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Strawberries decrease circulating levels of tumor necrosis factor and lipid peroxides in knee osteoarthritis in obese adults

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Abstract

Objective: Knee osteoarthritis (OA) is increasingly prevalent in obese people, who often have high cardio-metabolic risk factors. Among the few available non-surgical approaches, nutraceuticals have gained popularity, and dietary berries have mitigated arthritis symptoms in observational and animal studies. Clinical studies in OA are sparse, but recently we reported that strawberry supplementation can mitigate pain and reduce inflammatory markers in adults with knee OA. This study extends those observations.

Methods: We conducted a randomized cross-over double-blind placebo-controlled trial on the effects of dietary freeze-dried strawberries on obesity-related hormones, biomarkers of inflammation and lipid peroxidation. Seventeen subjects (4 men, 13 women; age 57 ± 3 y) were randomized to strawberry supplements (50g/day for 12 weeks) vs. placebo (50g/day, matched for calories and fiber), for two 12-wk intervention periods, separated by 2-wk washout phase.

Results: Among 24 biomarkers of inflammation examined (Bioplex-Pro human inflammation panel), 12 were detectable in all samples. Among these, high-sensitivity TNF- α (hs-TNF- α) and the soluble tumor necrosis factor receptor (sTNF-R2) were significantly decreased after strawberry consumption ($p < 0.05$). There were no changes in other biomarkers of the TNF super family, such as APRIL and BAFF. Among serum biomarkers of oxidative stress, 4-hydroxy-2-nonenal (4-HNE)

and conjugated dienes were also reduced ($p < 0.05$). No changes were observed in body weight, serum obesity-related hormones, or osteocalcin.

Conclusion: Strawberries lowered TNF- α , and lipid peroxidation products in obese adults with knee OA. Since, they also mitigate pain, these findings merit further investigation in larger trials.

Introduction

Based on the National Health Interview Survey (NHIS) years 2013–2015, approximately 54 million US adults have a diagnosis of arthritis, with prevalence projected to rise to 78 million by the year 2040.¹ Knee osteoarthritis (OA) is the most common form, and is one of the leading causes of disability among aging adults. The burden of knee OA has been rising in the past two decades as obesity and mean population age increase.²³ Despite the severity of the problem, few treatments are available to relieve pain or to reverse underlying pathological processes. Several inflammatory pathways underlie the onset and progression of OA, and the role of nutraceuticals, such as capsaicin, as anti-inflammatory agents is now being recognized in clinical care guidelines.⁴ Further research is needed to identify the role of nutraceuticals in alleviating inflammation, oxidative stress and pain in OA. Dietary interventions, especially those derived from plant-based foods, are gaining popularity in arthritis management, creating a need for rigorous nutrition research to assess efficacy.⁵⁶

Inflammatory biomarkers, including C-reactive protein (CRP), the interleukins, and members of the tumor necrosis factor (TNF) family have been associated with progression of knee OA.^{7–9} In addition, hormones associated with obesity such as leptin and visfatin,¹⁰¹¹ and markers of oxidative stress have may add to the pathological milieu.¹²¹³ In continued efforts to address the need for optimal non-pharmacologic interventions in OA, recent human studies have identified effects of polyphenol-containing foods and beverages, such as pomegranates,¹⁴ green tea¹⁵ and curcumin¹⁶ in reversing inflammation and pain in OA; these studies are preliminary and some results are conflicting. They are also of short duration, and in many cases do not report data on detailed markers of inflammation and oxidative stress. Experimental studies support these clinical observations and support the need for further controlled studies in OA.¹⁷¹⁸ We have previously demonstrated the effects of commonly-consumed dietary berries, such as blueberries and strawberries, in lowering blood pressure and improving dyslipidemia respectively, in obese adults with metabolic syndrome, and also observed a decrease in selected markers of oxidative stress (oxidized LDL, malondialdehyde).^{19–21} Since ‘metabolic OA’²² is now considered a phenotype of OA in the obese population, therapies targeting the metabolic syndrome may also be effective in improving OA symptoms.²²²³ To address the gap in this area of research, we undertook a pilot randomized controlled crossover trial and previously reported the effects of strawberries in decreasing knee pain, and selected circulating markers of inflammation and cartilage degradation, especially interleukin (IL)-6 and 1 β , and also matrix metalloproteinase (MMP)-3. There was no effect on CRP.²⁴ In the present study, we extend our findings on the effects of the strawberry intervention on biomarkers of hormones, inflammation and lipid peroxidation that underlie obesity in knee OA.

The overall objective of this study is to examine the effects of dietary freeze-dried strawberry supplementation on obesity-related hormones, systemic inflammatory profiles using multiplex assay, and lipid peroxidation that underlie both obesity and the metabolic OA, in a randomized controlled trial. In accordance with our previous report,²⁴ we hypothesize that strawberry supplementation decreases systemic inflammation and lipid peroxidation, and that this may explain the previously observed improvements in knee pain in our study.

Methods

Study design and participants:

As previously described,²⁴ using a randomized controlled crossover trial (Figure 1), we enrolled 17 obese adults with knee OA who finally completed the study. Adults were eligible to participate if they had waist circumference (>35 inches for women and >40 inches for men), body mass index (BMI) >30 kg/m², and radiographic evidence of degenerative OA on one or both knees. A diagnosis of knee OA was verified by a sports medicine specialist based on the radiological evidence of mild to moderate bilateral primary knee OA as defined by the American College of Rheumatology (ACR).²⁵ Participants were excluded if they had any of the following conditions: previous knee surgery, rheumatoid arthritis, abnormal blood values in the comprehensive metabolic panel test at screen, pregnant or lactating, use of corticosteroids and/or intra-articular injections during the preceding 3 months, use of fish oils and glucosamine, participation in a weight loss program in the preceding 6 months, and recent changes in physical activity levels, regular smoking, or allergy to strawberries. Participants who were unable to assess their pain for any reason were excluded from the study. The study was approved by the ethics committees at the University of Oklahoma Health Sciences Center (OUHSC) and at Oklahoma State University (OSU). All participants provided written informed consent prior to enrollment. The trial was registered with clinicaltrials.gov (NCT02518347).

Intervention:

The composition of the strawberry and the control powders obtained from the California Strawberry Commission (Watsonville, CA) has been previously reported.²⁴ The administered dose of 50g strawberry powder provided approximately 1585 mg total polyphenols, 66 mg anthocyanins, 220 mg ellagic acid, 160 kcal, and 8g dietary fiber. The control powder was matched for calories and fiber. Each intervention was for 12 weeks, separated by a two week washout phase, so that total study duration was 26 weeks. During the active treatment phase, the participants consumed 50 g of freeze-dried strawberry powder reconstituted in water twice a day: this is equivalent to 500 g of fresh strawberries, and was previously used in another study.²¹ The control powder was formulated to match the sensory properties, caloric value and macronutrient composition of the strawberry powder. The nutrient and phytochemical composition of the strawberry and control powders was determined at the Robert M. Kerr Food and Agricultural Products Center at Oklahoma State University (Stillwater, OK), and at the Brunswick Laboratories (Southborough, MA), respectively. The participants were asked to consume the strawberry or control beverage twice a day, at similar time points that were at least six to eight hours apart, and also to

consume the beverage as a snack by itself, and not with a meal or other snacks to prevent the confounding effects of other dietary factors. Compliance was assessed by the return of unused test agents and a mandatory three visits per week to the clinic for supervised consumption and a determination of plasma ellagic acid.²⁶

Serum obesity-related hormones and inflammatory factor assays:

Freshly drawn fasting blood samples were used for analyses of comprehensive metabolic panel (automated clinical analyzer, Abbott Architect Instruments, OU Medical Center laboratory). For other assays, sera were promptly frozen at -80°C for subsequent analyses of biomarkers. Obesity-related hormones [C-peptide, ghrelin, gastric inhibitory polypeptide (GIP), glucagon, insulin, leptin, plasminogen activator inhibitor-1 (PAI-1), glucagon-like peptide-1 (GLP-1), resistin, visfatin], and inflammatory factors [IL-11, 19, 20, 26, 27, 28A, 29, 32, 34, 35, TNFSF14, Thymic stromal lymphopoietin (TSLP), osteocalcin, pentraxin-3, interferon (IFN)- β , gp130, a proliferation-inducing ligand/tumor necrosis factor superfamily 13 (APRIL/TNFSF13), B cell activating factor of the TNF family (BAFF/TNFSF13B), chitinase 3-like 1 (CHI3L1), soluble CD30/TNF receptor superfamily member 8 (sCD30/TNFRSF8), sCD163, soluble tumor necrosis factor receptor (sTNF)-R1, R2, and TNF homologue with weak apoptosis-inducing activity (TWEAK)] were measured using Bio-Plex ProTM magnetic bead assays (Bio-Rad Laboratories, Hercules, CA), according to the manufacturer's instruction. High sensitivity TNF- α (hs-TNF- α) was determined using ELISA kits (R&D Systems, Minneapolis, MN) based on the manufacturer's protocol: inter-assay CV was 7.5%.

Detection of 4-hydroxy-2-nonenal(HNE)-modified proteins in serum:

The samples were prepared by mixing one μL sera, 23 μL water, and six μL Laemmli buffer. The heated protein samples (100°C for 5 min) were electrophoresed on a 4–20% gradient gel, transferred onto nitrocellulose membrane using a semi-dry transfer method, immunoblotted with anti-HNE rabbit sera (Alpha Diagnostic International, San Antonio, TX) and finally with anti-rabbit alkaline phosphatase conjugate (Jackson Immuno-research, West Grove, PA), prior to development with 1-step nitro-blue tetrazolium/5-bromo-4-chloro-3'-indolylphosphate substrate (ThermoFisher Scientific, Waltham, MA) as previously reported.²⁷

Conjugate diene assay:

Twenty-five μL sera were mixed well with 4 mL 2:1 chloroform-methanol in a glass test tube. The test tubes were centrifuged at $3000 \times g$ for 15 minutes. Two mL of the supernatant was transferred to another test tube and dried at 42°C . The dried lipid pellet was dissolved in 1 mL methanol. Conjugate dienes in the samples were analyzed by scanning from 200 to 360 nanometers using a spectrophotometer as previously reported.²⁸

Knee pain scores:

Knee pain scores were obtained from the ICOAP survey that has been used in several large studies, as previously described.²⁴ The ICOAP is an 11-item scale evaluating two pain domains: a 5-item scale evaluating constant pain and a 6-item scale evaluating intermittent

pain.²⁹ In addition, the Health Assessment Questionnaire-Disability Index (HAQ-DI) was used to assess functional ability using 20 items distributed across eight dimensions (dressing, arising, eating, walking, reach, grip, hygiene, and daily activity), rating each according to a four-level disability scale (range 0–3).³⁰ The participants were asked to fill out the questionnaires on the morning of their fasting study visits, at least 10–12 h following their last test dose of strawberry or control beverage.

Statistical analyses:

Continuous variables were expressed as means $\pm$ SE; discrete variables were presented as counts and proportions. Our main objective was to assess whether the selected biomarkers of inflammation and lipid peroxidation, in addition to knee pain scores, were different between the strawberry and control phases of 12-wk each. To test this hypothesis, we used a linear mixed-effects model (PROC MIXED) with time as within-subject factor and intervention group as a between-subject factor for each variable. Data were corrected for baseline values. The assumptions used in the sample size calculation were conservative, based on the report by Panahi et al. using curcumin supplementation in OA.³¹ Our original power calculation was based on a previous dietary intervention study in knee OA; we expected a decrease in serum IL-6 in the range of 0.39–0.45 pg/mL.³² All p -values < 0.05 were considered statistically significant and data were analyzed using SAS/STAT software (Version 9.4; SAS Institute Inc., Cary, NC, USA).

Results

In this randomized double-blind controlled trial, 35 participants were screened, and 17 qualified and completed the 26-week study (Figure 1). Baseline characteristics have been previously reported.²⁴ Participants had a baseline Kellgren-Lawrence grade score of 2.1 ± 0.7 (mean $\pm$ SE). Among the participants who completed the study, compliance was 100% for the strawberry group and 97% for the control group as assessed by mandatory thrice weekly visits, with the return of any unconsumed strawberry and control powder on the days the participants did not come to the clinic. No adverse events were reported. As an objective measure of compliance, plasma ellagic acid was detectable in all 17 participants upon completion of the strawberry phase (30.2 ± 3.6 ng/mL), whereas concentrations were not detectable at baseline, at the end of washout, or at the end of the control phase.

Table 1 shows effects of strawberries and control intervention on anthropometrics and obesity-related hormones, which were not significantly different between the two phases. Among the 24 serum factors of inflammation examined, 12 were detectable in all samples and these are listed in Table 2. Among these, only sTNF-R2 was significantly lowered after the strawberry vs. control phase ($p < 0.05$). We measured hsTNF- α using ELISA assay and this too was significantly lowered after the strawberry vs. the control phase (Table 2, $p < 0.05$).

Oxidative modification of serum lipids was measured by immunoblot assays of serum samples with anti-HNE rabbit sera, as well as by the determination of serum conjugated diene formation. In both measurements, strawberry supplementation was associated with

significantly lower HNE-modification of serum proteins (Figure 2) and conjugated diene formation after adjustment for baseline values (Figure 3) (both $p < 0.05$).

Discussion

We report the effects of dietary strawberries in decreasing circulating TNF- α and its soluble receptor (sTNF-R2), as well as lipid peroxidation products in obese adults with clinical diagnosis of knee OA. No differences in serum obesity-related hormones were noted, and as previously reported,²⁴ there were no changes in body weight or waist circumference at the end of the study. Our current findings further explain the changes in circulating biomarkers of inflammation and lipid peroxidation that may underlie the improvements in knee pain in our previous report.²⁴ Strawberries are a rich source of various phytochemicals, such as ellagic acids and anthocyanins, and have been identified in a survey to relieve symptoms in patients with rheumatoid arthritis.⁵ Consumption of other berries, such as blueberries and raspberries, have also been shown to exert anti-inflammatory effects in experimental models of collagen-induced arthritis.^{33,34} However, clinical studies on berry fruits and their phytochemicals in OA management are lacking, and our findings address this important gap in nutraceutical research in OA.

Inflammation plays a key role at all stages of OA, and has been positively correlated with knee pain and poor prognosis of this degenerative disease. The Foundation for the National Institutes of Health/Osteoarthritis Initiative (FNIH/OAI) biomarkers consortium has validated a panel of biomarkers for studies of OA, including the inflammatory biomarkers CRP, IL-6 and TNF- α , and the adipokine leptin, all reported in our study.^{35,36} In prior studies, reduction of serum levels of IL-6 and TNF- α by weight loss and herbal therapy was associated with significant improvements in OA symptoms.^{32,37} TNF- α increases catabolic effects of chondrocytes, upregulating expression of matrix metalloproteinases, and promoting OA.³⁸ In addition, mechanistic studies reveal higher expression of TNF- α receptors in OA cartilage compared to normal cartilage, thereby making the OA cartilage more susceptible to the catabolic effects of the pro-inflammatory cytokine.³⁹ Our findings on the effects of strawberries in lowering TNF- α and TNF-R2 provide mechanistic insights to explain the decrease in pain symptoms in our²⁴ and previously-reported studies of nutraceuticals, such as curcumin³¹ and green tea¹⁵ in OA. Soluble receptors of TNF- α as biomarkers of inflammation have not been reported in nutraceutical studies, though studies show their responses to exercise and alternative treatments such as mud pack therapy in patients with knee OA.^{40,41} Prior studies have demonstrated a role of sTNF-R2 in the pathophysiology of weight gain and insulin resistance in mice and adult humans,⁴² and this receptor has also been positively associated with dietary inflammatory scores in human cohort studies.⁴³ Thus, given that TNF- α is associated with the metabolic syndrome and diabetes, strawberries may be especially effective in ameliorating inflammation in 'metabolic OA',²² as manifest in our obese participants.

We also examined other and less commonly measured inflammatory markers, such as serum APRIL, BAFF, TWEAK, sCD30, sCD163, and CHI3L1; these have important immunological functions and have been implicated in autoimmune diseases and in chondrocyte dysfunction underlying OA.^{8,44,45} While most of these markers have not been

examined in clinical studies following administration of foods or nutraceuticals, and no effects were noted in our study, markers such as sCD30 has been reported to be altered by nutritional deficiencies in children.⁴⁶

We did not observe any changes in serum adipokines, especially leptin, and other biomarkers of inflammation, such as PAI-1, and osteocalcin. Our study was exploratory, and these null findings may be explained by the need for a different dose and duration of strawberry supplementation, or possibly a larger dose of anthocyanins than what was present in the current strawberry dose, in modulating biomarkers that affect OA. There lies the possibility that further dietary modifications that promote total body weight and fat loss, as well as interventions that affect bone and cartilage remodeling, may modulate these biomarkers in metabolic OA.

Lipid peroxidation-derived metabolites, especially 4-hydroxynonenal (HNE), have been associated with OA in experimental models.⁴⁷ Lipid peroxidation has been shown to aggravate OA principally by inducing apoptosis of chondrocytes and upregulating inflammation.¹³⁴⁸ Conjugated dienes, formed as a result of metal-induced oxidation of lipids, have been the principal measures of lipid peroxidation in studies of rheumatoid arthritis.⁴⁹ Curcumin has been shown to decrease malondialdehyde, a marker of lipid peroxidation, in patients with knee OA,⁵⁰ and ginger extracts have been demonstrated to decrease lipid peroxidation in chondrocytes.⁵¹ Our study demonstrates the antioxidant effects of strawberries in significantly decreasing conjugated diene formation, as well as HNE-modification of serum proteins in obese adults with knee OA. Mechanistic studies have implicated IL-6 in impaired antioxidant enzyme function in chondrocytes and as a contributor to oxidative stress.⁵²⁵³ Based on these observations, we may speculate that strawberry phytochemicals can inhibit lipid peroxidation by a direct antioxidant effect, quenching free radicals as reported in several studies,²¹⁵⁴⁵⁵ and secondly, by restoring the antioxidant enzyme activity as a result of lowering IL-6 as we previously reported.²⁴

Our exploratory study has limitations that must be taken into consideration. Firstly, we have a small sample representative sample of 'metabolic OA': further studies are essential to determine effects of berry nutrients and phytochemicals. These studies might include patients with post-traumatic OA, and those who are undergoing knee arthroplasty. Secondly, we did not measure all possible biomarkers of oxidative stress and antioxidant status, such as the antioxidant enzymes that may reverse OA progression. Finally, we used a large dose of strawberries that may not be feasible for consumption on a daily basis. Nonetheless, our findings support the use of processed strawberry powder that has a greater shelf life and is a more convenient mode of delivery of phytochemicals than equivalent amounts of fresh fruits. Use of such supplements may be helpful in the management of inflammation and oxidative damage, and our findings with those of others thus provide a platform for dialogue between food industry and health care research on increasing production and affordability of the freeze-dried berry powders for improving public health. Also, multiple statistical comparisons are inevitable in exploratory studies, but our clinical findings are consistent with previous pre-clinical and a few clinical studies that now deserve larger trials focusing on the biomarkers identified in our study. Future, more definitive studies should assess dose-response to inform dietary recommendations. Keeping in view the heterogeneous

characteristics of inflammatory biomarkers, there is certainly a need to identify effective therapies with minimum side effects that can decrease multiple biomarkers implicated in OA. Our current findings, in combination with our previous report²⁴ provides evidence in this direction.

Overall, our findings demonstrate the role of dietary strawberries, in addition to mitigating knee pain, have effects on TNF-related biomarkers of inflammation, and lipid peroxidation that play a substantial role in promoting OA. With the rise of ‘metabolic OA’ in the obese and aging population and concomitant increases in systemic inflammation and oxidative stress, it may be a prudent strategy to recommend the intake of strawberries as part of a healthy anti-inflammatory diet.

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Figure 1:
Study design

Figure 2:
Immunoblot analysis of serum samples with anti-HNE rabbit sera. Top: Control 12-wk;
bottom: Strawberry 12-wk

Figure 3: Absorption spectra of conjugated dienes in serum samples from participants at baseline, control phase (12-wk) and strawberry phase (12-wk) of the study at 200–320 nm. Values are expressed in terms of absorbance [OD]/25 μ L human sera. Each line represents three phases in our study: baseline, strawberry, control. Overall $P=0.001$ between strawberry and control adjusted for baseline.

Table 1

Anthropometrics and obesity-related hormones following strawberry and control interventions in a 26-week crossover trial in adults with symptomatic knee osteoarthritis (n=17/group)

Variables	Baseline	Strawberry 12-wk	Washout (2-wk)	Control 12-wk	P-value*
BMI (kg/m ²)	39.1±1.5	39.3±1.4	39.3±1.5	39.3±1.5	0.41
Body weight (lb)	246.4±7.3	245.6±7.4	245.1±7.1	245.0±7.2	0.32
Waist circumference (inches)	46.4 ±1.1	46.5± 1.1	46.1±1.0	46.8±1.1	0.26
C-Peptide (pg/mL)	96.8±31.0	84.1±21.6	36.6±4.7	103.2±35.8	0.52
Ghrelin (pg/mL)	89.2±15.5	96.3±10.3	100.7±18.9	82.4±10.5	0.73
GIP (pg/mL)	57.9±18.0	64.4±10.1	51.2±9.5	68.1±15.4	0.41
Glucagon (pg/mL)	186.5±17.8	200.9±18.4	183.2±20.8	180.5±16.5	0.68
Insulin (pg/mL)	261.2±33.1	426.3±136.9	333.6±76.7	352.7±71.6	0.53
Leptin (pg/mL)	3722.1±926.4	3811.5±712.3	3264.8±1196.1	3165.4±758.3	0.60
PAI-1 (pg/mL)	10382.1±951.3	12252.3±809.2	9854.3±1880.6	11675.7±1210.4	0.50
Resistin (pg/mL)	20.24±7.0	25.1±7.4	14.0±4.5	18.9±3.7	0.64
Visfatin (pg/mL)	1012.5±293.9	515.1±85.8	336.2±81.6	597.5±120.7	0.57

Values are means ± SEMs obtained from a linear mixed-effects model with time as within-subject factor and intervention group as a between-subject factor

* Strawberry 12-wk vs. Control 12-wk adjusted for Baseline; p<0.05 in bold

BMI: body mass index; GIP: gastric inhibitory polypeptide; plasminogen activator inhibitor-1

Table 2

Biomarkers of inflammation following strawberry and control interventions in a 26-week crossover trial in adults with symptomatic knee osteoarthritis (n=17/group)

Variables	Baseline	Strawberry 12-wk	Washout (2-wk)	Control 12-wk	P-value*
hs-TNF- α , pg/mL	5.5 $\pm$ 1.5	2.8 $\pm$ 2.6	4.5 $\pm$ 3.3	5.2 $\pm$ 2.1	0.02
APRIL/TNFSF13, pg/mL	18543.6 $\pm$ 1676.9	33110.9 $\pm$ 2719.6	25334.9 $\pm$ 2342.4	29291.8 $\pm$ 2286.6	0.25
BAFF/TNFSF13B, pg/mL	5433.1 $\pm$ 1935.9	6169.3 $\pm$ 451.5	57999.3 $\pm$ 541.5	6446.2 $\pm$ 545.6	0.33
sCD30/TNFRSF8, pg/mL	216.4 $\pm$ 24.7	379.6 $\pm$ 32.5	334.5 $\pm$ 38.5	358.5 $\pm$ 62.1	0.28
sTNF-R1, pg/mL	3086.0 $\pm$ 497.5	4410.2 $\pm$ 367.2	3675.4 $\pm$ 347.4	3988.2 $\pm$ 455.2	0.27
sTNF-R2, pg/mL	2384.2 $\pm$ 247.5	1685 $\pm$ 323.1	1994.5 $\pm$ 237.4	3047.2 $\pm$ 384.5	0.01
IL-19, pg/mL	28.4 $\pm$ 5.7	23.8 $\pm$ 3.2	22.6 $\pm$ 4.4	20.5 $\pm$ 1.2	0.14
TWEAK/TNFSF12, pg/mL	290.8 $\pm$ 38.1	314.1 $\pm$ 39.2	354.8 $\pm$ 45.7	316.5 $\pm$ 39.6	0.22
sCD163, pg/mL	76320.5 $\pm$ 19073.9	90093.2 $\pm$ 9344.9	89006.2 $\pm$ 9561.9	84157.7 $\pm$ 7767.7	0.28
CHI3L1, pg/mL	9428.8 $\pm$ 1172.6	13391.2 $\pm$ 1824.5	10056.6 $\pm$ 1211.6	13309.7 $\pm$ 1616.8	0.18
Osteocalcin, pg/mL	845.5 $\pm$ 62.1	1084.4 $\pm$ 90.3	995.5 $\pm$ 70.3	1074.7 $\pm$ 65.3	0.26
Pentraxin-3, pg/mL	171.8 $\pm$ 18.8	196.6 $\pm$ 26.8	205.6 $\pm$ 32.5	188.5 $\pm$ 22.6	0.19

Values are means $\pm$ SEMs obtained from a linear mixed-effects model with time as within-subject factor and intervention group as a between-subject factor

* Strawberry 12-wk vs. Control 12-wk adjusted for Baseline; p<0.05 in bold

APRIL/TNFSF13: a proliferation-inducing ligand/tumor necrosis factor superfamily 13; BAFF/TNFSF13B: B cell activating factor of the TNF family; CHI3L1: chitinase 3-like 1; hs-TNF- α : high-sensitivity tumor necrosis factor-alpha; IL-interleukin; sCD30/TNFRSF8: soluble CD30/TNF receptor superfamily member 8; sCD163: soluble CD163; sTNF-R: soluble tumor necrosis factor receptor; TWEAK: TNF homologue with weak apoptosis-inducing activity