Research Article

Tart Cherry Juice Reduces Plasma Triglycerides and CVD Risk Factor, But Does not Affect Indirect Measures of Insulin Resistance, in Overweight and Obese Subjects: A Randomized, Crossover Pilot Study

Keith R Martin[1]*, Jennifer Bopp[2] and Lacey Burrell[2]

[1]Center for Nutraceutical and Dietary Supplement Research, University of Memphis, Tennessee, United States

[2]Healthy Lifestyles Research Center, Arizona State University, Phoenix, AZ, United States

Corresponding Author: Keith R Martin, Center for Nutraceutical and Dietary Supplement Research, University of Memphis, Tennessee, United States.

Abstract

Background: Cardiovascular disease (CVD) is the leading cause of death for men and women globally in both developed and developing countries, thus is a significant health problem. Obesity and overweightness (BMI ≥ 25 kg/m^2), occurring in 67% of the U.S. population, and insulin insensitivity (pre-diabetes) are co-morbidities frequently occurring concomitantly with CVD. Accumulating epidemiological evidence shows that polyphenol-rich diets rich in fruits can significantly reduce CVD risk.

Methods: In this randomized, placebo-controlled crossover pilot study, we recruited 10 participants (38.1 ± 12.5 y; 9 females, 2 males) with BMI > 25.0 (32.2 ± 4.6 kg/m^2; 5 obese, 5 overweight) to consume 8 fl oz. daily of either 100% tart cherry juice (TCJ) or an alternate placebo beverage, for 4 weeks each with a 2-week intervening washout period. Fasting blood samples were collected at the beginning and end of each arm for measurement of biomarkers of dyslipidemia and glycemia.

Results: Total cholesterol (TC) was not different between treatments (p > 0.05) but plasma triglycerides (TG) and the CVD risk factor ratio TG/HDL-C were statistically decreased 10% and 17%, respectively (p < 0.05) after TCJ consumption. A trend existed for VLDL, which was reduced 15%. LDL-C and HDL-C were not different between treatments. Baseline fasting glucose (FG) and insulin levels were 99 ± 7 mg/dl and 12.8 ± 5.8 uIU/ml, respectively, with half having FG > 100 mg/dL. HOMA, QUICKI, and McAuley indices of insulin resistance were modulated suggesting pre-diabetes, but values were not significantly different between groups at study completion.

Conclusion: Collectively, the data suggest that 100% TCJ can reduce CVD risk by reducing plasma TG and some routinely used risk factors. ratios

Keywords: Tart Cherry Juice; Metabolic Syndrome; Insulin Resistance; Triglycerides; Overweight/Obese

Abbreviations

BMI: Body Mass Index; CVD: Cardiovascular Disease; FG: Fasting Glucose; FI: Fasting Insulin; HOMA: Homeostatic Model Assessment; QUICKI: Quantitative Insulin Sensitivity Check Index; TCJ: Tart Cherry Juice; TC: Total Cholesterol; TG: Triglycerides; TyG: Triglyceride and Glucose Index

Tart Cherry Juice Reduces Plasma Triglycerides and CVD Risk Factor, But Does not Affect Indirect Measures of Insulin Resistance, in Overweight and Obese Subjects: A Randomized, Crossover Pilot Study

68

Introduction

Cardiovascular disease (CVD) is the leading cause of death for men and women globally in both developed and developing countries, thus is a significant health problem. Hyperlipidemia and dysglycemia are risk factors for CVD and collectively damage the endothelium and contribute to atherogenesis and insulin resistance [1]. Obesity and overweightness (BMI ≥ 25 kg/m^2), occurring in approximately two thirds of the U.S. population, and insulin insensitivity (pre-diabetes) are co-morbidities frequently occurring concomitantly with CVD and often with elaboration of the same underlying processes and subsequent damage to the endothelium. Those with metabolic syndrome (MetS) are especially at significant, increased risk of CVD related to plaque buildup in artery walls (e.g., stroke and peripheral vascular disease) and type-2 diabetes.

In the U.S., it is estimated that over 50 million Americans have metabolic syndrome, which has now is a global epidemic [2-3]. Metabolic syndrome, known also as syndrome X, is defined by WHO and the American Heart Association as a pathologic condition characterized by three or more of the following conditions including abdominal obesity (waist circumference > 40" in males and > 35" in females), triglyceride level ≥ 150 mg/dL), HDL cholesterol < 40 mg/dL in males or < 50 mg/dL in females, systolic blood pressure ≥ 130 mmHg, or diastolic blood pressure ≥ 85 mmHg, and fasting glucose ≥ 100 mg/dL. Current medical treatments often fail to retard the progress of cardiovascular disturbances, and plant-derived polyphenols are increasingly being investigated as a possible way to provide safe and effective complementary therapy [4].

Epidemiologic studies support that high dietary polyphenol consumption from fruits and vegetables is inversely associated with CVD risk [6-8]. Cherries are a nutritionally dense fruit that are particularly high in polyphenols, specifically anthocyanins, thus are potentially protective against dyslipidemia and dysglycemia associated with CVD risk [9,11]. Several recent studies using hyperinsulinemic and hypercholesterolemic models supplemented with tart cherry powder demonstrated significantly decreased total cholesterol, triglycerides, insulin, and fasting glucose levels [7]. Moreover, dietary tart cherry powder (up to 10% w/w) reduced metabolic syndrome and oxidative stress, as well as triglyceride, total cholesterol, insulin, and the oxidative stress biomarker 8-isoprostane, while improving HDL [6]. TCJ also significantly increased plasma antioxidant activity and reduced risk factors for CVD and inflammation in other phenotypes of the metabolic syndrome [8-9]. In other studies, cherry-based diets reduced pro-inflammatory cytokines IL-6 and TNF-alpha up to 50% and improved insulin secretion in response to varying glucose loads and demonstrated glucose-lowering effects [9-11]. Collectively, considerable evidence suggests that TCJ can be effective in reducing myriad parameters associated with MetS such as dyslipidemia and dysglycemia. However, these results require additional clinical trials to corroborate the results.

In this study, we conducted a 12-week randomized, 2 x 2 crossover, placebo-controlled dietary intervention using overweight (25.0-29.9 kg/m^2) and obese (≥ 30.0 kg/m^2) participants. Study participants were randomized to consume 8 fl oz daily of either placebo (artificial cherry-flavored, anthocyanin-free beverage) or 100% tart cherry juice (TCJ) for 4-wk, followed by a 2-wk washout period, after which they consumed 8 fl oz daily of the alternate beverage for 4 wk. Biomarkers of dyslipidemia, CVD risk (using CVD risk ratios), and glucose homeostasis (insulin sensitivity using indirect indices) were measured or calculated at the beginning and end of both intervention phases to determine possible associations between parameters in an at-risk cohort (Figure 1).
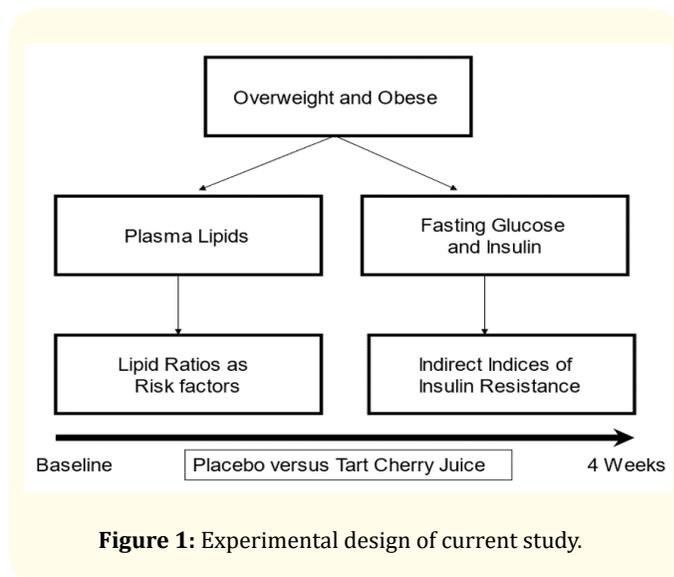

Figure 1: Experimental design of current study.

Methods

Participants, study design, and intervention

This study was a 10-week 2 x 2 crossover, randomized, placebo-controlled dietary intervention in overweight and obese partici-

Tart Cherry Juice Reduces Plasma Triglycerides and CVD Risk Factor, But Does not Affect Indirect Measures of Insulin Resistance, in Overweight and Obese Subjects: A Randomized, Crossover Pilot Study

69

pants (BMI ≥ 25.0 kg/m^2). Participants that were ≥ 18 years of age, not pregnant, not diabetic, with no unresolved infections or diseases (inflammation, diabetes, CVD, cancer and liver disease), and nonsmokers were recruited for this study. Histories of medication and dietary supplement use were collected and those taking anti-inflammatory or lipid-lowering medications were excluded. This study was approved by the Institutional Review Board at Arizona State University. Informed consent was obtained from each respondent for prior to screening and/or entering the study.

After enrollment, subjects were randomly assigned using an online computer randomizer program to consume either 8 oz of 100% TCJ (R.W. Knudsen, Chico, CA) or a generic anthocyanin-free fruit punch for 4 wk. After a 2-wk washout period, subjects were switched to the alternate beverage for an additional 4 wk. Participants were instructed to refrain from consuming any other anthocyanin-containing fruits and juices during the study period and provided a list of products to avoid and/or minimize. Dietary, medical, and physical activity questionnaires and records were collected and analyzed using Food Processor Nutrition and Fitness Software (ESHA, version 8.5; Salem OR) to determine any changes in food or beverage consumption.

Biomedical analysis and anthropometric measurement

After a ≥ 12-hour fast, fasting blood samples were drawn by standard venipuncture protocols into each of 3 evacuated tubes (Fisher Scientific, Hampton, NJ). Total, HDL-, and LDL-cholesterol, and TG were measured directly using a blood chemistry analyzer (Piccolo, Abaxis Inc., Union City, CA). VLDL-cholesterol was calculated as was the CVD risk ratios (i.e., TC/HDL, LDL/HDL). Plasma was separated by centrifugation at 1,100 x g at 4°C for 20 min and archived in 0.5 ml aliquots at -80°C until analysis.

Anthropometric measurements including body weight, height and body composition (body fat percentage, fat mass, fat-free mass, total body water, basal metabolic rate (BMR)) were measured at each visit by bioelectrical impedance (TBF 300A Tanita Body Composition Analyzer, Tokyo, Japan). Dietary records and physical activity questionnaires were collected and reviewed at each visit.

Analysis and calculation of insulin resistance

Fasting glucose and insulin were measured using COBAS (Roche Diagnostics, North America, Indianapolis, IN) and Immulite (Sie-mens USA, Tarrytown, NY) automated chemiluminescent blood chemistry analyzers. Insulin sensitivity was calculated using a panel of indices including 1) the homeostatic model assessment (HOMA) calculated as glucose (mg/dl) x insulin (μU/ml)/22.5, (2) the quantitative insulin-sensitivity check index (QUICKI) calculated as 1/(log [insulin in mU/l] + log [glucose in mg/dl]) and (3) the McAuley index calculated as exp[2.63 - 0.28 ln (insulin in mU/L) - 0.31 ln (triglycerides in mmol/L). The glucose/insulin ratio and 1/insulin reciprocal were also calculated. The triglyceride and glucose index (TyG index) has become a more frequently used option for evaluating insulin sensitivity due to accessible, inexpensive biochemical markers needed for its calculation. TgY index is calculated as Ln ((fasting glucose [mg/dL] x triglycerides [mg/dL])/2).

Statistical Analysis

All data obtained during this study were analyzed using the Statistical Package for the Social Sciences (SPSS) version 17.0.2, 2009. Values were expressed as means ± standard deviation (SD). Differences in participant plasma lipid, insulin, markers of insulin sensitivity, glucose, electrolyte and metabolite concentrations were considered significant at $P < 0.05$ and considered a trend at P $0.1 \leq P > 0.05$. Repeated measures and multiple paired-samples t-tests were conducted. All data were tested for normality and transformed by the Friedman Test and Wilcoxon Signed Ranked Test if required.

Results

Our initial screen of subjects in this study indicated the population was overweight and obese (BMI = 32.2 kg/m2) thus more likely to demonstrate dyslipidemia and insulin resistance (Table 1). The initial lipid panel suggested values were either within or close to the normal range for each parameter. We also noted that the average glucose concentration was 99 ± 7 mg/dL and insulin was 12.12 ± 9.89 uU/mL indicating both analyte values were at the upper ranges of the normal reference range suggesting movement towards insulin resistance. In essence, this cohort displayed existence of quasi-hyperlipidemia and quasi-hyperglycemia and increased risk for chronic disease. We enrolled 10 subjects with BMI > 25.0 mg/k^2 into the pilot study (mean ± SD age: 38 ± 12; 9:2 females: males). Fasting glucose was 99 ± 7 with half displaying values > 100 mg/dL indicative of glucose intolerance. LDL-C was 109 ± 19 and TG was 153 ± 58 mg/dL, respectively. There were no differences in average macronutrient intake or energy consumption between test groups (data not shown).

Tart Cherry Juice Reduces Plasma Triglycerides and CVD Risk Factor, But Does not Affect Indirect Measures of Insulin Resistance, in Overweight and Obese Subjects: A Randomized, Crossover Pilot Study

70

Characteristics		Baseline		
n (male/female)		10 (2/8)		
Age	y	38	±	12
BMI	kg/m²	32.2	±	4.6
Total cholesterol	mg/dL	188	±	21
	mM	4.86	±	0.54
LDL Cholesterol	mg/dL	109	±	19
	mM	2.82	±	0.49
HDL Cholesterol	mg/dL	48	±	10
	mM	1.24	±	0.03
VLDL Cholesterol	mg/dL	31	±	11
	mM	0.81	±	0.28
Non-HDL Cholesterol	mg/dL	140	±	15.5
	mM	3.62	±	0.4
Triglycerides	mg/dL	153	±	58
	mM	1.73	±	0.65
Blood glucose	mg/dL	99	±	7
	mM	5.5	±	0.4
Insulin	uU/mL	12.12	±	9.89
	pM	72.7	±	59.3

Table 1: Baseline characteristics of study cohort.

We next explored whether lipid panels would differ between pre and post samples when subjects consumed either the placebo beverage or tart cherry juice. We noted no significant differences between the two or with time for any of the major cholesterol particles including HDL-C, LDL-C, and VLDL-C (Table 2). We did, however, observe a significant reduction of plasma triglycerides from 141.7 to 127.2 mg/dL, a significant 10.1%.

The relative contribution of specific lipid particles is important in determining chronic disease risk. Thus, we next examined the impact on well-recognized and used ratios of lipids to determine if risk was present. There were no significant differences between pre and post values in either the placebo or tart cherry juice group and no differences between the two regarding a time effect (Table 3). However, we did observe that the TG/HDL was significantly lower ($p < 0.05$) after tart cherry juice consumption and at the upper limit of optimal for total cholesterol/HDL ratio and TG/HDL. The values for LDL/HDL and HDL/LDL were within normal range for relative risk. The data suggest that triglycerides had a major impact on triglyceride-dependent calculations. We noted a significant reduction in serum triglycerides after consumption for 4 weeks of tart cherry juice (147 ± 5 versus 127 ± 45) when compared to the placebo group. We noted a trend ($0.1 < p > 0.05$) for VLDL. There were no significant differences at the end of the study for TC, LDL-C and HDL-C between either beverage group. We also noted a significant reduction in the TG/HDL-C risk factor by 17% indicating a less atherogenic lipid profile and subsequently decreased risk for the development of coronary disease [10].

		Optimal		Pre			Placebo	Post			Pre			TCJ	Post		
	Units			Pre				Post			Pre				Post		
Total cholesterol	mg/dL	< 200	188.1	±	21.0	177.4	±	23.0	185.9	±	25	188.0	±	25.6
	mM	< 5.2	4.9	±	0.5	4.6	±	0.6	4.8	±	0.6	4.9	±	0.7
LDL Cholesterol	mg/dL	< 130	109.3	±	19.1	103.7	±	21.5	112.0	±	23	115.0	±	20.2
	mM	< 3.36	2.8	±	0.5	2.7	±	0.6	2.9	±	0.6	3.0	±	0.5
HDL Cholesterol	mg/dL	> 60	48.0	±	10.2	46.7	±	8.4	45.4	±	9.5	43.2	±	17.4
	mM	> 1.55	1.2	±	0.3	1.2	±	0.2	1.2	±	0.2	1.1	±	0.4
VLDL Cholesterol	mg/dL	< 30	30.6	±	11.7	27.3	±	13.0	28.2	±	8.7	25.6	±	7.7
	mM	< 0.78	0.8	±	0.3	0.7	±	0.3	0.7	±	0.2	0.7	±	0.2
Non-HDL-C	mg/dL	< 130	140.1	±	10.8	130.7	±	14.6	140.5	±	15	144.8	±	8.2
	mM	< 3.37	3.6	±	0.3	3.4	±	0.4	3.6	±	0.4	3.7	±	0.2
Triglycerides	mg/dL	< 150	153.4	±	58.4	135.9	±	65.3	141.7	±	43	127.2	±	37.3*
	mM	< 1.69	1.7	±	0.7	1.5	±	0.7	1.6	±	0.5	1.4	±	0.4*
*p < 0.05													

Table 2: Blood lipids of individuals cosuming placebo or tart cherry brverages for four weeks.

Citation: Keith R Martin*., et al.* "Tart Cherry Juice Reduces Plasma Triglycerides and CVD Risk Factor, But Does not Affect Indirect Measures of Insulin Resistance, in Overweight and Obese Subjects: A Randomized, Crossover Pilot Study". *Acta Scientific Nutritional Health* 6.9 (2022): 67-75.

Tart Cherry Juice Reduces Plasma Triglycerides and CVD Risk Factor, But Does not Affect Indirect Measures of Insulin Resistance, in Overweight and Obese Subjects: A Randomized, Crossover Pilot Study

71

	Optimal			Placebo						TCJ		
Ratio		**Pre**			**Post**			**Pre**			**Post**		
TG to HDL	< 1.3	3.4	±	1.5	3.1	±	1.8	3.4	±	1.6	2.8	±	1.1
Total to HDL	< 3.0, 3.5	4.0	±	0.8	3.9	±	0.8	4.2	±	0.8	4	±	0.7
LDL to HDL	< 2.5	2.4	±	0.6	2.6	±	0.6	2.5	±	0.6	2.5	±	0.5
HDL to LDL	> 0.4	0.4	±	0.5	0.5	±	0.4	0.4	±	0.4	0.4	±	0.9

Table 3: Calculated lipid ratios of individuals consuming placebo or tart cherry beverages for four weeks.

Glucose and insulin concentrations were at the upper level just under the upper limit for the normal range suggesting that half of participants were normoglycemic and half were hyperglycemic (Table 4). HOMA, McAuley, and G/I were elevated and indicated increased risk for chronic CVD risk. QUICKI, TyG index, and insulin ratio were all within the normal range. We measured fasting plasma insulin, glucose, and triglyceride concentrations and used these values to calculate the QUICKI and HOMA scores, McAuley index, and glucose/insulin and 1/insulin ratios to determined insulin sensitivity. We noted that all subjects displayed a shift in values consistent with an increase in insulin insensitivity or glucose intolerance. We calculated the McAuley index, which is dependent on triglyceride levels, and found all participants to exhibit values moving closer to the cutoff for insulin insensitivity. No significant effects between test beverages were noted for any of the indirect indices or plasma glucose and insulin. Glucose levels, however, did decrease by > 10% after tart cherry juice consumption, but not in the placebo group but fasting insulin did not change in either group. The results suggest pre-diabetes in this cohort with some indirect indices being better than others in revealing this occurrence.

		Optimal			Placebo						TCJ		
	Units		**Pre**			**Post**			**Pre**			**Post**		
Glucose	mg/dL	< 100	100.0	±	10.3	101.0	±	9.6	100.6	±	6.0	101.2	±	7.0
	mM	7.8-11.1	5.6	±	0.6	5.6	±	0.5	5.6	±	0.3	5.6	±	0.4
Insulin	uU/mL	5-15	12.8	±	5.8	13.2	±	6.8	13.3	±	7.9	13.3	±	5.8
	pM	34.7-138.9	89.2	±	40.6	92.0	±	47	92.5	±	55	92.4	±	40.2
HOMA		< 1	3.2	±	1.7	3.3	±	1.9	3.3	±	2.1	3.3	±	1.5
QUICKI		> 0.384	0.3	±	0.0	0.3	±	0	0.3	±	0	0.3	±	0.0
McAuley		> 6.3	3.9	±	0.5	3.7	±	0.4	3.5	±	0.4	3.9	±	0.4
TyG index		4.55-4.78	4.9	±	1.2	4.9	±	1.4	5.0	±	2.1	4.9	±	1.1
1/insulin		> 0.2	0.1	±	0.1	0.1	±	0.1	0.1	±	0.7	0.1	±	0.1
Glucose/insulin		> 16	9.5	±	5.3	10.0	±	5.4	12.1	±	8.4	10.9	±	5.7

Table 4: Plasma glucose and insulin concentrations and calculation of indirect indices of insulin resistance in individuals consuming either placebo or tart cherry beverages for four weeks.

Discussion

We demonstrate in this study a significant reduction of serum triglycerides in overweight and obese participants with impaired insulin sensitivity and at increased risk for CVD. We also observed a significant reduction in the TG/HDL-C risk ratio, an indicator of risk for cardiovascular events and all-cause mortality. We noted no significant changes in parameters of insulin sensitivity in a population that displayed increasing insulin resistance as determined by all six commonly used indirect indices. TG/HDL-C and total cholesterol/HDL-C supported dyslipidemia and increased chronic dis-

Tart Cherry Juice Reduces Plasma Triglycerides and CVD Risk Factor, But Does not Affect Indirect Measures of Insulin Resistance, in Overweight and Obese Subjects: A Randomized, Crossover Pilot Study

72

ease risk, whereas HDL-C/LDL-C and LDL-C/HDL-C ratios revealed borderline values, which would seem to correlate with half of participants being overweight and half being obese.

These data suggest that TCJ may reduce serum triglycerides and subsequently CVD risk in those persons already at increased risk. Although seemingly marginal, the contribution of triglyceride reduction to prophylaxis, in association with other dietary and lifestyle changes, may exert a considerable impact on reducing the long-term risk for chronic disease. Numerous other studies have shown similar effects on triglycerides in rodent models. Seymour., *et al.* showed a statistically significant 14% reduction in serum triglycerides, as well as plasma cholesterol and LDL in Dahl salt-sensitive rats after 90 days of freeze-dried cherries [8]. Whole tart cherry powder also significantly lowered fasting glucose and insulin, and a plasma marker of oxidative damage, while slightly raising HDL and significantly elevating blood antioxidant capacity [8]. The cherry-enriched diets also reduced the accumulation of triglyceride and cholesterol in the liver. Cherry extracts have also been shown to decrease hepatic triglycerides and cholesterol in numerous other rodent models [8,10,13].

We recruited individuals that were overweight and obese, an at-risk population, to test tart cherry juice. However, in two other human studies specifically using cherry products, lipids were unaltered after consumption of tart cherry juice supplement or Bing sweet cherries [7,14]. In these studies, test subjects were healthy adult male and females and perhaps not as amenable to the dietary intervention as adults at-risk for or with MetS. However, in mice fed a mixture of 25 polyphenol compounds from *Aspalathus linearis,* the source of Rooibus tea, hyperlipidemic mice, but not normal mice, displayed significant reductions in serum cholesterol, triglyceride and free fatty acid concentrations, as well as reductions in adipocyte size and number and dietary-induced hepatic steatosis [15-16]. These observations support that dietary supplementation may be effective in at-risk models but equivocal for healthy controls.

There is sufficient evidence to suggest that bioactive polyphenols, and anthocyanins, major components of tart cherry juice, might lower blood lipids such as serum triglycerides [13]. For example, a polyphenol-rich extract (2.5% in water) from flowers fed to rats for 9 weeks decreased body weight, size of epididymal fat,

serum triglycerides, and atherogenic index and hepatic lipids with reduced pancreatic lipase activity [14]. Male Wistar rats fed a high-fructose (65%) diet to induce metabolic syndrome and treated for 2 weeks with an ethyl acetate extract of amla (Indian gooseberry) displayed significant, reduced hypertriglyceridemia and hypercholesterolemia [15]. In mice fed a high-fat diet supplemented with 5% (w/w) polyphenol-rich black tea extract (BTPE) for 8 wk, BTPE inhibited pancreatic lipase activity *in vitro*, suppressed increases in body weight, parametrial adipose tissue mass, and liver lipid content. BTPE also suppressed increases in plasma triglyceride levels in a dose-dependent manner after oral administration of a lipid emulsion [16]. Polyphenol-rich pomegranate juice and one of its bioactive agents, viz., punicalagin, significantly and dose-dependently decreased triglyceride biosynthesis rate and content in macrophages by 30%, which appeared due to inhibition diacylglycerol acyltransferase, the rate-limiting enzyme in triglyceride biosynthesis [17]. In mice fed a high-fat diet (60% energy as fat), supplemented with dietary polyphenol (3.2 g/kg diet) for 16 wk, results showed reduced body weight gain, percent body fat, steatosis, hepatic stored triglycerides, and visceral fat weight compared with mice without treatment. EGCG, the most abundant polyphenolic flavonoid in tea, also reduced insulin resistance, blood glucose, and plasma cholesterol [18]. In a randomized crossover study of 12 normal weight men aged 22 + 3, antioxidant capacity and postprandial triglycerides were significantly lower after Montmorency cherries compared to placebo [19]. Thus, there is mechanistic and biological evidence to support the observed effects of tart cherry juice.

We did not note changes in other lipids including LDL-C, HDL-C, and TC. This agrees with the results of two other human studies showing no effect on plasma lipids in healthy humans. In these studies, consumption of sweet cherries or tart cherry concentrate did not change triglycerides, LDL-C, or VLDL-C concentrations, HDL-C, total cholesterol, lipoprotein particles, or lipoprotein size in healthy adults [7]. Several studies have demonstrated the ability of tart cherry juice to reduce various lipids including LDL-cholesterol and total cholesterol but the inconsistencies in observations among studies requires further elucidation [20-22].

In this study, we noted that all 10 participants displayed signs of insulin insensitivity using routinely employed indirect measures of insulin resistance. While the euglycemic hyperinsulinemic clamp

Tart Cherry Juice Reduces Plasma Triglycerides and CVD Risk Factor, But Does not Affect Indirect Measures of Insulin Resistance, in Overweight and Obese Subjects: A Randomized, Crossover Pilot Study

73

technique is the gold standard for determining insulin insensitivity, HOMA and QUICKI are the most widely used indirect indices for assessing insulin insensitivity with HOMA reportedly being less reproducible than QUICKI [23,24]. We used the McAuley index because it relies on serum triglycerides and insulin (versus glucose and insulin) for calculation and is argued to be the most sensitive and specific indirect method in subjects with normal glucose metabolism and reported to be predictive in those with hypertension and diabetes [22-24]. Subjects were normoinsulinemic but values for QUICKI, HOMA. 1/I and G/I were moving away from normal and towards insulin resistance. The most compelling marker was the McCauley index, which indicated a substantial degree of insulin insensitivity with all participants' values ranging from 4.8-4.9, which is considerably less than the 6.3 minimum value indicating normal insulin function. Fasting plasma glucose ranged from 88-117 mg/dL with 50% exhibiting values > 100 mg/dL, which meets the definition of impaired fasting glucose. In this study, we did not observe any changes in glycemia, either beneficial or detrimental, as reported by others after subjects consumed tart cherry juice, which may support the inclusion of tart cherry juice as a healthy dietary choice for those managing blood glucose [7,25]. Conversely, cherry extract fed to high fat diets in rodents suppressed circulating glucose, insulin, and hepatic triglycerides compared to control groups [8,11,26]. Bozzetto., *et al*. report that polyphenol-rich diets improved glucose metabolism by reducing plasma glucose AUC, increased early insulin secretion, and improved post-challenge oral glucose insulin sensitivity in people at high cardiometabolic risk in a controlled, randomized intervention trial [4]. In the current study, test subjects were at-risk with metabolic values in the high end of the normal range of values (with some participants exceeding the range), but not "high" risk as stated by Bozzetto., *et al*. for their cohort as a result, the latter may have been more amenable to intervention with perhaps more robust responses than the former cohort.

Triglyceride glucose (TyG) index is a novel inexpensive clinical surrogate marker for insulin resistance, which has been demonstrated to have high sensitivity and specificity in identifying metabolic syndrome [27,28]. Previous studies have shown that TyG index is associated with carotid atherosclerosis, coronary artery calcification and high risk of CVD [24]. Although TyG could potentially serve as a marker of insulin resistance (IR), epidemiological evidence on its relationship with the risk of atherosclerotic cardio-

vascular disease and long-term risk of mortality is limited [23]. In this study, we noted no differences between pre and post beverage consumption nor did we observe a difference between placebo ad TCJ groups. All means were within the normal range suggested for TyG suggesting this small cohort is not insulin resistant.

Tart cherry juice may contribute to euglycemia and minimize potential side effects of hyperglycemia. *In vitro*, anthocyanin extracts from tart cherries stimulated production of insulin by murine pancreatic cells by 50% compared to control cells [11]. In fact, a single dose of anthocyanins decreased fasting blood glucose levels by 19 percent and improved glucose tolerance by 29 percent [25]. Furthermore, fasting blood glucose levels had dropped by 50% compared to baseline and glucose tolerance had improved significantly after 12 weeks of flavonoid-rich green tea supplementation, rats displayed lower fasting plasma levels of glucose, insulin, triglyceride, and free fatty acid than the control rats consuming water. Insulin-stimulated glucose uptake of, and insulin binding to, adipocytes were increased as was basal and insulin-stimulated glucose uptake of adipocytes. Results demonstrated that polyphenol-rich green tea increased insulin sensitivity in rats [26-28]. In our study, we noted a directional, although non-significant, decrease in fasting glucose by 10.1% after consumption of tart cherry juice but no significant change in plasma insulin suggesting that tart cherry juice may have been decreasing fasting glucose, but was not yet at a level of significance.

In this study, we have shown a significant reduction in serum triglycerides and the CVD risk ratio TG/HDL in overweight and obese subjects who consumed anthocyanin-rich tart cherry juice, This agrees with the observations of Desai., *et al*. (2021) who showed that cherry juice consumption significantly improved blood pressure, fasting glucose, total cholesterol, and the total cholesterol: HDL ratio [29]. Results from numerous human, animal, and cell culture studies suggest that anthocyanins, a major component of tart cherries, may decrease blood glucose by slowing glucose production from complex carbohydrates, hepatic glucose output, decreasing production of glucagon by pancreas, and increasing hepatic glucose uptake and production of insulin by the pancreas [30]. The anthocyanin concentration may have been too low in juices to elicit a response or the time of consumption may have been too short in duration. Additionally, participants in this trial were displaying signs of developing insulin resistance but may not have progressed

Tart Cherry Juice Reduces Plasma Triglycerides and CVD Risk Factor, But Does not Affect Indirect Measures of Insulin Resistance, in Overweight and Obese Subjects: A Randomized, Crossover Pilot Study

74

far enough pathologically for the dietary intervention to exert beneficial effects in the four weeks of the intervention. Collectively, however, evidence exists suggesting that cherry consumption may promote healthy glucose regulation, but future studies are needed to confirm whether these findings translate to a reduced risk for diabetes.

Conclusions

We demonstrate in this study the significant 10% reduction of serum triglycerides in overweight and obese participates with impaired insulin sensitivity and at increased risk for CVD. We also observed a significant reduction in the TG/HDL-C risk ratio, an indicator of risk for cardiovascular events and all-cause mortality. We noted no significant changes in parameters of insulin sensitivity in a population that was progressing towards insulin resistance as determined by several commonly used indirect indices. These data suggest that TCJ may reduce serum triglycerides and subsequently CVD risk in those persons already at increased risk.

Authors' Contributions

KRM initiated and designed the study and secured the funding from the Cherry Marketing Institute. JB and LB recruited, screened, and provided informed consent to respondents under the supervision of KRM, as well as collected, processed, and analyzed, in part, data, samples and questionnaires. KRM interpreted the data and prepared the manuscript. All authors critically reviewed the manuscript.

Ethics Approval and Consent to Participate

This study was approved by the Arizona State University Institutional Review Board. Prior to entering nit the study, all respondents and participants provided written informed consent.

Competing Interests

The authors declare no competing financial and/or pesonal interests.

Bibliography

1. B Brannick., *et al.* "Prediabetes as a toxic environment for the initiation of microvascular and macrovascular complications". *Experimental Biology and Medicine* 241.12 (2016): 1323-1331.

2. MG Saklayen. "The Global Epidemic of the Metabolic Syndrome". *Current Hypertension Reports* 20.2. Current Medicine Group LLC (2018).

3. Edwards CM and Cusi K. "Prediabetes: A worldwide epidemic". *Endocrinology and Metabolism Clinics of North America* 45.4 (2016): 751-764.

4. L Bozzetto., *et al.* "Polyphenol-rich diets improve glucose metabolism in people at high cardiometabolic risk: a controlled randomised intervention trial". *Diabetologia* 58.7 (2015): 1551-1560.

5. JA Vita. "Polyphenols and cardiovascular disease: effects on endothelial and platelet function". *The American Journal of Clinical Nutrition* 81.1 (2005): 292-297.

6. DS Kelley., *et al.* "A review of the health benefits of cherries". *Nutrients* 10.3 (2018): 1-22.

7. DS Kelley., *et al.* "Consumption of bing sweet cherries lowers circulating concentrations of inflammation markers in healthy men and women". *The Journal of Nutrition* 136.4 (2006): 981-986.

8. EM Seymour., *et al.* "Regular tart cherry intake alters abdominal adiposity, adipose gene transcription, and inflammation in obesity-prone rats fed a high fat diet". *Journal of Medicinal Food* 12.5 (2009): 935-942.

9. Ghosh D and Scheepens A. "Vascular action of polyphenols". *Molecular Nutrition and Food Research* 53.3 (2009): 322-331.

10. PL Da Luz., *et al.* "High ratio of triglycerides to HDL-cholesterol predicts extensive coronary disease". *Clinics* 63.4 (2008): 427-432.

Tart Cherry Juice Reduces Plasma Triglycerides and CVD Risk Factor, But Does not Affect Indirect Measures of Insulin Resistance, in Overweight and Obese Subjects: A Randomized, Crossover Pilot Study

75

11. B Jayaprakasam., *et al*. "Amelioration of obesity and glucose intolerance in high-fat-fed C57BL/6 mice by anthocyanins and ursolic acid in cornelian cherry (Cornus mas)". *Journal of Agricultural and Food Chemistry* 54.1 (2006): 243-248.

12. RDRMIBDRJJASCAFG Iswaldi I. "Identification of phenolic compounds in aqueous and ethanolic rooibos extracts (Aspalathus linearis) by HPLC-ESI-MS (TOF/IT)". *Analytical and Bioanalytical Chemistry* 400.10 (2011): 3643-3654.

13. SP Shah K. "Effect of Anthocyanin Supplementations on Lipid Profile and Inflammatory Markers: A Systematic Review and Meta-Analysis of Randomized Controlled Trials". *Cholesterol* 22.20 (2018): 1-10.

14. CYHCLCLYLYLKCY Yang DJ. "Antiobesity and hypolipidemic effects of polyphenol-rich longan (Dimocarpus longans Lour.) flower water extract in hypercaloric-dietary rats". *Journal of Agricultural and Food Chemistry* 58.3 (2010).

15. Kim HY., *et al*. "The protective role of amla (Emblica officinalis Gaertn.) against fructose-induced metabolic syndrome in a rat model". *British Journal of Nutrition* 103.4 (2010): 502-512.

16. TYSAYAYH Uchiyama S. "Prevention of diet-induced obesity by dietary black tea polyphenols extract *in vitro* and *in vivo*". *Nutrition* 21.3 (2011): 287-292.

17. Rosenblatt M and Aviram M. "Pomegranate juice protects macrophages from triglyceride accumulation: inhibitory effect on DGAT1 activity and on triglyceride biosynthesis". *Annals of Nutrition and Metabolism* 58.1 (2011): 1-9.

18. LJJJRKSSCSY Bose M. "The major green tea polyphenol, (-)-epigallocatechin-3-gallate, inhibits obesity, metabolic syndrome, and fatty liver disease in high-fat-fed mice". *The Journal of Nutrition* 138.8 (2008): 1677-1683.

19. KR Polley., *et al*. "Tart cherry consumption with or without prior exercise increases antioxidant capacity and decreases triglyceride levels following a high-fat meal". *Applied Physiology, Nutrition, and Metabolism* 44.11 (2019): 1209-1218.

20. Chai SC., *et al*. "Effects of Tart Cherry Juice on Biomarkers of Inflammation and Oxidative Stress in Older Adults". *Nutrition* 11.2 (2019).

21. APBDERLRLMCJ and B JP. "How can we measure insulin sensitivity/resistance?" *Diabetes and Metabolism* 37.3 (2011): 179-188.

22. R Acaso., *et al*. "Diagnosing Insulin Resistance by Simple quantitative methods in subjects with normal glucose metabolism". *Diabetes Care* 26.12 (2003): 3320-3325.

23. F Zhang., *et al*. "The association of triglyceride and glucose index, and triglyceride to high-density lipoprotein cholesterol ratio with prehypertension and hypertension in normoglycemic subjects: A large cross-sectional population study". *The Journal of Clinical Hypertension* 23.7 (2021): 1405-14120.

24. HGLKHYFY Liu XC. "The Triglyceride-Glucose Index, an Insulin Resistance Marker, Was Non-linear Associated With All-Cause and Cardiovascular Mortality in the General Population". *Frontiers in Cardiovascular Medicine* 14 (2021): 1-10.

25. Cherian S., *et al*. "Antidiabetic effect of a glycoside of pelargonidin isolated from the bark of Ficus bengalensis Linn". *Indian Journal of Biochemistry and Biophysics* 29.4 (1992): 380-382.

26. Wolfram S., *et al*. "TEAVIGO (epigallocatechin gallate) supplementation prevents obesity in rodents by reducing adipose tissue mass". *Annual Review of Nutrition* 49.1 (2005): 54-63.

27. Wolfram S. "Effects of green tea and EGCG on cardiovascular and metabolic health". *Journal of the American College of Nutrition* 26.4 (2007): 373S-388S.

28. Wolfram S., *et al*. "Epigallocatechin gallate supplementation alleviates diabetes in rodents". *Journal of Nutrition* 136.10 (2006): 2512-2518.

29. Desai T., *et al*. "The effects of Montmorency tart cherry juice supplementation and FATMAX exercise on fat oxidation rates and cardio-metabolic markers in healthy humans". *European Journal of Applied Physiology* 118.12 (2018): 2523-2539.

30. PG Crepaldi G., *et al*. "Dipeptidyl peptidase 4 (DPP-4) inhibitors and their role in Type 2 diabetes management". *Journal of Endocrinological Investigation* 30 (2007): 610-614.