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Low-fat yogurt consumption reduces biomarkers of chronic inflammation and inhibits markers of endotoxin exposure in healthy premenopausal women: a randomised controlled trial

Ruisong Pei1,2, Diana M. DiMarco1, Kelley K. Putt2, Derek A. Martin1,2, Qinlei Gu2, Chureeporn Chitchumroonchokchai3, Heather M. White1, Cameron O. Scarlett5, Richard S. Bruno3 and Bradley W. Bolling1,2*

1Department of Nutritional Sciences, University of Connecticut, 3624 Horsebarn Rd. Ext., Unit 4017, Storrs, CT 06269, USA
2Department of Food Science, University of Wisconsin-Madison, 1605 Linden Dr, Madison, WI 53706, USA
3Human Nutrition Program, The Ohio State University, 1787 Neil Ave, Columbus, OH 43210, USA
4Department of Dairy Science, University of Wisconsin-Madison, 1675 Observatory Dr., Madison, WI 53706, USA
5School of Pharmacy, University of Wisconsin-Madison, 777 Highland Ave., Madison, WI 53705, USA

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Abstract
The anti-inflammatory mechanisms of low-fat dairy product consumption are largely unknown. The objective of this study was to determine whether low-fat yogurt reduces biomarkers of chronic inflammation and endotoxin exposure in women. Premenopausal women (BMI 18.5 – 27 and 30 – 40 kg/m^2) were randomised to consume 339 g of low-fat yogurt (yogurt non-obese (YN); yogurt obese (YO)) or 324 g of soya pudding (control non-obese; control obese (CO)) daily for 9 weeks (n 30/group). Fasting blood samples were analysed for IL-6, TNF-α/sTNF-RII, high-sensitivity C-reactive protein, 2-arachidonoylglycerol, anandamide, monocyte gene expression, soluble CD14 (sCD14), lipopolysaccharide (LPS), LPS binding protein (LBP), IgM endotoxin-core antibody (IgM EndoCAb), and zonulin. BMI, waist circumference and blood pressure were also determined. After 9-week yogurt consumption, YO and YN had decreased TNF-α/sTNF-RII. Yogurt consumption increased plasma IgM EndoCAb regardless of obesity status. sCD14 was not affected by diet, but LBP/sCD14 was lowered by yogurt consumption in both YN and YO. Yogurt intervention increased plasma 2-arachidonoylglycerol in YO but not YN. YO peripheral blood mononuclear cells expression of NF-κB inhibitor α and transforming growth factor β1 increased relative to CO at 9 weeks. Other biomarkers were unchanged by diet. CO and YO gained approximately 0.9 kg in body weight. YO had 3.6% lower diastolic blood pressure at week 3. Low-fat yogurt for 9 weeks reduced biomarkers of chronic inflammation and endotoxin exposure in premenopausal women compared with a non-dairy control food. This trial was registered as NCT01686204.

Key words: Obesity: Low-fat yogurt: Chronic inflammation: Endotoxin exposure: Dairy

Obesity is associated with increased risk of CVD, type 2 diabetes, hypertension and certain cancers(1). These comorbidities are associated with chronic inflammation(1). 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 cytokine release(2). Inflammation is exacerbated by chronic endotoxin exposure resulting from compromised intestinal barrier function in obese individuals(3,4). Endotoxin, especially the gram-negative-derived lipopolysaccharide (LPS) from gut microbiota, induces inflammation in humans(5). 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)/MD2 complex(6). The endocannabinoid (eCB) system also modulates intestinal barrier function and metabolic endotoxemia associated with obesity(4).

Yogurt consumption appears to be a promising strategy to improve obesity-associated intestinal barrier dysfunction and prevent chronic inflammation. Consumption of Lactobacillus-containing yogurt reduced surrogate markers of endotoxemia and decreased intestinal permeability in individuals with compromised gut barrier integrity(7,8). Smaller intervention studies

Abbreviations: 2-AG, 2-arachidonoylglycerol; AEA, anandamide; CO, Control obese; IgM EndoCAb, IgM endotoxin-core antibody; LBP, lipopolysaccharide-binding protein; LPS, lipopolysaccharide; sCD14, soluble CD14; TLR4, toll-like receptor 4; YO, yogurt obese.

* Corresponding author: B. W. Bolling, fax +608 262 6872, email bwbolling@wisc.edu
demonstrated that yogurt consumption reduced biomarkers of inflammation in elderly individuals and children\textsuperscript{(9,10)}. Epidemiological studies have also associated increased dairy product consumption with decreased risk of chronic diseases such as CVD, type 2 diabetes and hypertension\textsuperscript{(11–13)}. However, the effect of yogurt consumption on inflammation and endotoxin exposure in healthy premenopausal women is still largely unknown. We hypothesised that regular consumption of a commercial low-fat yogurt would reduce biomarkers of chronic inflammation and endotoxin exposure in healthy premenopausal women. We tested this hypothesis by conducting a randomised trial using a macronutrient- and texture-matched non-dairy food as a control.

### Methods

**Chemicals and reagents**

Anandamide (AEA), 2-arachidonoylglycerol (2-AG), anandamide-d4 (D4-AEA) and 2-arachidonoyl glycercol-d5 were purchased from Cayman Chemical. Acetonitrile, formic acid, acetic acid, water (Optima\textsuperscript{TM} LC/MS), isopropanol, ethanol and chloroform were purchased from Fisher Scientific. Histopaque\textsuperscript{10}·1077 was purchased from Sigma-Aldrich. PBS was purchased from MP Biomedicals. RNA Later and TRIzol reagent were purchased from Life Technologies. RNase-free water was purchased from Dot Scientific. RNase-free DNase was purchased from Qiagen.

**Participants**

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants were approved by the Institutional Review Boards at the University of Connecticut (no. H12-108) and University of Wisconsin-Madison (no. 2014-0669). Written informed consent was obtained from all participants. This trial was registered at clinicaltrials.gov as NCT01686204. The study inclusion criteria included: BMI from 18.5 to 27 kg/m\textsuperscript{2} or from 30 to 40 kg/m\textsuperscript{2}, age 21 to 55 years, stable body weight for the previous 2 months, willing to avoid yogurt and probiotic-containing foods and consume the provided 339 g of yogurt or 324 g soya 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 or prescribed oestrogen replacement therapy, not on slimming, 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.

**Dietary treatment**

The dietary intervention consisted of commercially available low-fat yogurt (Yoplait, General Mills, Inc.), and soya pudding (unfermented) as a macronutrient and micronutrient, energy content and texture-matched control food (ZenSoy). The nutrients provided daily by the two test foods are described in Table 1.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Low-fat yogurt (Yoplait)</th>
<th>Control food (ZenSoy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serving (g)</td>
<td>339</td>
<td>324</td>
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<tr>
<td>Energy content (kJ)</td>
<td>1381</td>
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<tr>
<td>Energy content (kcal)</td>
<td>330</td>
<td>330</td>
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<tr>
<td>Total fat (g)</td>
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<td>3</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>66</td>
<td>66</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>9</td>
<td>6–9\textsuperscript{‡}</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Na (mg)</td>
<td>180</td>
<td>165–210\textsuperscript{§}</td>
</tr>
<tr>
<td>Ca (mg)</td>
<td>450</td>
<td>180–450\textsuperscript{§}</td>
</tr>
<tr>
<td>Vitamin A (IU)</td>
<td>1500</td>
<td>600</td>
</tr>
<tr>
<td>Vitamin D (μg)</td>
<td>6.75</td>
<td>6.75</td>
</tr>
</tbody>
</table>

\textsuperscript{* Ingredients included: cultured pasteurised grade A low-fat milk, sugar, strawberries (or banana puree/peaches/raspberries, depending on the flavours), modified corn starch, non-fat milk, Kosher gelatin, citric acid, tricalcium phosphate, coloured with carmine, natural flavour, pectin, vitamin A acetate, vitamin D\textsubscript{3}. Contained disclaimer that product meets National Yogurt Association criteria for live and active culture yogurt (Contains Lactobacillus bulgaricus and Streptococcus thermophilus with at least 10\textsuperscript{8} culture/vg at manufacture).

\textsuperscript{†} Different flavours of control snack contained either 6 or 9 g of protein.

\textsuperscript{‡} Due to the reformulation by the manufacture during the intervention, the sodium content decreased from 210 to 165 mg; the calcium decreased from 450 to 180 mg (n 24, on the old formulation; n 36, on the new formulation).

**Experimental design**

We performed a randomised, controlled study of parallel design to examine how yogurt consumption affects chronic inflammation and intestinal barrier function relative to the consumption of a non-dairy control food. sCD14 has been used as a biomarker for endotoxin exposure and was thus chosen as the primary outcome\textsuperscript{(14)}. For the sample size calculation, an 8–2% margin of error for sCD14 was determined for a moderate group size (n 30/group) based on the standard deviation of sCD14 in a prior study\textsuperscript{(14)}. Apparently healthy premenopausal women were recruited from the Storrs, CT and Madison, WI area from October 2012 to April 2015. After the initial screening, 128 subjects were enrolled and randomly assigned to either the yogurt group or the soya pudding control group, in blocks of six\textsuperscript{\textsuperscript{15}}. Participants were randomised upon enrolment by assigning random numbers generated by Minitab 17 (Minitab Inc.). To avoid confounding by probiotic or dairy product consumption, the participants were instructed to restrict consumption of dietary supplements, fermented foods and limit their dairy product consumption to ≤4 servings/d for 2 weeks before the intervention (washout period, weeks −2–0) and throughout the intervention. From the beginning of week 0 to the end of week 9 (intervention period), the participants consumed 339 g of yogurt (12 oz.) or 324 g of control food daily. The lids of consumed yogurt and pudding cups were collected weekly to assess compliance. Participants visited the study centre at weeks 0, 3, 6 and 9 for anthropometric and blood pressure measurements. In addition, fasting blood samples were collected from the antecubital vein into evacuated tubes containing EDTA or sodium heparin (Becton, Dickinson and Company). At weeks 0 and 9, peripheral blood mononuclear cells (PBMC) were immediately isolated from 20 ml of sodium heparin blood samples for later mRNA analysis. For biomarker analysis, plasma was prepared.
Yogurt, intestinal barrier and inflammation

from blood samples held on ice and centrifuged (4°C, 15 min, 1500 g) within 20 min of collection. Aliquots of plasma were transferred to sterile cryogenic vials and snap-frozen in liquid N₂. All samples were stored at −80°C until analysis.

Anthropometric and blood pressure measurements

Body weight and height were measured on a digital physician scale to the nearest 0.1 kg and 1 cm (Rice Lake H150-10-5). Waist circumference was determined by placing a measuring tape evenly around a bare abdomen at the iliac crest. Blood pressure was measured using an Omron HEM-780 with ComFit cuff for non-obese participants, or an Omron BP710 with Omron H003D large adult cuff for obese participants (Ommor Healthcare, Inc.). Before 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. Blood pressure was measured twice, at least 5 min apart. If readings were more than 5 mmHg apart, a third reading was taken.

Inflammatory biomarkers

IL-6 and TNF-α in EDTA plasma were measured by human high-sensitive ELISA kits (IL-6, Cat no. S6600B; TNF-α, Cat no. SSTA00D; R&D System). Soluble TNF-Receptor II (sTNF-RII) was measured by a human ELISA kit (Cat no. SRT200; R&D System). High-sensitivity C-reactive protein (hsCRP) was measured by a human ELISA kit (Cat no. BC-T119; BioCheck Inc.). All the measurements were performed on a SpectraMax M2 microplate reader (Molecular Devices), following the manufacturers' instruction.

Biomarkers of endotoxin exposure and intestinal barrier function

LBP and Ig M Endotoxin-core antibody (IgM EndoCab) in EDTA plasma were measured by human ELISA kits (LBP, Cat no. HK315; IgM EndoCab, Cat no. HK504-IGM; Hycult Biotech). EDTA plasma LPS was measured by the PyroGene Recombinant Factor C endotoxin Detection System (Cat no. 50-658U; Lonza Group Ltd). EDTA plasma zonulin was measured by a human ELISA kit (Cat no. K5600; Immunodiagnostik AG). Sodium heparin plasma sCD14 was measured by a human ELISA kit (Cat no. DC140; R&D System). All the measurements were performed on a SpectraMax M2 microplate reader (Molecular Devices), following the manufacturers' instruction. The ratio between LBP and sCD14 was calculated as a marker for endotoxemia. Plasma AEA and 2-AG were quantitated by UPLC-MS/MS after protein precipitation and cleanup by Ostro pass-through sample preparation plate. These methods are described further in the online Supplementary Material.

Peripheral blood mononuclear cells isolation and mRNA analysis

PBMC were isolated from sodium heparin blood by gradient centrifugation using Histopaque®-1077 and stored at −80°C. RNA was extracted from PMBC by TRIzol reagent and further purified with an RNeasy mini kit (Qiagen). After subsequent complementary DNA synthesis, RT quantitaive PCR was performed using the iTaq™ Universal SYBR® Green Supermix (Bio-Rad), on a Bio-Rad CFX96 system (Bio-Rad). A detailed description of the methodology and primer sequences are in the online Supplementary Material and Table S1.

Dietary analysis

Participants were instructed to maintain their usual dietary pattern and caloric intake, accounting for the additional 1255 kJ (300 kcal) provided by the intervention foods. Dietary records were used to examine if the intervention changed patients' nutrients intakes or dietary patterns and to determine their compliance to the requested dietary restrictions. Participants were instructed to complete 3-d food records on two non-consecutive weekdays and 1 weekend day of their choice during the washout period and near the end of the intervention. Non-consecutive days were used to include a more representative record of foods in the dietary records. 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 analysed by 2013 Nutrition Data System for Research (NCC).

Statistical analysis

All results were expressed as mean values with their standard errors. Statistical analysis was conducted on SAS 9.4 software. The significance level was set at α=0.05 for all tests. At baseline, the differences of Obese v. Non-obese were analysed by independent t test (PROC TTEST). Pearson's correlation coefficients of pairwise variables were determined by linear regression modelling (PROC CORR). The effects of dietary treatment (yogurt v. control), obesity (obese v. non-obese) and treatment x obesity on BMI, waist circumference and blood pressure were determined by two-way repeated-measures ANOVA with time as a covariate (PROC MIXED). At week 9, the difference in biomarkers between yogurt and control treatment groups was analysed by ANCOVA with baseline as the covariate (PROC GLM). For data analysis that required normal distribution, Shapiro-Wilk test (PROC UNIVARIATE) was used for testing normality. If data failed the normality test, log-transformation was performed to achieve normality.

Results

In brief, 128 subjects were recruited. Eight subjects were excluded or dropped from the study due to personal or compliance issues. At the end of the study, sixty obese (30–40 kg/m²) and sixty non-obese (18.5–27 kg/m²) participants completed the study, with n=30/group (yogurt obese (YO); control obese (CO); yogurt non-obese (YN); control non-obese) (Fig. 1). Due to the...
sCD14 and LPS were not different between non-obese and obese participants, with non-obese participants, obese participants had higher IL-6, hsCRP (mg/l), BMI (kg/m2), sCD14 (ng/ml), sTNF-RII (pg/ml), and AEA (nM). Notably, baseline sCD14 and LPS were different between non-obese and obese groups (P=0.6579 and P=0.2005, respectively). All measured biomarkers for inflammation and endotoxin exposure except for sCD14 and LPS were correlated with BMI (online Supplementary Table S2).

### Fasting plasma markers of inflammation

Low-fat yogurt consumption for 9 weeks led to decreased TNF-α (P_{treatment} = 0.0219) and the TNF-α/sTNF-RII ratio (P_{treatment} = 0.0013) in both YN and YO (Table 3). Compared with baseline levels, YO had 7% decreased TNF-α/sTNF-RII ratio. The

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**Table 2. Baseline anthropometric of enrolled participants**

<table>
<thead>
<tr>
<th>Characteristics (unit)</th>
<th>CN</th>
<th>CO</th>
<th>YN</th>
<th>YO</th>
<th>Obese v. Lean</th>
<th>Significance (P)*</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25±3</td>
<td>1±1</td>
<td>31±9</td>
<td>1±6</td>
<td>24±8</td>
<td>0±8</td>
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<tr>
<td>Weight (kg)</td>
<td>62±3</td>
<td>1±6</td>
<td>93±5</td>
<td>2±2</td>
<td>64±7</td>
<td>1±5</td>
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<td>Height (m)</td>
<td>1.66</td>
<td>0±0.01</td>
<td>1±6</td>
<td>0±0.01</td>
<td>1.67</td>
<td>0±0.01</td>
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<td>BMI (kg/m²)</td>
<td>22.5</td>
<td>0±4</td>
<td>34.3</td>
<td>0±5</td>
<td>23.3</td>
<td>0±5</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>69.5</td>
<td>1±4</td>
<td>96±3</td>
<td>1±6</td>
<td>73±7</td>
<td>1±4</td>
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<tr>
<td>LBP (mmHg)</td>
<td>105±5</td>
<td>2±1</td>
<td>116±9</td>
<td>2±2</td>
<td>103±5</td>
<td>2±1</td>
</tr>
<tr>
<td>sCD14 (ng/ml)</td>
<td>1±0</td>
<td>0±0.07</td>
<td>12±5</td>
<td>0±1</td>
<td>1±14</td>
<td>0±0.09</td>
</tr>
<tr>
<td>hsCRP (mg/l)</td>
<td>1±24</td>
<td>0±0.26</td>
<td>2±97</td>
<td>0±0.31</td>
<td>1±15</td>
<td>0±0.21</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>2±1.10</td>
<td>2±1.25</td>
<td>0±1</td>
<td>1±14</td>
<td>0±0.09</td>
<td>1±52</td>
</tr>
<tr>
<td>sTNF-RII (pg/ml)</td>
<td>2±2125</td>
<td>2±72</td>
<td>2±2452</td>
<td>9±4</td>
<td>2±2028</td>
<td>6±9</td>
</tr>
<tr>
<td>LPS (EU/ml)</td>
<td>1±14±2</td>
<td>0±0.9</td>
<td>16±2</td>
<td>0±1.2</td>
<td>1±119</td>
<td>0±1.0</td>
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<tr>
<td>LBP (μg/ml)</td>
<td>9±9</td>
<td>0±0.9</td>
<td>12±4</td>
<td>0±0.7</td>
<td>1±9.3</td>
<td>0±0.6</td>
</tr>
<tr>
<td>sCD14 (ng/ml)</td>
<td>1±14±2</td>
<td>1±0</td>
<td>14±18</td>
<td>1±45</td>
<td>1±1420</td>
<td>2±59</td>
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<tr>
<td>LBP:sCD14</td>
<td>1±-1.14</td>
<td>1±0.69</td>
<td>8±62</td>
<td>0±58</td>
<td>6±89</td>
<td>0±4.4</td>
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<tr>
<td>IgM EndoCAb (MMU/ml)</td>
<td>1±-9.6</td>
<td>1±10</td>
<td>6±70</td>
<td>1±42</td>
<td>1±10±4</td>
<td>1±101</td>
</tr>
<tr>
<td>AEA (μM)</td>
<td>1±0.75</td>
<td>1±0.04</td>
<td>1±15</td>
<td>0±0.06</td>
<td>1±0.85</td>
<td>0±0.04</td>
</tr>
<tr>
<td>2-AG (μM)</td>
<td>1±-3.46</td>
<td>2±0.29</td>
<td>4±6</td>
<td>0±3.33</td>
<td>3±4.35</td>
<td>3±0.3</td>
</tr>
</tbody>
</table>

CN, control non-obese; CO, control obese; YN, yogurt non-obese; YO, yogurt obese; WC, waist circumference; SysBP, systolic blood pressure; DiaBP, diastolic blood pressure; LPS, lipopolysaccharide; LBP, lipopolysaccharide-binding protein; sCD14, soluble CD14; IL-6, IL-6; hsCRP, high-sensitivity C-reactive protein; sTNF-RII, soluble TNF-receptor II; IgM EndoCAb, Ig M endotoxin-core antibody; AEA, anandamide; 2-AG, 2-arachidonoylglycerol.

* Differences between obese and non-obese subjects were determined by independent t test (PROC TTEST).
obesity status had no effect on TNF-α or the TNF-α/sTNF-R-II ratio. On the other hand, neither plasma IL-6 nor hsCRP was affected by dietary treatment or obesity status.

### Fasting biomarkers of endotoxin exposure and intestinal barrier function

After 9 weeks intervention, IgM EndoCab increased in both YN and YO ($P_{treatment} = 0.0052$) (Table 3). The primary study outcome, fasting plasma sCD14 was unchanged (Table 3). Similarly, LBP was not affected by the dietary treatment over 9 weeks. The ratio of LBP:sCD14 was determined as an exploratory outcome, as recent data have indicated this may be a better marker of endotoxin exposure than either marker alone.$^{18,19}$ The LBP/sCD14 ratio was lower in the yogurt consumption groups than in the control groups ($P_{treatment} = 0.0477$) (Table 3). However, plasma LPS was not affected by the dietary treatment. A significant dietary treatment × obesity interaction effect on plasma 2-AG was detected ($P_{treatment × obesity} = 0.0372$). Subgroup analysis indicated yogurt consumption led to higher 2-AG in YO than in CN, control non-obese; CO, control obese; YN, yogurt non-obese; YO, yogurt obese; hsCRP, high-sensitivity C-reactive protein; sTNF-RII, soluble TNF II; LPS, lipopolysaccharide; LBP, lipopolysaccharide-binding protein; sCD14, soluble CD14; IgM EndoCab, Ig M endotoxin-core antibody; AEA, anandamide; 2-AG, 2-arachidonoylglycerol.
Gene expression was detected. The fold-change from baseline of 280 group. At baseline, YO and CO gene expression did not differ not changed.

Peripheral blood mononuclear cells mRNA expression

Endotoxin induces the production of inflammatory cytokines via TLR4/NF-κB pathway. We evaluated PBMC mRNA expression of key components of the NF-κB pathway in the obese group. At baseline, YO and CO gene expression did not differ (online Supplementary Fig. S1). After 9 weeks, YO NFκBIA (encoding NF-κB inhibitor α (IκBα)) and encoding transforming growth factor β1 (TGFB1) increased by 54% and 20% from baseline, respectively (Fig. 2). In contrast, the mRNA expression of these genes did not change in CO.

Anthropometric changes

After 9 weeks intervention, BMI was affected by obesity status, but not dietary treatment (P_{obesity} = 0.0084) (Table 4). BMI increased continuously in both YO and CO. However, dietary records did not indicate increased energy intake (online Supplementary Table S3). Despite this weight gain, YO and CO waist circumferences were unchanged. Yogurt consumption significantly reduced diastolic blood pressure (P_{treatment} = 0.0188), but not systolic blood pressure (Table 4). Within YO, diastolic blood pressure decreased by 2.82 (SEM 0.90) mmHg at week 3, with less decreases at weeks 6 and 9.

Discussion

The present study demonstrated that consuming two servings of low-fat yogurt daily for 9 weeks reduced fasting biomarkers of chronic inflammation and endotoxin exposure in apparently healthy premenopausal women. These findings are of significance because of the known role of compromised intestinal barrier function and subsequent endotoxin exposure as a mechanism of chronic inflammation, particularly in obesity(3,4).

Increased proinflammatory biomarkers such as IL-6, hsCRP and TNF-α have been associated with obesity in both adults and children(20,21). Consuming low-fat yogurt for 9 weeks resulted in a modest, but significant reduction of the levels of TNF-α, which may be partly explained by reduced activation of the TLR4-mediated inflammatory pathway. Upon TLR4 activation by endotoxin, a downstream signaling cascade is triggered that

Table 4. Changes from baseline in BMI, waist circumference (WC), and blood pressure (BP) of participants during the 9-week intervention

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Time (weeks)</th>
<th>Significance (P*)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
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<td>△BMI (kg/m²)</td>
<td>Group</td>
<td>0 3 6 9</td>
</tr>
<tr>
<td>CN</td>
<td>0.00</td>
<td>0.08</td>
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CN, control non-obese; CO, control obese; YN, yogurt non-obese; YO, yogurt obese; SysBP, systolic blood pressure; DiaBP, diastolic blood pressure.

* The effects of treatment, obesity, and treatment x obesity were determined by two-factor repeated-measures ANOVA with time as a covariate (PROC MIXED).
leads to activation of the NF-κB pathway. IκBα (encoded by NFKBIA) inhibits the NF-κB pathway by trapping the hetero-
dimeric complex in the cytosol. Since YO NFKBIA was
increased at the end of the intervention, yogurt consumption
may suppress TLR4 activation of NF-κB. TGF-β1 is an anti-
inflammatory and reparative cytokine that suppresses proin-
flammatory signaling from Toll-like receptors. YO TGFβ1
(encoding TGF-β1) expression was increased by the interven-
tion but other downstream genes of NF-κB including PTGS2,
NCF1, TNF and JNNG were not affected by the intervention. Given the reduction in YO plasma TNF-α, non-PBMC sources of
TNF such as the immunocytes resident in the intestine, adipose
tissue, or skeletal muscle may have contributed to this change.

Obesity is associated with subclinical endotoxemia which
increases chronic inflammation. Contrary to others, the
obese group did not have increased LPS relative to the non-
obese group in the present study. We expected plasma LPS to
be reduced by yogurt consumption, however non-significant
increases in plasma LPS were observed after the dietary inter-
vention. Direct quantitation of LPS by the LAL method is chal-
lenging, due to its short half-life, low blood concentrations and
the difficulty of removing interference from the blood matrix.

The LAL assay also does not account for lipoprotein-bound
LPS. In addition, other bacterial compounds such as glyco-
lipids and lipopolysaccharides derived from pathogenic Gram-positive
bacteria are pro-inflammatory. Therefore, it is likely that
quantitation of LPS by the LAL method does not account for
total bioactive endotoxin, and could be masked by lipoprotein
differences between experimental groups. Thus, the extent
fasting LPS values in the present study reflect intestinal barrier
function and true endotoxin load is unclear.

LBP and sCD14 have been proposed as surrogate biomarkers
of endotoxemia because of their roles in sequestering and
translocating LPS and other bacterial compounds to inflamma-
tory signaling pathways. Similar to the present study, LBP
was higher in overweight/obese individuals than in normal-
weight individuals, indicating low-grade chronic endotox-
emia. Serum LBP was also associated with increased
abdominal obesity and proinflammatory cytokines IL-6 and
IL-8. However, the originally proposed primary outcome,
fasting plasma sCD14, was not different between the obese and
non-obese groups in the present study. Similarly, sCD14 was
not associated with obesity in another study population (n = 420,
55% females, age 18–92 years). Therefore, sCD14 alone may
not be an appropriate biomarker for low-grade endotoxemia.

LBP and sCD14 act together to detoxify endotoxin. Healthy men
have postprandial plasma LBP/sCD14 ratios that are correlated
with plasma endotoxin. We observed that obese participants had 27% higher plasma LBP/sCD14 ratio than the non-
obese participants at baseline, which suggested increased
endotoxin exposure. Moderate increases in LBP/sCD14 ratio
were found in control but not the yogurt-consuming groups,
suggesting protective effects of yogurt against chronic
endotoxemia.

Another surrogate biomarker of endotoxemia is IgM Endo-
CAB, which can bind to the inner core of endotoxin and protect
against endotoxin. In a cross-sectional study involving
ninety-three age-matched middle-aged women, IgM EndoCAB
in obese and obese diabetic women was 55 and 30% of non-
obese participants. Similarly, IgM EndoCAB level in obese
participants was 69% of that in the non-obese in the present
study. Yogurt consumption increased the IgM EndoCAB level in
both obese and non-obese participants, suggesting decreased
level of endotoxin exposure resulting from the yogurt
intervention.

Intestinal barrier function is regulated in-part by eCB. 2-AG
improves the intestinal barrier, whereas AEA is associated with
decreased intestinal barrier function. After the 9-week inter-
vention, a modest decrease in 2-AG was observed in CO, but
not in YO. This may suggest protective effects of yogurt on
intestinal barrier function relative to the control snack.

Yogurt directly increased tight junction proteins and
improved barrier function in Caco-2 cells. A few dietary
interventions have improved intestinal barrier function in vari-
ous populations. For example, consumption of 300 g/d yogurt
containing Lactobacillus johnsonii for 4 weeks decreased
plasma LBP and intestinal permeability in elderly adults with
intestinal bacterial overgrowth. Intervention studies on the
anti-inflammatory effects of yogurt consumption are also lim-
ited. In elderly individuals, consumption of 100 g/d yogurt
containing Lactobacillus acidophilus and Bifidobacterium
lactis for 2 weeks decreased faecal haptoglobin, but
plasma inflammatory biomarkers were not determined.

In children with Helicobacter pylori, 400 ml/d yogurt containing
L. acidophilus and B. lactis for 4 weeks decreased serum
IL-6. The yogurt in the present study is a commercially
available product that is more representative of typical yogurt
products in the USA. Thus, probiotic fortification may not be
necessary in yogurt to modulate chronic inflammation in
apparently healthy women. Nevertheless, dietary intervention
alone might not be sufficient to manage obesity-associated
inflammation. Other strategies such as weight management and
pharmaceutical approaches should be incorporated.

In this study, obese participants gained approximately 1 kg
body weight, even though dietary records did not reveal
increased caloric intake. Most obese participants reported an
energy intake below the calculated estimated energy require-
ments, indicating underreporting. The interventions supplied
54 g of sugar, which contributed to the increased sugar con-
sumption by YO and CO. It is estimated that individuals having
higher sugar intake have 0.75 kg (95% CI 0.30, 1.19; P = 0.001)
more body weight than those consuming less sugar during
short-term interventions. Diastolic blood pressure was lower
in YO at week 3, but rebounded at later weeks, possibly
because of weight gain at later weeks. Thus, it is possible that
sustained increased sugar intake diminished the beneficial
effect of yogurt consumption. This study adds to the existing
evidence that increased dairy product consumption might
reduce blood pressure in obese individuals. A 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).

Several limitations should be considered when interpreting
the results of this study. Since only women were included as
participants, the results cannot be directly extended to men.
Furthermore, we did not recruit obese participants on the basis
of 200 g of low-fat dairy products with decreased risk of
hypertension (RR 0.96; 95% CI 0.93, 0.99).
of metabolic syndrome status, which could affect response to dairy product interventions. In addition, participants self-selected days to complete food records, which helps to improve participant compliance to study procedures but may introduce bias in selecting convenient days to record food intake (e.g., low diversity of foods or skipped meals). Further studies are needed to establish the extent regular yogurt consumption contributes to nutrient intake and diet quality. A strength of this study was that yogurt intervention consisted of commercially available products within the recommended dietary guidance for dairy product consumption. Notably, the yogurt was not fortified with probiotics, but this study was not designed to test the effects of fermentation. Other studies have demonstrated specific anti-inflammatory and intestinal barrier-promoting activity of probiotics.

A number of factors could have contributed to the benefits of yogurt consumption in the present study. Preclinical studies suggest milk oligosaccharides and lactoferrin promote intestinal barrier function and have anti-inflammatory properties. Dairy product fermentation also liberates peptides with hypotensive activity, as reviewed elsewhere. Other simultaneous dietary changes occurring with the intervention cannot be ruled out for their effect on biomarkers of chronic inflammation and intestinal barrier function.

In summary, this study demonstrated that consuming yogurt daily for 9 weeks modestly reduced chronic inflammation and inhibited markers of endotoxemia in apparently healthy premenopausal women. The anti-inflammatory effect of yogurt consumption was partially attributable to improved intestinal barrier function indicated by EndoCaB and LBP:sCD14 in comparison to the non-dairy product control.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114517003038

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B. W. B. and R. S. B. designed the research. R. P., D. M. D., K. K. P. and D. A. M. recruited and screened participants. R. P., D. M. D., Q. G., C. C. and C. O. S. performed research and data analysis. B. W. B., R. P., R. S. B., H. M. W. and C. O. S. supervised data analysis. R. P., B. W. B. and R. S. B. were responsible for data interpretation and had primary responsibility for final content. R. P. and B. W. B. wrote the paper. All authors have approved the final manuscript.

The authors declare that there are no conflicts of interest.

References

outcome may depend on LPS transporters LBP and sCD14.

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