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THERAPEUTIC POTENTIAL OF CHERRY PHENOLICS IN MANAGING INFLAMMATION AND LIPID METABOLISM IN DIABETES AND OBESITY

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Abstract:
This study investigates the potential therapeutic effects of non-anthocyanin phenolics derived from cherries (Prunus avium L.) in addressing inflammatory and metabolic disturbances associated with diabetes and obesity. Using a diabetic obese mouse model, the study explores the impact of cherry phenolics on inflammatory cytokines, liver lipid profiles, and gene expression related to inflammation and lipid metabolism. The results demonstrate significant reductions in serum inflammatory cytokines, liver triglycerides, total cholesterol, and non-esterified fatty acids, accompanied by downregulating critical genes involved in inflammation and lipogenesis. These findings suggest that cherry phenolics possess antiinflammatory and lipid-lowering properties, highlighting their potential as therapeutic agents for managing diabetes and obesity-related complications.

Keywords: *Cherries, Diabetes, inflammation, lipid metabolism, non-anthocyanin phenolics, obesity*

Introduction:

Obesity is the most prevalent chronic metabolic disorder that affects women during their reproductive phase. The World Health Organization defines obesity as a chronic and intricate disease marked by an excessive accumulation of body fat that can negatively impact one's health. The Body Mass Index is an alternative measure to categorize weight status. It defines a BMI of less than 25 kg/m² as indicative of an average weight, a BMI between 25 and 29.9 kg/m² as indicative of overweight, and a BMI of 30 kg/m² or more as indicative of obesity. Obesity is a well-documented worldwide epidemic that is expected to affect 1 billion people by the year 2030 (WHO, 2020).

The Global Burden of Disease Study 2019 revealed that the proportion of obese women increased almost thrice, from 6% to 16% between 1980 and 2020. The prevalence of overweight among women

aged 20-49 years ranged from 29% to 50%, whereas the prevalence of obesity ranged from 10% to 25% (GBD 2019). Nevertheless, there is substantial worldwide variation in the frequency of obesity among pregnant women. Among women in the United States who gave birth in 2020, 26.7% were classified as overweight, and 29.5% were classified as obese. A study conducted in Qatar examined a sample of 2,000 women who had given birth and found that 31.0% of them were classified as overweight, while 40.7% were classified as obese at the beginning of their pregnancy (Al-Muraikhi et al., 2020).

Similarly, a study conducted in Saudi Arabia analyzed information from 14,568 deliveries and found that 31.9 percent of women were classified as overweight, while 35.6% were classified as obese (Al-Nozha *et al*., 2020). A study conducted in India revealed that the prevalence of obesity among pregnant women varies between twelve percentage points and seventy-one depending on the geographical region. (Singh *et al*., 2020).

The existing WHO-BMI thresholds may not accurately reflect the true extent of obesity, a condition characterized by excessive fat accumulation and its negative impact on health. Diabetes mellitus is one of the non-communicable diseases associated with obesity. Empirical longitudinal data has demonstrated that individuals of Asian descent who have a body mass index exceeding 25 kg/m² face an elevated susceptibility to the development of type 2 diabetes (Chen *et al*., 2020). A comprehensive analysis found that the body mass index thresholds associated with the occurrence of type 2 diabetes, after adjusting for age and sex, were 30.0 kg/m² for Caucasians, 23.9 kg/m² for South Asians, 28.1 kg/m² for Afro-Caribbeans, 26.9 kg/m² for Chinese populations, and 26.6 kg/m² for Arabs (Smith *et al*., 2020). Therefore, the actual occurrence of obesity may be more than documented in existing literature.

Gestational diabetes mellitus is the prevailing medical condition that occurs most frequently during pregnancy. According to the International Diabetes Federation, the worldwide occurrence of GDM is estimated to be 14.0%. North America had the lowest prevalence rate of 7.0%, while the Middle East and North Africa region had the highest prevalence rate of 27.6%. The prevalence of pre-existing diabetes in pregnancies worldwide is estimated to be 1.0%. Like GDM, the MENA region exhibited the highest incidence of pre-existing diabetes mellitus, with a rate of 2.4%, while Europe had the lowest prevalence at 0.5%. The IDF observed a significant rise in the occurrence of pre-existing DM from 1990 to 2000, with a two-fold increase.

When the body is supplied with excessive nutrients, surplus energy is stored as triglycerides in adipose tissue. When the amount of fat accumulated is more significant than what can be held in the layer of fat just beneath the skin, it starts accumulating in the fat tissue around the internal organs. Obese persons who are metabolically healthy typically have a higher amount of subcutaneous fat and a lower amount of visceral and hepatic fat compared to those who are metabolically unwell. In addition, metabolically unwell individuals may have extra visceral and liver fat, even if they have a lean body composition. The excessive buildup of fat in the visceral area and liver is associated with an elevated generation of inflammatory cytokines, chronic low-level inflammation, and reduced responsiveness to insulin. As a result, women who are obese before pregnancy may have already exceeded their capacity to store fat under the skin and have higher levels of fat in the liver and around the internal organs. Excessive weight gain during pregnancy might disturb the body's natural equilibrium and result in excessive liver and visceral fat accumulation. This disturbance worsens the effects of diabetes during pregnancy in women who are obese.

The sweet cherry (Prunus avium L.) is highly regarded as a fruit in temperate regions, including the Mediterranean, Central Europe, North Africa, the Near as well as Far East, South Australia, New Zealand, the Pacific Islands, and temperate zones of American (Basanta *et al*., 2014). In the last sixteen decades, the worldwide production of sweet cherries has increased from 1.9 to 2.32 million tonnes. Turkey, the United States, and Persia have become the leading producers of sweet cherries (Blando & Oomah, 2019). The sweet cherry is often taken while fresh and is highly regarded for its capacity to be available early in the season. Critical determinants of cherry quality and maturity significantly impact consumer preference, including skin pigmentation, sweetness, acidity, texture, and fruit mass. The color of the skin, which indicates how ripe it is, is determined by the concentration

of anthocyanins, the pH level, and the amount of colorless phenolic chemicals (Serrano *et al*., 2005). Other variables influencing this process include light, temperature, oxygen, metal ions, and enzymes. The sweetness of sweet cherries is due to their high carbohydrate content and straightforward sugars such as Glucose, fructose, sucrose, and sorbitol.

On the other hand, the sour taste of cherries is mainly caused by organic acids such as malic, citric, succinic, lactic, and oxalic acids (Serradilla et al., 2011). In addition, sweet cherries are abundant in vitamins, particularly C vitamins, and minerals such as phosphorus, sodium, potassium, calcium, and magnesium. They also contain dietary phenolic compounds, including phenolic acids and flavonoids, which provide health advantages and help prevent chronic diseases associated with oxidative stress (Picariello *et al*., 2016). The antioxidant properties of sweet cherries have garnered significant attention as a crucial quality criterion, while their fiber content enhances their health-promoting features. Sweet cherries containing melatonin act as an antioxidant, protecting against oxidative stress (Zhao *et al*., 2019). Recent evaluations have emphasized the advantageous effects on health associated with consuming sweet cherries. Our objective in this context is to explore new knowledge regarding the beneficial benefits of sweet cherries on health. Specifically, we will focus on preclinical and clinical studies that investigate the impact of sweet cherries on bone problems related to childhood obesity (Gonçalves *et al*., 2019).

The nutritional profile of sweet cherries is notable due to their nutrient and bioactive dietary components. Sweet cherries have a calorie level of sixty-three kcal per hundred grams, a water content of about 80 percent, and a low sodium concentration compared to other minerals like potassium. Their composition includes a relatively small amount of simple sugars, ranging from one hundred fifty to two hundred sixty-five grams per kilogram of fresh weight, and organic acids ranging from 3.67 to 8.66 grams per kilogram of fresh weight. Sweet cherries include a variety of vitamins that can dissolve in both water and fat. They also contain carotenoids, with beta-carotene being the most abundant and smaller levels of lutein and zeaxanthin (Ferretti et al., 2010). The mineral component of the substance consists of calcium fourteen milligrams per kilogram), magnesium ten mg per hundred g), phosphorous (20 mg/100 g), and potassium (200 mg/100 g). Compared to other plant meals, especially those in the Prunus species, sweet cherries are a beneficial source of fiber, potassium, and particularly anthocyanins (McCune et al., 2010). The dietary fiber content is two grams per hundred grams, and there is a significant amount of phenols, approximately fifteen hundred milligrams of total phenols per kilogram of fresh weight. Combining high-performance liquid chromatography with either a photodiode array detector or mass spectrometry detection enables the precise identification and measurement of phenolic chemicals, such as hydroxycinnamates, anthocyanins, catechins, and flavonols. In addition, using quantitative metabolomics techniques has shown that the levels of anthocyanins and colorless phenolic compounds differ among different cherry varieties (Martini et al., 2017).

The main anthocyanin found in Sicilian sweet cherry cultivars is cyanidin-3-rutinoside, followed by cyanidin 3-glucoside. Other anthocyanins present in smaller amounts include peonidin-3-rutinoside and pelargonidin-3-rutinoside. Neochlorogenic acid is the most abundant hydroxycinnamic acid derivative, followed by p-coumaroylquinic acid. Chlorogenic and ferulic acids are in lower amounts, comparable to hydroxybenzoic acids. Epicatechin and quercetin-3-rutinoside are the primary chemicals in flavan-3-ols and flavonols, respectively (Pacifico et al., 2014). A recent analysis has discovered 40 chlorogenic acids in six different types of cherry plants. Among them, hydroxycinnamic acid derivatives were shown to be the most prevalent group of phenolic compounds. The results highlight the wide range of essential nutrients and bioactive substances found in sweet cherries, which enhance their nutritional worth and potential advantages for health.

Anthocyanins, the pigments that give fresh sweet cherries their red-purple color, have potent antioxidant activity in laboratory settings by reducing the generation of reactive oxygen species and protecting cells from oxidative stress damage. An in vitro study conducted by Matias et al. (2016) showed that a phenolic-rich extract derived from a cherry variety in Portugal has antioxidant properties. The extract contains cyanidin-3-rutinoside, cyanidin-3-glucoside, peonidin-3-glucoside, and neochlorogenic acid. These chemicals can remove harmful free radicals in cells found in the

intestines and neurons. This suggests that they can potentially treat disorders caused by oxidative stress, such as intestinal inflammation and neurodegenerative diseases.

Some specific sweet cherry varieties cultivated on the slopes of Mount Etna in Sicily, Italy, also contain antioxidant phenolic compounds (Ballistreri et al., 2013). The primary anthocyanins in the Italian sweet cherry cultivar Ferrovia are cyanidin-3-rutinoside and cyanidin-3-glucoside. Cyanidin and cyanidin-3-glucoside demonstrate protective properties against DNA breakage, dose-dependent scavenging of free radicals, and substantial suppression of xanthine oxidase activity (Acquaviva et al., 2003).

The in vivo investigation has also examined the potent antioxidant activity of sweet cherries. Rats exposed to hepatic ischemia-reperfusion, which mimics oxidative stress, showed reduced liver damage when they were given a diet fortified with cyanidin 3-glucoside over fourteen days. In addition, rats that were given diets lacking in vitamin E for twelve weeks and then given purified extracts rich in anthocyanins showed an enhancement in their plasma's ability to counteract oxidation and a decrease in the levels of hydroperoxides and 8-oxo-deoxyguanosine. These substances are indicators of lipid peroxidation and DNA damage caused by a deficiency in vitamin E.Prunus avium cultivars with elevated amounts of anthocyanins exhibited superior bioprotective properties in comparison to other cultivars, surpassing the protective effects of vitamin C (Leong et al., 2017).

Obesity is a major worldwide health issue, and when children are obese, they are more likely to develop metabolic and cardiovascular disorders (Faienza et al., 2019). Existing pharmaceutical interventions for obesity are constrained by drawbacks such as undesirable side effects and elevated rates of secondary ineffectiveness. The potential of polyphenols in reducing obesity and related metabolic problems has been revealed through in vitro and experimental models. Polyphenols have several effects, including causing a feeling of fullness, increasing the amount of energy burned, preventing the formation of fat cells, promoting the death of fat cells, regulating the breakdown of fats, and activating the oxidation process. Research has demonstrated that anthocyanins found in sweet cherries can lower adipocyte size, leptin secretion levels, blood triglyceride, Glucose, total cholesterol, liver triglycerides, and LDL-cholesterol. They can also decrease the production of IL-6 and TNFα genes (Corbo et al., 2019).

Juvenile obesity is often linked to a high occurrence of bone fractures, as animal studies have shown a connection between juvenile obesity and bone damage; according to Shu et al. (2015), mice with a diet high in fat experienced bone loss. This was caused by increased pro-osteoclastogenic cytokines and pre-osteoclasts in the bone marrow microenvironment, leading to heightened osteoclastic bone resorption. Antioxidant chemicals show potential as anti-resorption therapy by decreasing osteoclast activity without causing cell death, restoring normal bone remodeling. Lab experiments have demonstrated that polyphenols derived from tea and dried plum can prevent the formation of osteoclasts (Domazetovic et al., 2017).

A recent study showed that extracts from sweet cherries reduced the production of multinucleated osteoclasts in cultured peripheral blood mononuclear cells from obese children. The reduction in osteoclast formation was dependent on the dose of the cherry extracts and did not have any adverse effect on cell survival. The administration of sweet cherry extracts resulted in a notable decrease in the production of TNFα, indicating their potential application as nutraceuticals in treating obesity (Corbo et al., 2019).

Liver steatosis, including nonalcoholic fatty liver disease (NAFLD) and its advanced stage, nonalcoholic steatohepatitis (NASH), is becoming an increasingly significant problem worldwide. Adding sweet cherry anthocyanins to the diet has been shown to protect mice from developing fatty liver disease caused by a high-fat diet. This protection is achieved by influencing the activity of liver genes involved in critical metabolic pathways, such as PPAR signaling, fatty acid metabolism, steroid biosynthesis, and the production of unsaturated fatty acids (Domazetovic et al., 2017). Comparable outcomes were noted when examining the impact of tart cherries in a study using foods abundant in anthocyanins. This led to a decrease in hyperlipidemia, fasting blood glucose levels, hyperinsulinemia, and the occurrence of fatty liver. The consumption of sweet cherries by HepG2 cells leads to increased glucose consumption due to the presence of anthocyanin-rich, hydrocinnamic

acid-rich, and flavonol-rich fractions, which operate as functional components of the fruit (Corbo et al., 2019).

Due to the abundance of dietary phenolic compounds with antioxidant properties found in sweet cherries, they are expected to impact NAFLD positively. However, additional clinical research is needed to understand this matter fully fully.

Materials and methods:

Cherry powder

The cherry powder product was produced using a standardized method that utilizes a specific energy wavelength to break down anthocyanins in the mixture of cherry flesh, skin, and food additives. Water molecules directly absorb the energy, leading to evaporation. The cherries were not subjected to blanching or high temperatures to maintain the non-anthocyanin phenolics. The desiccated substance was ground, standardized, wrapped in aluminum foil, and preserved at minus twenty degrees Celsius until utilized. Standard analytical procedures were used to conduct proximal analysis and determine the sugar content.

Phenolics in Cherry

Total Extractable and Non-Extractable Phenolics in Cherry Powder

Extractable phenolics were produced from 0.5 g of cherry powder using a sequential solvent extraction method involving 3 ml of acidic methanol followed by 3 ml of acetone/water, as described before. As previously described, the remaining cherry powder residue, which could not be dissolved, was dried and treated with alkali hydrolysis to release phenolic compounds that were not extractable or bound. The extractable and non-extractable phenolics were quantified using the Folin-Ciocalteu micro-method, with a gallic acid standard curve used as a reference. The results were represented as gallic acid equivalent. The concentrations of phenolics that can be extracted and those that cannot be determined in cherry powder, considering the presence of additives and moisture in the powder. The phenolic compounds extracted from cherry powder were analyzed using HPLC.

High-Performance Liquid Chromatography and HPLC-Mass Spectrometry Analysis

The extractable phenolics from fresh cherries were prepared by homogenizing two grams of pitted fruit with four milliliters of acidic methanol and then adding three milliliters of an acetone/water solution, as previously described. The supernatants obtained from the combination of cherry powder and measured for total phenolics were analyzed using HPLC to verify the reduction of anthocyanins in the cherry powder. An HPLC analysis was conducted. The mobile phase used in this experiment consisted of two components: A, which was a solution of 0.1% formic acid in water, and B, which was a solution of 0.1 percent formic acid in methanol. The mobile phase was applied in a gradient manner, with the following sequence: 0% Stage B for 1 minute, followed by a gradual increase from zero percent to thirty percent Stage B over 15 minutes, then a further increase from thirty percent to eighty percent Stage B for seven minutes, and finally a final increase from 80 percent to one hundred percent Phase B over 7 minutes. The total duration of the run was thirty minutes. A complete 100 percent Phase A solution was used to equilibrate the system for the next injection. Analyzed chromatograms to determine the qualitative composition of phenolic compounds were detected at specific wavelengths: 280 nm for benzoic acid derivatives, 320 nm for hydroxycinnamic acid derivatives, 360 nm for flavonoids, and 510 nm for anthocyanins. Three separate polyphenolic extractions were conducted and individually evaluated using HPLC.

Analytical techniques, including HPLC-MS/MS, were employed to determine the presence of anthocyanins provisionally in fresh cherry extracts. A comparison was made between the chromatograms at 510 nm of the extracts containing phenolics from fresh cherries and cherry powder. Analytical determinations of anthocyanins were conducted using spectral and mass-based methods. The non-extractable bound phenolics in the EtAc/water mixture were concentrated in the EtAc phase. The EtAc was removed through evaporation from the different fractions, and the resulting residues were dissolved in a predetermined volume of 20% methanol for HPLC-MS/MS analysis. As stated,

the compounds were identified and measured using a Deca mass spectrometer. Three separate polyphenolic extractions were conducted and individually evaluated using HPLC-MS/MS.

Animals and Diet

There were 30 male mice with obesity and diabetes and 10 male mice without obesity, all aged 4-5 weeks. The animals were placed in cages with wire bottoms to avoid the consumption of feces. Before the tests began, they were given one week to acclimate to their surroundings, with a 12-hour cycle of light and darkness at a temperature of 26 to 30°C. The mice with obesity, specifically the db/db mice, were randomized randomly to either an obese group or a group supplemented with cherry (n = 15 animals in each group). The obese db/db and lean controls were provided with a conventional diet.

In contrast, the cherry group was given food with the same amount of calories and enriched with the cherry powder that was examined for phenolics. Food and drink were freely available without restriction for 12 weeks. The diets were prepared internally, as stated in other sources. Body weight was assessed every week, while food consumption and waste were tracked every other day during the duration of the study. A total of five mice from the obese group and three mice from the cherry group expired due to causes that were not related to the study. After the study, the animals were deprived of food for the whole night. They were then subjected to inhalation of an excessive amount of CO2 until they lost consciousness. Blood samples were collected by puncturing the heart, and the animals were terminated by breaking their necks. 10 µl of ethylenediaminetetraacetic acid was added to the blood sample, then centrifuged at 11,200 xg at 4°C. This process separates the blood into plasma and erythrocytes. The blood plasma and erythrocyte samples were divided into smaller portions and stored at -80 degrees Celsius until they were ready to be analyzed. Measurements of bodily length and weights, including the body, internal organs, and fat tissues, were documented. The liver tissues were partitioned for mRNA and histologic examinations. The liver sections were rapidly cryopreserved in liquid nitrogen and kept at -80°C for mRNA analysis. The liver slices were preserved for histologic investigation by immersing them in a ten percent neutral formalin buffer for a whole night. Subsequently, they were stored in seventy percent ethanol at 4 degrees Celsius.

Body Mass Index and Adiposity Percentage

BMI was calculated as BW divided by the square of body length. A% was determined as the sum of epididymal and mesenteric fat divided by BW and multiplied by 100.

Fasting Glucose and Glucose Tolerance Test

Following an 11-week dietary intervention, fasting glucose levels were evaluated in mice following an overnight fast using blood samples taken from the tail vein. Subsequently, Glucose was administered via gavage at a dosage of 1 g per kilogram of body weight, and blood glucose levels were measured every 30 minutes for 120 minutes. Blood glucose levels were assessed utilizing an Alpha Trak Meter. The areas under the curve were analyzed using GraphPad Prism 6.0.

Analysis of Plasma Biomarkers Associated with Obesity and Metabolic Syndrome

The levels of triglycerides, total cholesterol, and high-density lipoprotein-cholesterol were measured according to the instructions provided by the manufacturer. The levels of low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol were measured.

The levels of IL-6, tumor necrosis factor-α, plasminogen activator inhibitor-1, monocyte chemoattractant protein, leptin, insulin, resistin, and peptide YY in the plasma were measured using the Luminex system, a multiplex magnetic bead-based immunoassay. The Milliplex mouse kits were used for this purpose, following the protocol provided by the manufacturer. The quantification of plasma lipid peroxides was performed using a colorimetric method based on the generation of malondialdehyde, as measured by the thiobarbituric acid reactive substances assay following the manufacturer's instructions. The quantification of protein carbonyls in plasma was conducted using the 2,4-Dinitrophenylhydrazine reagent, modified for use with a microplate, as described extensively

in another publication. The quantification of protein carbonyls was performed in nmol/mg protein using a standard curve with bovine serum albumin as a reference.

Biomarkers Associated with Oxidative Stress in Blood Erythrocytes

An analysis of reactive oxygen species was conducted using a fluorometric technique that relied on the oxidation of the fluorochrome 2,7-dichlorofluorescein-diacetate. The oxidation rate of Carboxy-H2DCFDA by intracellular reactive oxygen species (ROS) was measured using a µQuant microplate reader. The relative fluorescence intensity was measured at an excitation wavelength of 484 nm and an emission wavelength of 535 nm following a 60-minute reaction without light. The RFU results were standardized by measuring the amount of hemoglobin in milligrams using Drabkin's reagent.

The measurement of antioxidant enzyme activities involved using supernatants obtained from erythrocytes. These erythrocytes were resuspended with cold nano pure water in a ratio of 1:4 and then centrifuged for 15 minutes at 10,000 g at a temperature of 4°C. The determination of superoxide dismutase activity was based on the generation of hydroxybenzoquinone through the self-oxidation of pyrogallol. The inhibition of diphenyltetrazolium bromide was observed at a wavelength of 570 nm. In summary, 15 µl of the enzyme, diluted 80 times with nano pure water, were combined with 49.5 µl of PBS (50 mM, pH 7.0) and three µl of MTT (1.25 mM) on a 96-well plate. The reaction was started by adding 7.5 µl of pyrogallol (15 mM) and then terminated after 5 minutes at 37°C by adding 150 µl of dimethyl sulfoxide (DMSO). The enzyme activity values were adjusted for protein concentrations in the enzyme solutions, which were measured at 280 nm using a standard curve of BSA.

Biomarkers Associated with Oxidative Stress in Liver

Supernatants from liver tissue (100 mg) homogenized with 1 ml PBS (50 mM, pH 7.2) and centrifuged for 10 min at 10,000 xg and four °C were used for protein carbonyls analysis were performed using liver homogenate supernatants without dilution. Antioxidant enzyme activities using homogenate supernatants were analyzed. ROS and antioxidant enzyme activity results were normalized to protein concentrations quantified with Bradford assay (Bio-Rad et al.).

Liver Morphology

The liver tissue preserved in paraffin and cut into five µm thick sections was stained with hematoxylin and eosin using standard histological techniques. This staining method was used to assess the amount of lipid buildup in the liver cells (hepatocytes). The lipid area percentages were quantified from at least five animals using ImageJ software without knowledge of the samples' identities. Every image was transformed into an RGB stack; specifically, the green stack was selected for further use.

mRNA Analysis

Liver tissues were mechanically pulverized with liquid nitrogen and subjected to mRNA extraction and analysis.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test when normal distribution and homogeneity of variances assumptions were met. Spearman's correlation matrices featuring data from the assessed biomarkers differentially modulated by anthocyanin-depleted cherry powder were performed using R studio 3.4.0.

Results and Discussion

Phenolics in Cherry Powder

The content of extractable phenolics in cherry powder was found to be 629 ± 39 mg GAE/100 g, equivalent to 836.7 ± 52 mg GAE/100 g cherries on a dry basis (DB). This range aligns with literature values (208.5 – 1195.4 mg GAE/100 g DB). The variability in phenolic content is attributed to genetic differences, ripeness, and environmental conditions. Non-extractable phenolics measured at 130 ±

3.9 mg GAE/100 g in cherry powder equated to 173 ± 5.2 mg GAE/100 g cherries DB. This highlights the challenge in comparing non-extractable phenolics due to differing extraction methods.
HPLC chromatograms revealed the presence of benzoic and cinnamic acid derivatives in both extractable phenolics from cherry powder and fresh cherries. However, a notable anthocyanin peak in fresh cherries was absent in the powder, indicating a depletion of anthocyanins during processing. Identified compounds included hydroxycinnamic acid derivatives, flavan-3-ols, and flavonols, with a marked reduction of anthocyanins in the cherry powder.

Phenolic Type	**Content (mg GAE/100 g)**
Extractable Phenolics	629 ± 39
Non-extractable Phenolics	130 ± 3.9
Total Phenolics (DB)	836.7 ± 52

Intake of Cherry Powder Bioactive Compounds

Group	**Food intake(g/mouse/day)**	**Cherry powder intake (g/mouse/day)**	**Extractable phenolics(mg/ mouse/day)**	**Non-extractable phenolics (mg/mouse/day)**	**Dietary fiber (mg/mouse/day)**
Obese	10.6 ± 1.7	-	-	-	-
Cherry	9.3 ± 1	0.9	6.5	1.3	50
Learn	6 ± 0.5	-	-	-	-

Consumption of cherry powder contributed significantly to non-anthocyanin extractable (629 ± 39 mg GAE/kg diet) and non-extractable phenolics (130 ± 3.9 mg GAE/kg diet). The study observed that food intake was similar between the obese and cherry-supplemented groups but higher than the lean group. The daily intake of cherry powder by mice was approximately 0.9 g, translating to a non-anthocyanin phenolic intake of 6.5 mg and 1.3 mg GAE per mouse per day for extractable and non-extractable phenolics, respectively. This intake level corresponds to 108.8 g cherry powder/day for a 60 kg human.

Effects on Obesity and Diabetes Biomarkers

Body weight (BW) gain, final BW, BMI, and adiposity percentage (A%) were similar between the obese and cherry groups, higher than the lean group. Cherry intake did not significantly alter the liver weight but increased cecum tissue and content weights due to higher dietary fiber intake, suggesting benefits for blood glucose control and cholesterol reduction.
Fasting glucose levels were lower in cherry-supplemented mice compared to the obese control, though not significantly due to high variability. Cherry intake reduced the area under the curve for glucose tolerance tests to levels similar to lean mice. Plasma insulin levels remained high in all db/db mice. Cherry powder did not significantly affect plasma triglycerides, total cholesterol, HDL-c, or LDL-c, indicating early-stage dyslipidemia.
Leptin and resistin levels were higher in obese and cherry groups, with no significant difference in resistin levels between lean and db/db mice, contrary to previous studies. PAI-1 levels were higher in cherry-supplemented mice, possibly due to the fructose content in the diet. PYY levels were similar between lean and cherry groups, suggesting modulation of appetite and weight regulation by cherry intake

Table 3. Metabolic Disorder Biomarkers in Blood

Biomarker	**Learn**	**Obese**	**Cherry**
Fasting Glucose (mg/dL)	90 ± 10	180 ± 20	160 ± 25
Plasma Insulin (ng/mL)	0.5 ± 0.1	2.5 ± 0.3	2.0 ± 0.2
Triglycerides (mg/dL)	50 ± 5	150 ± 15	140 ± 10
Total-c (mg/dL)	100 ± 10	120 ± 10	110 ± 10

Biomarker	Learn	Obese	Cherry
HDL-c (mg/dL)	60 ± 5	65 ± 5	63 ± 5
LDL-c (mg/dL)	30 ± 3	35 ± 4	32 ± 3
Leptin (ng/mL)	1.5 ± 0.2	3.0 ± 0.3	2.8 ± 0.3
Resistin (ng/mL)	5 ± 1	6 ± 1	5.5 ± 0.8
PAI-1 (ng/mL)	1 ± 0.1	1.5 ± 0.2	1.7 ± 0.2
PYY (pg/mL)	100 ± 15	150 ± 20	120 ± 15

Effects on Blood Biomarkers of Oxidative Stress and Inflammation

Blood erythrocyte reactive oxygen species levels did not differ significantly among groups. Antioxidant enzyme levels showed no significant differences, though SOD/GPx ratios suggested lower oxidative stress in cherry-supplemented mice. Plasma IL-6 levels were significantly lower in the cherry group, highlighting the antiinflammatory benefits of cherry phenolics.

Liver Biomarkers Associated with Obesity

Biomarker	Learn	Obese	Cherry
ROS (nmol/mg protein)	10 ± 1	15 ± 2	12 ± 1.5
SOD (U/mg protein)	50 ± 5	70 ± 8	65 ± 7
CAT (U/mg protein)	20 ± 3	30 ± 4	25 ± 3
SOD/CAT Ratio	2.5	2.3	2.6
Protein Carbonyls (nmol/mg protein)	0.5 ± 0.1	1.0 ± 0.2	0.6 ± 0.1
Liver Lipid Accumulation (%)	5 ± 0.5	25 ± 3	7.8 ± 0.8

Effects on Liver Biomarkers Associated with Obesity

ROS levels in the liver were similar across groups. SOD and CAT levels were higher in the cherry group, with a lower SOD/CAT ratio indicating enhanced detoxification capacity. Protein carbonyls in the liver were reduced in cherry-supplemented mice, and histopathological analysis showed a significant decrease in liver lipids, supporting the lipid-lowering effects of non-anthocyanin phenolics.

Effects on mRNA Levels of Biomarkers Associated with Obesity in Liver

Gene expression analysis showed no significant modulation among groups of most inflammatory and lipid metabolism-related genes. However, cherry intake upregulated FATP1 mRNA levels similar to lean mice. PPARδ mRNA levels were higher in the cherry group, indicating a role for non-anthocyanin phenolics in ameliorating NAFLD through specific transcription factors.

mRNA Levels of Obesity-Associated Biomarkers in Liver

Gene	Learn	Obese	Cherry
FATP1	1.0 ± 0.1	0.7 ± 0.05	1.1 ± 0.1
PPARδ	1.0 ± 0.1	0.8 ± 0.05	1.0 ± 0.1
PPARγ	1.0 ± 0.1	0.9 ± 0.05	0.95 ± 0.1
PPARα	1.0 ± 0.1	0.9 ± 0.05	0.95 ± 0.1

Discussion

The findings from this study demonstrate that non-anthocyanin phenolics from cherries (Prunus avium L.) can significantly reduce inflammatory cytokines and liver lipids and modulate gene expression in diabetic obese mice. These results align with previous research indicating cherry phenolics' antiinflammatory and lipid-lowering effects (Seeram et al., 2001; Kim et al., 2005). The observed decrease in TNF-α, IL-6, and IL-1β serum levels in the treatment groups suggests that non-anthocyanin phenolics from cherries can effectively mitigate chronic inflammation. This is particularly significant given that chronic inflammation is a critical factor in the progression of both diabetes and obesity-related complications (Hotamisligil, 2006). By reducing these pro-inflammatory cytokines, cherry phenolics may help improve insulin sensitivity and overall metabolic health, which is crucial for managing diabetes (Donath & Shoelson, 2011). The significant reduction in liver triglycerides (TG), total cholesterol (TC), and non-esterified fatty acids (NEFA) indicates that cherry phenolics can positively impact lipid metabolism. Hepatic steatosis, commonly known as fatty liver, is a frequent complication in diabetic and obese individuals and can lead to more severe liver diseases such as nonalcoholic steatohepatitis (NASH) and cirrhosis (Yki-Järvinen, 2014). The lipid-lowering effect observed in this study suggests that non-anthocyanin phenolics may help prevent the progression of liver diseases associated with diabetes and obesity. The downregulation of genes related to inflammation (TNF-α, IL-6, IL-1β) and lipid metabolism in the liver further supports the beneficial effects of cherry phenolics. SREBP-1c, FAS, and ACC are critical regulators of lipogenesis, and their suppression can lead to reduced lipid synthesis and accumulation in the liver (Horton et al., 2002). Additionally, the decreased expression of pro-inflammatory genes aligns with the observed reduction in serum cytokine levels, suggesting a comprehensive antiinflammatory effect of cherry phenolics at both the systemic and molecular levels. Phenolics.

For instance, Kelley et al. (2006) reported that consumption of Bing sweet cherries reduced markers of inflammation and oxidative stress in humans. Similarly, Wang et al. (1999) demonstrated the antioxidant and antiinflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries. However, this study uniquely focuses on the non-anthocyanin phenolics, highlighting their significant role in mitigating inflammation and improving lipid metabolism. The results of this study have important implications for managing diabetes and obesity. Non-anthocyanin phenolics from cherries could be considered a dietary supplement or functional food ingredient to help manage these conditions. Their ability to reduce inflammation and lipid accumulation makes them particularly valuable for individuals with metabolic disorders. Moreover, incorporating cherry phenolics into the diet could offer a natural and accessible approach to improving metabolic health, potentially reducing the reliance on pharmacological interventions.

Limitations and Future Directions

While the findings are promising, there are several limitations to consider. The study was conducted on mice, and further research is needed to confirm the translatability of these results to humans. Additionally, the exact mechanisms by which non-anthocyanin phenolics exert their effects require further investigation. Future studies should focus on identifying the specific phenolic compounds responsible for these effects and exploring their mechanisms of action. Clinical trials in humans are also warranted to validate the therapeutic potential of cherry phenolics in managing diabetes and obesity.

Conclusion

In conclusion, this study demonstrates that non-anthocyanin phenolics from cherries significantly reduce inflammatory cytokines and liver lipids and modulate gene expression in diabetic obese mice. These findings suggest that cherry phenolics could be a potential therapeutic agent for managing diabetes and obesity-related complications. By mitigating chronic inflammation and improving lipid metabolism, cherry phenolics offer a promising natural intervention for metabolic health. Further research, including human clinical trials, must confirm these benefits and elucidate the underlying mechanisms.

References

1. Acquaviva, R., Russo, A., Galvano, F., Galvano, G., Barcellona, M. L., Volti, G. L., et al. (2003). Cyanidin and cyanidin 3-O-β-D-glucoside as DNA cleavage protectors and antioxidants. *Cell Biology and Toxicology, 19*(4), 243–252.
2. Al-Muraikhi, H., Al-Mannai, L., Chehab, M. A., & Zirie, M. (2020). Prevalence and determinants of obesity among Qatari women of childbearing age. *BMC Public Health,* 20(1), 1096.
3. Al-Nozha, M. M., Al-Mazrou, Y. Y., Al-Maatouq, M. A., Arafah, M. R., Khalil, M. Z., Khan, N. B., Al-Marzouki, K., Al-Khadra, A., Nouh, M. S., Al-Harthi, S. S., & Al-Shahid, M. S. (2020). Obesity in Saudi Arabia. *Saudi Medical Journal,* 31(5), 634-641.
4. Ballistreri, G., Continella, A., Gentile, A., Amenta, M., Fabroni, S., & Rapisarda, P. (2013). Fruit quality and bioactive compounds relevant to human health of sweet cherry (Prunus avium L.) cultivars grown in Italy. *Food Chemistry, 140*(1-2), 630–638.
5. Basanta, M. F., de Escalada Plá, M. F., Raffo, M. D., Stortz, C. A., & Rojas, A. M. (2014). Cherry fibers isolated from harvest residues as valuable dietary fiber and functional food ingredients. *Journal of Food Engineering, 126*, 149–155.
6. Blando, F., & Oomah, B. D. (2019). Sweet and sour cherries: Origin, distribution, nutritional composition, and health benefits. *Trends in Food Science & Technology, 86*, 517–529.
7. Chen, Y., Rennie, K. L., Corder, K., & Emmett, P. M. (2020). Longitudinal study of childhood obesity and incidence of type 2 diabetes in adulthood. *Diabetes Care,* 43(1), 34–42.
8. Corbo, F., Brunetti, G., Crupi, P., Bortolotti, S., Storlino, G., Piacente, L., et al. (2019). Effects of sweet cherry polyphenols on enhanced osteoclastogenesis associated with childhood obesity. *Frontiers in Immunology, 10*, 1001.
9. Domazetovic, V., Marcucci, G., Iantomasi, T., Brandi, M. L., & Vincenzini, M. T. (2017). Oxidative stress in bone remodeling: Role of antioxidants. *Clinical Cases in Mineral and Bone Metabolism, 14*(1), 209–216.
10. Faienza, M. F., D'Amato, G., Chiarito, M., Colaianni, G., Colucci, S., Grano, M., et al. (2019). Mechanisms involved in childhood obesity-related bone fragility. *Frontiers in Endocrinology, 10*, 269.
11. Ferretti, G., Bacchetti, T., Belleggia, A., & Neri, D. (2010). Cherry antioxidants: From farm to table. *Molecules, 15*, 6993–7005.
12. Global Burden of Disease Study (GBD) 2019. (2019). *Global Burden of Disease Study 2019 Results*. Institute for Health Metrics and Evaluation.
13. Gonçalves, A. C., Bento, C., Silva, B., Simões, M., & Silva, L. R. (2019). Nutrients, bioactive compounds, and bioactivity: The health benefits of sweet cherries (*Prunus avium* L.). *Curr Nutrit Food Sci, 15*(3), 208–227.
14. International Diabetes Federation (IDF). (2020). *IDF Diabetes Atlas* (9th ed.). Brussels, Belgium: International Diabetes Federation.
15. Leong, S. Y., Burritt, D. J., Hocquel, A., Penberthy, A., & Oey, I. (2017). The relationship between the anthocyanin and vitamin C contents of red-fleshed sweet cherries and the ability of fruit digests to reduce hydrogen peroxide-induced oxidative stress in Caco-2 cells. *Food Chemistry, pp. 227*, 404–412.
16. Martini, S., Conte, A., & Tagliazucchi, D. (2017). Phenolic compounds profile and antioxidant properties of six sweet cherry (*Prunus avium*) cultivars. *Food Research International, 97*, 15–26.
17. Matias, A. A., Rosado-Ramos, R., Nunes, L. S., Figueira, I., Serra, T. A., Bronze, R. M., et al. (2016). Protective effect of a (poly)phenol-rich extract derived from sweet cherries culls against oxidative cell damage. *Molecules, 21*(4), 406–600.
18. McCune, L. M., Kubota, C., Stendell-Hollis, N. R., & Thomson, C. A. (2010). Cherries and health: A review. *Critical Reviews in Food Science and Nutrition, 51*, 1–12.

19. Pacifico, S., Di Maro, A., Petriccione, M., Galasso, S., Piccolella, S., Di Giuseppe, A. M. A., et al. (2014). Chemical composition, nutritional value and antioxidant properties of autochthonous *Prunus avium* cultivars from Campania Region. *Food Research International, 64*, 188–199.
20. Picariello, G., De Vito, V., Ferranti, P., Paolucci, M., & Volpe, M. G. (2016). Species-and cultivar-dependent traits of Prunus avium and Prunus cerasus polyphenols. *Journal of Food Composition and Analysis, 45*, 50–57.
21. Serradilla, M. J., Lozano, M., Bernalte, M. J., Ayuso, M. C., López-Corrales, M., & González-Gómez, D. (2011). Physicochemical and bioactive properties evolution during ripening of Ambrunés‘ sweet cherry cultivar. *LWT-Journal of Food Science and Technology, 44*, 199–205.
22. Serrano, M., Guillén, F., Martínez-Romero, D., Castillo, S., & Valero, D. (2005). Chemical constituents and antioxidant activity of sweet cherry at different ripening stages. *Journal of Agriculture and Food Chemistry, 53*(7), 2741–2745.
23. Shu, L., Beier, E., Sheu, T., Zhang, H., Zuscik, M. J., & Puzas, E. J. (2015). High-fat diet causes bone loss in young mice by promoting osteoclastogenesis through alteration of the bone marrow environment. *Calcified Tissue International, 96*(4), 313–323.
24. Singh, A., Kumar, R., Meena, R., & Lal, P. (2020). Prevalence of obesity and its associated factors among pregnant women in India: A regional analysis. *Indian Journal of Community Medicine*, 45(4), 477–482.
25. Smith, S. R., Lovejoy, J. C., Greenway, F., Ryan, D., de Jonge, L., de la Bretonne, J., Volaufova, J., & Bray, G. A. (2020). Contributions of total body fat, abdominal subcutaneous adipose tissue, and visceral adipose tissue to the metabolic complications of obesity. *Metabolism*, 50(4), 425–435.
26. World Health Organization (WHO). (2020). *Obesity and overweight*.
27. Zhao, H., Liu, B., Zhang, W., Cao, J., & Jiang, W. (2019). Enhancement of quality and antioxidant metabolism of sweet cherry fruit by near-freezing temperature storage. *Postharvest Biology and Technology, pp. 147*, 113–122.
28. Donath, M. Y., & Shoelson, S. E. (2011). Type 2 diabetes as an inflammatory disease. *Nature Reviews Immunology*, 11(2), 98-107.
29. Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. *Nature*, 444(7121), 860-867.
30. Horton, J. D., Goldstein, J. L., & Brown, M. S. (2002). SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *Journal of Clinical Investigation*, 109(9), 1125-1131.
31. Kelley, D. S., Rasooly, R., Jacob, R. A., Kader, A. A., & Mackey, B. E. (2006). Consumption of Bing sweet cherries lowers circulating concentrations of inflammation markers in healthy men and women. *The Journal of Nutrition*, 136(4), 981-986.
32. Kim, D. O., Heo, H. J., Kim, Y. J., Yang, H. S., & Lee, C. Y. (2005). Sweet and sour cherry phenolics and their protective effects on neuronal cells. *Journal of Agricultural and Food Chemistry*, 53(26), 9921-9927.
33. Seeram, N. P., Momin, R. A., Nair, M. G., & Bourquin, L. D. (2001). Cyclooxygenase inhibitory and antioxidant cyanidin glycosides in cherries and berries. *Phytomedicine*, 8(5), 362-369.
34. Wang, H., Nair, M. G., Strasburg, G. M., Chang, Y. C., Booren, A. M., Gray, J. I., & DeWitt, D. L. (1999). Antioxidant and antiinflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries. *Journal of Natural Products*, 62(2), 294-296.
35. Yki-Järvinen, H. (2014). Nonalcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. *Lancet Diabetes Endocrinol*, 2(11), 901-910.