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# Blueberry intake included in hypocaloric diet decreases weight, glucose, cholesterol, triglycerides and adenosine levels in obese subjects

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ABSTRACT

Obesity is a disease characterized by an excessive accumulation of fat in the body and it has been linked the enhancement of inflammation-related endogenous molecules, such as adenosine (AD). Since blueberries may induce anti-obesity effects, we tested the hypothesis that blueberries consumption contained in hypocaloric diet would decrease weight, BMI as well as glucose, cholesterol, triglycerides and AD levels in obese subjects. The baseline conditions of obesity-related variables were collected for all subjects prior the implementation of blueberries intake. Later, participants received a hypocaloric diet that included the consumption of blueberries (50 g/day) during 30 days. We found that male obese subjects that consumed blueberries showed a decrease in weight, glucose, cholesterol, triglycerides and AD whereas female obese subjects that ate blueberries in hypocaloric diet showed no differences in weight, BMI, glucose and triglycerides but displayed a diminution in cholesterol and AD levels. Data suggest that intake of blueberries seems to decrease some of the obese-linked parameters in male or female subjects. Importantly, blueberry consumption decreased the inflammation-related compound AD in both sexes.

## 1. Introduction

Obesity is a health disturbance characterized by multiple symptoms, including excessive accumulation of fat in the body, as well as high body mass index (BMI) ($> 30\ Kg/m^2$; WHO, 2012). In addition, obesity is linked with several health problems, such as hypertension, type 2 diabetes, and cancer, just to mention a few. Moreover, obesity causes the enhancement of pro-inflammatory-related compounds including adenosine (AD) (Kaysen, 2009; Pandolfi et al., 2015; Rabkin & Campbell, 2015). One clinical approach to control obesity is the use of specific diets. In line with this, several fruits included in the diet have been associated with health benefits. For example, blueberries contain antioxidant polyphenols that seem to control obesity and inflammation processes (Burton-Freeman, Sandhu, & Edirisinghe, 2016; Jakobsdottir et al., 2013; Jiao et al., 2018; Lee et al., 2016; Liu et al., 2017; Noratto, Chew, & Ivanov, 2016; Wu, Gao, Guo, Zhang, & Gong, 2018).

*Abbreviations:* AD, adenosine; BMI, Body Mass Index; TEE, Estimation of the Total Energy; HPLC, high-performance liquid chromatography

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Nevertheless, as the beneficial health-promoting effects of blueberries on obesity and AD levels remains to be determined. Thus, the present study investigated whether consumption of blueberries included in a hypocaloric diet and given to obese subjects may decrease anthropometric measurements such as weight and BMI and exert positive effects on dysregulated glucose, cholesterol, and triglycerides and AD levels.

## 2. Materials and methods

### 2.1. Bioethical considerations

The Committee for Research and Bioethics of our Institution approved the experimental protocol. During the development of the study, personal information was ensured to be used strictly for the purposes of the analysis of data. In addition, the management of biological samples followed the Guidelines of the Official Mexican Norm (NOM-253-SSA1-2012) as well as the Guidelines of Hazardous Waste Management Biological-Infectious (derived from the Mexican Official Norm [NOM-087-ECOL-SSA1-200]). Furthermore, the study was conducted in accordance with the World Medical Association (Declaration of Helsinki) considering the subjects' approved participation by a written informed consent. The data were analyzed anonymously throughout the study.

### 2.2. Participants

#### 2.2.1. Recruitment

We recruited participants by using advertisements in the Universidad Anáhuac Mayab (students and staff), and by word-of-mouth.

#### 2.2.2. Screening and inclusion

We screened interested volunteers over the telephone and/or social media (email, Facebook, WhatsApp) to be scheduled for the first interview. In session 1, they met with the principal investigator and research staff who gave them a written and verbal detailed description of the research to assess their willingness to participate. Prior to the comprehensive screening assessment, the informed consent was obtained from all participants if they met the inclusion criteria.

#### 2.2.3. Inclusion criteria for participants

To be eligible for inclusion in the study, participants met the obesity criteria (Body Mass Index [BMI]; ≥30 Kg/m2). Therefore, subjects were scheduled for Session 2 (09:00 h) to collect anthropometric measurements such as weight and BMI at the Nutrition Clinic at Universidad Anáhuac Mayab using a body composition analyzer (INBODY®, Body Composition Analyzer, model Inbody 720. Korea, Japan). The procedures used in this section of the study met the internationally accepted criteria (Cáceres-Medina et al., 2015; WHO, 2012). The anthropometric variables collected from Session 2 were considered as baseline condition (pre-diet). Exclusion criteria included self-report of volunteers having previous or recent diagnosis of cancer, endocrine disorders, higher blood pressure (> 140/90 mm Hg), diabetes mellitus, inflammatory, autoimmune disorders, or similar diseases. In addition, female participants were excluded if they were pregnant or lactating. Self-reported medical conditions such as anxiety, chronic pain, sleep disturbances, and depression, under pharmacological prescription or drug (alcohol, other substances) use were also conditions considered as exclusion criteria.

#### 2.2.4. Participants

From the total participants ($n$ = 40, men; $n$ = 20, women), only those who successfully completed the study were included in the report (> 18 years old; men [$n$ = 5] and women [$n$ = 9]). Those who did not complete the study (men [$n$ = 35] and women [n = 11]) were excluded from final report since they failed to follow the diet. Study completers were from Metropolitan area of Mérida City and self-identified as Hispanic.

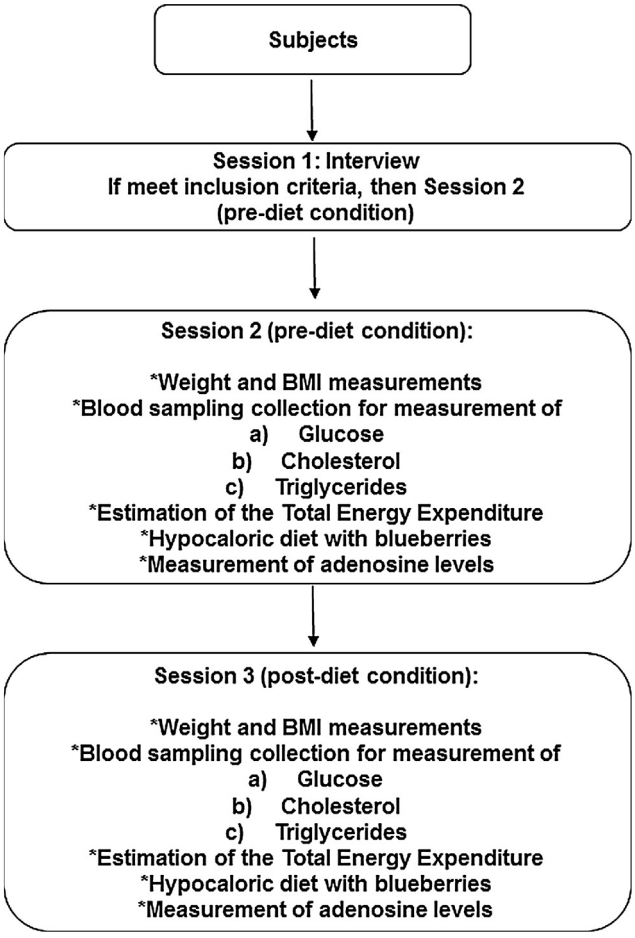


**Fig. 1.** Schematic representation of the experimental design used in the current report.

### 2.3. Chemicals

Reagents, chemicals, and materials were purchased from Sigma-Aldrich (St Louis, MO. USA).

### 2.4. Interventions

The study design flow chart is shown in Fig. 1. In detail: Participants (men [n = 5] and women [n = 9]) were diagnosed in Session 2 (baseline condition: Pre-diet) as obese according to the World Health Organization criteria for BMI (Oviedo-Solís, Cornejo-Manzo, & Murillo-Ortiz, 2018). The Session 2 included the collection of anthropometric measurements (see section *Anthropometric Measurements*), and biochemical sampling (see section *Blood sampling collection*). Moreover, in this session, the estimation of total energy expenditure was determined (see section *Estimation of the Total energy expenditure)* and the hypocaloric diet was given to the participants (see section *Hypocaloric diet with blueberries consumption*). Thirty days later, volunteers were appointed once again for Session 3 (treatment: Post-diet with blueberries consumption) at 09:00 h to obtain anthropometric measurements as well as blood sample collection.

#### 2.4.1. Anthropometric measurements

The weight was calculated by using a standardized balance whereas BMI (BMI ≥ 30 Kg/m$^2$) was obtained by dividing weight by squared height (Kg/m$^2$) using a body composition analyzer (INBODY®, Body Composition Analyzer, Inbody 720. Korea, Japan). The procedures used

in this section of the study were developed as previously reported (Cáceres-Medina et al., 2015; Houska et al., 2018). All measurements were obtained from all participants at Session 2 (baseline condition: Pre-diet) and Session 3 (treatment: Post-diet with blueberries consumption). All the equipments used for obtaining weight and BMI were calibrated after every single measurement for each subject.

#### 2.4.2. Blood sampling collection

Once obtaining the anthropometric measurements from participants, blood was collected (6 mL) into pyrogen-free tubes (Vacutainer TM, BD Diagnostics, NJ. USA) containing EDTA anticoagulant (Sigma Chemicals; St. Louis, MO. USA). Later, blood was centrifuged (1000*g*/4 °C) for 30 min, and serum samples were obtained and stored (−80 °C) until analysis. This procedure was developed in Session 2 (baseline condition: Pre-diet) and Session 3 (treatment: Post-diet with blueberries consumption) following previous reports (Cáceres-Medina et al., 2015).

#### 2.4.3. Estimation of the total energy expenditure (TEE)

To design the diet plan, TEE was determined by the number of Kcal that subjects consumed using the indirect calorimetric measurement (KORR, Reevue. Salt Kale, UT. USA) estimating the rest energy expenditure [REE]). This procedure was used as previously reported by other groups (Cáceres-Medina et al., 2015; Houska et al., 2018; Steinberg et al., 2017).

#### 2.4.4. Hypocaloric diet including blueberries intake

In Session 2 (baseline condition: Pre-diet), a dietary guidance was provided to subjects consisting in a hypocaloric diet (reduction of 500 kcal of total energy requirement) with a distribution of macronutrients as follows: Carbohydrates 50%, protein 22% and fat 28%. The diet was applied during 30 days and it was based on previous reports (Benito et al., 2015; Cáceres-Medina et al., 2015; Houska et al., 2018; Steinberg et al., 2017).

Literature suggests that blueberries consumption has been associated with amelioration of obesity as well as diminution of obesity-related pro-inflammatory molecules (Badillo et al., 2017; Burnstock & Gentile, 2018; Cáceres-Medina et al., 2015; Margalef et al., 2017; Noratto et al., 2016; Oviedo-Solís, Cornejo-Manzo, & Murillo-Ortiz, 2018; Pandolfi et al., 2015; Wyckoff, Evans, Manasse, Butryn, & Forman, 2017). Thus, we included blueberries as beneficial health-promoting food in hypocaloric diet as suggested by others (Wu, Yang, et al., 2018; Zhang, Ma, Luo, & Li, 2018). Therefore, to evaluate whether a pathological state of obesity would be influenced by blueberries consumption (50 g/day), this food item was included in the group of subjects. Lastly, reminders of the diet were made to all subjects through phone calls once a week across the duration of the study.

### 2.5. Blood sample collection and determination of glucose, cholesterol, triglycerides

Blood sample procedure was developed according to standardized medical practices in Session 2 (baseline condition: Pre-diet) and Session 3 (treatment: Post-diet with blueberries consumption) following previous reports (Cáceres-Medina et al., 2015). The blood samples were obtained as follows: The subjects were seated in a chair for venipuncture, while resting both arms. In order to collect blood properly, the site to be punctured was selected avoiding areas such as bruises, burns, or scars, and one of the veins to be punctured was visually identified (ulnar, cephalic or basilic). Once the vein was identified and selected, a tourniquet 3–4 in. above the selected site was placed on the subject's arm (indistinct). The tourniquet remained for a time no longer than 5 min. Subsequently, the area to be punctured was decontaminated with 70% ethyl or isopropyl alcohol using a cotton swab. To perform the blood extraction, the arm preferably remained horizontal, the subject was requested to open, and close the fist 3–5 times to pump blood, and then keep the fist closed. Subsequently, the tip of the needle was placed at an angle of 15–30 degrees on the surface of the chosen vein and the needle was inserted with a firm movement into the lumen of the vein. The blood was collected until required volume was obtained (6 mL). The blood samples were used to analyze glucose, cholesterol, and triglyceride levels by following standard procedures (Kaysen, 2009; Tucková et al., 2017). Blood sample procedure was developed in Session 2 (baseline condition: Pre-diet) and Session 3 (treatment: Post-diet with blueberries consumption) following previous reports (Cáceres-Medina et al., 2015).

### 2.6. Measurement of adenosine levels by HPLC

The blood samples collected in Session 2 (baseline condition: Pre-diet) and Session 3 (treatment: Post-diet with blueberries consumption) were processed for analysis of contents of AD using the high-performance liquid chromatography (HPLC; Modular Prominence HPLC, Shimadzu. Japan). The separation of molecule was achieved by using a C18 column (5 µm, 150 × 4.6 mm; Restek. Bellefonte, PA. USA) within controlled temperature (35 °C; oven CTO-20A. Shimadzu. Japan). The detection of AD was obtained by using an UV detector (260 nm; SPD-20A Prominence. Shimadzu. Japan) coupled to HPLC. The mobile phase (20 mM of ammonium acetate, pH 5.8: methanol [97.5:2.5]) was perfused (flow rate 1 mL/min) using a pump (LC-20AT. Prominence HPLC, Shimadzu. Japan). The samples were automatically injected (SIL-20A HT Prominence HPLC, Shimadzu. Japan) and chromatographic data was stored in a personal computer (via computer controller. CBM-20A. Shimadzu. Japan). The levels of AD were calculated by using the HPLC software (LC LabSolution. Shimadzu. Japan) comparing the sample peaks with known external standards. The procedure for analysis of the concentrations of AD was carried out as previously reported as well as by manufacturer's suggestions (Cáceres-Medina et al., 2015; Murillo-Rodríguez et al., 2017).

### 2.7. Statistical analysis

The results are represented as mean ± standard error of the mean. To determine whether blueberries intake would induced changes in weight, BMI, glucose, cholesterol, triglycerides as well as AD levels, variables were compared between baseline and blueberries consumption condition. Statistical differences were identified by a Student *t*–test with a significance level of $P < 0.05$. All statistical analyses were determined using StatView (version 5.0.0; SAS Institute. USA).

## 3. Results and discussion

### 3.1. Male anthropometric, biochemical and AD measurements in pre-diet vs male post-diet with blueberries consumption

Table 1 shows that comparison between male pre-diet vs male post-diet with blueberries consumption displayed statistical differences in weight ($P < 0.05$) whereas BMI did not show significant changes

**Table 1**
Weight, BMI, glucose, cholesterol, triglycerides and adenosine measurements from male pre-diet vs. male post-diet with blueberries consumption.

| | Male pre-diet | Male post-diet with blueberries consumption | *P*-value |
|---|---|---|---|
| Weight (Kg) | 93.20 ± 2.4 | 80.26 ± 2.0 | *P < 0.05* |
| BMI (Kg/m$^2$) | 30.64 ± 1.3 | 29.26 ± 1.3 | *P = 0.4* |
| Glucose (mg/dL) | 92.04 ± 3.7 | 83.14 ± 1.6 | *P < 0.05* |
| Cholesterol (mg/dL) | 210.45 ± 14.1 | 152.70 ± 6.6 | *P < 0.006* |
| Triglycerides (mg/dL) | 190.69 ± 54.7 | 112.16 ± 2.4 | *P < 0.05* |
| Adenosine | 257.66 ± 170.1 | 70.61 ± 5.8 | *P < 0.0005* |

**Table 2**
Weight, BMI, glucose, cholesterol, triglycerides and adenosine measurements from female pre-diet vs. female post-diet with blueberries consumption.

| | Female pre-diet | Female post- diet blueberries consumption | *P*- value |
|---|---|---|---|
| Weight (Kg) | 72.66 ± 2.8 | 71.60 ± 3.2 | $P = 0.8$ |
| BMI (Kg/m$^2$) | 28.29 ± 0.9 | 27.91 ± 1.0 | $P = 0.7$ |
| Glucose (mg/dL) | 93.19 ± 3.7 | 95.96 ± 3.4 | $P = 0.5$ |
| Cholesterol (mg/dL) | 189.35 ± 3.7 | 170.70 ± 3.5 | $P < 0.05$ |
| Triglycerides (mg/dL) | 133.57 ± 28.1 | 137.11 ± 13.0 | $P = 0.9$ |
| Adenosine | 1566.25 ± 148.6 | 59.46 ± 3.3 | $P < 0.0001$ |

($P = 0.4$). However, it is worthy to mention that glucose ($P < 0.05$), cholesterol ($P < 0.006$) and triglycerides ($P < 0.05$) levels were diminished after blueberries consumption. Importantly, we found that intake of blueberries contained in a hypocaloric diet caused a decrease in AD levels in male obese subjects ($P < 0.0005$).

### 3.2. Female anthropometric, biochemical and AD measurements in pre-diet vs female post-diet blueberries intake

Comparison of anthropometric variables in female obese subjects before and after blueberries intake showed no significant differences were found in weight ($P = 0.8$), BMI ($P = 0.7$), glucose ($P = 0.5$) and triglycerides ($P = 0.9$). However, cholesterol was decreased ($P < 0.05$) as well as AD levels ($P < 0.0001$) (see Table 2).

Dietary plans replacing high caloric food items have become a clinical approach for treating obesity. However, diet plan changes show limited successful outcomes (Amiot, Riva, & Vinet, 2016; Asghari, Mirmiran, Yuzbashian, & Azizi, 2017; Curioni & Lourenco, 2005; Lichtenstein et al., 2014; Martin & Gadde, 2014; Moosavi, Hosseini, Saso, & Firuzi, 2016; Noratto et al., 2016; Rabkin & Campbell, 2015). The positive impact on obese-related measurements by including the consumption of blueberries in diets has opened a new horizon revealing the putative role of these fruits consumption in endogenous obesity-related molecules (Burton-Freeman et al., 2016; Jakobsdottir et al., 2013; Lee et al., 2016; Liu et al., 2017; Noratto et al., 2016; Oviedo-Solís, Cornejo-Manzo, & Murillo-Ortiz, 2018). In this study, we examined the hypothesis that consumption of blueberries included in hypocaloric diet given to obese subjects would decrease anthropometric measurements, biochemical elements as well as the pro-inflammatory molecule AD.

A dietary pattern comprising hypocaloric restriction as well as blueberries (50 g/day/30 days) caused differences in male weight, glucose, cholesterol, triglycerides and AD whereas BMI remained with no significant changes compared to pre-diet condition. On the other hand, female obese subjects that consumed blueberries in hypocaloric diet showed no statistical differences in weight, BMI, glucose and triglycerides while cholesterol and AD levels were decreased. We speculate that differences in effects observed in experimental variables such as weight, BMI, glucose and triglycerides between sexes might be related with metabolic aspects proper of male or female subjects (Dearden, Bouret, & Ozanne, 2018; Barstad et al., 2018).

The current results are in line with previous reports by showing the positive effects of consuming berries in obesity control as well as in decreasing inflammatory-related molecules (Kowalska, Olejnik, Szwajgier, & Olkowicz, 2017; Leu et al., 2017; Noratto et al., 2016; Vendrame, Tsakiroglou, Kristo, Schuschke, & Klimis-Zacas, 2016; Wu et al., 2013; Wu, Yang, et al., 2018). In this regard, rat models of obesity respond positively if animals are under diet enriched with blueberries (Aranaz et al., 2017). Similar outcome shave been reported in obese humans suggesting that functional foods such as blueberries exert positive effects in obese-related parameters (Basu et al., 2010; Novotny, Baer, Khoo, Gebauer, & Charron, 2015; Stull, Cash, Johnson, Champagne, & Cefalu, 2010).

However, some limitations from current report are important to mention. For example, we recognize that number of subjects reported in our study could represent a limitation. In this regard, it is assumed that participants in clinical research are responsible for following the agreement when they accept to be enrolled in a study. These responsibilities of fulfilling instructions are based on promise-keeping. However, participants also have the right of withdrawal at any time and without giving any reason and research staff are unable to “push” subjects to remain in the study by any mean (Schaefer & Wertheimer, 2010; Resnik & Ness, 2012; Hug & Johansson, 2017). Perhaps in future trials using financial incentives, subjects might remain for meeting study requirements. In addition, we are aware about the likely influence of hormonal status in our study in female subjects that might affect the metabolism as reported by others (Bryant, Truesdale, & Dye, 2006). Thus, next studies should include hormonal control in female subjects. Another limitation identified includes the unknown mechanism of action of some of the compounds contained in blueberries, such as the polyphenols on weight, BMI, glucose, cholesterol, triglycerides and AD. Indeed, future reports should aim the description of the polyphenols in modulation of obese-related biomedical parameters. In this regard, nutritional effects caused by blueberries seem to be related with flavonoids. Several pieces of evidence have suggested that consumption of fruits containing flavonoids exert a positive effect in obesity. Based in the evidence, one might hypothesize that intake of blueberries may exert anti-inflammatory effects by increasing the degradation of AD, compound that has been involved in the pathophysiology of obesity (Babu, Liu, & Gilbert, 2013; Gentile et al., 2018; Singh & Venkatesan, 2018). Thus, the effects of blueberries consumption in the synthesis and/or degradation of AD in obese subjects should be addressed as well. Moreover, it remains to be elucidated if the effects on obesity-related variables induced by blueberries consumption are long-term. Moreover, it is possible that additional biochemical elements related to inflammation and glucose metabolism, including cytokines, might be under the influence of blueberry supplementation (Gonzalez, Garrie, & Turner, 2018; Kern, Mittenbühler, Vesting, Ostermann, Wunderlich, & Wunderlich, 2018; Zhou, Li, & Pathak, 2019). Future studies should address whether contents of TNFα and IL6 in obese subjects respond to blueberry intake in diet.

## 4. Conclusions

In this study, we found that consumption of blueberries in male obese subjects decreased weight, glucose, cholesterol, triglycerides as well as AD levels whereas female obese subjects displayed no statistical changes in weight, BMI, glucose, and triglycerides but decreased AD contents. This study could provide preliminary evidence regarding the effects of consuming blueberries as part of a diet given to obese subjects.

## Declaration of Competing Interest

No competing financial or other interest to declare for the study.

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