention between yogurt and control treatment groups in non-obese [*P (C vs. Y)*] and obese individuals [*P (CO vs. YO)*] was determined by ANCOVA with baseline intakes as covariate (PROC GLM).

[2] alpha-tocopherol.

Table 3.8 Self-reported food groups consumption at start of intervention, determined by 3-day dietary records (mean ± standard error).

Food group	Group				*Significance (P)*[1]		
	C (n=29)	CO (n=30)	Y (n=30)	YO (n=30)	*C vs. Y*	*CO vs. YO*	*Obese vs. Lean*
Fruit	1.73 ± 0.22	1.48 ± 0.15	1.84 ± 0.24	1.47 ± 0.25	*0.9877*	*1.0000*	*0.1482*
Vegetable	3.85 ± 0.32	3.99 ± 0.45	3.36 ± 0.26	3.38 ± 0.38	*0.7866*	*0.6277*	*0.8250*
Grains	5.59 ± 0.39	5.99 ± 0.45	5.69 ± 0.37	6.51 ± 0.53	*0.9985*	*0.8310*	*0.1667*
Meat, egg, nuts	5.19 ± 0.50	6.50 ± 0.65	5.91 ± 0.50	6.02 ± 0.51	*0.7835*	*0.9211*	*0.1971*
Dairy[2]	1.40 ± 0.13	1.62 ± 0.13	1.19 ± 0.13	1.52 ± 0.22	*0.7795*	*0.9662*	*0.0800*
Fat	3.29 ± 0.46	4.44 ± 0.59	3.45 ± 0.49	4.63 ± 0.42	*0.9954*	*0.9934*	*0.0185*
Sweet	1.17 ± 0.19	1.43 ± 0.28	1.24 ± 0.18	1.66 ± 0.35	*0.9970*	*0.9241*	*0.2003*
Beverages	4.60 ± 0.58	6.35 ± 0.57	6.17 ± 0.70	6.90 ± 0.59	*0.2743*	*0.9190*	*0.0473*

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese.

[1] Differences between yogurt and control treatment groups in non-obese [*P (C vs. Y)*] and obese individuals [*P (CO vs. YO)*], and the differences between obese and lean subjects [*P (Obese vs. Lean)*] were determined by independent T-test (PROC TTEST).

[2] Included various types of milk, cheese, yogurt, dairy dessert, and certain nondairy alternative, etc.

Table 3.9 Self-reported food groups consumption near the end of 9-wk consumption of 12 oz. yogurt or control snack (mean ± standard error).

Food group	**Group**				***Significance** (P)*[1]	
	C (n=29)	CO (n=30)	Y (n=30)	YO (n=30)	*C vs. Y*	*CO vs. YO*
Fruit	1.50 ± 0.21	0.97 ± 0.15 ↓	1.05 ± 0.20 ↓	0.82 ± 0.16 ↓	*0.0749*	*0.4892*
Vegetable	2.80 ± 0.27 ↓	3.22 ± 0.33	2.64 ± 0.26 ↓	3.96 ± 0.47	*0.9065*	*0.0218*
Grains	5.47 ± 0.32	5.60 ± 0.48	5.08 ± 0.38	5.45 ± 0.45 ↓	*0.3980*	*0.4651*
Meat, egg, nuts	4.36 ± 0.48	4.94 ± 0.41 ↓	4.91 ± 0.59	4.82 ± 0.45 ↓	*0.7894*	*0.9710*
Dairy[2]	1.19 ± 0.14	1.11 ± 0.18 ↓	2.41 ± 0.13 ↑	2.56 ± 0.14 ↑	*<.0001*	*<.0001*
Fat	3.61 ± 0.54	3.96 ± 0.54	2.96 ± 0.32	4.09 ± 0.37	*0.2199*	*0.8884*
Sweet	1.00 ± 0.34	1.27 ± 0.37	0.87 ± 0.18 ↓	0.86 ± 0.18 ↓	*0.6352*	*0.1500*
Beverages	5.19 ± 0.60	5.81 ± 0.62	7.02 ± 0.95	7.60 ± 0.99	*0.5630*	*0.1511*

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese.

↓ Significant decrease from baseline determined by paired T-test (PROC TTEST).

↑ Significant increase from baseline determined by paired T-test (PROC TTEST).

[1] Food group consumption at the end of intervention between yogurt and control treatment groups in non-obese [*P (C vs. Y)*] and obese individuals [*P (CO vs. YO)*] was determined by ANCOVA with baseline intakes as covariate (PROC GLM).

[2] NDSR includes various types of milk, cheese, yogurt, dairy dessert, and certain dairy replacements, etc. The control food was not considered a dairy serving for this analysis.

3.5 Discussion

3.5.1 Anthropometric changes

In this study, obese and lean women consumed an additional 1.5 servings of yogurt or a control snack for 9 weeks, while maintaining usual dietary patterns. While yogurt consumption decreased WC initially in lean women, the magnitude of change might not be clinically significant. A WC less than 88 cm is considered to be the cutoff point for increased chronic disease (Klein, Allison et al. 2007).

Yogurt consumption over 4 years was associated with a 0.82 lb weight loss in a prospective cohort study of 120,877 US adults ($P \leq 0.005$) (Mozaffarian, Hao et al. 2011). However, data from human intervention studies suggest that weight-loss from increased dairy consumption is dependent on energy restriction (Abargouei, Janghorbani et al. 2012, Chen, Pan et al. 2012). The present study suggests that obese individuals could be at risk for weight gain when consuming additional calories from yogurt or similar non-dairy control food.

Yogurt and control food consumption increased body weight in obese participants by a similar amount. The interventions supplied an additional ~ 300 kcal/day, but dietary records near the end of the intervention did not indicate increased caloric intake among participants. It is possible that the timing of dietary record collection may not have captured increased caloric intake throughout the study. Underreporting is found to be the most prevalent cause of inaccurate dietary reports among women (Archer, Hand et al. 2013). In addition, the degree of underreporting is associated with higher BMI (Nielsen, Nielsen et al. 2009, Meng, Kerr et al. 2013). Most obese participant reported a calorie intake below the calculated Estimated Energy Requirements (EER) based on their age, weight, height and physical activity in the present study.

YO and CO had increased ratios of carbohydrate intake at the expense of lipid intake. The interventions supplied 54 g of added sugar, which contributed to the increased added sugar consumption by YO and CO. It is estimated that individuals having higher sugar intake have 0.75 kg (0.30 - 1.19 kg, 95% CI; $P = 0.001$) more body weight than those consuming lower sugars intake during short-term interventions (Morenga, Mallard et al. 2012).

As we did not determine body composition, it is unclear if YO and CO weight gains are associated with changes to fat or lean mass. Also, the weight gain did not appear to be detrimental as it was not correlated with the reduction in the antihypertensive effect of yogurt in YO at week 6 and 9 (data not shown). The mechanism by which increased body weight can affect hypertension is well-described. Obesity is associated with long-term over-activation of sympathetic nervous system which could raise arterial pressure by inducing peripheral vasoconstriction and increasing sodium reabsorption in the renal tubules (Rahmouni, Correia et al. 2005). Furthermore, activated renin-angiotensin system and increased plasma aldosterone levels found in obesity also contribute to the development of hypertension (Massiéra, Bloch-Faure et al. 2001, Goodfriend and Calhoun 2004).

3.5.2 Blood pressure

Obese individuals had lower BP after yogurt consumption. Even small BP decreases might promote cardiovascular health. A decrease as small as of 5.6 mm Hg in systolic BP and 2.2 mm Hg in diastolic BP may reduce the relative risk of major macrovasular or microvacular event by 9% (Patel, MacMahon et al. 2007).

This study adds to the existing evidence that increased dairy consumption can reduce BP in obese individuals. The Dietary Approaches to Stop Hypertension (DASH) trial demonstrated

that inclusion of low-fat dairy products with reduced saturated and total fat augmented the antihypertensive effects of a diet rich in fruits and vegetables (Appel, Moore et al. 1997). A meta-analysis of ~ 45,000 subjects determined an inverse association between dairy consumption and development of elevated BP, defined as ≥ 130 mm Hg systolic and/or ≥ 84 mm Hg diastolic BP (RR 0.87, 0.81 - 0.94, 95% CI) (Ralston, Lee et al. 2012). Another meta-analysis of prospective cohort studies associated an increased daily intake of 200 g of low-fat dairy products with decreased risk of hypertension (RR 0.96, 95% CI, 0.93 - 0.99) (Soedamah-Muthu, Verberne et al. 2012).

Intervention studies with dairy or fermented dairy consumption for BP management of prehypertension or hypertension are equivocal. Consumption of 3 daily servings of dairy products decreased mean daytime ambulatory systolic BP by 2 mm Hg ($P = 0.05$) in men with mild to moderate essential hypertension (Drouin-Chartier, Gigleux et al. 2014). However, ingestion of 300 mL fermented milk for 12 weeks did not reduce BP in patients with type 2 diabetes and high systolic baseline BP (143 ± 18 mm Hg) (Hove, Brøns et al. 2015). Furthermore, individuals with prehypertension or stage 1 hypertension did not have a reduction in BP after consuming 2 servings of low-fat dairy products for 5 weeks (Maki, Rains et al. 2013).

The potential antihypertensive mechanism of yogurt has not been adequately investigated. Some peptides (e.g. Val-Pro-Pro and Ile-Pro-Pro) derived from bacterial hydrolysis of milk protein may have hypotensive effects via inhibiting angiotensin-converting-enzyme (Nakamura, Mizutani et al. 2009). Low potassium intake might contribute to the genesis of high BP (Whelton, He et al. 1997). However, the potassium content of yogurt does not appear to be sufficient to solely be responsible for reduce BP in obese individuals. Potassium consumption in the YO

group increased by 9.0% during the intervention, and was higher than the CO group (2695 ± 101 vs. 2264 ± 96 mg/d, $P = 0.0113$). However, this level was still well below the Recommended Dietary Allowance (RDA) of 4700 mg/d for potassium in adults.

3.5.3 Dietary intakes

At baseline, the average intakes of total carbohydrate and total protein in each group met Dietary Reference Intake recommendations (Otten, Hellwig et al. 2006). However, the total fiber intake was below 25 g/d in all participants. YO had higher cholesterol intake exceeding 300 mg/d, while the other groups were below this level. For vitamins, all groups met vitamin A, C, thiamin, riboflavin, niacin, B6, folate, and B12 requirements. However, the vitamin D and E intakes were considerably below the recommended intake in all groups. For minerals, the intakes of calcium, magnesium, potassium, iron were moderately lower than recommended, while the average intakes of sodium in each group were above the upper limit. All groups met the recommended intakes of phosphorus, zinc, copper, selenium, and manganese.

Although both the yogurt and control food provided 6.75 μg of vitamin D per day, final vitamin D intake was less than 15 μg/d. YO and Y calcium intakes reached 1000 mg/d. The increase might be partly from the yogurt intervention which provided 450 mg calcium/d. C and CO had lower calcium intakes than the corresponding yogurt treatment groups. The original control snack contained the same level of calcium with yogurt. However, the control was reformulated by the manufacturer during the intervention, which decreased the calcium content from 150 to 60 mg/cup. This partially explained the difference in calcium intakes between the yogurt and treatment groups.

3.5.4 Intakes by food group

At baseline, YO and CO consumed more servings of dietary fat and beverages than Y and C, but no difference in any food group category between the yogurt and control treatment groups was found. Both Y and YO increased the dairy consumption by about 1 serving/d, which was moderately lower than the 1.5 servings yogurt intervention. This suggested participants substituted the yogurt for part of their usual dairy consumption. Y and YO but not C and CO had decreased sweet consumption. It was possible that yogurt was more likely to be considered as a sweet than the soy pudding, despite equivalent sugar content. Incorporating yogurt into diets might help to curb sweet consumption, although this needs replication in other studies.

Both Y and YO had decreased in fruit consumption after the intervention. The fruit flavor of yogurt may have impacted the tendency of participants to replace the fruit with yogurts. The lean, but not obese groups had decreased vegetable consumption after consuming the intervention foods. Previous studies supported the health benefits of adequate consumption of fruits and vegetables (Liu, Manson et al. 2000, Bazzano, He et al. 2002). Therefore, it will be important to advise participants to maintain the adequate consumption of fruits and vegetables when conducting yogurt intervention studies in the future. However, it is important to consider the inadequacy of NDSR to categorize all recipes and foods into food groups. Nevertheless, it provided insight on how the participants adapted to the intervention.

3.6 Conclusion

This study demonstrated 9-week consumption of 12 oz. of low-fat yogurt lowered diastolic blood pressure in obese premenopausal women within 3 wk, but this effect decreased by 9 wk, which coincided with modest weight gain. Yogurt consumption led to a slight decrease

in waist circumference but had no effect on blood pressure in the lean premenopausal women. Although yogurt consumption increased calcium and vitamin D intake, reduction of fruit and vegetables is potentially concerning and should be monitored in future studies to avoid the possible interfering effects on study outcomes. Potential metabolic differences resulting from increased weight gain in obese individuals will be considered in later research chapters. Although the soy pudding control food caused an increase in body weight in obese participants, a similar increase was observed in obese participants consuming yogurt. The soy pudding did not affect other study outcomes such as BP and inflammatory biomarkers which will be discussed subsequent chapters. Therefore, soy pudding should be considered as an adequate control food for low-fat yogurt consumption.

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Chapter 4: Effect of Pre-meal Low-fat Yogurt Consumption on Postprandial Metabolism Following a High-fat, High-carbohydrate Meal

4.1 Abstract

Background: Postprandial dysmetabolism causes inflammation and is associated with increased risk for insulin resistance and atherosclerosis.

Objective: Our objective was to determine the effects of pre-meal yogurt consumption on postprandial intestinal barrier function, inflammation, glycemia, lipidemia, and insulin secretion.

Design: Apparently healthy premenopausal women with body mass index (BMI) values of 18.5 to 27 (lean) or 30 to 40 kg/m^2 (obese) participated in a 9-week randomized, controlled study. Participants in each BMI group were randomly assigned to either a low-fat yogurt-supplemented group (Y, yogurt lean; YO, yogurt obese) or a soy pudding-supplemented control group (C, control lean; CO, control obese) with n = 30/group. Participants were advised to consume 12 oz. of yogurt or the control food daily for 9 weeks while maintaining their usual caloric intake. At the start and end of the intervention, participants consumed 8 oz. of the yogurt or control food, followed by a high-fat, high-carbohydrate challenge meal. Plasma concentrations of glucose, triglyceride (TG), insulin, interleukin 6 (IL-6), lipopolysaccharide-binding protein (LBP) and soluble CD14 (sCD14) were measured at baseline and at 1, 2, 3, and 4 h after the meal.

Results: The obese participants had fasting baseline values indicative of metabolic dysfunction, with 5% higher glucose, 32% higher TG, 89% higher insulin, 111% higher IL-6, 28% higher LBP, and 27% higher LBP/sCD14 ratio than the lean participants. The two challenge meal tests had similar results. The postprandial LBP/sCD14 ratio increased by an average of 10% in CO, but did not change in YO. Y and C did not have a consistent change in LBP/sCD14 ratio. The challenge meal induced an average of 80% increase in CO IL-6 at 4 h compared to 34% increase in YO IL-6. YO had a 15% decrease in IL-6 area under the curve $(AUC)_{0\text{-}4h}$ at the second

challenge meal test. CO postprandial glucose increased by an average of 16% at 1 h, but was unchanged in YO. Glucose decreased by 14% - 21% in C and 8% - 13% in Y within two hours. Y had less postprandial insulin over-secretion than C, while postprandial insulin was similar in YO and CO. Postprandial TG changes were similar between YO and CO, as well Y and C.

Conclusion: Yogurt consumption before a challenge meal improves metabolic dysfunction in lean and obese women. In obese participants, yogurt consumption reduces postprandial inflammation and improves biomarkers of intestinal barrier function. This trial was registered at clinicaltrials.gov as NCT01686204.

4.2 Introduction

Obesity leads to metabolic dysfunction and chronic inflammation. Consumption of high-calorie challenge meals can be used to assess the role of diet in preventing inflammation and metabolic dysfunction (Ghanim, Abuaysheh et al. 2009, Ghanim, Sia et al. 2010, Ghanim, Sia et al. 2011). Insulin resistance and arteriosclerosis are closely associated with postprandial inflammation (Burdge and Calder 2005, O'Keefe, Gheewala et al. 2008). Low-grade postprandial inflammation is usually transient and results from postprandial hyperglycemia and hyperlipidemia (Nappo, Esposito et al. 2002, Ceriello, Quagliaro et al. 2004, Ceriello, Assaloni et al. 2005). In the postprandial state, increased glucose and free fatty acids enter the Krebs cycle, overcoming the capacity for oxidative phosphorylation, leading to oxidative stress (O'Keefe and Bell 2007). Oxidative stress is a strong stimulator of inflammation, via activating the mitogen-activated protein kinases (MAPK) family and nuclear factor kappa B (NF-κB) pathway (Rahman 2002, Leung and Chan 2009).

Acute, low-grade endotoxemia also contributes to postprandial inflammation (Erridge, Attina et al. 2007). Bacterial endotoxins could circumvent the intestinal barrier and induce systemic inflammation (Kemna, Pickkers et al. 2005, Andreasen, Larsen et al. 2010). Lipopolysaccharide (LPS) inflammatory signaling is mediated by binding to lipopolysaccharide-binding protein (LBP), and membrane-bound or soluble CD14 (sCD14) before activating Toll-like receptor 4 (TLR4)/MD2 complex (Van Bossuyt, De Zanger et al. 1988, Park, Song et al. 2009, Manco, Putignani et al. 2010). The intestinal barrier function of obese individuals is compromised, leading to increased endotoxin exposure and chronic inflammation (Sun, Yu et al. 2010). The obesity-associated endotoxemia is characterized by two- to threefold increase in circulating endotoxin, usually referred as metabolic endotoxemia (Cani, Amar et al. 2007).

Dairy proteins and calcium attenuate postprandial hyperlipidemia and hyperglycemia by insulinotropic activity, delaying gastric emptying, and decreasing fat absorption (Westphal, Kästner et al. 2004, Lorenzen, Nielsen et al. 2007, Mortensen, Hartvigsen et al. 2009). In addition, prior studies suggest that yogurt may improve intestinal barrier function by modifying gut microbiota, stimulating the production of intestinal mucins, increasing secretory immunoglobulin A and antimicrobial peptides secretion, and maintaining function of tight junctions (Pei, Martin et al. 2015). Therefore, we hypothesized that pre-meal yogurt consumption could alleviate postprandial inflammation by reducing postprandial dysmetabolism and endotoxemia.

4.3 Materials and Methods

4.3.1 Study participants

Obese and lean women were recruited to participate in the study as described in Section **3.3.1**.

4.3.2 Postprandial challenge meal and treatment

The composition of the challenge meal was formulated to induce metabolic dysfunction as previously described (Ghanim, Abuaysheh et al. 2009, Ghanim, Sia et al. 2010). The high-fat, high-carbohydrate challenge meal consisted of two sausage, egg and cheese sandwiches (Jimmy Dean, Peoria, IL) and two hash browns (obtained from local stores). The meal provided 56-60 g of total fat (~ 54% of total energy), 82 g of carbohydrates (~ 34% of total energy), and 28-30 g of protein (~ 12% of total energy), supplying a total of ~ 960 kcal.

The intervention consisted of commercially available low-fat yogurt (Yoplait, General Mills, Inc. Minneapolis, MN) and soy pudding as control food (ZenSoy, South Hackensack, NJ)

as described in **Section 3.3.2**. Prior to the challenge meals, participants consumed 8 oz. of the intervention foods, providing an additional 330 kcal. Throughout the 9-week intervention, participants ate 12 oz. of yogurt or the control food daily.

4.3.3 Experimental design

A randomized, controlled study was used to characterize the effects of yogurt consumption on intestinal barrier function and chronic inflammation relative to consumption of a non-dairy control food. This study was powered to detect an 8.2% difference in sCD14, the primary outcome (Laugerette, Vors et al. 2011). The recruitment, initial prescreening, enrollment, compliance, and completion rate of 128 subjects were previously described in **Section 3.3.3**. Briefly, n = 30 participants from each group: YO, yogurt obese; CO, control obese; Y, yogurt lean; C, control lean completed all study visits.

Two identical challenge meal tests were conducted at the beginning (wk 0) and end of intervention (wk 9) as depicted in **Figure 4.1**. On each test day, participants were instructed to arrive at the study center in the fasted state (10 - 12 h) in the morning. Fasting blood samples (0 h) were collected from the antecubital vein into evacuated tubes containing sodium heparin or ethylenediaminetetraacetic acid (EDTA) (Becton, Dickinson and Company, Franklin Lakes, NJ). The participants were instructed to consume one serving (8 oz.) of yogurt or control food followed by the challenge meal. Blood samples were collected at 1, 2, 3 and 4 h after the test meal was finished. All blood samples were placed in ice bath immediately and centrifuged (4°C, 15 min, 1500 × g) within 20 min of collection. Aliquots of the upper layer of plasma were snap-frozen in liquid nitrogen. All samples were stored at -80 °C until analysis. During the postprandial phase, the subjects were asked to stay at the study center and avoid exercise.

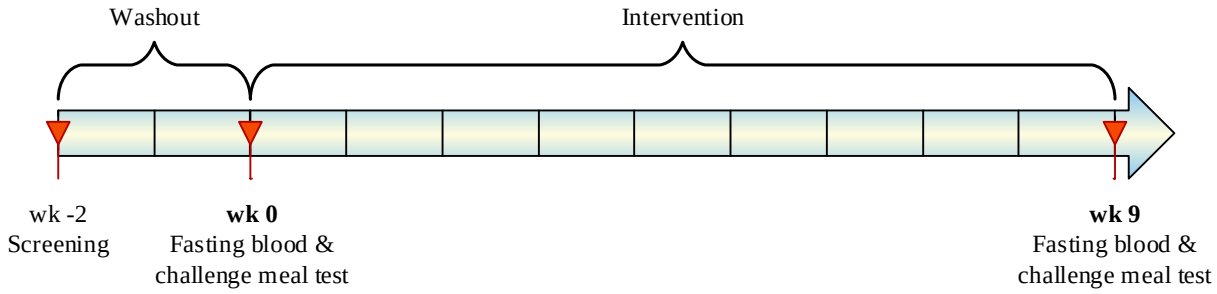

Figure 4.1 Experimental design of challenge meal tests.

After 2 weeks of washout, lean and obese participants were randomly assigned to either yogurt or the control treatment for 9 weeks. At the beginning and end of intervention, participants consumed challenge meals. At each test, the participants were instructed to consume one serving of yogurt or control food, followed by a high-fat, high-carbohydrate test meal. Blood samples were collected at baseline (0), 1, 2, 3, and 4 h after the meal.

4.3.4 Biomarker analysis

Glucose was determined in sodium heparin plasma by a commercial colorimetric enzymatic assay kit (Cat # 10009582; Cayman Chemical, Ann Arbor, MI). Total TG in sodium heparin plasma was measured by a commercial enzymatic kit (Cat # 461-08992 and 461-09092; Wako Diagnostics, Richmond, VA). Insulin in sodium heparin plasma was determined by immunoassay (Cat # 80-INSHU-E01.1; Alpco Diagnostics, Salem, NH). IL-6 and sCD14 were measured by immunoassay in EDTA and sodium heparin plasma, respectively (IL-6, Cat # SS600B, high-sensitivity; sCD14, Cat # DC140; R&D System, Minneapolis, MN). LBP was determined in EDTA plasma by ELISA (Cat # HK315-02; Hycult Biotech, Uden, Netherlands). All the measurements were performed on a SpectraMax M2 microplate reader (Molecular Devices, Sunnyvale, CA) according to manufacturers' instructions. To estimate insulin resistance, the homeostatic model assessment of insulin resistance (HOMA-IR) index was calculated as

fasting insulin concentration (μIU/mL) × fasting glucose concentration (mg/dL)/405 (Wallace, Levy et al. 2004). The ratio between LBP and sCD14 was calculated as a marker for endotoxemia.

4.3.5 Statistical analysis

All results were expressed as mean ± standard error. Statistical analysis was conducted on SAS 9.4 software (Cary, NC). The significance level was set at $\alpha = 0.05$ for all statistical tests. At baseline, the differences between Y and C, and YO and CO were analyzed by independent T-test. The effects of treatment (yogurt vs. control), time (0, 1, 2, 3 and 4 h) and treatment × time on glucose, TG, insulin, IL-6, LBP, sCD14, and LBP/sCD14 ratio were determined by repeated-measures ANOVA. Time-dependent changes in each group were determined by repeated-measures ANOVA with Dunnett's test for multiple comparisons. Area under the curve (AUC) was calculated using the trapezoidal rule and compared by independent T-test for group comparisons or paired T-test for time-dependent changes.

4.4 Results

At baseline, obese participants had 5% higher glucose, 32% higher TG, 89% higher insulin, 111% higher IL-6, 28% higher LBP, and 27% plasma LBP/sCD14 ratio in fasting plasma than lean participants (**Table 4.1**). However, the fasting plasma glucose, TG, insulin, IL-6, LBP, sCD14, and LBP/sCD14 ratio in the yogurt treatment group did not differ from that in the control treatment group within lean and obese groups.

The average postprandial concentrations of glucose, TG, insulin, IL-6, LBP, sCD14 and the ratio of LBP/sCD14 for each group and challenge meal are presented in **Appendix A.2 Supplementary Table 4.1-4.7**.

4.4.1 Postprandial glucose changes

In YO and CO, treatment, time and treatment × time effects were all significant at both challenge meals (**Figure 4.2 A** and **B**). At 1 h, CO plasma glucose increased by 15% after the first challenge meal, and by 17% after the second challenge meal. In contrast, YO postprandial plasma glucose did not change from baseline at any of the time points examined, up to 4 h. Chronic consumption of yogurt did not markedly improve postprandial glucose response. At 1 h, CO had 16% and 20% higher glucose than YO at the first and second challenge meal tests, respectively.

Y and C plasma glucose had significant time and treatment × time effects after the first challenge meal, and significant treatment and time effects after the second challenge meal (**Figure 4.2 C** and **D**). C plasma glucose decreased a maximum of 21% and 14% at 2 h after the first and second challenge meal, respectively. Y exhibited a smaller decrease in plasma glucose of 13% and 8% from baseline at the first and second challenge meal, respectively. Y postprandial plasma glucose returned to baseline by 2 h, whereas C returned at 3 h.

The mean postprandial glucose $AUC_{0\text{-}4h}$ of YO and CO was 17% and 19% higher than that of Y and C after the first and second challenge meals, respectively (**Table 4.2**). YO postprandial glucose $AUC_{0\text{-}4h}$ was 8% lower than CO after the first challenge meal and tended to be lower after the second challenge meal (349.4 ± 9.2 vs. 391.3 ± 19.0 mg/dL·h, $P = 0.0543$). The plasma glucose $AUC_{0\text{-}4h}$ of Y was not significantly different than that of C after both challenge meals.

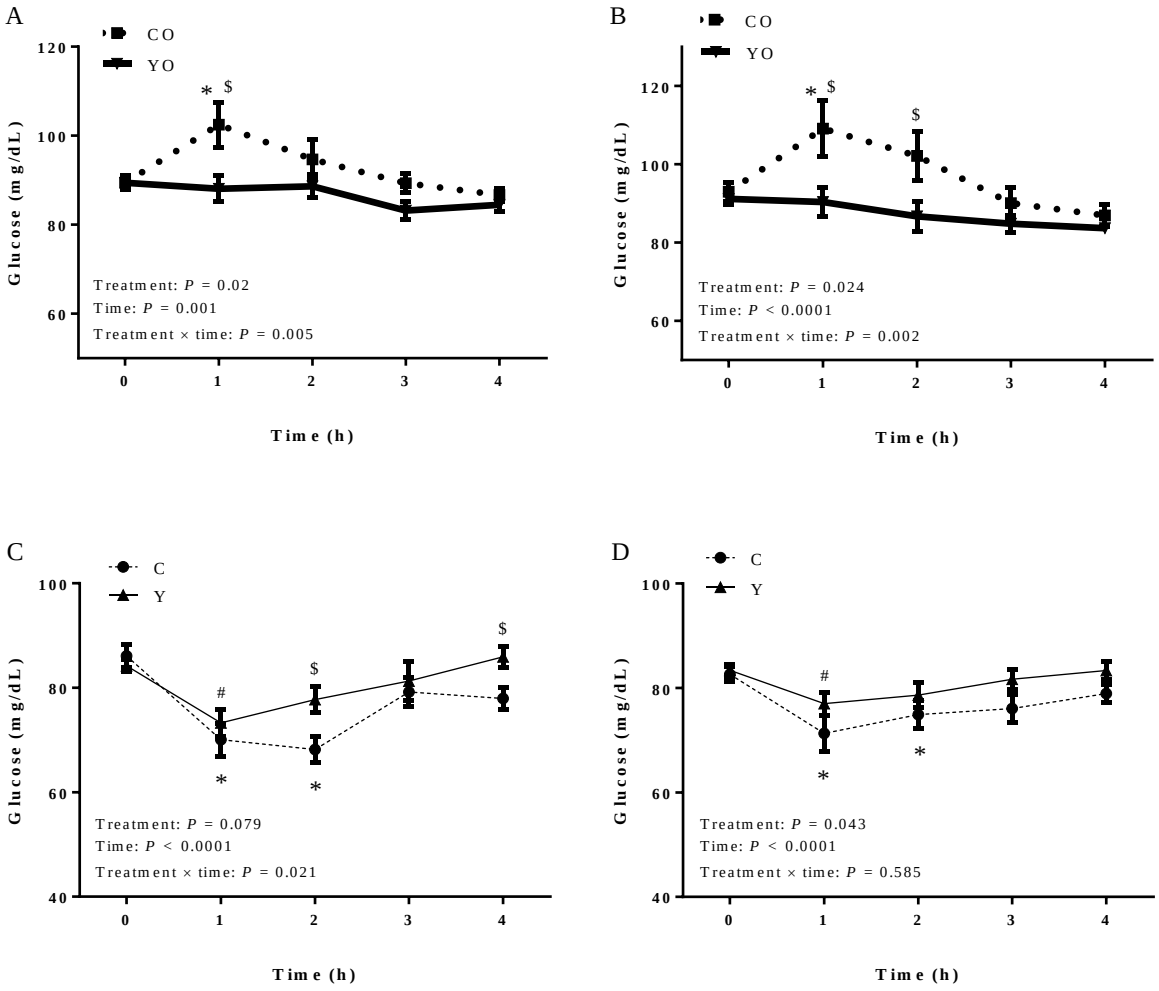

Figure 4.2 Change of plasma glucose in the obese groups at the first (A) and second (B) challenge meal, and in the lean groups at the first (C) and second (D) challenge meal.

C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese. Repeated-measures ANOVA with Dunnett's test for multiple comparison was performed to evaluate time and treatment effects. * Significantly different from baseline in the control treatment groups, $P < 0.05$. # Significantly different from baseline in yogurt treatment groups, $P < 0.05$. $ Significantly different between the yogurt and control treatment groups at different time points, $P < 0.05$.

Table 4.1 Baseline fasting characteristics of participants completing all visits (mean ± standard error, n = 30/group).

Characteristic (unit)	**Group**				***Significance** (P)*[1]		
	C	CO	Y	YO	*P vs. C*	*CO vs. YO*	*Obese vs. Lean*
Age (year)	25.3 ± 1.1	31.9 ± 1.6	24.8 ± 0.8	36.7 ± 2.0	*0.7603*	*0.0609*	*<.0001*
Weight (kg)	62.3 ± 1.6	93.5 ± 2.2	64.7 ± 1.5	94.1 ± 1.9	*0.2788*	*0.8336*	*<.0001*
Height (m)	1.66 ± 0.01	1.65 ± 0.01	1.67 ± 0.01	1.65 ± 0.01	*0.7534*	*0.6489*	*0.2883*
BMI (kg/m^2)	22.5 ± 0.4	34.3 ± 0.5	23.3 ± 0.5	34.4 ± 0.7	*0.2603*	*0.9670*	*<.0001*
Glucose (mg/dL)	86.1 ± 2.2	89.4 ± 1.6	84.2 ± 1.1	89.4 ± 1.4	*0.4498*	*0.9871*	*0.0104*
TG (mg/dL)	66.9 ± 5.1	94.9 ± 7.4	72.0 ± 7.1	90.9 ± 7.6	*0.5684*	*0.5934*	*0.0014*
Insulin ($\mu IU/mL$)[2]	7.0 ± 0.5	13.6 ± 1.3	7.7 ± 0.7	14.2 ± 1.5	*0.7607*	*0.4180*	*<.0001*
HOMA-IR[2]	1.48 ± 0.11	3.25 ± 0.43	1.69 ± 0.18	3.21 ± 0.32	*0.3364*	*0.9389*	*<.0001*
IL-6 (pg/mL)	0.74 ± 0.08	1.56 ± 0.13	0.88 ± 0.13	1.86 ± 0.22	*0.3941*	*0.2487*	*<.0001*
LBP ($\mu g/mL$)	9.9 ± 0.9	12.4 ± 0.7	9.3 ± 0.6	12.3 ± 0.9	*0.6044*	*0.9302*	*0.0010*
sCD14 (ng/mL)	1421 ± 50	1481 ± 45	1402 ± 59	1388 ± 45	*0.7993*	*0.1539*	*0.6579*
LBP/sCD14	7.14 ± 0.69	8.62 ± 0.58	6.89 ± 0.44	9.13 ± 0.73	*0.7584*	*0.5881*	*0.0032*

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese; BMI, body mass index; TG, triglyceride; HOMA-IR, homeostatic model assessment of insulin resistance; IL-6, interleukin 6; LBP, lipopolysaccharide-binding protein; sCD14, soluble CD14.

[1] Baseline differences between yogurt and pudding treatment groups in non-obese [*P (C vs. Y)*] and obese participants [*P (CO vs. YO)*], and the differences between obese and non-obese participants [*P (Obese vs. Lean)*] were determined by independent T-test (PROC TTEST).

[2] Determined at the second challenge meal (wk 9).

Table 4.2 AUC comparisons (mean ± standard error).

Markers' AUC (unit)	**Group**	**$AUC_{0\text{-}4\ h}$**		***Significance** (P)*		
		wk 0	wk 9	*Group difference[1] at wk 0*	*Group difference[1] at wk 9*	*Time difference[2] (wk 0 vs. wk 9)*
Glucose $AUC_{0\text{-}4\ h}$ (mg/dL·h)	C (n = 30)	299.5 ± 7.9	303.0 ± 8.0	*0.1245*	*0.0813*	*0.8129*
	Y (n= 30)	317.4 ± 8.3	320.7 ± 5.9			*0.6204*
	CO (n = 30)	374.5 ± 10.5	391.3 ± 19.0	*0.0307*	*0.0543*	*0.1359*
	YO (n = 30)	346.8 ± 6.7	349.4 ± 9.2			*0.9833*
	Lean (n = 60)	308.4 ± 5.8	311.8 ± 5.1	*<.0001*	*<.0001*	*0.1909*
	Obese (n = 60)	360.4 ± 6.4	370.0 ± 10.7			*0.5414*
TG $AUC_{0\text{-}4\ h}$ (mg/dL·h)	C (n = 30)	390.0 ± 25.7	390.7 ± 23.2	*0.9316*	*0.6959*	*0.7961*
	Y (n= 30)	393.8 ± 35.3	375.8 ± 30.3			*0.5965*
	CO (n = 30)	528.9 ± 39.4	538.5 ± 36.8	*0.8453*	*0.7348*	*0.2952*
	YO (n = 30)	540.6 ± 45.1	560.3 ± 53.2			*0.4654*
	Lean (n = 60)	392.0 ± 21.9	383.4 ± 18.8	*0.0002*	*<.0001*	*0.5941*
	Obese (n = 60)	534.7 ± 29.6	549.0 ± 31.7			*0.4097*
IL-6 $AUC_{0\text{-}4\ h}$ (pg/mL·h)	C (n = 30)	4.04 ± 0.38	4.05 ± 0.60	*0.7151*	*0.734*	*0.9213*
	Y (n= 30)	4.24 ± 0.40	4.01 ± 0.37			*0.5283*
	CO (n = 30)	7.96 ± 0.66	7.55 ± 0.54	*0.9249*	*0.4946*	*0.4522*
	YO (n = 30)	8.05 ± 0.77	6.87 ± 0.83			*0.0232*
	Lean (n = 60)	4.14 ± 0.27	4.03 ± 0.35	*<.0001*	*<.0001*	*0.4619*
	Obese (n = 60)	8.00 ± 0.50	7.21 ± 0.49			*0.0355*

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese; TG, triglyceride; IL-6, interleukin 6.

[1] Group differences of AUCs [*P (C vs. Y)*, *P (CO vs. YO)*, and *P (Lean vs. Obese)*] at wk 0 and wk 9 were compared by independent T-test.

[2] Time difference of AUCs between wk 0 and wk 9 in each group were compared by paired T-test.

4.4.2 Postprandial TG changes

Consumption of yogurt or the control food did not significantly change the postprandial TG response for any group at either challenge meal (**Figure 4.3**). Although there was a significant treatment × time effect ($P = 0.0350$) on postprandial plasma TG in the obese groups after the second challenge meal, group differences were not significant at any time point. Postprandial plasma TG increased continuously and remained above the baseline at all selected time points in all groups. At 4 h after the two challenge meals, plasma TG was increased by 48% - 77% within groups.

Obese participants had higher postprandial lipid exposure than lean participants. Relative to lean groups, obese groups had 36% and 43% greater plasma TG $AUC_{0\text{-}4h}$ after the first and second challenge meals, respectively (**Table 4.2**). Postprandial TG $AUC_{0\text{-}4h}$ was not significantly different between treatment groups within each BMI group or between challenge meals in any group.

4.4.3 Postprandial insulin changes

Because the postprandial changes in plasma glucose and TG for each group were similar after both challenge meals, plasma insulin was characterized only at the second challenge meal (**Figure 4.4**). YO and CO postprandial plasma insulin were largely similar. CO insulin was increased from baseline at 1, 2, and 3 h, and YO at 1 and 2 h. YO and CO postprandial insulin were similar and peaked 1 h at around 6.5-fold of baseline. In lean individuals, yogurt consumption reduced the postprandial insulin secretion relative to the control food. Y and C plasma insulin was increased from baseline at 1, 2, and 3 h. Y and C postprandial plasma insulin was highest at 1 h, at 7.2-fold of baseline in C and 5.4-fold of baseline in Y. At 3 h, C had 30%

higher insulin than Y. Also, insulin $AUC_{0\text{-}4h}$ of C tended to be higher than that of Y (128.9 ± 15.3 vs. 100.5 ± 5.8 μIU/mL·h, $P = 0.0907$). Obesity also increased postprandial insulin. Relative to lean individuals, the obese groups had 1.3-fold greater insulin $AUC_{0\text{-}4h}$.

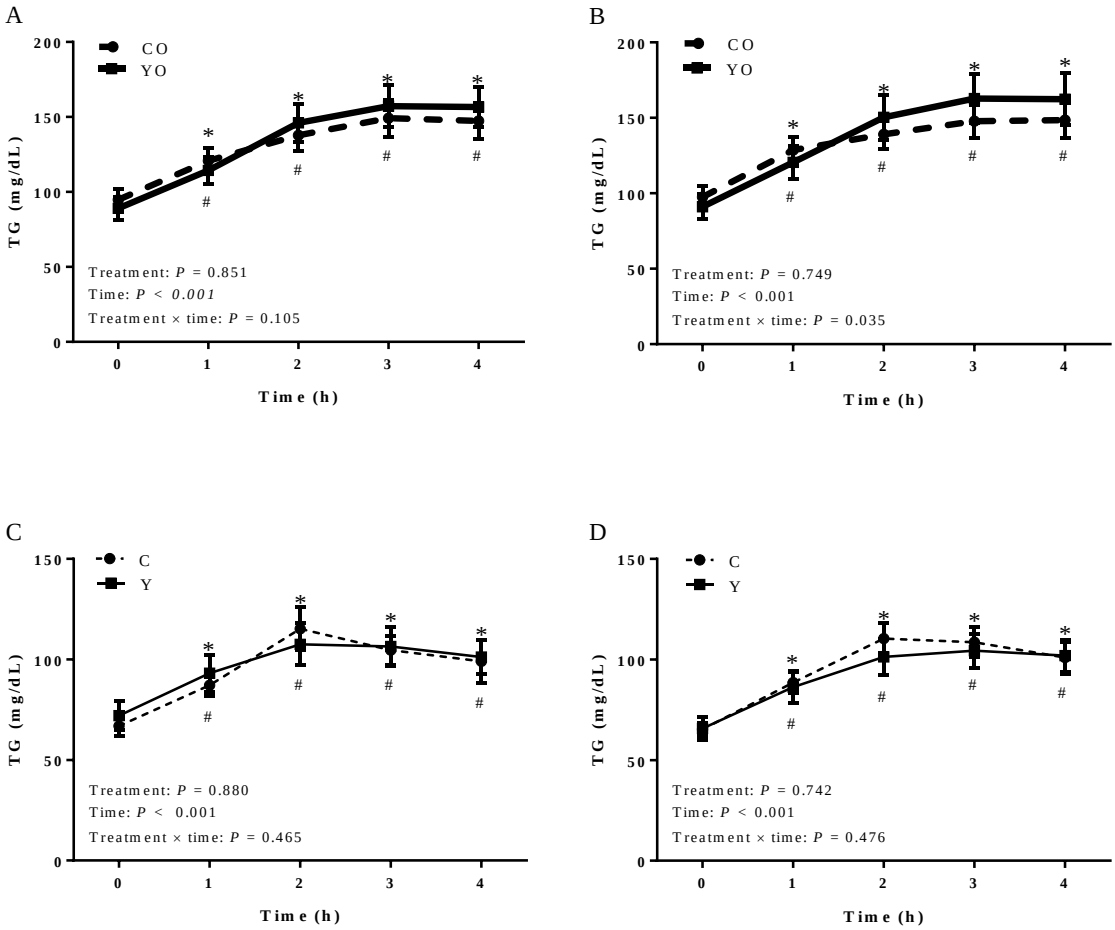

Figure 4.3 Postprandial plasma TG in obese participants after the first (A) and second (B) challenge meal, and in lean participants after the first (C) and second (D) meal.

C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese. Repeated-measures ANOVA with Dunnett's test for multiple comparison was performed to evaluate time and treatment effects. *

Significantly different from baseline in the control treatment groups, $P < 0.05$. # Significantly different from baseline in the yogurt treatment groups, $P < 0.05$.

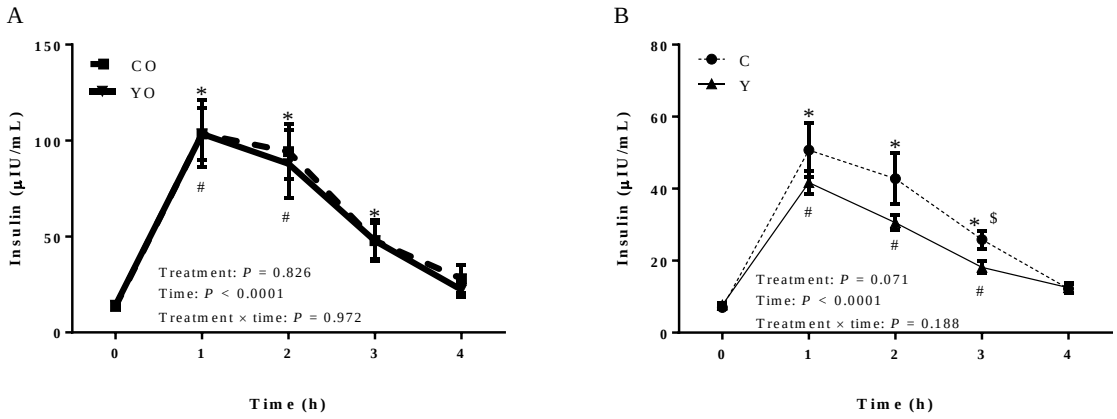

Figure 4.4 Postprandial plasma insulin in obese participant (A) and in the lean groups (B), after the second challenge meal.

C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese. Repeated-measures ANOVA with Dunnett's test for multiple comparison was performed to evaluate time and treatment effects. * Significantly different from baseline in the control treatment groups, $P < 0.05$. # Significantly different from baseline in the yogurt treatment groups, $P < 0.05$. $ Significantly different between the yogurt and control treatment groups at different time points, $P < 0.05$.

4.4.4 Postprandial IL-6 changes

Yogurt consumption inhibited postprandial increases in IL-6 in obese participants. YO and CO postprandial plasma IL-6 had a significant treatment × time effect at the first challenge meal ($P = 0.037$) and a significant treatment effect after the second challenge meal ($P = 0.021$) (**Figure 4.5**). CO plasma IL-6 was increased above baseline by 3 and 4 h after both challenge meals. YO plasma IL-6 was also increased above baseline at 3 and 4 h after the first challenge meal, but was only increased at 4 h after the second challenge meal. At 4 h, the increase of IL-6 in YO was 42% and 53% of that in CO after the first and second challenge meal, respectively. In contrast to obese participants, Y and C postprandial IL-6 were not significantly different. Both challenge meals increased lean postprandial plasma IL-6 at 3 or 4 h. For example, in the second test, C and Y IL-6 increased 62% and 49% from baseline at 4 h, respectively.

Obesity also increased chronic postprandial inflammation. Relative to lean participants, obese participants had 93% and 79% greater plasma IL-6 $AUC_{0\text{-}4h}$ after the first and second challenge meals, respectively (**Table 4.2**). In each BMI group, IL-6 $AUC_{0\text{-}4h}$ was not different between the yogurt and control treatment groups after either challenge meal. Daily low-fat yogurt consumption appeared to decrease postprandial inflammation in YO, as indicated by 15% less IL-6 $AUC_{0\text{-}4h}$ at wk 9 than at wk 0.

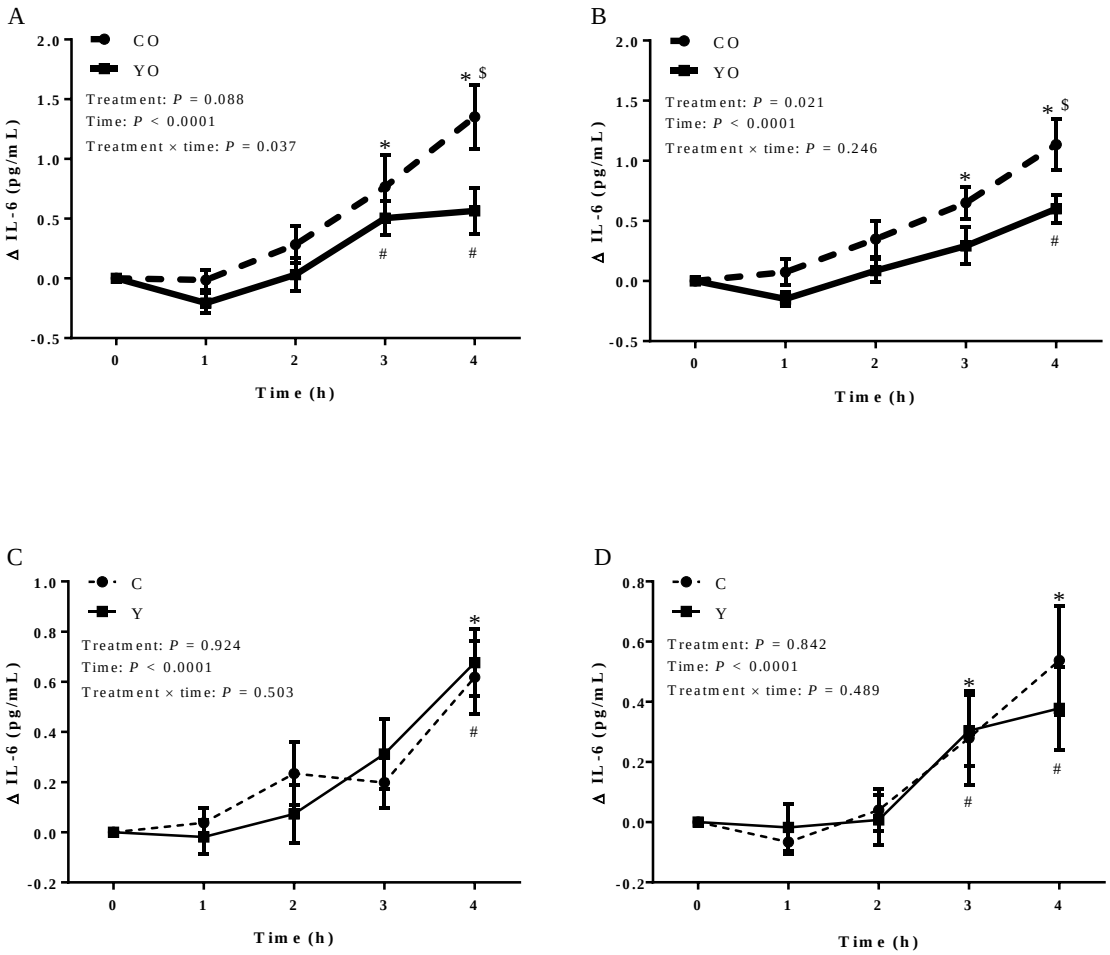

Figure 4.5 Postprandial plasma IL-6 change from baseline (0 h) in obese participants at the first (A) and second (B) challenge meal, and in lean participants after the first (C) and second (D) challenge meal.

C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese. Repeated-measures ANOVA with Dunnett's test for multiple comparison was performed to evaluate time and treatment effects. * Significantly different from baseline in the control treatment groups, $P < 0.05$. # Significantly different from baseline in the yogurt treatment groups, $P < 0.05$. $ Significantly different between the yogurt and control treatment groups at different time points, $P < 0.05$.

4.4.5 Postprandial LBP and sCD14 changes

YO and CO had similar postprandial LBP responses (**Figure 4.6**). After both challenge meals, YO plasma LBP was lower than CO, but this difference was not statistically significant. YO plasma LBP decreased 3% and 6% at 2 and 3 h at the second challenge meal. In Y and C, postprandial plasma LBP did not change after the first challenge meal. After the second challenge meal, Y LBP was 5% - 9% below baseline at 1, 3, and 4 h and was lower than C at 1 to 4 h.

YO and CO had different postprandial plasma sCD14 responses after both challenge meals (**Figure 4.7**). After both meals, CO plasma sCD14 was decreased at 1 and 2 h, with an average of 5% decrease at 2 h; in contrast, YO plasma sCD14 was unchanged. Also, CO had less plasma sCD14 than YO at 1 and 2 h. These differences were apparent in obese participants only. Y and C postprandial sCD14 were not significantly different after either challenge meal. However, within Y, sCD14 decreased by around 4% at 1 h after both challenge meals.

Yogurt consumption changed LBP/sCD14 ratio in obese participants relative to the control (**Figure 4.8**). CO LBP/sCD14 increased postprandially by a maximum of 12% and 7% after the first and second challenge meals, respectively. YO postprandial LBP/sCD14 did not change after either challenge meal. Y and C postprandial LBP/sCD14 changes were not affected by treatments.

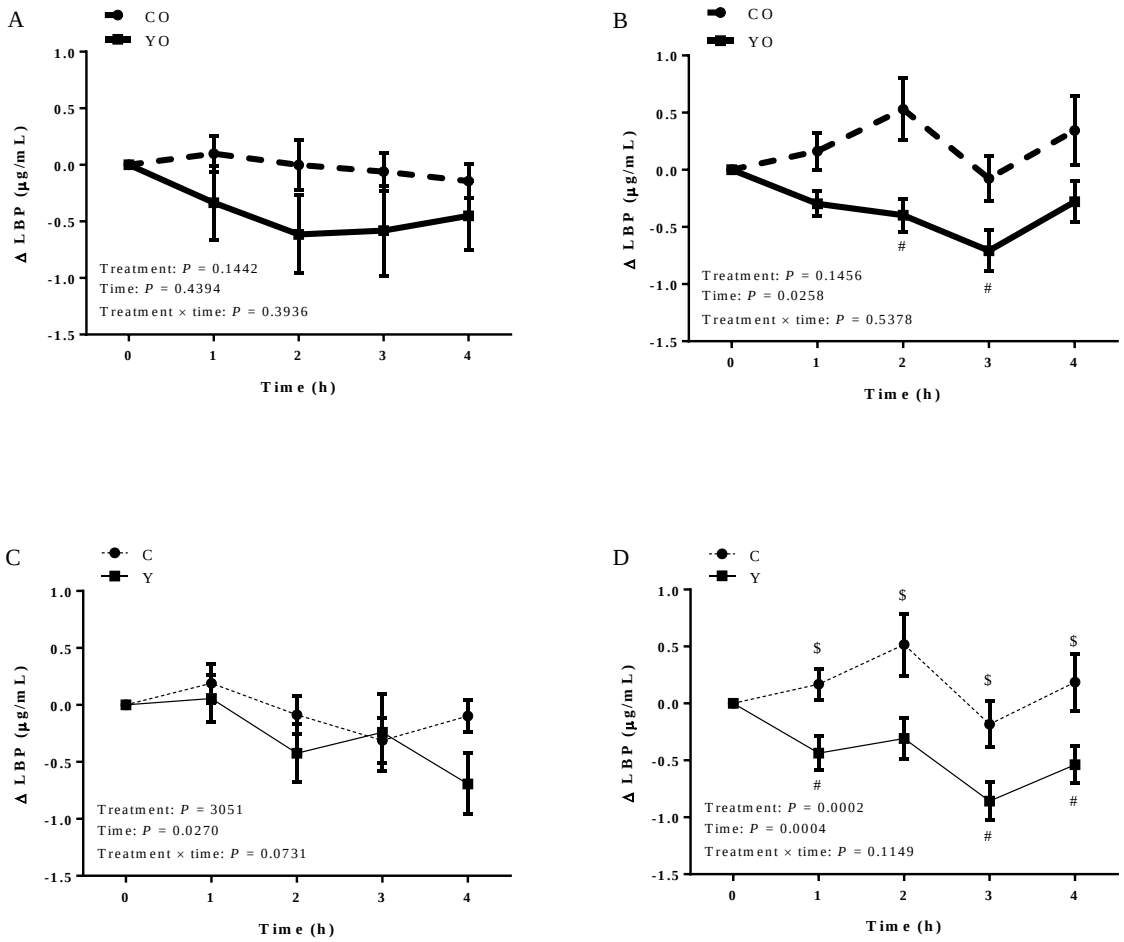

Figure 4.6 Relative change of plasma LBP from baseline (0 h) in the obese groups at the first (A) and second (B) challenge meal, and in the lean groups at the first (C) and second (D) challenge meal.

C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese. Repeated-measures ANOVA with Dunnett's test for multiple comparison was performed to evaluate time and treatment effects. # Significantly different from baseline in the yogurt treatment groups, $P < 0.05$. $ Significantly different between the yogurt and control treatment groups at different time points, $P < 0.05$.

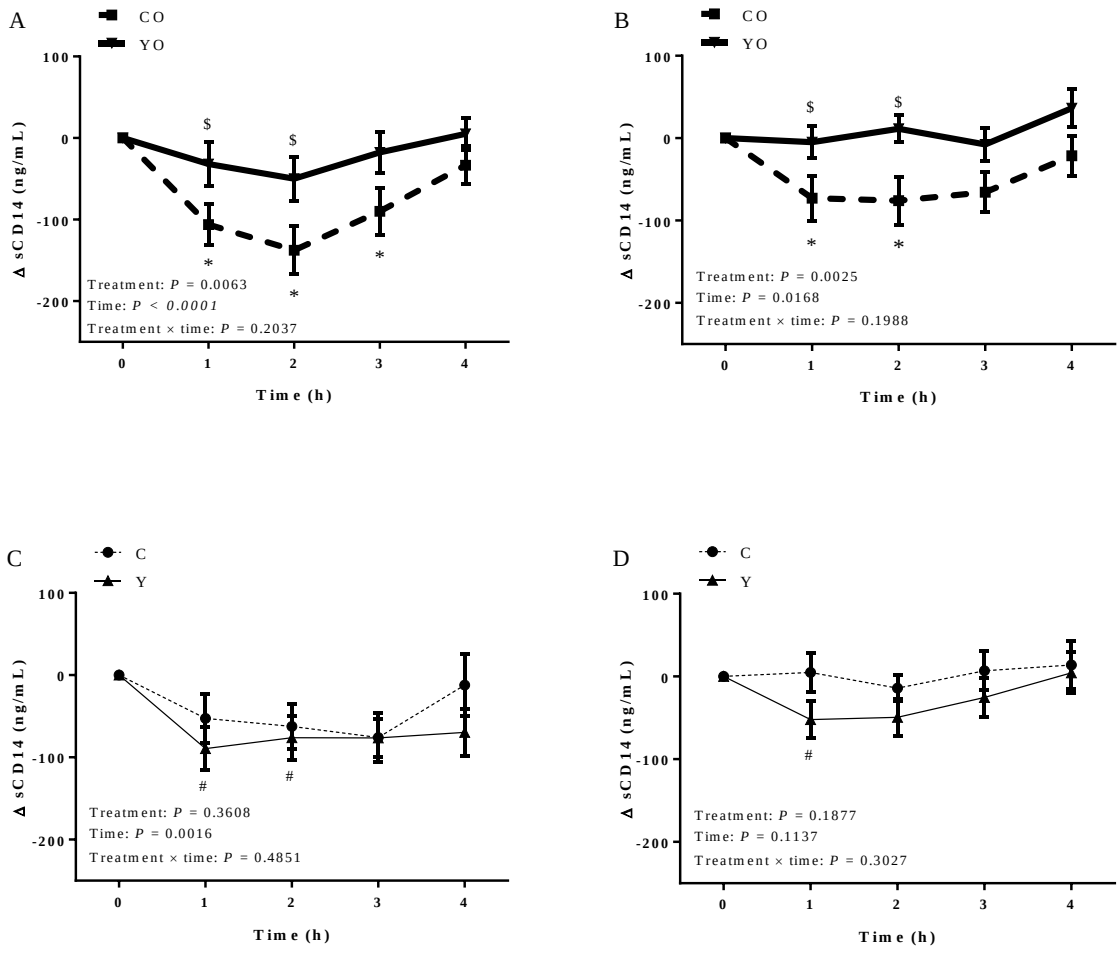

Figure 4.7 Relative change of plasma sCD14 from baseline (0 h) in the obese groups at the first 1 (A) and second (B) challenge meal, and in the lean groups at the first (C) and second (D) challenge meal.

C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese. Repeated-measures ANOVA with Dunnett's test for multiple comparison was performed to evaluate time and treatment effects. * Significantly different from baseline in the control treatment groups, $P < 0.05$. # Significantly different from baseline in the yogurt treatment groups, $P < 0.05$. $ Significantly different between the yogurt and control treatment groups at different time points, $P < 0.05$.

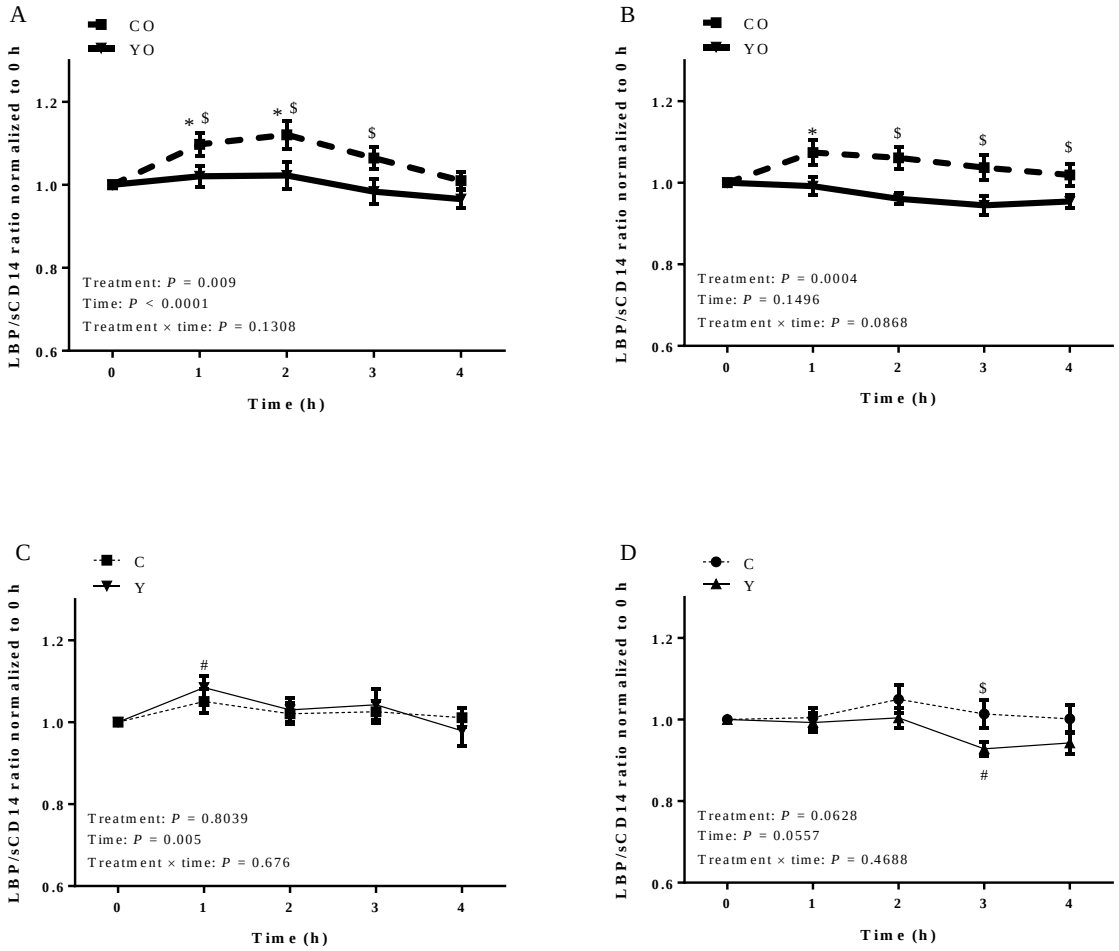

Figure 4.8 Changes in LBP/sCD14 ratio normalized to 0 h in the obese groups at the first (A) and second (B) challenge meal, and in the lean groups at the first (C) and second (D) challenge meal.

C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese. Repeated-measures ANOVA with Dunnett's test for multiple comparison was performed to evaluate time and treatment effects. * Significantly different from baseline in the control treatment groups, $P < 0.05$. # Significantly different from baseline in the yogurt treatment groups, $P < 0.05$. $ Significantly different between the yogurt and control treatment groups at different time points, $P < 0.05$.

4.5 Discussion

We examined the effects of pre-meal consumption of yogurt on postprandial intestinal barrier function, inflammation, glycemia, lipidemia, and insulin secretion in apparently healthy premenopausal women. In obese participants, low-fat yogurt consumption reduced postprandial increases of glucose, IL-6 and LPB/sCD14 ratio, suggesting lower inflammatory status and reduced endotoxemia. In lean participants, yogurt consumption attenuated postprandial hypoglycemia, possibly by reducing insulin over-secretion.

4.5.1 Postprandial glycemia, lipidemia and insulin secretion

The data from the present study support prior observations that obese individuals have dysregulated postprandial metabolism and insulin resistance (Esposito, Ciotola et al. 2007, Calder, Ahluwalia et al. 2011). After consuming the same high-fat, high-calorie challenge meal, the obese participants secreted more insulin than lean participants. Despite increased insulin, plasma glucose was also increased in obese compared to lean participants. This hyperglycemia was suppressed by pre-meal consumption of yogurt, although yogurt did not affect the TG response and insulin secretion.

Dairy protein alone may improve postprandial metabolism. In healthy, normal-weight adults, co-ingestion 50 g of casein, the major protein in dairy products, reduced the increase of free fatty acids after a fatty meal (Westphal, Kästner et al. 2004). In healthy, normal-weight men, a preload of whey protein as low as 10 g reduced postprandial blood glucose concentration and the ratio of cumulative blood glucose to insulin AUCs (Akhavan, Luhovyy et al. 2010). In type-2 diabetics, co-ingestion of 45 g of whey protein improved postprandial response by suppressing the increases in glucose, TG and free fatty acids (Mortensen, Hartvigsen et al. 2009).

Pre-meal consumption low-fat yogurt consumption might attenuate postprandial hyperglycemia by several mechanisms. Dairy calcium, but not calcium carbonate, diminished postprandial lipidemia, possibly via inhibiting fat absorption (Lorenzen, Nielsen et al. 2007). Casein may also be insulinotropic (Westphal, Kästner et al. 2004). An anti-inflammatory effect might also improve insulin resistance. IL-6 induces suppressor of cytokine signaling-3 (SOCS-3), which could interfere with insulin signaling (Ueki, Kondo et al. 2004, Ghanim, Abuaysheh et al. 2009). However, these do not appear to be the primary mechanisms by which yogurt inhibits postprandial hyperglycemia, as YO and CO postprandial TG and insulin were similar and plasma IL-6 increased after the hyperglycemia was evident.

Alternatively, yogurt consumption has the potential to modulate incretin function. Dairy proteins inhibit dipeptidyl peptidase-4 (DPP4) activity, a serine protease that inactivates Glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) (Lacroix and Li-Chan 2013, Silveira, Martínez-Maqueda et al. 2013). GLP-1 increases glucose uptake and decreases hepatic glucose production (Opinto, Natalicchio et al. 2013). GIP is not only insulinotropic, but also shows insulin-like activity on glucose metabolism (Hauner, Glatting et al. 1988, Gault, O'Harte et al. 2003).

Consumption of yogurt or dairy protein may also decrease the gastric emptying rate, which is regulated by GLP-1 (Sanggaard, Holst et al. 2004, D'Alessio, Sandoval et al. 2005, Akhavan, Luhovyy et al. 2010, Opinto, Natalicchio et al. 2013). Gastric emptying rate affects the magnitude and timing of postprandial glucose and insulin response by influencing the absorption of ingested nutrients (Rayner, Samsom et al. 2001). Rapid gastric emptying is also associated with postprandial hypoglycemia induced by excessive insulin release (Permutt 1976).

Pre-meal yogurt consumption might prevent over-secretion of insulin in lean individuals, thus attenuating the postprandial hypoglycemia. However, it is noteworthy that we did not observe the postprandial glucose peak in YO, C or Y. It is possible that the peak occurred between 0 and 1 h and was not captured by the selected study time points. In a postprandial study using a challenge meal that was similar to the present study, the glucose peaked at 30 min, and returned to baseline at 60 min (Dandona, Ghanim et al. 2015).

4.5.2 Postprandial inflammation

Circulating IL-6 increases postprandially within 4 hours in many postprandial studies using different challenge meals (Alvarez, Higgins et al. 2009, Laugerette, Vors et al. 2011, Burton-Freeman, Talbot et al. 2012). On the other hand, other classical inflammatory markers like TNF-α and hsCRP did not increase postprandially, possibly due to their delayed response (Poppitt, Keogh et al. 2008, Alvarez, Higgins et al. 2009, Biasillo, Leo et al. 2010, Schmid, Petry et al. 2015). Obesity-associated postprandial metabolic dysregulation is accompanied by increased inflammation compared to lean individuals, as indicated by greater incremental AUC of IL-6 (Manning, Sutherland et al. 2008). Similarly, obese participants in the present study had higher postprandial IL-6 exposure than lean participants. Pre-meal low-fat yogurt consumption was able to suppress the increased postprandial IL-6 in obese participants.

The yogurt-induced suppression IL-6 in obese participants may be related to reduced postprandial hyperglycemia in this group. Postprandial glucose induces oxidative stress (O'Keefe and Bell 2007). Yogurt consumption also inhibited postprandial plasma malondialdehyde, a biomarker of lipid oxidation, in obese women (DiMarco 2014). Postprandial oxidative stress and inflammation have been reported in many intervention studies (Anderson, Evans et al. 2001,

Nappo, Esposito et al. 2002, Ceriello, Quagliaro et al. 2004, Ceriello, Assaloni et al. 2005, Monnier, Mas et al. 2006). Oxidative stress stimulates inflammation by increasing MAPK and NF-κB signaling (Rahman 2002, Leung and Chan 2009). Also, antioxidant consumption reduces postprandial oxidative stress and inflammation (Edirisinghe, Banaszewski et al. 2011, Burton-Freeman, Talbot et al. 2012). It is plausible that yogurt and its bioactive components such as probiotics and protein exhibit antioxidant activity (Ebringer, Ferenčík et al. 2008).

4.5.3 Postprandial endotoxemia

Reduced endotoxin exposure may also explain the reduction of YO postprandial IL-6. LPS is a potent proinflammatory molecule (Cani, Amar et al. 2007, Andreasen, Larsen et al. 2010). Pre-meal consumption of low-fat yogurt might reduce endotoxin exposure in obese individuals, as indicated by lower postprandial LBP, LBP/sCD14 ratio, but higher sCD14 in the yogurt-consuming group than in the control group. LBP and sCD14 have been proposed as surrogate markers for endotoxemia, due to their roles in translocating LPS and other bacterial compounds to inflammatory signaling pathways (Schroder and Schumann 2005). Healthy overweight/obese individuals have higher LBP than lean individuals (Sun, Yu et al. 2010). Similarly, obese LBP was higher than lean in the present study.

A few intervention studies report sCD14 and LBP as biomarkers for low-grade, postprandial endotoxemia. A high-fat, high-calorie meal increased plasma LBP in 10 healthy, normal weight adults, which was suppressed by supplemental grape polyphenols (Ghanim, Sia et al. 2011). A mixed meal with emulsified fat increased plasma sCD14 and LPS in 12 healthy young normal weight men (Laugerette, Vors et al. 2011). However, such increases in plasma sCD14 and LBP were not observed in some other challenge meals studies (Clemente-Postigo,

Queipo-Ortuno et al. 2013, Laugerette, Alligier et al. 2014). In the present study, postprandial LBP decreased in both Y and YO, suggesting reduced endotoxin exposure. However in obese individuals, postprandial sCD14 also decreased upon in those consuming the control, but was unchanged after yogurt consumption. In the presence of LPS and LBP, sCD14 can form a ternary sCD14·LBP·LPS complex which is bound and internalized by cells (Tapping and Tobias 1997). Ingesting a meal containing 40 g fat induced higher postprandial LPS than after a meal containing 10 g fat in healthy obese adults (Vors, Pineau et al. 2015). The decreased sCD14 observed in the obese control group may have resulted from increased LPS and LBP which was complexed with sCD14 and subsequently cleared from circulation.

Circulating sCD14 is positively associated with insulin sensitivity in morbidly obese individuals (de Courten, Moreno-Navarrete et al. 2015). This is supported by one animal study that recombinant human sCD14 improved insulin action in high-fat-fed mice and *ob/ob* mice (Fernandez-Real, del Pulgar et al. 2011). On the other hand, circulating LBP is associated with insulin resistance and inflammatory marker in cross-sectional studies (Gubern, Lopez-Bermejo et al. 2006, Moreno-Navarrete, Ortega et al. 2012). Therefore, the improved postprandial glucose response in obese participants consuming yogurt might be partly explained by the higher level of postprandial sCD14 and lower LBP than those consuming the control.

In mice, a higher plasma LBP/sCD14 ratio is correlated with higher level of inflammation and higher endotoxin exposure (Laugerette, Furet et al. 2012). Similarly, a high-fat, high-calorie challenge meal increased the postprandial LBP/sCD14 ratio (Laugerette, Alligier et al. 2014). We observed a postprandial increase in CO LBP/sCD14 only. In contrast, the YO LBP/sCD14 did not increase, indicating suppressed postprandial endotoxemia. Yogurt consumption might

improve the impaired intestinal barrier function of obese individuals and reduce postprandial endotoxemia. Probiotics and whey peptides enhanced mucin secretion *in vitro* (Mack, Michail et al. 1999, Martínez-Maqueda, Miralles et al. 2012). In addition, yogurt consumption increased sIgA production in both adults and children (Link-Amster, Rochat et al. 1994, Yang and Sheu 2012). Moreover, yogurt and its associated probiotics could help maintain tight junction integrity. For example, in a rat model, yogurt with *B. lactis* prevented the increase in intestinal permeability induced by partial restraint stress by restoring expression of occludins and junctional adhesion molecules (Agostini, Goubern et al. 2012). Supplemental calcium also inhibited loss of tight junction function in diabetic rats (Stuart, Sun et al. 1994, Leal, Martins et al. 2010).

Data on plasma LPS would improve our ability to interpret LBP and sCD14 changes. However, direct measurement of low-levels of plasma LPS is challenging, due to its short half-life, low blood concentration and the difficulty of removing interferences in blood (Novitsky 1998, Munford 2005). LPS measurement also does not take into account of other bacterial compounds such as glycolipids and lipoproteins derived from pathogenic Gram-positive bacteria that can stimulate inflammation (Schroder and Schumann 2005). In addition, direct measure of gut permeability with sugar (lactulose and mannitol) absorption test, and the expression of tight junction proteins might also facilitate the assessment of endotoxemia and intestinal barrier function (Gummesson, Carlsson et al. 2011, Teixeira, Souza et al. 2012).

4.6 Conclusion

Yogurt consumption suppressed the postprandial increases of IL-6 and LBP/sCD14 ratio in obese participants. Pre-consumption of yogurt also reduced the postprandial increase in

glucose in the obese participants and attenuated the postprandial hypoglycemia in lean participants. A further 9-week consumption of yogurt seemed to further improve the acute benefits of pre-meal yogurt consumption on postprandial inflammation. Thus, pre-meal yogurt consumption is a feasible strategy to improving postprandial metabolism in lean and obese individuals. Because increased postprandial dysmetabolism and endotoxin exposure contribute to chronic inflammation, we examined the effect of yogurt consumption on chronic inflammatory status, as discussed in Chapter 5.

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Chapter 5: Effects of Low-fat Yogurt Consumption on Chronic Inflammation and Endotoxemia in Apparently Healthy Premenopausal Women

5.1 Abstract

Background: Diet may prevent low-grade endotoxemia and chronic inflammation associated with obesity. Increased dairy consumption prevents chronic inflammation in obese individuals, but the mechanisms for this effect are unknown.

Objective: Our objective was to determine how increased low-fat yogurt consumption affects chronic inflammation and endotoxemia in apparently healthy, obese women.

Design: Apparently healthy premenopausal women with BMI of 18.5 to 27 (lean) and 30 to 40 kg/m^2 (obese) participated in a 9-week randomized, controlled study. Participants in each BMI group were randomly assigned to either a low-fat yogurt-supplemented group (Y, yogurt lean; YO, yogurt obese) or a soy pudding-supplemented control group (C, control lean; CO, control obese) with n = 30/group. Participants were advised to consume 12 oz. of yogurt or the control food daily for 9 weeks while maintaining their usual caloric intake. Participants provided fasting blood samples at wk 0, 3, 6, and 9 for determination of inflammation and endotoxemia. The mRNA expressions of genes related to inflammation were further determined in peripheral blood mononuclear cells (PBMC) of obese participants at wk 0 and 9.

Results: At baseline, obese participants had increased plasma biomarkers of chronic inflammation and endotoxemia compared to lean individuals. After 9 weeks, YO biomarkers of chronic inflammation decreased from baseline: interleukin-6 (IL-6) by 17%, high-sensitivity C-reactive protein (hsCRP) by 9%, tumor necrosis factor alpha (TNF-α) by 7%, TNF-α/soluble tumor necrosis factor receptor II (sTNF-RII) by 7%. After 9 weeks, YO had 5% increased plasma immunoglobulin M endotoxin-core antibody (IgM EndoCAb), as well as 54% increased nuclear factor kappa B inhibitor alpha (IκB-α) and 20% increased transforming growth factor

beta 1 (TGFβ1) mRNA expression in PBMC. Y also had decreased TNF-α, TNF-α/sTNF-RII and increased IgM EndoCAb from baseline. Biomarkers of inflammation were unchanged in C and CO at 9 weeks. CO had moderate decrease in soluble CD14 (sCD14) and increase in lipopolysaccharide-binding protein (LBP)/sCD14 ratio.

Conclusion: Consuming low-fat yogurt for 9 weeks reduces biomarkers of chronic inflammation and endotoxemia in apparently healthy premenopausal women compared to a non-dairy control food. This trial was registered at clinicaltrials.gov as NCT01686204.

5.2 Introduction

Chronic inflammation resulting from obesity has been well characterized. Obesity increases adipose excretion or plasma accumulation of proinflammatory cytokines tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), interleukin 8 (IL-8), monocyte chemoattractant protein-1 (MCP-1), and C-reactive protein (CRP) (Kern, Ranganathan et al. 2001, Kim, Park et al. 2006). In diet-induced obese animal models, mRNA expression and protein secretion of proinflammatory markers are up-regulated in adipose tissue, pancreatic islets, and the hypothalamus (De Souza, Araujo et al. 2005, Brake, Smith et al. 2006, Ehses, Perren et al. 2007). Increased chronic inflammation is proposed to contribute to comorbidity of chronic disease with obesity (Lumeng and Saltiel 2011).

Unresolved low-grade obesity-associated inflammation originates from the interplay between immune cells and metabolic tissues such as adipose, liver, muscle, pancreas, and brain in response to excessive nutrient intake, leading to increased proinflammatory cytokines release (Gregor and Hotamisligil 2011). Inflammation is exacerbated by chronic endotoxin exposure resulting from compromised intestinal barrier function in obese individuals (Cani, Bibiloni et al. 2008, Sun, Yu et al. 2010). Endotoxin, especially the Gram-negative-derived lipopolysaccharides (LPS) from gut microbiota, can effectively induce inflammation in humans (Kemna, Pickkers et al. 2005, Andreasen, Larsen et al. 2010). LPS inflammatory signaling is mediated by its translocation by lipopolysaccharide-binding protein (LBP) and membrane-bound or soluble CD14 (sCD14) to Toll-like receptor 4 (TLR4) and MD2 complex (Van Bossuyt, De Zanger et al. 1988, Park, Song et al. 2009, Manco, Putignani et al. 2010). Furthermore, persistent postprandial inflammation and oxidative stress is exacerbated by obesity and contribute to chronic

inflammation (Burdge and Calder 2005, Esposito, Ciotola et al. 2007, Calder, Ahluwalia et al. 2011).

Yogurt consumption appears to be a promising strategy to improve obesity-associated intestinal barrier dysfunction and prevent chronic inflammation. Consuming yogurt containing lactobacillus reduces surrogate markers of endotoxemia and decreases intestinal permeability (Zeng, Li et al. 2008, Schiffrin, Parlesak et al. 2009). Consumption of yogurt containing *L. acidophilus* and *B. lactis* decreases fecal haptoglobin in elderly individuals (Matsumoto, Ohishi et al. 2001). Consumption of yogurt containing *L. acidophilus* and *B. lactis* decreases serum IL-6 in children (Yang and Sheu 2012). Epidemiological studies show increased dairy consumption is associated with decreased risk of chronic diseases such as cardiovascular diseases, type 2 diabetes and hypertension (Warensjö, Jansson et al. 2010, Tong, Dong et al. 2011, Ralston, Lee et al. 2012, Sluijs, Forouhi et al. 2012). However, the effect of yogurt consumption on inflammation and endotoxemia in obese population is still largely unknown. We hypothesized that regular consumption of a commercial low-fat yogurt would reduce biomarkers of chronic inflammation and low-grade endotoxemia in healthy premenopausal women. We tested this hypothesis by conducting a randomized trial using a macronutrient- and texture-matched non-dairy food as a control.

5.3 Material and Methods

5.3.1 Study participants

Obese and lean women were recruited to participate in the study as described in Section **3.3.1**.

5.3.2 Experimental design

A randomized, controlled study was used to determine the effects of yogurt consumption on chronic inflammation and intestinal barrier function relative to consumption of a non-dairy control snack (**Figure 5.1**). This study was powered to detect an 8.2% difference of sCD14, the primary outcome (Laugerette, Vors et al. 2011). The recruitment, initial prescreening, enrollment, compliance, and completion rate of 128 subjects were previously described in **Section 3.3.3**. Briefly, n = 30 participants from each group: YO, yogurt obese; CO, control obese; Y, yogurt lean; C, control lean completed all study visits.

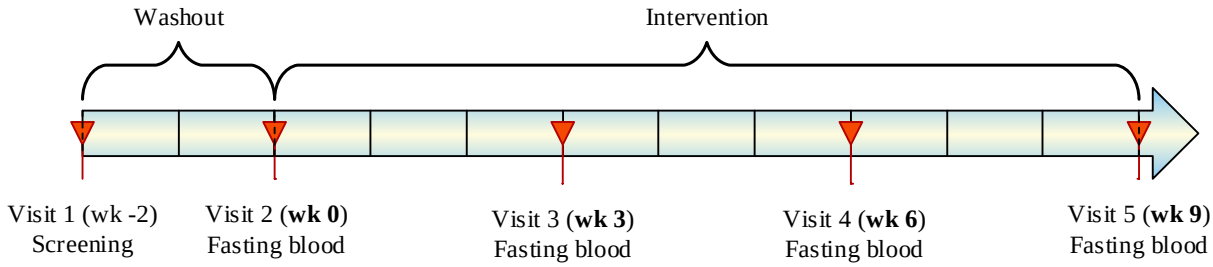

Figure 5.1 Experimental design of chronic study.

After 2 weeks of washout, lean and obese subjects were randomly assigned to either consume 12 oz. of yogurt or a non-dairy control food for 9 weeks. Starting at wk 0, participants visited the study center every 3 weeks till the end of wk 9 to provide a fasting blood sample.

To avoid confounding by probiotic or dairy consumption, the participants restricted consumption of dietary supplements, fermented foods and limited their dairy consumption to ≤ 4 servings/day for 2 weeks before the intervention (washout period, wk -2 to wk 0) and throughout the intervention. From the beginning of wk 0 to the end of wk 9 (intervention period), the subjects consumed 12 oz. of yogurt or the control food daily. The composition of the

intervention foods were described in **Section 3.3.2**. Participants visited the study center at wk 0, 3, 6 and 9 to provide fasting blood samples. Fasting blood samples were collected from the antecubital vein into evacuated tubes containing ethylenediaminetetraacetic acid (EDTA) or sodium heparin (Becton, Dickinson and Company, Franklin Lakes, NJ). At wk 0 and 9, peripheral blood mononuclear cells (PBMC) were immediately isolated from 20 mL of sodium heparin-blood samples for later mRNA analysis as described in **Section 5.3.6,** below. For biomarker analysis, plasma was prepared from blood samples held on ice and centrifuged (4°C, 15 min, 1500 × g) within 20 min of collection. Aliquots from the upper layer of plasma were snap-frozen in liquid nitrogen. All samples were stored at -80 °C until analysis.

5.3.3 Inflammatory markers

IL-6 and TNF-α in EDTA plasma were measured by human high-sensitive ELISA kits (IL-6, Cat # SS600B; TNF-α, Cat # SSTA00D; R&D System, Minneapolis, MN). Soluble TNF-Receptor II (sTNF-RII) was measured by a human ELISA kit (Cat# SRT200; R&D System, Minneapolis, MN). High-sensitivity C-reactive protein (hsCRP) was measured by a human ELISA kit (Cat # BC-1119; BioCheck Inc., Foster City, CA). All the measurements were performed on a SpectraMax M2 microplate reader (Molecular Devices, Sunnyvale, CA), following the manufacturers' instruction.

5.3.4 Endotoxemia markers

LBP and immunoglobulin M Endotoxin-core antibody (IgM EndoCAb) in EDTA plasma were measured by human ELISA kits (LBP, Cat # HK315; IgM EndoCAb, Cat # HK504-IGM; Hycult Biotech, Uden, Netherlands). Sodium heparin plasma sCD14 was measured by a human ELISA kit (Cat # DC140; R&D System, Minneapolis, MN). All the measurements were

performed on a SpectraMax M2 microplate reader (Molecular Devices, Sunnyvale, CA), following the manufacturers' instruction. The ratio between LBP and sCD14 was calculated as a marker for endotoxemia.

5.3.5 PBMC isolation and mRNA analysis

PBMC were isolated from sodium heparin blood by gradient centrifugation using Histopaque®-1077 (Sigma-Aldrich, St. Louis, MO), following the manufacturer's instruction with minor modifications. Briefly, 20 mL blood was diluted with 15 mL isotonic, sterile, pyrogen-free phosphate buffered saline (PBS) (MP Biomedicals, Santa Ana, CA) and then carefully loaded onto 15 mL Histopaque®-1077 in a clear, 50 mL centrifuge tube. After centrifugation at 400 × g for 30 min at room temperature, the interface layer containing mononuclear cells was collected and washed twice with PBS. Cell number was then determined on a TC10™ automated cell counter (Bio-Rad, Hercules, CA) and around 1×10^6 cells were transferred to a new centrifuge tube and pelleted at 250 × g for 10 min at room temperature. The pellet was suspended in 1 mL RNAlater (Life Technologies, Carlsbad, CA), mixed well by vortexing, and stored at 4 °C overnight. The next day, the cells were pelleted at 8000 × g for 5 min at 4 °C and stored at -80 °C until RNA extraction.

RNA was extracted by TRIzol reagent (Life Technologies, Carlsbad, CA) following the manufacturer's recommended protocol. Briefly, cells were homogenized by pipetting up and down in TRIzol reagent. Chloroform (Fisher Scientific, Pittsburgh, PA) was added for phase separation, and aqueous phase was removed and mixed with isopropanol (Fisher Scientific, Pittsburgh, PA) to precipitate RNA. RNA was pelleted by centrifugation, washed with 75% ethanol (Fisher Scientific, Pittsburgh, PA), and dissolved in RNase-free water (Dot Scientific,

Burton, MI). The extracted RNA was further purified using an RNeasy mini kit (Qiagen, Valencia, CA). Briefly, the RNA solution was mixed with the supplied lysis buffer and ethanol, and then loaded onto a column, digested with an RNase-free DNase (Qiagen, Valencia, CA) and RNA was eluted with RNase-free water. RNA concentration was then determined by fluorometric quantitation on a Qubit (Life Technologies, Carlsbad, CA). Then, cDNA was synthesized from 1 μg RNA using an iScript™ cDNA synthesis kit (Bio-Rad, Hercules, CA) on a Veriti® thermal cycler (Life Technologies, Carlsbad, CA), resulting in a 20 μL reaction volume. Then, 5 μL of the newly synthesized cDNA product was pooled for preparation of a standard curve.

Reverse transcription quantitative PCR (RT-qPCR) was performed using the iTaq™ Universal SYBR® Green Supermix (Bio-Rad, Hercules, CA), on a Bio-Rad CFX96 system (Bio-Rad, Hercules, CA), following the manufacture's protocol. To prevent amplification of genomic DNA, specific primers were designed by the NCBI primer design tool to span an intron-exon boundary of genes (http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome) (Altschul, Gish et al. 1990). Primers were manufactured by Integrated DNA Technologies (Coralville, IA) at 25 nmol scale. The genes and primer sequences determined by RT-qPCR are listed in **Table 5.1**. The controls consisted of *RNA18S5* and *RPRL0* genes that are stably expressed in PBMC (Bas, Forsberg et al. 2004, Dheda, Huggett et al. 2004). The RT-qPCR reaction volume was 10 μL, consisting of 4 μL of 20-times diluted cDNA and 6 μL of master mix (5 μL of SYBR® Green Supermix; 0.5 μL of each 2.5 μM primer). Each RT-qPCR run included no cDNA and no reverse transcriptase as negative controls. A 6-point standard curve was prepared using the 4-fold serial dilution of the samples cDNA pool and the unknown samples were quantified by the relative standard curve

method using Bio-Rad CFX manager 2.1 software (Bio-Rad, Hercules, CA) (Grala, Roche et al. 2014).

5.3.6 Statistical analysis

All results were expressed as mean ± standard error. Statistical analysis was conducted on SAS 9.4 software (Cary, NC). The significance level was set at $\alpha = 0.05$ for all tests. At baseline, the differences of Y vs. C, and YO vs. CO were analyzed by independent T-test. Pearson correlation coefficients of paired markers were analyzed by linear regression modeling. Time-dependent changes of IL-6 and sCD14 in each group were determined by repeated-measures ANOVA with Dunnett's test for multiple comparisons. At wk 9, the difference of biomarkers between yogurt and control treatment groups was analyzed by analysis of covariance (ANCOVA) with baseline as the covariate. The difference between wk 0 and wk 9 in each group was analyzed by paired T-test.

5.4 Results

At baseline, obese participants had levels of biomarkers indicative of chronic inflammation and endotoxemia. Relative to lean participants, obese had increased fasting plasma IL-6 (111%), hsCRP (135%), TNF-α (23%), LBP (28%), LBP/sCD14 ratio (27%), but 31% less plasma IgM EndoCAb than the lean participants (**Table 5.2)**. However, the baseline biomarkers within lean or obese groups were not different. Baseline plasma biomarkers of chronic inflammation, IL-6, hsCRP, TNF-α, and sTNF-RII were correlated in all study participants (**Table 5.3**). Among the markers for endotoxemia, LBP and IgM EndoCAb were negatively correlated ($r = -0.3431$, $P = 0.0002$). No other correlation between endotoxemia markers were

identified. Furthermore, fasting plasma LBP was more strongly correlated to proinflammatory biomarkers markers than sCD14 and IgM EndoCAb.

The average concentrations of IL-6 and sCD14 at wk 0, 3, 6, and 9 in each group are presented in **Appendix A.3 Supplementary Table 5.1**. The average concentrations of hsCRP, TNF-α, sTNF-RII, TNF-α/sTNF-RII ratio, LBP, LBP/sCD14 ratio, and IgM EndoCAb at wk 0 and 9 in each group are presented in **Appendix A.3 Supplementary Table 5.2**.

5.4.1 Fasting plasma inflammation markers

YO plasma IL-6 decreased by 17% from baseline after 3 weeks, which was further maintained at wk 6 and 9. However, at wk 3, 6 and 9, YO and CO IL-6 were not significantly different, given the lower baseline CO IL-6 (**Figure 5.2**). Y, C, and CO IL-6 was unchanged from baseline during 9 weeks. Similar to IL-6, YO plasma hsCRP decreased by 9% from baseline at wk 9 (**Figure 5.3**). After adjusting for baseline, YO tended to have lower hsCRP than CO (2.42 ± 0.32 vs. 2.98 ± 0.32 mg/L, $P = 0.1088$). In contrast, Y, C, and CO hsCRP were unchanged at wk 9. YO and Y plasma TNF-α decreased by 7% and 9%, respectively at wk 9 (**Figure 5.4**). In addition, Y had 13% lower TNF-α than C at wk 9. YO plasma sTNF-RII increased by 4%. Because of these changes, YO and Y TNF-α/sTNF-RII ratio decreased by 7% each.

5.4.2 Fasting plasma endotoxemia biomarkers

Plasma LBP was unchanged in all groups at wk 9 (**Figure 5.5 A** and **B**). At wk 3, 6, and 9, CO fasting plasma sCD14 decreased by 6%, 10%, and 8%, respectively from baseline. C fasting plasma sCD14 decreased by 7% temporarily at wk 3 and returned to baseline at wk 6 and 9. In contrast, YO and Y sCD14 was unchanged by the intervention (**Figure 5.5 C** and **D**). The

LBP/sCD14 ratio, a marker of endotoxemia, increased by 17% in C, and by 11% in CO but did not change in Y and YO (**Figure 5.5 E** and **F**). The LBP/sCD14 ratio was 9% higher in CO than in YO. Y and YO plasma IgM EndoCAb increased by 6% and 5%, respectively (**Figure 5.6**). Y also had 10% higher IgM EndoCAb than C at wk 9.

5.4.3 Expression of mRNA in PBMC

We determined the change in PBMC mRNA expression of several key factors and downstream target genes involved in the TLR4/NF-κB pathway including *TLR4* (encoding Toll-like receptor 4), *RELA* (encoding p65 subunit of nuclear factor kappa B), *NFKBIA* (encoding nuclear factor kappa B inhibitor alpha), *PTGS2* (encoding cyclooxygenase-2), *NCF1* (encoding the p47 subunit of nicotinamide adenine dinucleotide phosphate-oxidase, or NADPH oxidase), *TNF* (encoding tumor necrosis factors), and *IFNG* (interferon-γ) and the mRNA expression of *TGFB1* (encoding transforming growth factor beta 1, or TGFβ1), a key factor in the resolution of inflammation. The analysis focused on obese individuals, given the greater magnitude of changes in biomarkers of endotoxemia and chronic inflammation. At baseline, YO and CO gene expression did not differ (**Figure 5.7**). After 9 weeks, YO *NFKBIA* and *TGFB1* increased by 54% and 20% from baseline, respectively (**Figure 5.8**). In addition, YO had 36% more increase in *TGFB1* than CO. In contrast, CO mRNA expression of the genes in the present study was unchanged at wk 9 from baseline.

Table 5.1 Primer sequences of target and reference PBMC genes.

Name	GenBank accession number	Forward (primer sequence 5′–3′)	Reverse (primer sequence 5′–3′)
RNA18S5	NR_003286.2	CTG AGA AAC GGC TAC CAC ATC	GCC TCG AAA GAG TCC TGT ATT G
RPLP0	NM_001002.3	CTC GTG GAA GTG ACA TCG TCT	GCT TGG AGC CCA CAT TGT CT
TLR4	NM_003266.3	GTC CCT GAA CCC TAT GAA CTT T	AAC CAG CCA GAC CTT GAA TAC
RELA	NM_001145138.1	CCA GAC CAA CAA CAA CCC CT	GGG GGC ACG ATT GTC AAA GA
NFKBIA	NM_020529.2	GAG CTC CGA GAC TTT CGA GG	TGT AGA CAC GTG TGG CCA TT
PTGS2	NM_000963.3	AAC TGC TCA ACA CCG GAA T	CCC TTG AAG TGG GTA AGT ATG TAG
NCF1	NM_000265.5	CTG AGC CCA ACT ATG CAG GT	TGA CGT CGT CTT TCC TGA TG
TNF	NM_000594.3	CCT CTC TCT AAT CAG CCC TCT	AGG GTT TGC TAC AAC ATG GG
IFNG	NM_000619.2	GGC TTT TCA GCT CTG CAT CG	TCT GTC ACT CTC CTC TTT CCA
TGFB1	NM_000660.5	CGA CTC GCC AGA GTG GTT AT	CGG TAG TGA ACC CGT TGA TGT

RNA18S5, encoding 18s rRNA; *RPLP0*, encoding ribosomal protein large P0; *TLR4*, encoding Toll-like receptor 4; *RELA*, encoding p65 subunit of nuclear factor kappa B; *NFKBIA*, encoding nuclear factor kappa B inhibitor alpha; *PTGS2*, encoding cyclooxygenase-2, or COX-2; *NCF1*, encoding the p47 subunit of nicotinamide adenine dinucleotide phosphate-oxidase, or NADPH oxidase; *TNF*, encoding tumor necrosis factors, or TNF; IFNG, encoding interferon-γ; *TGFB1*, encoding transforming growth factor beta 1, or TGFβ1.

Table 5.2 Fasting baseline characteristics enrolled participants (mean ± standard error, n = 30/group).

Characteristic (unit)	**Group**				***Significance (P)*[1]**		
	C	CO	Y	YO	*P VS. C*	*CO VS. YO*	*Obese VS. Lean*
Age (year)	25.3 ± 1.1	31.9 ± 1.6	24.8 ± 0.8	36.7 ± 2.0	*0.7603*	*0.0609*	*<.0001*
Height (m)	1.66 ± 0.01	1.65 ± 0.01	1.67 ± 0.01	1.65 ± 0.01	*0.7534*	*0.6489*	*0.2883*
Weight (kg)	62.3 ± 1.6	93.5 ± 2.2	64.7 ± 1.5	94.1 ± 1.9	*0.2788*	*0.8336*	*<.0001*
BMI (kg/m^2)	22.5 ± 0.4	34.3 ± 0.5	23.3 ± 0.5	34.4 ± 0.7	*0.2603*	*0.9670*	*<.0001*
IL-6 (pg/mL)	0.74 ± 0.08	1.56 ± 0.13	0.88 ± 0.13	1.86 ± 0.22	*0.3941*	*0.2487*	*<.0001*
LBP (μg/mL)	9.9 ± 0.9	12.4 ± 0.7	9.3 ± 0.6	12.3 ± 0.9	*0.6044*	*0.9302*	*0.0010*
sCD14(ng/mL)	1421 ± 50	1481 ± 45	1402 ± 59	1388 ± 45	*0.7993*	*0.1539*	*0.6579*
LBP/sCD14	7.14 ± 0.69	8.62 ± 0.58	6.89 ± 0.44	9.13 ± 0.73	*0.7584*	*0.5881*	*0.0032*
hsCRP (mg/L)	1.24 ± 0.26	2.97 ± 0.31	1.15 ± 0.21	2.63 ± 0.37	*0.7872*	*0.4840*	*<.0001*
TNF-α (pg/mL)	1.10 ± 0.07	1.25 ± 0.1	1.14 ± 0.09	1.52 ± 0.12	*0.7755*	*0.0815*	*0.0104*
sTNF-RII (pg/mL)	2125 ± 72	2452 ± 94	2028 ± 69	2550 ± 101	*0.3362*	*0.4827*	*<.0001*
IgM EndoCAb (MMU/mL)	99.6 ± 10.6	70.7 ± 4.2	101.4 ± 10.1	68.7 ± 7.1	*0.9078*	*0.8091*	*0.0004*

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese; BMI, body mass index; IL-6, interleukin 6; LBP, lipopolysaccharide-binding protein; sCD14, soluble CD14; hsCRP, high-sensitivity C-reactive protein; TNF-α, tumor necrosis factor alpha; sTNF-RII, soluble tumor necrosis factor receptor II; IgM EndoCAb, immunoglobulin M endotoxin-core antibody.

[1] Differences between yogurt and pudding treatment groups in non-obese [*P (C vs. Y)*] and obese individuals [*P (CO vs. YO)*], and the differences between obese and lean subjects [*P (Obese vs. Lean)*] were determined by independent T-test (PROC TTEST).

Table 5.3 Pearson correlation coefficients between paired markers of all participants at baseline.

		IL-6	hsCRP	TNF-α	sTNF-RII	LBP	sCD14
hsCRP	r[1]	0.3788					
	P[2]	<.0001					
TNF-α	r[1]	0.2880	0.1965				
	P[2]	0.0017	0.0337				
sTNF-RII	r[1]	0.3140	0.3017	0.3891			
	P[2]	0.0005	0.0008	<.0001			
LBP	r[1]	0.3634	0.5411	0.0663	0.2392		
	P[2]	<.0001	<.0001	0.4754	0.0088		
sCD14	r[1]	0.1590	0.1819	-0.0864	0.2855	0.0979	
	P[2]	0.0854	0.0467	0.3523	0.0016	0.2874	
IgM EndoCAb	r[1]	-0.1426	-0.1336	-0.0671	-0.2500	-0.3431	-0.0908
	P[2]	0.1234	0.1473	0.4701	0.0061	0.0002	0.3281

Abbreviations: IL-6, interleukin 6; LBP, lipopolysaccharide-binding protein; sCD14, soluble CD14; hsCRP, high-sensitivity C-reactive protein; TNF-α, tumor necrosis factor alpha; sTNF-RII, soluble tumor necrosis factor receptor II; IgM EndoCAb, immunoglobulin M endotoxin-core antibody.

[1] Pearson correlation coefficient.

[2] P value.

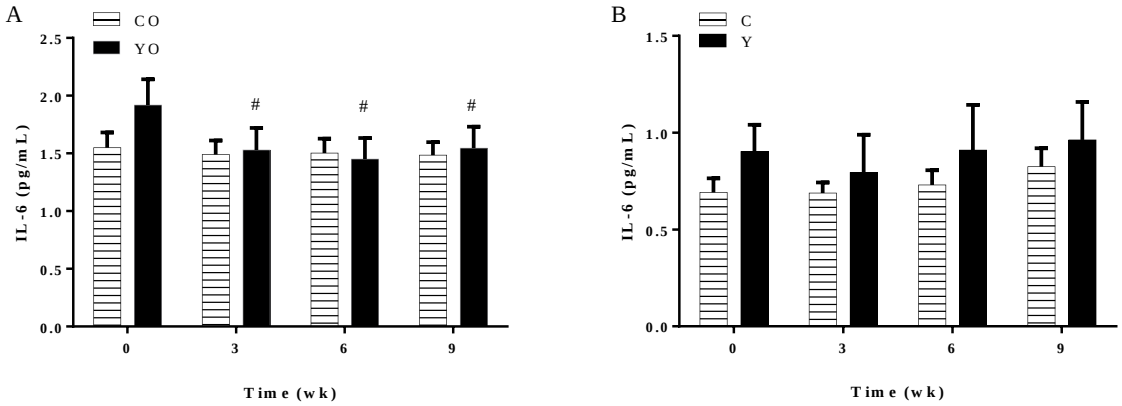

Figure 5.2 Fasting plasma interleukin 6 (IL-6) of (A) obese and (B) lean participants at baseline and after consuming 12 oz. of yogurt or a non-dairy control food daily for 9 weeks.

C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese (n = 30/group). Group differences at wk 9 were tested for significance by ANCOVA using baseline level as a covariate. Within-group differences between wk 0 and wk 9 means were tested by paired T-test. # Significantly different from YO baseline, $P < 0.05$.

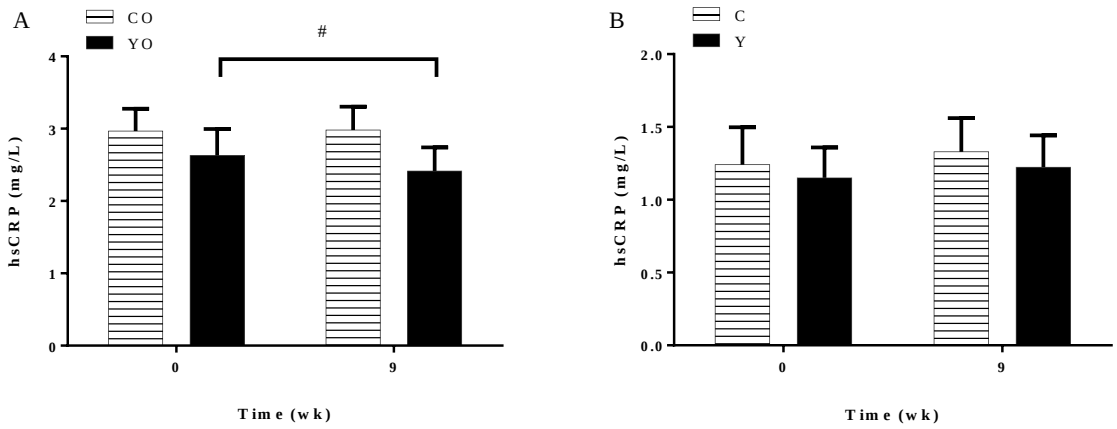

Figure 5.3 Fasting plasma high-sensitivity C-reactive protein (hsCRP) in (A) obese and (B) lean participants at baseline and after consuming 12 oz. of yogurt or a non-dairy control food daily for 9 weeks.

C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese (n = 30/group). Between-group differences in wk 9 means were assessed by ANCOVA with baseline as a covariate. Within-group differences between wk 0 and wk 9 means were compared by paired T-test. # Significantly different from YO baseline, $P < 0.05$.

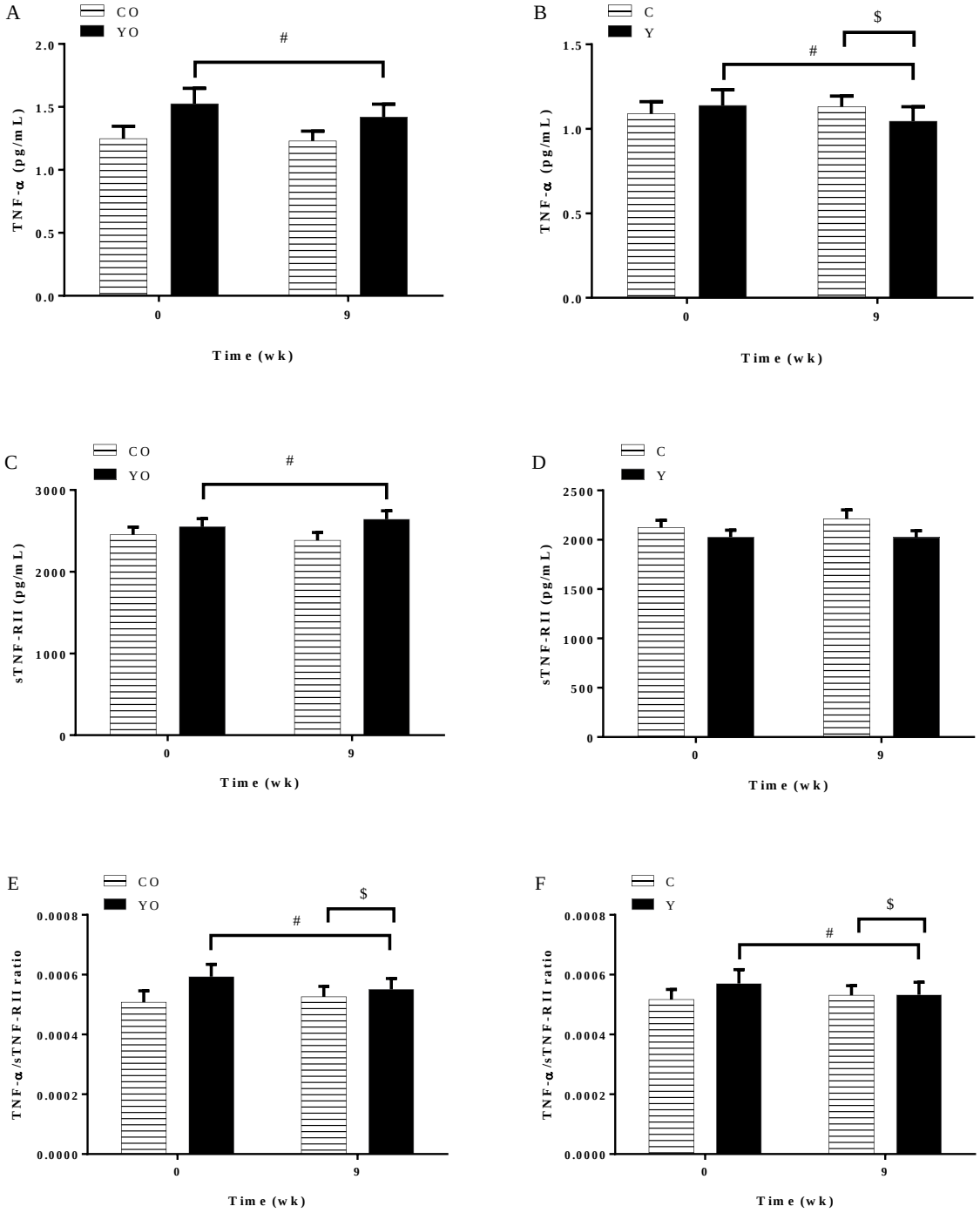

Figure 5.4 Fasting plasma tumor necrosis factor alpha (TNF-α) of (A) obese and (B) lean, soluble tumor necrosis factor receptor II (sTNF-RII) of (C) obese and (D) lean, and TNF-

α/sTNF-RII ratio of (E) obese and (F) lean participants at baseline and after consuming 12 oz. of yogurt or a non-dairy control food daily for 9 weeks.

C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese (n = 30/group). Between-group difference of wk 9 means were compared by ANCOVA with baseline as a covariate. Within-group differences between wk 0 and wk 9 means were compared by paired T-test. # Significantly different from YO or Y baseline, $P < 0.05$. $ Significantly different between the YO and CO, or Y and C, $P < 0.05$.

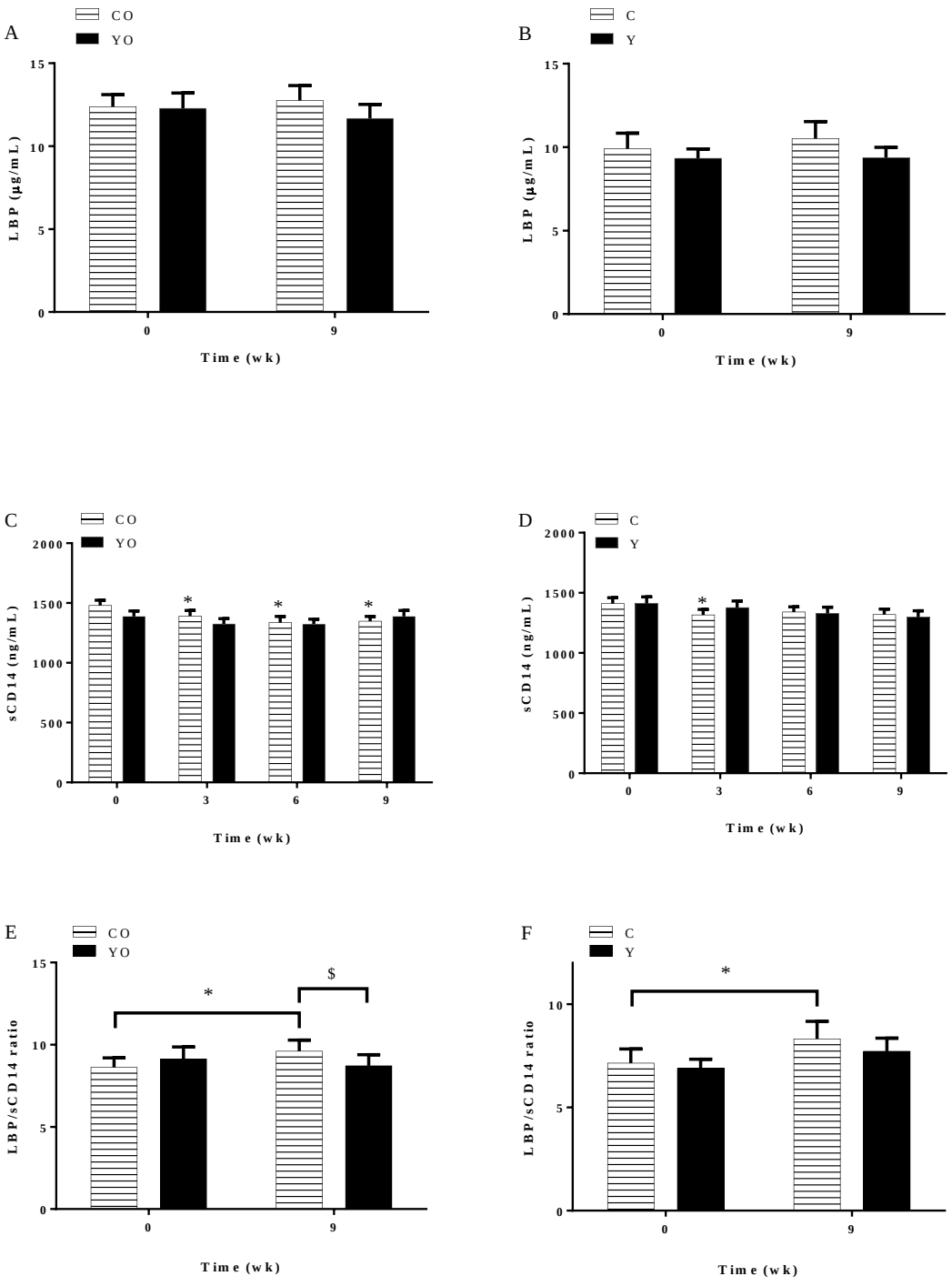

Figure 5.5 Fasting plasma lipopolysaccharide-binding protein (LBP) of (A) obese and (B) lean, soluble CD14 (sCD14) of (C) obese and (D) lean, and LBP/sCD14 ratio of (E) obese and (F)

lean participants at baseline and after consuming 12 oz. of yogurt or a non-dairy control food daily for 9 weeks.

C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese. Between-group differences of wk 9 means were compared by ANCOVA with baseline as a covariate. Within-group differences between wk 0 and wk 9 means were compared by paired T-test. * Significantly different from C or CO baseline, $P < 0.05$. $ Significantly different between CO and YO, $P < 0.05$.

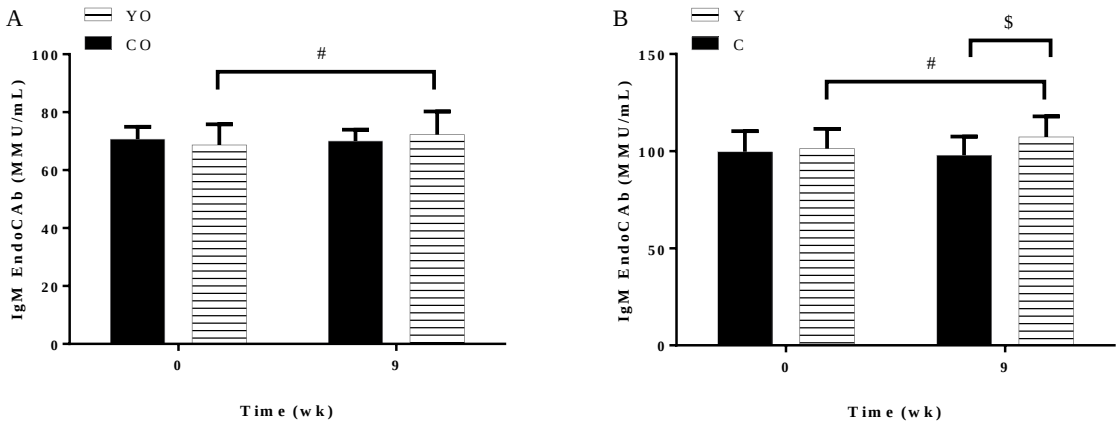

Figure 5.6 Fasting plasma immunoglobulin M endotoxin-core antibody (Ig M EndoCAb) in (A) obese and (B) lean participants, after consuming 12 oz. of yogurt or a control food daily for 9 weeks.

C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese (n = 30/group). Group differences at wk 9 were compared by ANCOVA with baseline as the covariate. The means of wk 0 and wk 9 within each group were compared by paired T-test. $P < 0.05$. # Significantly different from YO or Y baseline, $P < 0.05$. $ Significantly different between Y and C, $P < 0.05$.

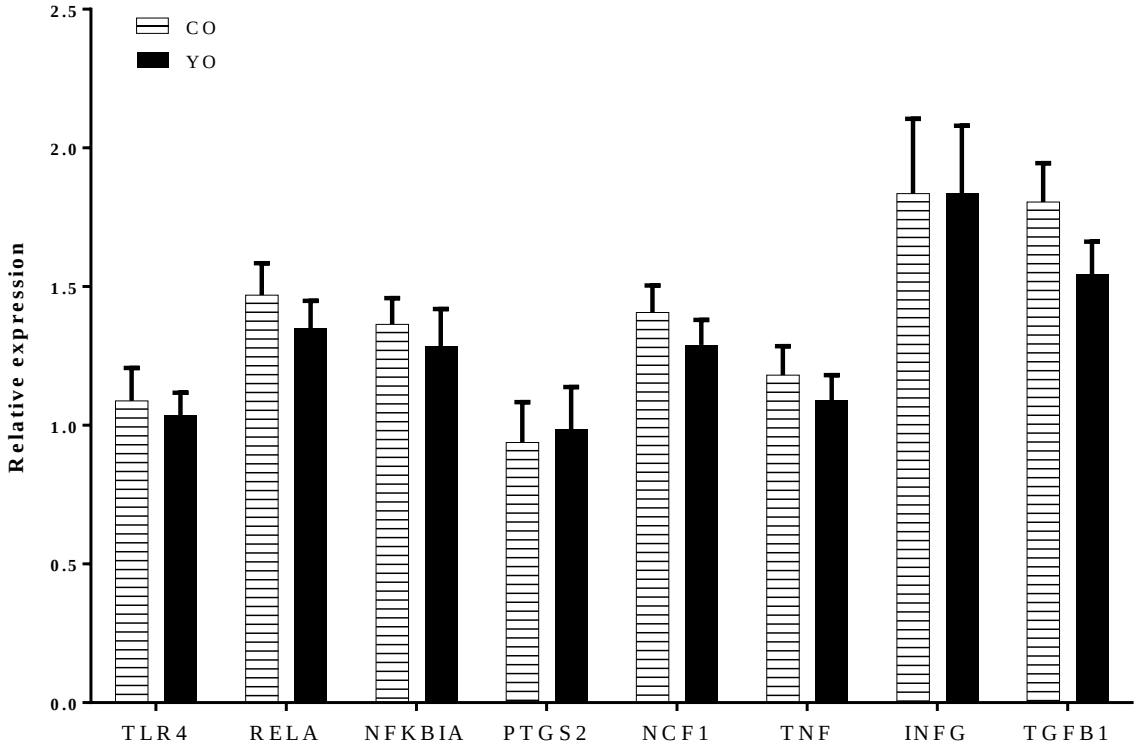

Figure 5.7 Baseline fasting PBMC relative gene expression, normalized to 18s rRNA and RPLP0.

CO, control obese; YO, yogurt obese (n = 30/group). The relative expression of target genes between groups was compared by independent T-test. No significant differences between YO or CO gene expression was detected. *TLR4*, encoding Toll-like receptor 4; *RELA*, encoding p65 subunit of nuclear factor kappa B; *NFKBIA*, encoding nuclear factor kappa B inhibitor alpha; *PTGS2*, encoding cyclooxygenase-2, or COX-2; *NCF1*, encoding the p47 subunit of nicotinamide adenine dinucleotide phosphate-oxidase, or NADPH oxidase; *TNF*, encoding tumor necrosis factors, or TNF; IFNG, encoding interferon-γ; *TGFB1*, encoding transforming growth factor beta 1, or TGFβ1.

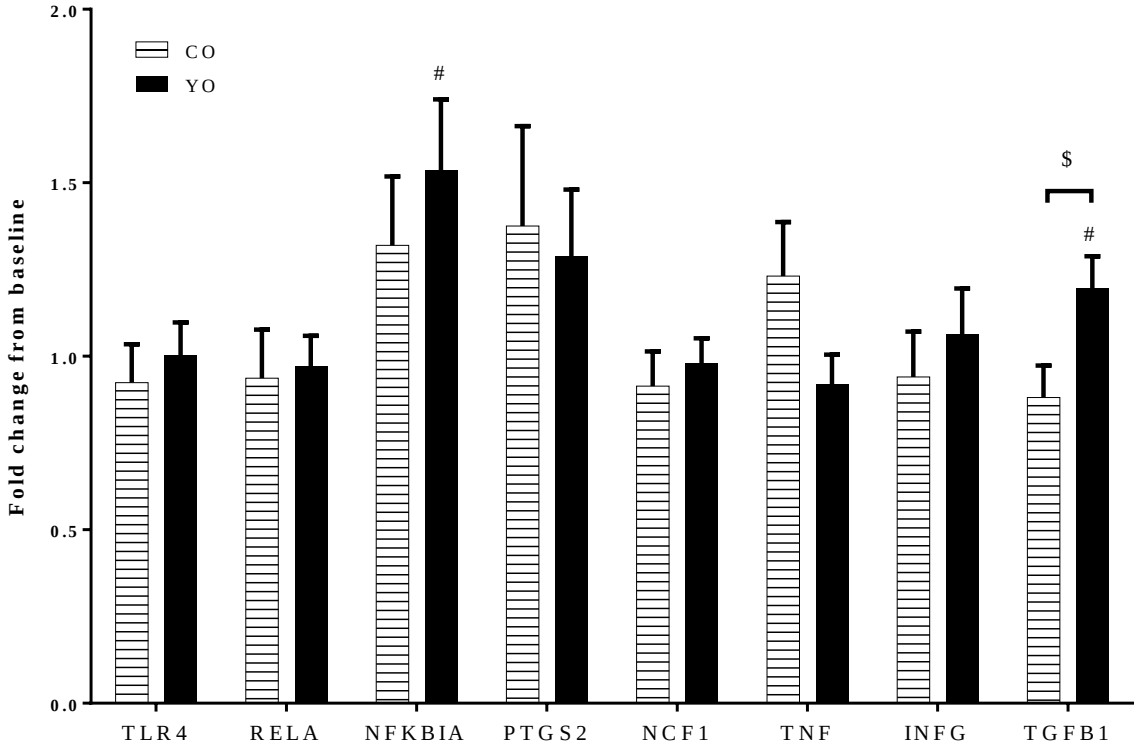

Figure 5.8 Fold-change of fasting PBMC gene expression between wk 0 to wk 9, during which obese participants consumed 12 oz. of yogurt or control food daily.

CO, control obese; YO, yogurt obese (n = 30/group). The fold-change from baseline of target genes between groups was compared by independent T-test. The difference between wk 0 and wk 9 in each group was compared by paired T-test. # Significantly different from YO baseline, $P < 0.05$.
$ Significantly different between YO and CO, $P < 0.05$. *TLR4*, encoding Toll-like receptor 4; *RELA*, encoding p65 subunit of nuclear factor kappa B; *NFKBIA*, encoding nuclear factor kappa B inhibitor alpha; *PTGS2*, encoding cyclooxygenase-2, or COX-2; *NCF1*, encoding the p47 subunit of nicotinamide adenine dinucleotide phosphate-oxidase, or NADPH oxidase; *TNF*, encoding tumor necrosis factors, or TNF; IFNG, encoding interferon-γ; *TGFB1*, encoding transforming growth factor beta 1, or TGFβ1.

5.5 Discussion

Daily consumption of 12 oz. of low-fat yogurt for 9 weeks reduced biomarkers for inflammation and low-grade endotoxemia in both lean and obese women relative to those consuming a non-dairy control snack. Yogurt consumption reduced plasma TNF-α and TNF-α/sTNF-RII ratio but increased IgM EndoCAb in both lean and obese individuals, and further reduced IL-6 and hsCRP in obese individuals. Yogurt consumption also upregulated IκB-α and TGFβ1 mRNA expression in PBMC in obese participants. These improvements occurred despite ~ 0.9 kg weight gain in YO and CO, and were concurrent with reduced postprandial metabolic dysregulation in YO (**Chapters 3** and **4**).

5.5.1 Chronic inflammation

Inflammation has a pivotal role in linking obesity to various metabolic diseases (Lumeng and Saltiel 2011). Increased IL-6, hsCRP, and TNF-α have been associated with obesity in both adults and children (Panagiotakos, Pitsavos et al. 2005, Mauras, DelGiorno et al. 2010). This is corroborated by higher baseline plasma IL-6, hsCRP, and TNF-α in obese compared to lean participants in the present study. The reported levels of IL-6, hsCRP and TNF-α in obese non-diabetic population ranges from 2.00 to 4.30 pg/mL, 0.59 to 5.98 mg/L, and 1.42 to 2.69 pg/mL, respectively (Bastard, Jardel et al. 2000, Esposito, Pontillo et al. 2003, Rexrode, Pradhan et al. 2003, Maachi, Piéroni et al. 2004, Park, Park et al. 2005). The variation might be partly attributed to the different ELISA kits. Fasting concentrations of these biomarkers were reduced in individuals consuming low-fat yogurt for 9 weeks. Absolute TNF-α levels may not correspond to pro-inflammatory status, as sTNF-RII and other circulating TNF receptors can bind to TNF and inhibit its activity (Van Meijl and Mensink 2010). Thus, it is notable that low-fat yogurt consumption also increased plasma sTNF-RII in obese participants, and decreased the TNF-

α/sTNF-RII ratio in both obese and lean participants. Similarly, 8-week consumption of 500 mL low-fat milk and 150 g low-fat yogurt increased the sTNF-RII and decreased the TNF-α/sTNF-RII ratio in overweight and obese individuals (Van Meijl and Mensink 2010). Weight loss by restricted diet and exercise also led to decreased TNF-α and increased sTNF-RII (Zahorska-Markiewicz, Olszanecka-Glinianowicz et al. 2008). The recombined form of sTNF-RII namely etanercept is used to improve inflammatory conditions in patients with metabolic syndrome (Bernstein, Berry et al. 2006). The function and regulation of sTNF-RII is not yet fully understood. sTNF-RII is shed from the membrane-bound forms in response to inflammatory stimuli which also induce TNF-α secretion (Porteu and Nathan 1990). The shedding results in decreased membrane-bound TNF receptor, thus inhibiting TNF-α activity (Van Zee, Kohno et al. 1992). Given the reproducible effect of dairy products to modulate sTNF-RII, it may be worthwhile to elucidate the mechanism by which this occurs.

Intervention studies on the immune-modulating and anti-inflammatory effects of yogurt consumption are limited. In elderly individuals, consumption of 100 g/day yogurt containing *L. acidophilus* and *B. lactis* for 2 weeks decreased fecal haptoglobin, but plasma inflammatory biomarkers were not determined (Matsumoto, Ohishi et al. 2001). In children with *H. pylori*, 400 mL/day yogurt containing *L. acidophilus* and *B. lactis* for 4 weeks decreased serum IL-6 (Yang and Sheu 2012). In inflammatory bowel disease (IBD) patients, consumption of 125 g of yogurt containing *L. rhamnosus* and *L. reuteri* for 30 days led to increase in regulatory $CD4^+$ $CD25^{high}$ T cells and decreases the percentage of TNF-α- or IL-12-producing monocytes and dendritic cells (Lorea Baroja, Kirjavainen et al. 2007). Although yogurt composition, dosage, intervention period, and participant population have varied in these studies, it appears these data support the intestinal and systemic anti-inflammatory activity of yogurt. The yogurt in the present study is a

commercially available product that contains conventional yogurt starter cultures (**Section 3.3.2**) and is more representative of typical yogurt products in the U.S. Thus, the present data suggest that probiotic fortification is not necessary in yogurt to modulate chronic inflammatory biomarkers in apparently healthy women. It is worth of noting that although yogurt consumption decreased the levels of inflammatory biomarkers in obese participants, the resulting levels were still higher than that in the lean participants. Thus, dietary intervention might be assisted by other strategies such as losing weight, exercise and pharmaceutical approaches to manage obesity-associated inflammation.

5.5.2 Chronic endotoxemia

Obesity is associated with subclinical endotoxemia, which increases chronic inflammation (Cani, Amar et al. 2007, Andreasen, Larsen et al. 2010). Direct quantitation of LPS is challenging, due to its short half-life, low blood concentrations and the difficulty of removing interference from the blood matrix (Novitsky 1998, Munford 2005). In addition, determination of LPS alone neglects other proinflammatory bacterial compounds such as glycolipids and lipoproteins derived from pathogenic Gram-positive bacteria (Schroder and Schumann 2005). LBP and sCD14 have been proposed as surrogate markers of endotoxemia because of their roles in sequestering and translocating LPS and other bacterial compounds to inflammatory signaling pathways (Schroder and Schumann 2005). LBP also accelerates LPS-lipoprotein binding *in vitro*, especially to chylomicrons, which reduces LPS toxicity of LPS and PBMC proinflammatory cytokines increase (Vreugdenhil, Rousseau et al. 2003). Moreover, LBP promotes the detoxification of lipoteichoic acid, a cell wall component of Gram-positive bacteria, by chylomicrons (Vreugdenhil, Rousseau et al. 2003). Subclinically low LBP is considered to

enhance the LPS signaling while high LBP inhibits LPS-induced toxicity (Kitchens and Thompson 2005).

LBP is higher in overweight/obese individuals than in normal-weight individuals, indicating low-grade chronic endotoxemia (Sun, Yu et al. 2010). Serum LBP is also associated with increased abdominal obesity, proinflammatory cytokine IL-6 and IL-8, but low concentrations of HDL-cholesterol (Gonzalez-Quintela, Alonso et al. 2013). Indeed, baseline plasma LBP of obese participants was 28% higher than lean participants, and was positively associated with IL-6 and hsCRP in the present study. On the other hand, fasting plasma sCD14 was similar between the obese and lean in the present study. Similarly, sCD14 is not associated with obesity in another study population (n = 420, 55% females, age 18-92 year) (Gonzalez-Quintela, Alonso et al. 2013). The increased endotoxemia in obese women is partly attributed to increased intestinal permeability. In healthy women, visceral adiposity is positively associated with intestinal permeability assessed by the ratio of urinary excretion of orally ingested sucralose to mannitol (Gummesson, Carlsson et al. 2011). Increased intestinal permeability assessed by similar sugar absorption test is associated with increased blood insulin, the homeostasis model assessment of insulin resistance (HOMA-IR) index, and decreased HDL concentration in obese women (Teixeira, Souza et al. 2012).

Healthy men have postprandial plasma LBP/sCD14 ratios that are correlated with plasma endotoxin (Laugerette, Furet et al. 2012, Laugerette, Alligier et al. 2014). We observed that obese participants had 27% higher plasma LBP/sCD14 ratio than the lean participants at baseline, which suggested increased endotoxin exposure. Moderate increases in LBP/sCD14 ratio were found in CO and C, but not the yogurt-consuming groups, suggesting protective effects of yogurt

against chronic endotoxemia. The increase of LBP/sCD14 ratio in the control groups was not consistent with the lack of changes in IgM EndoCAb and the inflammatory markers. Reliable measurement of LPS might help to clarify whether LBP/sCD14 ratio is a good measure of changes in fasting endotoxin level.

Fasting plasma sCD14 decreased from baseline in CO but not YO at 9 wk. Plasma sCD14 is found to be correlated with central adiposity (de Courten, Moreno-Navarrete et al. 2015), but not in the present study participants and others (Gonzalez-Quintela, Alonso et al. 2013). Thus, it is difficult to interpret the association of this change with chronic endotoxemia. Plasma sCD14 in healthy adults is ~ 1000-fold molar more than the LPS level in fatal septic shock patients so the magnitude of change in the present study bears consideration (Wright 1991, Bas, Gauthier et al. 2004). Also, sCD14 improves insulin sensitivity in obese individuals (de Courten, Moreno-Navarrete et al. 2015), which is consistent with reduced postprandial glucose levels in YO relative to CO (**Chapter 4**).

The inner core of endotoxin is considered as the only molecular pattern that is conserved across various gram-negative bacteria. IgM EndoCAb can bind to this molecular pattern, thus protecting against endotoxin. (Poxton 1995). Decreased EndoCAb has been proposed as a marker of endotoxin exposure (Barclay 1995). In a cross-sectional study involving 93 age-matched middle-aged women, IgM EndoCAb is lower in the obese (55% of lean women) and obese diabetic women (30% of lean women) (Hawkesworth, Moore et al. 2013). Similarly, IgM EndoCAb level in obese participants was 69% of the level in lean participants in the present study. Yogurt consumption increased the IgM EndoCAb level in both obese and lean participants, suggesting lower level of endotoxin exposure resulting from the yogurt intervention.

Other dietary interventions have improved intestinal barrier function in various populations. For example, consumption of 300 g/day yogurt containing *L. johnsonii* for 4 weeks decreased plasma LBP, sCD14 and surrogate markers of LPS permeability in elderly adults with intestinal bacterial overgrowth (Schiffrin, Parlesak et al. 2009). In 30 diarrhea-predominant irritable bowel syndrome patients, consumption of 400 g/day fermented milk containing *L. acidophilus* and *B. longum* for 4 weeks decreased small bowel permeability assessed by sugar absorption test (Zeng, Li et al. 2008). In 93 healthy overweight volunteers, a gut microbiota-targeted dietary intervention for 9 weeks (BMI $\geq$ 28 kg/m^2) decreased gut permeability, concurrent with reduced LBP, TNF-α and IL-6 (Xiao, Fei et al. 2014).

5.5.3 Gene expression in PBMC

PBMC gene expression can be used as a biomarker of immune function and chronic inflammation (de Bont, Netea et al. 1998, O'Rourke, Kay et al. 2006, Holven, Retterstol et al. 2013). To better understand the effect of low-fat yogurt consumption on chronic inflammation, we examined the expression of genes involved in TLR4-activitated cytokine expression in PBMC from obese participants. TLR4 is located on the surface of various immune cells such as monocytes, macrophages, Kupffer cells, and preadipocytes, and plays an essential role in sensing endotoxin and activating the inmate immunity (Beutler 2000, Manco, Putignani et al. 2010). TLR4 mRNA expression is increased in the monocytes of patients with metabolic syndromes, and its level is upregulated by increased exposure to LPS (Muzio, Bosisio et al. 2000, Jialal, Huet et al. 2012, Parajuli, Sonobe et al. 2012). Although our results suggested YO had reduced endotoxemia, the PBMC *TLR4* was unchanged from baseline at wk 9.

Upon TLR4 endotoxin stimulation, a downstream signaling cascade is triggered that leads to activation of the NF-κB pathway (Manco, Putignani et al. 2010). Nuclear factor kappa B inhibitor alpha (IκBα, encoded by *NFKBIA*) inhibits the NF-κB pathway by trapping the heterodimeric complex containing p50 and p65 (encoded by *RELA*) in the cytosol (Scherer, Brockman et al. 1995). YO *NFKBIA* increased by 54% from baseline at wk 9 of the intervention. However, genes downstream of NF-κB including *PTGS2* (encoding cyclooxygenase-2, or COX-2), *NCF1* (encoding the p47 subunit of nicotinamide adenine dinucleotide phosphate-oxidase, or NADPH oxidase), *TNF* (encoding tumor necrosis factors, or TNF), and *IFNG* (encoding interferon-γ) were not affected by the intervention. Given the reduction in YO plasma TNF-α, non PBMC sources of TNF such as the intestine, adipose tissue, or skeletal muscle may have contributed to this change. TGF-β1 is an anti-inflammatory and reparative cytokine that suppresses proinflammatory signaling from Toll-like receptors (Serhan and Savill 2005). YO *TGFB1* (encoding TGF-β1) expression increased by 20% by the intervention. Whether PBMC is a major source of circulating proinflammatory cytokine and whether TGF-β1 excretion by PBMC reduces plasma IL-6 and hsCRP need further examination.

5.5.4 Study limitation

The mechanism by which yogurt improves intestinal barrier function and prevents chronic inflammation requires further investigation. Yogurt components, dairy proteins, calcium, microbes, and vitamin D are associated with decreased chronic inflammation (Lorea Baroja, Kirjavainen et al. 2007, Zemel and Sun 2008, Pal and Ellis 2010, Shab-Bidar, Neyestani et al. 2012). Constant postprandial stress may increase chronic inflammation and atherosclerosis (Burdge and Calder 2005). Pre-meal yogurt consumption reduced postprandial hyperglycemia and inflammation in obese participants (**Chapter 4**). This effect might contribute to suppression

of chronic inflammation. However, we did not specify how participants should consume yogurt or the control food. A further analysis of three-day food records indicated that Y and YO consumed yogurt with 69% and 66% of meals (**Appendix A.3 Supplementary Table 5.3**), respectively. This may still not capture the yogurt-eating pattern of participants. Therefore, the extent the postprandial response and timing of yogurt consumption contributes to long-term anti-inflammatory effect of yogurt consumption needs further investigation.

5.6 Conclusion

In summary, our work demonstrated that consuming 12 oz. of yogurt daily reduced chronic inflammation and endotoxemia in apparently healthy premenopausal women, which was in accordance with our hypothesis. Yogurt consumption decreased the plasma TNF-α and TNF-α/sTNF-RII ratio in both obese and lean participants. Furthermore, plasma IL-6 and hsCRP decreased in obese participants, suggesting a further benefit of yogurt consumption on the obese participants. Plasma IgM EndoCAb increased in both obese and lean participants, suggesting decreased endotoxin exposure. Lastly, yogurt intervention upregulated *NFKBIA* and *TGFB1* expression in PBMC, although it did not affect the NF-κB downstream pro-inflammatory genes. These improvements occurred despite ~0.9 kg weight gain in YO and CO during intervention, and were concurrent with reduced postprandial metabolic dysregulation in YO (**Chapters 3** and **4**).

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Chapter 6: Conclusions and Future Directions

6.1 Conclusions

In the present study, we tested the hypothesis that low-fat yogurt consumption would reduce postprandial and chronic inflammation associated with obesity by decreasing endotoxemia. This hypothesis was tested by a randomized, controlled, isocaloric study design in apparently healthy premenopausal women. In addition, the effects of yogurt consumption on body weight, blood pressure were examined. We found that obese participants had increased baseline plasma biomarkers of chronic inflammation and endotoxemia compared to lean individuals, indicative of metabolic dysfunction. Compared to lean individuals, obese participants had exacerbated postprandial inflammation, accompanied by hyperglycemia and increased endotoxemia after a high-fat, high-carbohydrate challenge meal. Such increases in inflammation, glucose and endotoxemia were suppressed concurrently by pre-meal consumption of yogurt in the obese. In addition, yogurt consumption reduced postprandial hypoglycemia in lean individuals, possibly by preventing insulin over-secretion. After 9-week daily consumption of 12 oz. of yogurt, obese individuals had decreased plasma biomarkers of chronic inflammation and endotoxemia, despite ~ 0.9 kg weight gain during intervention. Obese individuals experienced a temporary decrease in diastolic blood pressure after 3 weeks, but such decrease did not maintain at the end of intervention. These results support that low-fat yogurt consumption reduces postprandial and chronic inflammation, possibly by decreasing endotoxemia in obese individuals. This is in accordance with epidemiological studies associating increased dairy consumption with decreased risk of chronic diseases such as cardiovascular diseases, type 2 diabetes and hypertension (Warensjö, Jansson et al. 2010, Tong, Dong et al. 2011, Ralston, Lee et al. 2012, Sluijs, Forouhi et al. 2012). In addition, the present study included a non-dairy dietary control food and non-obese individuals in a relatively large number

of participants with an isocaloric design. Our study outcomes provide increasing evidence of the anti-inflammatory effect of yogurt reported in prior intervention studies (Matsumoto, Ohishi et al. 2001, Yang and Sheu 2012). More importantly, we contribute novel evidence on the beneficial effects of low-fat yogurt consumption on postprandial metabolism in both obese and lean apparently healthy premenopausal women. Because inflammation links obesity to metabolic diseases, our findings on the anti-inflammatory effect of a commercially available yogurt is a feasible dietary recommendation for management of obesity-associated chronic inflammation. Diet plays a pivotal role in prevention and treatment of the metabolic consequences of obesity. For example, various low-carbohydrate or energy restricted diets have been developed to promote health in obese populations. Although some of these diets were effective during short-term interventions, such benefits might not be sustainable due to poor adherence (Bonow and Eckel 2003). The good adherence to the present study intervention suggests it might be feasible to incorporate low-fat yogurt into habitual diet to achieve longer-term benefit.

High-fat, high-carbohydrate foods contribute to modern diets and a "constant feeding" has become common due to increased food availability. Therefore, postprandial stress might occur regularly and pose a risk for health, especially in obese individuals who experience exacerbated postprandial dysmetabolism. We demonstrated that one serving of a commercially available yogurt before a meal could effectively reduce postprandial hyperglycemia and inflammation in obese individuals. After 9-week yogurt consumption, obese individuals had decreased levels of biomarkers for chronic inflammation, but those levels were still higher than that in lean participants. Therefore, other strategies such as losing weight, exercise, or pharmaceutical approaches should also be considered to reduce obesity-associated chronic inflammation. It is worth noting the lower postprandial IL-6 increases in the second challenge

meal test than in the first one, suggesting extra benefits on postprandial metabolism from regular yogurt consumption. A large cohort prospective study finds long-term yogurt consumption is associated with decreased body weight (Mozaffarian, Hao et al. 2011). Therefore, habitual yogurt consumption might deliver extra health benefits for obese individuals. Interestingly, yogurt consumption also protects lean individuals from hypoglycemia after a high-fat, high-carbohydrate meal, possibly by preventing insulin over-secretion.

Increased added sugar consumption has been linked to weight gain during short-term interventions (Morenga, Mallard et al. 2012). We also observed ~ 0.9 kg weight gain in obese participants, providing that the interventions supplied 54 g of added sugar. Although we did not observe negative metabolic outcomes related to the weight gain, long-term weight gain may be problematic. Yoplait has recently reformulated the yogurt by reducing added sugar by 25%. Whether such change conveys extra benefits needs further examination.

This study was powered on soluble CD14 (sCD14), originally proposed as the primary biomarkers of chronic endotoxemia (Laugerette, Vors et al. 2011). However, we did not find an association between sCD14 and obesity at baseline. Therefore, it might not be a sensitive biomarker for chronic endotoxemia in this study population. Reliable direct measurement of endotoxin might help to determine whether sCD14 reflects chronic endotoxemia. Nevertheless, the increase in immunoglobulin M endotoxin-core antibody at the end of intervention supports the anti-endotoxemia effect of low-fat yogurt. Our results suggest that lower endotoxemia by yogurt consumption is accompanied by decreased inflammation both postprandially and chronically. To the best of our knowledge, this is the first report that consuming a low-fat conventional yogurt could reduce the risk of metabolic endotoxemia in apparently healthy

premenopausal women. Dietary intervention aimed at improving intestinal barrier function appears to be a promising target for reducing endotoxemia and thus inflammation. It needs to be pointed out that the obesity-associated endotoxemia is characterized by a two- to three times threshold increase in circulating endotoxin, due to impaired intestinal barrier function (Cani, Amar et al. 2007). The protective effects of low-fat yogurt against metabolic endotoxemia might not apply to clinical endotoxemia that is associated with to septic shock.

6.2 Future directions

We demonstrated that low-fat yogurt consumption could reduce postprandial and chronic inflammation, possibly by decreasing endotoxemia in obese individuals. However, the mechanism by which yogurt improves acute postprandial and chronic intestinal barrier function is still unclear. Animal or *in vitro* models might be better tools to examine the effects of low-fat yogurt consumption on change in intestinal barrier function. For example, whether the beneficial effect is mediated by modulating gut microbiota or the tight junction integrity might be study of interest. In addition, direct assessment of gut permeability such as sugars (lactulose and mannitol) absorption test might be incorporated in future studies. Furthermore, we found that pre-meal consumption reduced postprandial hyperglycemia in obese individuals without affecting insulin secretion. Prior studies suggest such insulin-independent effect might be related to stomach emptying rate. But such hypothesis needs to be tested. It is also worth of exploring other underlying mechanisms by which low-fat yogurt modulates postprandial glycemia. For example, *in vitro* model could be used to test whether yogurt components regulate glucose transporter, which has been proposed as potential mechanism by which polyphenols improve glucose response (Kobayashi, Suzuki et al. 2000, Manzano and Williamson 2010). Lastly, we observed ~ 0.9 kg weight gain in obese participants and increased added sugar intakes in all participants

after 9-week intervention. Although no negative metabolic effects associated with the weight gain was observed, how to avoid the weight and dietary changes in similar isocaloric study design needs to be considered in future studies.

In the present study, low-fat yogurt consumption also affected the biomarker levels of inflammation and metabolic endotoxemia in lean participants who are considered relatively healthy compared to the obese individuals. The implication of such modulating effects of low-fat yogurt on cytokines and immune function in otherwise healthy population needs further investigation.

6.3 References

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Appendix

A.1 Appendix Materials for Chapter 3

A.1.1 Analysis of isoflavones in soy pudding by HPLC

Chemicals

Hydrogen chloride (HCl) (37%) was from Acros Organics (Morris Plains, NJ). Daidzein, glycitein, genistein, and butylated hydroxytoluene (BHT) were from Sigma-Aldrich (St. Louis, MO). Ethanol, acetic acid, and acetonitrile were from Fisher Scientific (Fairlawn, NJ). Soy puddings of chocolate, vanilla, chocolate/vanilla swirl flavor were from ZenSoy (South Hackensack, NJ).

Isoflavones extraction

Isoflavones were extracted according to Franke et al. with modifications (Franke, Custer et al. 1994). Briefly, 1 g soy pudding was added to 6.25 mL of 37% HCl and 30 mL of 96% ethanol (containing 0.05% BHT as antioxidant). This slurry was vortexed for 10 s followed by 10 min of sonication at room temperature (FS140H, Fisher Scientific, Pittsburg, PA). After refluxing the mixture for 2 h at 90 °C, the mixture was cooled to room temperature and the ethanol lost during refluxing was replaced. Then, 1.2 mL of the above mixture was removed and centrifuged at 12,000 × g for 10 min.

HPLC analysis

Isoflavones were analyzed by HPLC as previously described, with minor modifications (Franke, Custer et al. 1994). A Shimadzu LC-20XR system was equipped with an SPD-20AV detector set to 260 nm. A 10 μL injection was eluted through a 250 mm × 4.6 mm i.d., 5 μm Waters Xterra MS C_{18} column (Waters Corporation, Milford, MA, USA) via a binary gradient of acetic acid/water (10/90 V/V) (A) and acetonitrile (B). The proportion of B increased from 23 to 70% over 8 min, then decreased to 23% until 9 min, and held at 23% until 21 min. Isoflavones were detected using authentic internal standards.

Results

Table A.3.1 Isoflavones contents in soy pudding determined HPLC (mg/100g)

Flavor	Daidzein	Glycitein	Genistein
Chocolate	2.00 ± 0.09	1.64 ± 0.01	5.34 ± 0.16
Vanilla	1.84 ± 0.24	1.29 ± 0.16	4.38 ± 0.61

Isoflavones were quantified by reverse phase resolution at 260 nm. Data are expressed as mean ± standard deviation of triplicate determination.

Reference

Franke, A. A., L. J. Custer, C. M. Cerna and K. K. Narala (1994). "Quantitation of Phytoestrogens in Legumes by Hplc." Journal of Agricultural and Food Chemistry **42**(9): 1905-1913.

A.1.2 NDSR nutrient content of the alternative substitute

Nutrient	Values
Primary Energy Sources	
Energy (kilocalories)	347 kcal
Energy (kilojoules)	1452 kj
Total Fat	3.674 g
Total Carbohydrate	64.807 g
Available Carbohydrate	63.786 g
Total Protein	14.866 g
Animal Protein	14.866 g
Vegetable Protein	0.000 g
Alcohol	0.000 g
% Calories from Fat	9.31%
% Calories from Carbohydrate	72.28%
% Calories from Protein	18.29%
% Calories from Alcohol	0.00%
Fat and Cholesterol	
Cholesterol	14 mg
Total Saturated Fatty Acids (SFA)	2.371 g
Total Monounsaturated Fatty Acids (MUFA)	1.010 g
Total Polyunsaturated Fatty Acids (PUFA)	0.105 g
Total Trans-Fatty Acids (TRANS)	0.102 g
Total Conjugated Linoleic Acid (CLA 18:2)	0.017 g
Omega-3 Fatty Acids	0.031 g
% Calories from SFA	6.01%
% Calories from MUFA	2.56%
% Calories from PUFA	0.27%
Polyunsaturated to Saturated Fat Ratio	0.044
Cholesterol to Saturated Fatty Acid Index	3.075
Carbohydrates	
Total Sugars	55.826 g
Fructose	7.518 g
Galactose	3.062 g
Glucose	8.641 g
Lactose	12.145 g
Maltose	1.021 g
Sucrose	23.439 g
Starch	0.000 g
Added Sugars (by Total Sugars)	14.118 g
Added Sugars (by Available Carbohydrate)	42.728 g
Fiber	

Total Dietary Fiber	1.021 g
Soluble Dietary Fiber	0.269 g
Insoluble Dietary Fiber	0.735 g
Pectins	0.000 g
Vitamins	
Total Vitamin A Activity (Retinol Equivalents)	448 mcg
Total Vitamin A Activity (International Units)	1500 IU
Total Vitamin A Activity (Retinol Activity Equivalents)	448 mcg
Beta-Carotene Equivalents (derived from provitamin A carotenoids)	7 mcg
Retinol	447 mcg
Vitamin D (calciferol)	9.999 mcg
Vitamin D2 (ergocalciferol)	0.000 mcg
Vitamin D3 (cholecalciferol)	9.999 mcg
Vitamin E (International Units)	0 IU
Vitamin E (Total Alpha-Tocopherol)	0.068 mg
Natural Alpha-Tocopherol (RRR-alpha-tocopherol or d-alpha-tocopherol)	0.068 mg
Synthetic Alpha-Tocopherol (all rac-alpha-tocopherol or dl-alpha-tocopherol)	0.000 mg
Total Alpha-Tocopherol Equivalents	0.068 mg
Beta-Tocopherol	0.000 mg
Gamma-Tocopherol	0.000 mg
Delta-Tocopherol	0.000 mg
Vitamin K (phylloquinone)	0.340 mcg
Vitamin C (ascorbic acid)	2.381 mg
Thiamin (vitamin B1)	0.126 mg
Riboflavin (vitamin B2)	0.606 mg
Niacin (vitamin B3)	0.323 mg
Niacin Equivalents	1.741 mg
Pantothenic Acid	1.664 mg
Vitamin B-6 (pyridoxine, pyridoxyl, & pyridoxamine)	0.136 mg
Total Folate	31 mcg
Dietary Folate Equivalents	31 mcg
Natural Folate (food folate)	31 mcg
Synthetic Folate (folic acid)	0 mcg
Vitamin B-12 (cobalamin)	1.599 mcg
Carotenoids	
Beta-Carotene (provitamin A carotenoid)	7 mcg
Alpha-Carotene (provitamin A carotenoid)	0 mcg
Beta-Cryptoxanthin (provitamin A carotenoid)	0 mcg
Lutein + Zeaxanthin	0 mcg
Lycopene	0 mcg
Minerals	
Calcium	1000 mg
Phosphorus	300 mg

Magnesium	51 mg
Iron	0.238 mg
Zinc	2.517 mg
Copper	0.272 mg
Manganese	0.221 mg
Selenium	10.546 mcg
Sodium	197 mg
Potassium	460 mg
Fatty Acids	
SFA 4:0 (butyric acid)	0.109 g
SFA 6:0 (caproic acid)	0.075 g
SFA 8:0 (caprylic acid)	0.048 g
SFA 10:0 (capric acid)	0.105 g
SFA 12:0 (lauric acid)	0.126 g
SFA 14:0 (myristic acid)	0.388 g
SFA 16:0 (palmitic acid)	1.000 g
SFA 17:0 (margaric acid)	0.000 g
SFA 18:0 (stearic acid)	0.357 g
SFA 20:0 (arachidic acid)	0.000 g
SFA 22:0 (behenic acid)	0.000 g
MUFA 14:1 (myristoleic acid)	0.000 g
MUFA 16:1 (palmitoleic acid)	0.082 g
MUFA 18:1 (oleic acid)	0.840 g
MUFA 20:1 (gadoleic acid)	0.000 g
MUFA 22:1 (erucic acid)	0.000 g
PUFA 18:2 (linoleic acid)	0.075 g
PUFA 18:3 (linolenic acid)	0.031 g
PUFA 18:4 (parinaric acid)	0.000 g
PUFA 20:4 (arachidonic acid)	0.000 g
PUFA 20:5 (eicosapentaenoic acid [EPA])	0.000 g
PUFA 22:5 (docosapentaenoic acid [DPA])	0.000 g
PUFA 22:6 (docosahexaenoic acid [DHA])	0.000 g
TRANS 16:1 (trans-hexadecenoic acid)	0.000 g
TRANS 18:1 (trans-octadecenoic acid [elaidic acid])	0.068 g
TRANS 18:2 (trans-octadecadienoic acid [linolelaidic acid]; incl. c-t, t-c, t-t)	0.000 g
CLA cis-9, trans-11	0.014 g
CLA trans-10, cis-12	0.003 g
Amino Acids	
Tryptophan	0.085 g
Threonine	0.609 g
Isoleucine	0.810 g
Leucine	1.497 g
Lysine	1.334 g

Methionine	0.439 g
Cystine	0.136 g
Phenylalanine	0.810 g
Tyrosine	0.752 g
Valine	1.232 g
Arginine	0.449 g
Histidine	0.367 g
Alanine	0.636 g
Aspartic Acid	1.180 g
Glutamic Acid	2.912 g
Glycine	0.357 g
Proline	1.762 g
Serine	0.922 g
Isoflavones and Similar	
Daidzein	0.000 mg
Genistein	0.000 mg
Glycitein	0.000 mg
Coumestrol	0.000 mg
Biochanin A	0.000 mg
Formononetin	0.000 mg
Sugar Alcohols (polyols)	
Erythritol	0.000 g
Inositol	0.024 g
Isomalt	0.000 g
Lactitol	0.000 g
Maltitol	0.000 g
Mannitol	0.000 g
Pinitol	0.000 g
Sorbitol	0.058 g
Xylitol	0.007 g
Other	
Acesulfame Potassium	0.000 mg
Aspartame	0.000 mg
Saccharin	0.000 mg
Sucralose	0.000 mg
Tagatose	9.866 mg
Caffeine	0 mg
Phytic Acid	2.279 mg
Oxalic Acid	1.783 mg
3-Methylhistidine	0.000 mg
Sucrose Polyester	0.000 g
Choline	47.627 mg
Betaine	2.722 mg

Glycemic Index (glucose reference)	33
Glycemic Index (bread reference)	47
Glycemic Load (glucose reference)	21
Glycemic Load (bread reference)	30
Nitrogen	2.347 g
Ash	3.470 g
Water	253.377 g
Grams	340.194 g

A.2 Appendix Materials for Chapter 4

Supplementary Table 4.1 Postprandial plasma glucose (mg/dL) changes in two separate challenge meal tests (mean ± standard error, n = 30/group).

			Time (h)					*Significance (P)*[1]		
			0	1	2	3	4	*Treatment*	*Time*	*Treatment × Time*
Test 1 (wk 0)	Lean	C	86.1 ± 2.2	70.1 ± 3.1 *	68.2 ± 2.5 *$	79.2 ± 2.8	77.9 ± 2.2 $	*0.0793*	*<.0001*	*0.0207*
		Y	84.2 ± 1.1	73.3 ± 2.7 #	77.8 ± 2.5 $	81.3 ± 3.7	85.9 ± 2.0 $			
	Obese	CO	89.4 ± 1.6	102.5 ± 5.1 *$	94.7 ± 4.4	89.3 ± 2.1	86.7 ± 1.4	*0.0204*	*0.0007*	*0.0050*
		YO	89.4 ± 1.4	88.1 ± 2.9 $	88.6 ± 2.6	83.2 ± 2.0	84.5 ± 1.5			
Test 2 (wk 9)	Lean	C	82.6 ± 1.4	71.3 ± 3.4 *	74.9 ± 2.6 *	76.1 ± 2.7	78.9 ± 1.8	*0.0432*	*<.0001*	*0.5848*
		Y	83.4 ± 1.1	77.0 ± 2.2 #	78.6 ± 2.4	81.7 ± 1.9	83.4 ± 1.7			
	Obese	CO	93.0 ± 2.4	109.1 ± 7.2 *$	102.1 ± 6.4 $	90.1 ± 4.1	87.0 ± 2.9	*0.0241*	*<.0001*	*0.0015*
		YO	91.2 ± 1.6	90.4 ± 3.8 $	86.7 ± 3.9 $	84.8 ± 2.2	83.7 ± 1.2			

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese.

[1] The effects of treatment, time and treatment × time were determined by repeated-measures ANOVA (PROC MIXED) with treatment, time and treatment × time as independent variables.

* Significantly different from baseline in pudding treatment group, P < 0.05, determined by repeated-measures ANOVA (PROC MIXED) with time as independent variable. # Significantly different from baseline in yogurt treatment groups, P < 0.05, determined by repeated-measures

ANOVA (PROC MIXED) with time as independent variable. $ Significantly different between control and yogurt treatment at different time points, determined by ANOVA with baseline as covariate (PROC GLM).

Supplementary Table 4.2 Postprandial plasma TG changes (mg/dL) after challenge meals (mean ± standard error, n = 30/group).

			Time (h)					*Significance (P)*[1]		
			0	1	2	3	4	*Treatment*	*Time*	*Treatment × Time*
Test 1 (wk 0)	Lean	C	66.9 ± 5.1	87.1 ± 5.2 *	115.3 ± 10.7 *	104.6 ± 7.3 *	99.1 ± 10.8 *	*0.8804*	*<.0001*	*0.4650*
		Y	72.0 ± 7.1	93.2 ± 9.1 #	107.5 ± 10.4 #	106.5 ± 9.6 #	101.2 ± 8.5 #			
	Obese	CO	94.9 ± 7.4	120.8 ± 8.9 *	137.7 ± 10.2 *	149.2 ± 12.3 *	147.4 ± 12.1 *	*0.8506*	*<.0001*	*0.1052*
		YO	89.1 ± 7.7	114.4 ± 9.3 #	146.1 ± 12.8 #	157.3 ± 14 #	156.6 ± 13.3 #			
Test 2 (wk 9)	Lean	C	65.3 ± 3.1	88.6 ± 5.0 *	110.4 ± 7.6 *	108.6 ± 7.3 *	101.0 ± 7.9 *	*0.7417*	*<.0001*	*0.4763*
		Y	65.7 ± 5.8	86.3 ± 8.1 #	101.3 ± 9.0 #	104.4 ± 8.4 #	102.0 ± 7.9 #			
	Obese	CO	97.5 ± 7.4	128.6 ± 8.9 *	139.1 ± 9.6 *	147.8 ± 10.8 *	148.5 ± 12.0 *	*0.7489*	*<.0001*	*0.0348*
		YO	91.1 ± 8.3	120.5 ± 10.9 #	150.3 ± 14.6 #	162.8 ± 16.4 #	162.3 ± 17.2 #			

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese.

[1] The effects of treatment, time and treatment × time were determined by repeated-measures ANOVA (PROC MIXED) with treatment, time and treatment × time as independent variables.

* Significantly different from baseline in control treatment group, P < 0.05, determined by repeated-measures ANOVA (PROC MIXED) with time as independent variable. # Significantly different from baseline in yogurt treatment groups, P < 0.05, determined by repeated-measures ANOVA (PROC MIXED) with time as independent variable.

Supplementary Table 4.3 Relative postprandial plasma insulin changes (Δ μIU/mL) from baseline at second challenge meal (mean ± standard error, n = 30/group).

			Time (h)					Significance (P)[1]		
			0	1	2	3	4	*Treatment*	*Time*	*Treatment × Time*
Test 2 (wk 9)	Lean	C	0.0 ± 0.0	43.7 ± 7.4 *	35.8 ± 7.2 *	18.9 ± 2.4 *$	5.2 ± 1.1	*0.0357*	*<.0001*	*0.1792*
		Y	0.0 ± 0.0	34.0 ± 2.8 #	22.9 ± 2.0 #	10.4 ± 1.4 #$	4.8 ± 1.1			
	Obese	CO	0.0 ± 0.0	89.9 ± 12.8 *	80.7 ± 13.7 *	34.3 ± 9.6 *	14.4 ± 6.3	*0.7605*	*<.0001*	*0.9716*
		YO	0 ± 0	89.3 ± 16.5 #	73.7 ± 17.0 #	33.5 ± 8.6	8.1 ± 2.5			

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese.

[1] The effects of treatment, time and treatment × time were determined by repeated-measures ANOVA (PROC MIXED) with treatment, time and treatment × time as independent variables.

* Significantly different from baseline in pudding treatment group, P < 0.05, determined by repeated-measures ANOVA (PROC MIXED) with time as independent variable. # Significantly different from baseline in yogurt treatment groups, P < 0.05, determined by repeated-measures ANOVA (PROC MIXED) with time as independent variable. $ Significantly different between pudding and yogurt treatment at different time points, determined by independent T-test (PROC TTEST).

Supplementary Table 4.4 Relative postprandial plasma IL-6 changes (Δ pg/mL) from baseline after challenge meals (mean ± standard error, n = 30/group).

			Time (h)					*Significance (P)*[1]		
			0	1	2	3	4	*Treatment*	*Time*	*Treatment × Time*
Test 1 (wk 0)	Lean	C	0.0 ± 0.0	0.04 ± 0.06	0.23 ± 0.13	0.20 ± 0.10	0.62 ± 0.15 *	*0.9236*	*<.0001*	*0.5026*
		Y	0.0 ± 0.0	-0.02 ± 0.07	0.07 ± 0.12	0.31 ± 0.14	0.68 ± 0.13 #			
	Obese	CO	0.0 ± 0.0	-0.01 ± 0.08	0.28 ± 0.15	0.77 ± 0.27 *	1.35 ± 0.27 *$	*0.0879*	*<.0001*	*0.0374*
		YO	0.0 ± 0.0	-0.21 ± 0.08	0.03 ± 0.14	0.50 ± 0.14 #	0.57 ± 0.19 #$			
Test 2 (wk 9)	Lean	C	0.0 ± 0.0	-0.07 ± 0.04	0.04 ± 0.07	0.28 ± 0.16 *	0.54 ± 0.18 *	*0.8424*	*<.0001*	*0.4887*
		Y	0.0 ± 0.0	-0.02 ± 0.08	0.01 ± 0.08	0.30 ± 0.12 #	0.38 ± 0.14 #			
	Obese	CO	0.0 ± 0.0	0.07 ± 0.11	0.35 ± 0.15	0.65 ± 0.13 *	1.13 ± 0.21 *$	*0.0209*	*<.0001*	*0.2459*
		YO	0.0 ± 0.0	-0.15 ± 0.06	0.09 ± 0.10	0.29 ± 0.15	0.60 ± 0.12 #$			

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese; IL-6, interleukin 6

[1] The effects of treatment, time and treatment × time were determined by repeated-measures ANOVA (PROC MIXED) with treatment, time and treatment × time as independent variables.

* Significantly different from baseline in control treatment group, P < 0.05, determined by repeated-measures ANOVA (PROC MIXED) with time as independent variable. # Significantly different from baseline in yogurt treatment groups, P < 0.05, determined by repeated-measures ANOVA (PROC MIXED) with time as independent variable. $ Significantly different between control and yogurt treatment at different time points, determined by independent T-test (PROC TTEST).

Supplementary Table 4.5 Relative postprandial plasma LBP changes (Δ μg/mL) from baseline after challenge meals (mean ± standard error, n = 30/group).

			Time (h)					*Significance (P)*[1]		
			0	1	2	3	4	*Treatment*	*Time*	*Treatment × Time*
Test 1 (wk 0)	Lean	C	0.00 ± 0.00	0.19 ± 0.17	-0.09 ± 0.17	-0.31 ± 0.20	-0.10 ± 0.14	*0.3051*	*0.0270*	*0.0731*
		Y	0.00 ± 0.00	0.06 ± 0.20	-0.42 ± 0.25	-0.24 ± 0.34	-0.69 ± 0.27			
	Obese	CO	0.00 ± 0.00	0.10 ± 0.16	0.00 ± 0.22	-0.06 ± 0.17	-0.14 ± 0.15	*0.1442*	*0.4394*	*0.3936*
		YO	0.00 ± 0.00	-0.34 ± 0.33	-0.62 ± 0.34	-0.58 ± 0.40	-0.45 ± 0.31			
Test 2 (wk 9)	Lean	C	0.00 ± 0.00	0.17 ± 0.14 $	0.52 ± 0.27 $	-0.18 ± 0.20 $	0.19 ± 0.25 $	*0.0002*	*0.0004*	*0.1149*
		Y	0.00 ± 0.00	-0.44 ± 0.15 #$	-0.31 ± 0.18 $	-0.86 ± 0.16 #$	-0.54 ± 0.16 #$			
	Obese	CO	0.00 ± 0.00	0.08 ± 0.27	-0.04 ± 0.24	-0.37 ± 0.27	-0.26 ± 0.18	*0.1456*	*0.0258*	*0.5378*
		YO	0.00 ± 0.00	-0.30 ± 0.11	-0.40 ± 0.14 #	-0.71 ± 0.18 #	-0.28 ± 0.18			

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese; LBP, lipopolysaccharide-binding protein.

[1] The effects of treatment, time and treatment × time were determined by repeated-measures ANOVA (PROC MIXED) with treatment, time and treatment × time as independent variables.

* Significantly different from baseline in control group, P < 0.05, determined by repeated-measures ANOVA (PROC MIXED) with time as independent variable. # Significantly different from baseline in yogurt groups, P < 0.05, determined by repeated-measures ANOVA (PROC MIXED) with time as independent variable. $ Significantly different between control and yogurt at different time points, determined by independent T-test (PROC TTEST).

Supplementary Table 4.6 Relative postprandial plasma sCD14 changes (Δ ng/mL) from baseline after challenge meals (mean ± standard error, n = 30/group).

			Time (h)					*Significance (P)*[1]		
			0	1	2	3	4	*Treatment*	*Time*	*Treatment × Time*
Test 1 (wk 0)	Lean	C	0.0 ± 0.0	-52.7 ± 29.7	-62.4 ± 27.2	-75.9 ± 30.1	-12.2 ± 37.4	*0.3608*	*0.0016*	*0.4851*
		Y	0.0 ± 0.0	-89.3 ± 25.8 #	-76.2 ± 26.9 #	-76.3 ± 23.2	-69.7 ± 28.8			
	Obese	CO	0.0 ± 0.0	-106 ± 24.7 *$	-137.5 ± 29.4 *$	-89.8 ± 28.7 *	-33.4 ± 23.0	*0.0063*	*<.0001*	*0.2037*
		YO	0.0 ± 0.0	-31.8 ± 27.0 $	-50 ± 27.2 $	-17.6 ± 24.7	5.2 ± 19.3			
Test 2 (wk 9)	Lean	C	0.0 ± 0.0	4.9 ± 23.7	-14.0 ± 16.1	7.0 ± 23.7	13.9 ± 28.6	*0.1877*	*0.1137*	*0.3027*
		Y	0.0 ± 0.0	-52.0 ± 22.2 #	-49.4 ± 21.9	-25.3 ± 23.5	4.5 ± 24.6			
	Obese	CO	0.0 ± 0.0	-72.8 ± 27.4*$	-75.8 ± 29.2 *$	-65.6 ± 24.3	-21.4 ± 24.5	*0.0025*	*0.0168*	*0.1988*
		YO	0.0 ± 0.0	-4.9 ± 19.6$	11.3 ± 16.3 $	-7.7 ± 20.0	36.4 ± 22.9			

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese; sCD14, soluble CD14.

[1] The effects of treatment, time and treatment × time were determined by repeated-measures ANOVA (PROC MIXED) with treatment, time and treatment × time as independent variables.

* Significantly different from baseline in control group, P < 0.05, determined by repeated-measures ANOVA (PROC MIXED) with time as independent variable. # Significantly different from baseline in yogurt groups, P < 0.05, determined by repeated-measures ANOVA (PROC MIXED) with time as independent variable. $ Significantly different between control and yogurt at different time points, determined by independent T-test (PROC TTEST).

Supplementary Table 4.7 Postprandial plasma LBP/sCD14 ratio changes after challenge meals (mean ± standard error, n = 30/group).

			Time (h)					*Significance (P)*[1]		
			0	1	2	3	4	*Treatment*	*Time*	*Treatment × Time*
Test 1 (wk 0)	Lean	C	1.00 ± 0.00	1.05 ± 0.03	1.02 ± 0.03	1.03 ± 0.03	1.01 ± 0.02	*0.8039*	*0.0050*	*0.6760*
		Y	1.00 ± 0.00	1.08 ± 0.03 #	1.03 ± 0.03	1.04 ± 0.04	0.98 ± 0.04			
	Obese	CO	1.00 ± 0.00	1.10 ± 0.03 *$	1.12 ± 0.03 *$	1.06 ± 0.03 $	1.01 ± 0.02	*0.0090*	*<.0001*	*0.1308*
		YO	1.00 ± 0.00	1.02 ± 0.03 $	1.02 ± 0.03 $	0.98 ± 0.03 $	0.97 ± 0.02			
Test 2 (wk 9)	Lean	C	1.00 ± 0.00	1.00 ± 0.02	1.05 ± 0.03	1.01 ± 0.03 $	1.00 ± 0.03	*0.0628*	*0.0557*	*0.4688*
		Y	1.00 ± 0.00	0.99 ± 0.02	1.00 ± 0.02	0.93 ± 0.02 #$	0.94 ± 0.03			
	Obese	CO	1.00 ± 0.00	1.07 ± 0.03 *	1.06 ± 0.03 $	1.04 ± 0.03 $	1.02 ± 0.03$	*0.0004*	*0.1496*	*0.0868*
		YO	1.00 ± 0.00	0.99 ± 0.02	0.96 ± 0.01 $	0.94 ± 0.02 $	0.95 ± 0.02$			

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese; LBP, lipopolysaccharide-binding protein; sCD14, soluble CD14.

[1] The effects of treatment, time and treatment × time were determined by repeated-measures ANOVA (PROC MIXED) with treatment, time and treatment × time as independent variables.

* Significantly different from baseline in control group, P < 0.05, determined by repeated-measures ANOVA (PROC MIXED) with time as independent variable. # Significantly different from baseline in yogurt groups, P < 0.05, determined by repeated-measures ANOVA (PROC MIXED) with time as independent variable. $ Significantly different between control and yogurt at different time points, determined by independent T-test (PROC TTEST).

A.3 Appendix Materials for Chapter 5

Supplementary Table 5.1 Fasting plasma concentration of IL-6 and sCD14 measured at wk 0, 3, 6, and 9 (mean ± standard error, n = 30/group).

Biomarkers (unit)	**Group**	**Time** (wk)				***Significance (P)***[1]		
		0	3	6	9	*Time*	*Treatment*	*Treatment × Time*
IL-6 (pg/mL)	C	0.74 ± 0.08	0.69 ± 0.05	0.73 ± 0.08	0.89 ± 0.11	*0.1432*	*0.3846*	*0.7722*
	Y	0.88 ± 0.13	0.80 ± 0.19	0.91 ± 0.23	0.87 ± 0.10			
	CO	1.56 ± 0.13	1.49 ± 0.12	1.50 ± 0.12	1.47 ± 0.11	*0.0040*	*0.4018*	*0.0726*
	YO	1.86 ± 0.22	1.53 ± 0.19 #	1.44 ± 0.18 #	1.59 ± 0.19 #			
sCD14 (ng/mL)	C	1421 ± 50	1316 ± 46 *	1340 ± 44	1323 ± 48	*0.0003*	*0.8631*	*0.6751*
	Y	1402 ± 59	1378 ± 54	1330 ± 52	1287 ± 55			
	CO	1481 ± 45	1391 ± 46 *	1337 ± 51 *	1365 ± 44 *	*0.0182*	*0.6642*	*0.0182*
	YO	1388 ± 45	1326 ± 44	1324 ± 42	1388 ± 49			

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese; IL-6, interleukin 6; sCD14, soluble CD14.

[1] The effects of treatment, time and treatment × time were determined by repeated-measures ANOVA (PROC MIXED) with treatment, time and treatment × time as independent variables.

* Significantly different from baseline in control group, $P < 0.05$, determined by repeated-measures ANOVA (PROC MIXED) with time as independent variable. # Significantly different from baseline in yogurt groups, $P < 0.05$, determined by repeated-measures ANOVA (PROC MIXED) with time as independent variable.

Supplementary Table 5.2 Fasting plasma concentration of biomarkers measured at wk 0 and wk 9 (mean ± standard error, n = 30/group).

Biomarkers (unit)	**Group**	**Time** (wk)		***Significance** (P)*	
		0	9	*Time*[1]	*Treatment*[2]
hsCRP (mg/L)	C	1.24 ± 0.26	1.33 ± 0.23	*0.6434*	*0.8471*
	Y	1.15 ± 0.21	1.22 ± 0.22	*0.6428*	
	CO	2.97 ± 0.31	2.98 ± 0.32	*0.9173*	*0.1088*
	YO	2.63 ± 0.37	2.42 ± 0.32	*0.0057*	
TNF-α (pg/mL)	C	1.10 ± 0.07	1.21 ± 0.09	*0.1404*	*0.0149*
	Y	1.14 ± 0.09	1.05 ± 0.09	*0.0201*	
	CO	1.25 ± 0.10	1.23 ± 0.08	*0.7099*	*0.6922*
	YO	1.52 ± 0.12	1.42 ± 0.10	*0.0128*	
sTNFRII (pg/mL)	C	2125 ± 72	2211 ± 92	*0.2683*	*0.1941*
	Y	2028 ± 69	2022 ± 69	*0.8940*	
	CO	2452 ± 94	2385 ± 95	*0.1857*	*0.0100*
	YO	2550 ± 101	2644 ± 103	*0.0296*	
TNF-α/ sTNFRII (pg/mL)	C	0.00052 ± 0.00003	0.00053 ± 0.00003	*0.3239*	*0.0338*
	Y	0.00057 ± 0.00005	0.00053 ± 0.00004	*0.0275*	
	CO	0.00051 ± 0.00004	0.00053 ± 0.00004	*0.2404*	*0.0173*
	YO	0.00059 ± 0.00004	0.00055 ± 0.00004	*0.0049*	
LBP (μg/mL)	C	9.9 ± 0.9	10.5 ± 1.0	*0.2746*	*0.2869*
	Y	9.3 ± 0.6	9.4 ± 0.6	*0.7795*	
	CO	12.4 ± 0.7	12.9 ± 0.9	*0.6000*	*0.1586*
	YO	12.3 ± 0.9	11.7 ± 0.8	*0.1828*	
LBP/sCD14 ratio	C	7.14 ± 0.69	8.32 ± 0.85	*0.0109*	*0.5985*
	Y	6.89 ± 0.44	7.72 ± 0.64	*0.0679*	
	CO	8.62 ± 0.58	9.60 ± 0.68	*0.0116*	*0.0360*
	YO	9.13 ± 0.73	8.71 ± 0.68	*0.3701*	
EndoCab IgM (MMU/mL)	C	99.6 ± 10.6	97.9 ± 9.6	*0.5571*	*0.0286*
	Y	101.4 ± 10.1	107.3 ± 10.6	*0.0059*	
	CO	70.7 ± 4.2	70 ± 4	*0.6528*	*0.0622*
	YO	68.7 ± 7.1	72.2 ± 8	*0.0455*	

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese; hsCRP, high-sensitivity C-reactive protein; TNF-α, tumor necrosis factor alpha; sTNF-RII, soluble tumor necrosis factor receptor II; LBP, lipopolysaccharide-binding protein; IgM EndoCAb, immunoglobulin M endotoxin-core antibody.

[1] The difference between wk 0 and wk 9 was compared by paired T-test (PROC TTEST).

[2] The group difference at wk 9 was compared by ANCOVA with baseline (wk 0) as covariate (PROC GLM).

Supplementary Table 5.3 Self-reported yogurt or soy pudding time of consumption, determined by analysis of 3-day food records.

Group	Consumption time	
	With meal	without meal
C (n = 29)	58%	42%
Y (n = 30)	69%	31%
CO (n = 30)	46%	54%
YO (n = 30)	66%	34%

Abbreviations: C, control lean; CO, control obese; Y, yogurt lean; YO, yogurt obese.