

## Regular Tart Cherry Intake Alters Abdominal Adiposity, Adipose Gene Transcription, and Inflammation in Obesity-Prone Rats Fed a High Fat Diet

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**ABSTRACT** Obesity, systemic inflammation, and hyperlipidemia are among the components of metabolic syndrome, a spectrum of phenotypes that can precede the development of type 2 diabetes and cardiovascular disease. Animal studies show that intake of anthocyanin-rich extracts can affect these phenotypes. Anthocyanins can alter the activity of tissue peroxisome proliferator-activated receptors (PPARs), which affect energy substrate metabolism and inflammation. However, it is unknown if physiologically relevant, anthocyanin-containing whole foods confer similar effects to concentrated, anthocyanin extracts. The effect of anthocyanin-rich tart cherries was tested in the Zucker fatty rat model of obesity and metabolic syndrome. For 90 days, rats were pair-fed a higher fat diet supplemented with either 1% (wt/wt) freeze-dried, whole tart cherry powder or with a calorie- and macronutrient-matched control diet. Tart cherry intake was associated with reduced hyperlipidemia, percentage fat mass, abdominal fat (retroperitoneal) weight, retroperitoneal interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) expression, and plasma IL-6 and TNF- $\alpha$ . Tart cherry diet also increased retroperitoneal fat PPAR- $\alpha$  and PPAR- $\gamma$  mRNA ( $P = .12$ ), decreased IL-6 and TNF- $\alpha$  mRNA, and decreased nuclear factor  $\kappa$ B activity. In conclusion, in at-risk obese rats fed a high fat diet, physiologically relevant tart cherry consumption reduced several phenotypes of metabolic syndrome and reduced both systemic and local inflammation. Tart cherries may reduce the degree or trajectory of metabolic syndrome, thereby reducing risk for the development of type 2 diabetes and heart disease.

**KEY WORDS:** • anthocyanins • anti-inflammation • diabetes • nuclear factor  $\kappa$ B • peroxisome proliferator-activated receptor

### INTRODUCTION

**O**BESITY, HYPERLIPIDEMIA, and systemic inflammation are among several clinical parameters of prediabetes of “metabolic syndrome,” and these phenotypes are positively correlated with the incidence of type 2 diabetes and cardiovascular disease.<sup>1</sup> Fruit and vegetable intake is inversely correlated with cardiovascular morbidity and mortality.<sup>2–4</sup> Furthermore, fruits and vegetables contain non-nutritive phytochemicals that may contribute to their health-promoting effects.

Anthocyanins are a major subclass of phytochemical flavonoids that are principally found in red-, blue-, and purple-pigmented fruits and vegetables. Several studies have suggested that anthocyanin-rich botanical extracts can modify lipid metabolism *in vitro* and can reduce hyperlipidemia *in vivo*.<sup>5–10</sup> Reductionistic approaches using *in vitro* approaches and/or isolated phytochemicals can attempt to

reveal mechanisms of effect. For example, isolated anthocyanins and anthocyanin-rich extracts have been shown to modify the activity of the peroxisome proliferator-activated receptor (PPAR).<sup>11,12</sup> PPAR is a transcription factor that controls genes related to metabolism and inflammation, and PPAR agonist drugs are currently prescribed to reduce risk and/or manage type 2 diabetes.<sup>13–15</sup> Tart cherries (*Prunus cerasus*) are a rich edible fruit source of anthocyanins.<sup>16,17</sup> In lean rats, we previously demonstrated that inclusion of whole tart cherry powder (1% wt/wt) into a low fat diet significantly reduced total cholesterol and triglycerides, reduced fatty liver, and increased liver PPAR isoform mRNA.<sup>18</sup> However, it is uncertain what disease phenotypes would be lost, sustained, or amplified if studied in an obesity-prone model fed a higher fat diet. This model is increasingly relevant to our growing at-risk population with metabolic syndrome and to typical patterns of human diets, which include elevated saturated fat and cholesterol.

Zucker fatty rats develop obesity, hyperlipidemia, insulin resistance, and systemic inflammation. Importantly, Zucker fatty rats are responsive to and benefited by PPAR agonist drugs.<sup>19–22</sup> We then tested the effect of 90-day diet provision of a high fat diet containing whole tart cherry powder.

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TABLE 1. PROFILES OF THE TART CHERRY-SUPPLEMENTED (CHE) DIET AND CARBOHYDRATE-SUPPLEMENTED CONTROL (CON) DIET

	CHE	CON
% of calories		
Total protein	20.0	20.0
Total carbohydrate	33.0	33.0
Total fat	47.0	47.0
kCal/g of diet	3.9	3.9
g/kg of diet		
Casein	200	200
Protein from cherry	0.09	0
DL-Methionine	3	3
Cornstarch	75	75
Sucrose	250	250
Sugar from cherry	7.3	0
Dextrose	0	3.6
Fructose	0	3.6
Cellulose	50	50
Fiber from cherry	0.9	0
Corn oil	25	25
Cholesterol	860 ppm	860 ppm
Stripped lard	178	178
Choline bitartrate	2	2
Vitamin Mix #300050	10	10
Mineral Mix #200000	35	35
Vitamin C	0.002	0
Vitamin A	441.4 IU	0
$\alpha$ -Carotene	44 RE	0
Phosphorus	0.013	0
Potassium	0.11	0
Sodium	0.015	0

Base diet is Dyet number 102121 from Dyets, Inc. IU, international units; RE, retinol equivalence.

We measured blood lipids, systemic inflammation as measured by plasma tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL-6), and percentage fat mass. Because abdominal adipose tissue is a significant source of pro-inflammatory cytokines, we surveyed mRNA related to inflammation in the abdominal retroperitoneal fat pad. We also measured abdominal fat levels of IL-6 and TNF- $\alpha$  and the activity of the transcription factor nuclear factor  $\kappa$ B (NF $\kappa$ B).

## MATERIALS AND METHODS

### Animal care

Male Zucker fatty rats (6 weeks old) were acquired from Harlan (Indianapolis, IN, USA) and were housed two per

cage. Rats were housed on 12-hour light:dark cycles. At 7 weeks of age, rats were randomized to two groups ( $n = 12$  each): 1% tart cherry powder by weight and 0.85% additional carbohydrate (dextrose:fructose 1:1) by weight to control for the additional carbohydrate provided by the tart cherry powder. Tart cherry powder or the dextrose:fructose mixture was incorporated into powdered higher fat diet as described in Table 1 (diet number 102121, Dyets, Bethlehem, PA, USA). The cherry product was a freeze-dried powder from individually quick-frozen tart cherries, harvested in northern Michigan and prepared by VanDrunen Farms (Momence, IL, USA). Tart cherry powder nutrient analysis was conducted by VanDrunen Farms and its subsidiary Futureceuticals (Momence), and further anthocyanin analysis was conducted by our group using liquid chromatography-mass spectrometry (Table 2). Diets were mixed weekly, vacuum-sealed, and stored at 4°C. Rats were provided 20 g of diet per head/day; this was approximately 10% below *ad libitum* intake to ensure complete consumption and equal food intake among all rats in the study. Water was provided *ad libitum*. This protocol was approved by the University of Michigan's University Committee on the Use and Care of Animals.

### Liquid chromatography-mass spectrometry analysis of tart cherry powder

Individually quick-frozen tart cherry powder (1 g) was extracted with 10 mL of methanol/water/acetic acid (85:15:0.5 by volume) in a 15-mL screw-cap tube and shaken overnight in the dark at 4°C. The sample was vortex-mixed and then sonicated for 10 minutes at 25°C and filtered (pore size, 0.45  $\mu$ m). An Alliance 2695 HPLC (Waters, Milford, MA, USA) was used to generate a binary gradient with 0.05% trifluoroacetic acid in water as the aqueous solvent (solvent A) and 0.05% trifluoroacetic acid in acetonitrile as the organic modifier (solvent B). Chromatographic separation was achieved with a Gemini 5- $\mu$ m (particle size) C18 150- $\times$ 2.00-mm (Phenomenex, Torrance, CA, USA) reverse-phase column held at 35°C using a flow rate of 0.19 mL/minute. The column was initially equilibrated to 8% solvent B, increased to 18% solvent B over 10 minutes, 28% solvent B over the next 8 minutes, 40% solvent B in 1 minute, 60% solvent B in 3 minutes, and then returned to initial conditions. The sample chamber was cooled to 10°C, and the injection volume was 10  $\mu$ L. Effluent from the high-performance liquid chromatography column was directed into the electrospray ionization probe

TABLE 2. TART CHERRY POWDER ANTHOCYANIN PROFILE AS MEASURED BY LIQUID CHROMATOGRAPHY (LC)-MASS SPECTROMETRY (MS)

Anthocyanin	LC retention time	MS/MS (m/z)	mg/g of dry weight
Cyanidin 3-sophoroside	12.668	611 $\rightarrow$ 287	0.0041
Cyanidin 3-glucosylrutinoside	13.618	757 $\rightarrow$ 611, 287	0.3757
Cyanidin 3-glucoside	14.416	449 $\rightarrow$ 287	0.0071
Cyanidin 3-rutinoside	15.096	595 $\rightarrow$ 287	0.2261
Peonidin 3-glucoside	17.333	463 $\rightarrow$ 301	0.0388
Pelargonidin	16.959	271 $\rightarrow$ 121	0.0086

of a TSQ Quantum Ultra AM triple quadrupole mass spectrometer (ThermoFinnigan, San Jose, CA, USA). Positive ions were generated with the following parameters: spray voltage, 3,000 V; sheath gas, 40 arbitrary units; auxiliary gas, 10 arbitrary units; and capillary temperature, 250°C. Tube lens voltages were optimized for each compound. Data were collected in centroid mode. Single reaction monitoring was used for mass analysis and quantification, with authentic standards for verification. Data analysis was performed with Xcalibur quantitation software (version 1.4 SR1, ThermoFinnigan).

#### Dual X-ray absorptiometry (DEXA)

Lean body mass and fat mass were measured every 4 weeks using DEXA on an Eclipse Peripheral DEXA Scanner with pDEXA Sabre software version 3.9.4 (Norland Medical Systems, Fort Atkinson, WI, USA) in research mode. Rats were anesthetized with 4% isoflurane and maintained with 1% isoflurane. After calibration, each rat was placed on the platform and scanned from nose to anus at 30 mm/second with a resolution of 1.0×1.0 mm. Results for percentage fat mass and lean body mass were determined relative to body weight on the day of the scan.

#### Sample collection

After 90 days of feeding, rats were fasted for 18 hours and sacrificed by decapitation. Trunk blood was collected in 0.6-mL capacity serum-separator tubes, allowed to clot at room temperature, and spun at 5,000 g for 7 minutes at 4°C. Serum was aliquoted and stored at -80°C until further analysis. A further fraction of trunk blood was collected in a 7-mL EDTA-containing Vacutainer® (Becton Dickinson, Franklin Lakes, NJ, USA) and spun at 4°C at 1,500 g. Plasma was then aliquoted and stored at -80°C until further analysis. Tissues were harvested and weighed, including heart, liver, kidneys, epididymal fat, and retroperitoneal fat. Tissues were snap-frozen in liquid nitrogen and stored at -80°C until further analysis.

#### Serum and plasma analysis

Serum lipids and glucose were measured using the automated IDEXX VetTest® Chemistry Analyzer from IDEXX Laboratories (Westbrook, ME, USA). Plasma TNF- $\alpha$  and IL-6 were measured by immunoassay (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.<sup>23</sup>

#### Tissue inflammation

A fragment of frozen retroperitoneal fat was powdered and homogenized with T-Per Solution (Pierce, Rockford, IL, USA) mixed with Complete Protease Inhibitor Mini-Tab cocktail (Roche, Indianapolis, IN, USA), according to the manufacturer's protocol. Levels of TNF- $\alpha$  and IL-6 were measured by enzyme-linked immunosorbent assay following the manufacturer's protocols (Quantikine®, R&D Sys-

tems). Both experimental samples and cytokine standards were assayed in duplicate, and the level of TNF- $\alpha$  and IL-6 was calculated from the standard curve and expressed as the average pg of TNF- $\alpha$  and IL-6 per mg of total protein. Protein concentrations were determined using a BCA Protein Assay kit (Pierce).

#### Real-time reverse transcription polymerase chain reaction (PCR)

Total RNA from frozen fragments of retroperitoneal fat was isolated with RNeasy™ Lipid Tissue Midi Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. Samples ( $n = 4$  per group) were analyzed by real time-PCR using a custom RT<sup>2</sup> Profiler PCR Array and its proprietary reagents (SABiosciences, Frederick, MD, USA). First, cDNA was prepared using the RT<sup>2</sup> First Strand Kit. cDNA was then added to the RT<sup>2</sup> qPCR Master Mix, which contains SYBR Green and a reference dye. The mixture is then added to a provided 96-well plate, which has 48 replicate wells containing preoptimized, species-specific primer sets for controls (housekeeping genes, genomic DNA control, reverse transcription control, and positive PCR control) and the experimental genes of interest (PPAR- $\alpha$ , PPAR- $\gamma$ , NF $\kappa$ B, IKB $\alpha$ , IL-6, and TNF- $\alpha$ ). Each primer set of the array has undergone quality assurance/quality control analysis by real-time reverse transcription PCR and gel electrophoresis to confirm the generation a single gene product, with no additional peak and/or bands that would indicate primer dimer formation. Relative expression of PPAR- $\alpha$ , PPAR- $\gamma$ , NF $\kappa$ B, IKB $\alpha$ , IL-6, and TNF- $\alpha$  was determined by the  $\Delta\Delta C_t$  method as described by Livak and Schmittgen.<sup>24</sup>  $\Delta C_t$  for each gene (averaged across four animals per group) was calculated relative to the average of five housekeeping genes (P1 large ribosomal protein, hypoxanthine guanine phosphoribosyl transferase, ribosomal protein L13A, lactate dehydrogenase A, and  $\beta$ -actin).  $\Delta\Delta C_t$  was then determined as  $\Delta C_t$  (group 2, cherry) minus  $\Delta C_t$  (group 1, control), and the values were then back-transformed ( $2^{-\Delta\Delta C_t}$ ) to calculate the fold change of each transcript.

#### Tissue NF $\kappa$ B activity

A fragment of frozen retroperitoneal fat was subjected to subcellular fractionation ( $n = 6$  per group) using the technique of Kutoh *et al.*<sup>25</sup> NF $\kappa$ B activity was measured in both nuclear and cytosolic extracts using the high-throughput TransAM™ enzyme-linked immunosorbent assay (Active Motif, Carlsbad, CA, USA). The TransAM kit contains a 96-well plate coated with immobilized oligonucleotide that contains the NF $\kappa$ B consensus site (5'-GGGACTTCC-3'). The active form of NF $\kappa$ B contained in the cell fractions then binds to the oligonucleotide. The provided primary antibody recognizes an epitope on the NF $\kappa$ B subunit p65 that is only accessible when NF $\kappa$ B is activated and bound to  $\kappa$ B consensus sites. A horseradish peroxidase-conjugated secondary antibody provides colorimetric detection of the bound complex at 450 nm. NF $\kappa$ B activity requires nuclear translocation, so the simultaneous assessment of NF $\kappa$ B activity

TABLE 3. SERUM AND PLASMA MEASURES IN RATS FED TART CHERRY-SUPPLEMENTED (CHE) DIET AND CARBOHYDRATE-SUPPLEMENTED CONTROL (CON) DIET

	CON	CHE
Total cholesterol (mg/dL)	207 ± 14	185 ± 15*
Total triglycerides (mg/dL)	284 ± 23	241 ± 18*
Glucose (mg/dL)	139 ± 8	117 ± 7*
Insulin (pg/mL)	8.9 ± 0.5	7.1 ± 0.5*
IL-6 (pg/mL)	289 ± 31	191 ± 22*
TNF-α (pg/mL)	43 ± 5	26 ± 4*

Data are mean ± SEM values (n = 12 per group). \*P < .05, versus CON.

in the cytosolic fraction served as a control for successful cell fractionation.

Statistical methods

Transcript differences are determined as ± SD using the ΔΔCt method as described by Livak and Schmittgen,<sup>24</sup> using the PCR Array data analysis web portal of SABiosciences (<http://www.sabiosciences.com/pcr/arrayanalysis.php>). All other experimental values are expressed as ± SEM and compared using a two-tailed Student's *t* test. For all measures, a value of *P* < .05 was considered statistically significant.

RESULTS

Fasting glucose, insulin, total cholesterol, and triglyceride were significantly reduced in the tart cherry-fed animals. Plasma IL-6 and TNF-α were reduced by 44% and 40%, respectively (Table 3). Despite pair-feeding and matched caloric intake, tart cherry intake was associated with a lower body weight (Table 4). This difference was not significant until week 8 of the 12-week study. DEXA scan results indicated that by week 12, tart cherry reduced relative fat mass (-18%, *P* < .05) and increased lean body mass (+14%, *P* < .05). These treatment results were apparent after week 4 (Table 4).

Tart cherry was associated with a nonsignificant increase in terminal heart and kidney weight but a statistically sig-

TABLE 4. SERIAL BODY WEIGHT AND DEXA MEASURES IN RATS FED TART CHERRY-SUPPLEMENTED (CHE) DIET AND CARBOHYDRATE-SUPPLEMENTED CONTROL (CON) DIET

	Week 0	Week 4	Week 8	Week 12
Body weight				
CON	188 ± 9	334 ± 17	445 ± 17	517 ± 25
CHE	191 ± 6	343 ± 19	433 ± 22	486 ± 32*
% lean body mass				
CON	58 ± 4	44 ± 2	33 ± 2	26 ± 2
CHE	55 ± 3	47 ± 3	38 ± 3*	34 ± 2*
% fat mass				
CON	29 ± 3	44 ± 2	55 ± 3	63 ± 3
CHE	33 ± 2	41 ± 3	48 ± 3*	54 ± 4*

Data are mean ± SEM values (n = 6 per group). \*P < .05, versus time-matched CON.

TABLE 5. TERMINAL ORGAN AND TISSUE WEIGHTS EXPRESSED RELATIVE TO TIBIAL LENGTH IN RATS FED TART CHERRY-SUPPLEMENTED (CHE) DIET AND CARBOHYDRATE-SUPPLEMENTED CONTROL (CON) DIET

	g/tibial length (cm)	
	CON	CHE
Heart weight	0.29 ± 0.04	0.35 ± 0.04
Liver weight	6.8 ± 0.3	8.5 ± 0.4*
Kidney weight	0.9 ± 0.1	1.1 ± 0.1
Retroperitoneal fat pad weight	6.4 ± 0.3	5.3 ± 0.2*
Epididymal + perirenal fat pad weight	3.9 ± 0.2	4.1 ± 0.3

Data are mean ± SEM values (n = 12 per group). \*P < .01, versus CON.

nificant increase in liver weight (Table 5). These effects are also observed in rats given PPAR agonist drugs, which causes proliferation of cellular peroxisomes and increased organ weight.<sup>26-29</sup> Retroperitoneal fat pad weight was reduced (-17%, *P* < .05), whereas the combined epididymal fat plus perirenal fat pad weight was not significantly affected by cherry (Table 5).

We further explored treatment effect on the retroperitoneal fat pad. Tart cherry diet significantly reduced retroperitoneal fat expression of TNF-α (3.2-fold reduction, *P* < .01) and IL-6 (3.6-fold reduction, *P* < .01) (Fig. 1). Gene expression in retroperitoneal fat was also altered by cherry. PPAR-α mRNA is significantly increased (3.4-fold, *P* = .014) by tart cherry diet. PPAR-γ mRNA was also increased (2.7-fold), and this change approached statistical significance (*P* = .122). Both NFκB and its activation inhibitor IKBα are reduced by cherry. IKBα transcription is regulated by PPARs, and reduced adipose tissue expression and activity of IKBα could reduce NFκB-related inflammation. In fact, several NFκB-regulated transcripts are expectedly reduced in retroperitoneal fat by tart cherry, including TNF-α (2.9-fold, *P* = .006) and IL-6 (-4.5-fold, *P* = .013) (Table 6). Finally, NFκB activity in nuclear extracts of retroperitoneal fat was significantly reduced by cherry (Fig. 2).

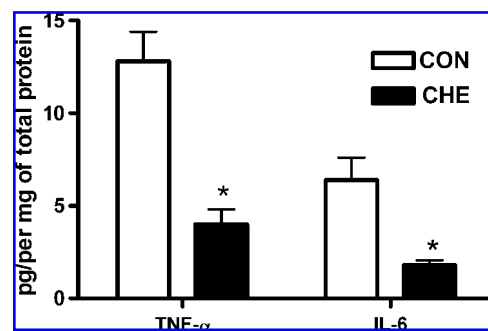


FIG. 1. Retroperitoneal fat IL-6 and TNF-α expression in rats fed tart cherry-supplemented (CHE) diet and carbohydrate-supplemented control (CON) diet. Data are mean ± SEM values (n = 6 per group). \*P < .05 versus CON.

TABLE 6. PCR ARRAY DATA IN EXPRESSED IN FOLD CHANGE IN mRNA IN RETROPERITONEAL FAT IN RATS FED TART CHERRY DIET, RELATIVE TO CONTROL DIET

Gene	Fold change by cherry diet	P value
PPAR- $\alpha$	+3.4	.014
PPAR- $\gamma$	+2.7	.122
NF $\kappa$ B	-3.8	.130
IKB $\alpha$	-4.4	.022
TNF- $\alpha$	-2.9	.006
IL-6	-4.5	.013

Data are mean values ( $n = 4$  per group).

## DISCUSSION

Excessive fat accumulation in adipose tissue, liver, and other organs precipitates the development of metabolic changes that increase overall risk for morbidity. Abnormalities that often accompany obesity include hypertension, impaired glucose tolerance, insulin resistance, hyperinsulinemia, and dyslipidemia. Visceral obesity, which is characterized by excess fat storage in and around the abdomen, is the prime cause of the metabolic abnormalities and therefore represents an important target in the treatment of metabolic syndrome. Adipose tissue is now clearly understood to be an endocrine organ and a major site of chronic low-grade inflammation that is associated with obesity. Adipose tissue-derived cytokines and “adipokines” play important roles in energy homeostasis, local and systemic energy inflammation, and insulin resistance. Specifically, abdominal fat is a primary source of adipokines and is therefore associated with greater pathologic consequence compared to other fat depots.

Within white adipose tissue, TNF- $\alpha$  is expressed in adipocytes, stromovascular cells, and macrophages. However, TNF- $\alpha$  produced by the adipocytes has only a local effect because it cannot be secreted; it is macrophage-produced TNF- $\alpha$  that is secreted and thus contributes to plasma TNF- $\alpha$ .<sup>30</sup> The expression of TNF- $\alpha$  in white adipose tissue correlates with obesity and insulin resistance.<sup>31</sup> Besides its role in white adipose tissue, TNF- $\alpha$  increases the expression of genes involved in the *de novo* synthesis of fatty acids and decreases mRNA transcripts involved in fatty acid oxidation

in liver. TNF- $\alpha$  also regulates the expression of other adipokines in white adipose tissue, such as IL-6. Approximately one-third of plasma IL-6 is produced by adipocytes, and IL-6 synthesis and secretion are approximately three times greater in visceral fat compared to subcutaneous fat.<sup>32</sup> The plasma levels of IL-6 positively correlate with fat mass, obesity, impaired glucose tolerance, and insulin resistance and thus could be used to predict the development of type 2 diabetes and cardiovascular disease.<sup>32,33</sup> IL-6, like TNF- $\alpha$ , modulates the insulin sensitivity of the liver and of skeletal muscle, thereby supporting the notion that cytokines produced by the adipose tissue influence whole-body insulin sensitivity.

### Anthocyanins and PPAR activation

The ligand-activated transcription factors of the PPAR family are involved in the regulation of inflammation and energy homeostasis and represent important targets for obesity, obesity-induced inflammation, and metabolic syndrome. The PPAR isoforms ( $\alpha$ ,  $\gamma$ , and  $\delta$ ) share a common mode of action that involves heterodimerization with the nuclear receptor retinoid X receptor and subsequent binding to specific PPAR-responsive elements in the promoter region of target genes.

In abdominal fat, we observed here that cherry intake reduced inflammation and reduced expression of inflammation-related genes. This effect may be related to bioavailable tart cherry anthocyanin effects on adipose PPARs. Current results show that the cherry-enriched diet was associated with enhanced PPAR isoform mRNA in retroperitoneal fat. The effect of tart cherry on PPAR isoforms is supported by previous findings with concentrated anthocyanins or anthocyanin-rich botanical extracts. Munoz-Espada and Watkins<sup>11</sup> showed in prostate cancer cells that cyanidin increases PPAR- $\gamma$  expression. Xia *et al.*<sup>12</sup> showed that isolated anthocyanins induced cholesterol efflux from macrophages in a PPAR- $\gamma$ -dependent manner and that anthocyanins increased both PPAR- $\gamma$  expression and PPAR- $\gamma$  transcriptional activity in a dose-dependent fashion. Park *et al.*<sup>9</sup> showed in diabetic mice that diets enriched with anthocyanin-rich mulberry leaf extract reduced glucose, insulin, and triglyceride while increasing liver and fat tissue expression of PPAR- $\gamma$ , PPAR- $\alpha$ , and liver lipoprotein lipase.

In the current study, tart cherry diet was associated with significantly increased abdominal fat PPAR- $\alpha$  mRNA expression. PPAR- $\alpha$  affects multiple target genes involved in lipid metabolism and inflammation. We previously demonstrated in lean animals fed a low fat diet that tart cherry intake was associated with increased liver PPAR- $\alpha$  mRNA, reduced liver neutral lipid fat content, enhanced hepatic acyl-coenzyme A oxidase activity, and reduced fatty acid synthase activity.<sup>18</sup> In the current model, the effect of tart cherry intake on reduced body weight, total fat mass, hyperlipidemia, abdominal fat mass, and inflammation may be derived from one of more of these PPAR- $\alpha$ -related mechanisms. PPAR- $\alpha$  agonists have a clear anorexic effect resulting in decreased food intake,<sup>34</sup> but the pair-feeding

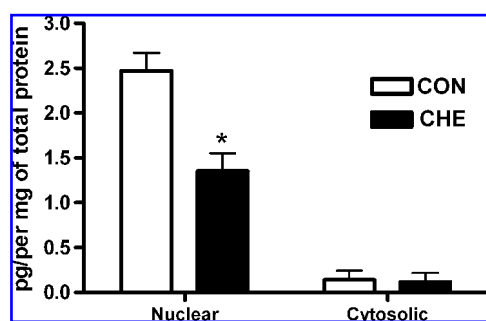


FIG. 2. Retroperitoneal fat NF $\kappa$ B activity in nuclear and cytosolic extracts from rats fed tart cherry-supplemented (CHE) diet and carbohydrate-supplemented control (CON) diet. Data are mean  $\pm$ SEM values ( $n = 6$  per group). \* $P < .05$  versus CON.

paradigm used here effectively eliminates that parameter from the possible mechanisms of effect. The mechanisms of PPAR effect on inflammation is likely multifaceted. PPAR- $\alpha$  activation reduces adipocyte size,<sup>35</sup> and smaller adipocytes secrete less inflammatory cytokines versus larger adipocytes. PPAR- $\alpha$  activation also enhances the transcription of an inhibitor of pro-inflammatory NF $\kappa$ B, IKB $\alpha$ , a PPAR-regulated transcript that limits the nuclear translocation and subsequent activation of NF $\kappa$ B. Additionally, PPAR activation may result in systemic events likely originating from liver, such as altered hepatic production of pro-inflammatory acute-phase proteins.

In the current study, tart cherry diet was also associated with increased abdominal fat PPAR- $\gamma$  mRNA expression. Although the degree of change approached statistical significance ( $P = .122$ ), the difference may still carry biological significance. We previously demonstrated in lean animals fed a low fat diet that tart cherry intake increased liver PPAR- $\gamma$  mRNA.<sup>18</sup> PPAR- $\gamma$  regulates genes important for adipogenesis, lipid metabolism, and glucose control.<sup>36-42</sup> Because we observed reduced rather than enhanced fat mass, the lipid oxidation-promoting effects of PPAR- $\alpha$  agonism appear to have overcome the effects of PPAR- $\gamma$  agonism on lipid balance. Similar to PPAR- $\alpha$ , PPAR- $\gamma$  is involved in governing the inflammatory response. Inflammatory adipokines mainly originate from macrophages residing within adipose tissue. PPAR- $\gamma$  agonists can alter macrophage phenotype from "classically activated," pro-inflammatory macrophages to the "alternatively activated," anti-inflammatory phenotype,<sup>43</sup> and PPAR- $\gamma$  agonists may also induce macrophage-specific cell death.<sup>44</sup> Finally, PPAR- $\gamma$  activation could alter downstream NF $\kappa$ B activity and the resulting pro-inflammatory genes, as is found with PPAR- $\alpha$  activation.

In correlation with effects on PPARs, cherry diet was associated with reduced retroperitoneal fat nuclear NF $\kappa$ B. Because NF $\kappa$ B is a vital regulator of inflammation, including the expression of TNF- $\alpha$  and IL-6, the diet effect on NF $\kappa$ B may be responsible for the reduced expression of tissue TNF- $\alpha$  and IL-6 observed here (as shown in Fig. 1). However, we cannot rule out the involvement of additional transcription factors in this effect, such as activator protein 1. As TNF- $\alpha$  and IL-6 are regulated by multiple transcription factors, a broader assessment of transcription factor activation in the fat tissue is warranted. Also, because whole fragments of adipose tissue were analyzed, it is uncertain which cell types within the retroperitoneal fat were most affected. For example, cherry diet could have affected NF $\kappa$ B activity in adipocytes, resident macrophages, and/or endothelial cells. However, the collective result is clear—the addition of tart cherry reduced inflammation in retroperitoneal fat. The determination of affected cell subtypes requires further investigation.

### Results in context

The current study uses an anthocyanin-rich whole food. However, the results may be impacted by the complex

polypharmacy of diverse bioavailable cherry phytochemicals and by their varied sugar moieties, which affect absorption, distribution, metabolism, and excretion. Available literature is sparse when specifically relating whole foods containing anthocyanins to the phenotypes of obesity, hyperlipidemia, insulin resistance, glucose tolerance, and inflammation. We previously reported that intake of tart cherry (1% wt/wt of diet) significantly reduced fasting glucose, insulin, total cholesterol, and triglyceride in the lean Dahl-salt sensitive rat model.<sup>18</sup> Prior *et al.*<sup>45</sup> examined the effect of anthocyanin-rich whole blueberry and strawberry consumption on dietary fat-induced obesity. Mice were fed a diet containing 10%, 45%, or 60% kilocalories from fat, with or without freeze-dried whole berry powders at 10% (wt/wt) of diet. Animals were fed *ad libitum*, and after 80 days, blueberry-fed mice had 14% higher body weight, 27% higher percentage body fat, and 32% higher perigonadal (epididymal) fat weight than controls. Strawberry-fed mice had 1% higher body weight and 11% higher percentage body fat but no change in epididymal fat weight versus control. However, parallel studies using matched content of their respective isolated berry anthocyanins showed reduced body weight and reduced percentage body fat. This difference between whole fruit and extract was evident despite the matched carbohydrate-derived calories and total calorie content per gram between diets. The difference between *in vivo* effects of the whole berry and isolated anthocyanins is unknown. The specific profile of sugars present in the whole foods and the presence of other phytochemicals may alter bioavailability and *in vivo* effects. In addition, a pair-feeding approach, as we used in the current study, may be required to eliminate the confounder of food intake. For example, whole blueberry-fed rats ate roughly 12% more calories per day than control rats. Given the length of the study, this may have contributed to weight gain or adiposity. However, the whole strawberry diet increased percentage body fat in the absence of greater average daily food intake, showing further diverging effects between the fruits. Indeed, the bioavailability and *in vivo* effects of anthocyanin-containing fruits are not universal, which requires detailed comparative studies like those of Prior *et al.*<sup>45</sup> and Joseph and co-workers.<sup>46,47</sup>

Studies with anthocyanin-rich extracts tend to support our current results. Tsuda *et al.*<sup>48</sup> showed that in high-fat fed mice, an anthocyanin-rich extract from purple corn significantly reduced fat mass, hyperlipidemia, hyperinsulinemia, and blood glucose. The anthocyanin extract-enriched diet also significantly reduced tissue lipid accumulation and the activity of enzymes that promote fat storage. Xia *et al.*<sup>8,49</sup> showed in atherosclerosis-prone mice that diets supplemented with an anthocyanin-rich extract from black rice significantly reduced atherosclerosis, total cholesterol, triglyceride, and tissue cholesterol and increased high-density lipoproteins. Jayaprakasam *et al.*<sup>50</sup> conducted a study in mice using Cornelian "cherry." This is not a true cherry of the agriculturally employed *Prunus* species, but is rather the red fruit of an ornamental dogwood tree (*Cornus mas*). These researchers reported that C57Bl/6 mice ingesting

Cornelian cherry-derived anthocyanin extract with a high-fat diet showed a significant decrease in weight gain and hepatic lipid accumulation while maintaining blood glucose control. Our findings in a whole-food model reflect those obtained with anthocyanin-rich botanical extracts. However, we cannot exclude the benefits of other tart cherry-derived phytochemicals, including quercetin, melatonin, isorhamnetin, and kaempferol. These compounds may exert independent or synergistic effects towards our observed phenotypes.

In summary, tart cherry-enriched diets were associated with significantly reduced body weight, abdominal fat, reduced blood lipids, reduced plasma inflammation, and reduced fasting glucose. In addition, tart cherry intake was associated with increased expression of PPAR isoforms and PPAR-related genes in abdominal fat, reduced abdominal fat IL-6 and TNF- $\alpha$ , and reduced NF $\kappa$ B activity. Future studies will histologically examine the abdominal fat for the effect of tart cherry on macrophage presence and on the size and number of adipocytes; these parameters affect the levels of both local and systemic adipokines. As a rich whole-food source of anthocyanins, tart cherry-enriched diets may modify several key risk indicators for type 2 diabetes. Further studies are needed in human subjects with metabolic syndrome to ascertain the degree and spectrum of tart-cherry derived clinical benefits.

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