Published in final edited form as:

Int J Cardiol. 2018 July 15; 263: 111–117. doi:10.1016/j.ijcard.2018.04.040.

Circulating metabolites of strawberry mediate reductions in vascular inflammation and endothelial dysfunction in *db/db* mice^{1–,5}

Chrissa Petersen¹, Divya Bharat¹, Brett Ronald Cutler¹, Samira Gholami¹, Christopher Denetso¹, Jennifer Ellen Mueller¹, Jae Min Cho¹, Ji-Seok Kim¹, J. David Symons^{1,2}, and Pon Velayutham Anandh Babu^{1,*}

¹Department of Nutrition and Integrative Physiology, College of Health, University of Utah, Salt Lake City, Utah 84112, USA

²Division of Endocrinology, Metabolism, and Diabetes; and Molecular Medicine Program, University of Utah, Salt Lake City, UT 84112, USA

Abstract

Background—Cardiovascular disease is 2–4-fold more prevalent in patients with diabetes. Human studies support the cardiovascular benefits of strawberry consumption but the effects of strawberry on diabetic vasculature are unknown. We tested the hypothesis that dietary strawberry supplementation attenuates vascular inflammation and dysfunction in diabetic mice.

Methods—Seven-week-old diabetic *db/db* mice that consumed standard diet (*db/db*) or diet supplemented with 2.35% freeze-dried strawberry (*db/db*+SB) for ten weeks were compared to non-diabetic control mice (*db/+*). Indices of vascular inflammation and dysfunction were measured. Endothelial cells (ECs) were isolated from the vasculature to determine the influence of strawberry on them. The effect of metabolites of strawberry on endothelial inflammation was

¹Supplementary Methods, Tables and Figures are available from the “Online Supporting Material”

⁵All above authors takes responsibility of all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation. Authors declare no potential conflict of interest.

*Corresponding author: Pon Velayutham Anandh Babu, Department of Nutrition and Integrative Physiology, College of Health, University of Utah, Salt Lake City, Utah 84112. Tel: +1-801-581-8376, Fax: +1-801-585-3874, anandh.velayutham@utah.edu.

⁴Current address for J.K.: Department of Physical Education, Gyeongsang National University, South Korea.

²Abbreviations: BSA, bovine serum albumin; CAECs, carotid artery endothelial cells; DAF, 4-amino-5-methylamino-2',7'-difluorofluorescein diacetate; *db/+*, non-diabetic control mice; *db/db*, diabetic mice; *db/+*+SB, strawberry fed control mice; *db/db*+SB, strawberry fed diabetic mice; DCFDA, 2',7'-Dichlorofluorescein diacetate; eNOS, endothelial nitric oxide synthase; HG, 25 mM glucose; IL-8, interleukin-8; ICAM-1, intercellular adhesion molecule-1; IκBβ, inhibitor κB kinase; MAECs, mouse aortic endothelial cells; MCP1, monocyte chemoattractant protein-1; NFκB, nuclear factor κB; L-NAME, L-N^G-Nitroarginine methyl ester; NO, nitric oxide; NOX, NADPH Oxidases; Pal, 100 μM palmitate-BSA; ROS, reactive oxygen species; TNF-α, Tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1.

³Supported by research funds from the University of Utah research start-up fund, University of Utah Seed Grant, and College of Health Pilot Grant (to P.V.A.B.); the University of Utah Undergraduate Research Opportunities Program award (to B.C., S.G., J.E.M.); Native American Research Internship (to C.D.); American Heart Association (AHA:16GRNT31050004) and National Institute of Health (NIH: R03AGO52848) (to J.D.S.).

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determined by incubating mouse aortic ECs (MAECs) with $\pm$ 5% serum, obtained from strawberry fed mice (metabolites serum) or standard diet fed mice (control serum) $\pm$ 25 mM glucose and 100 μ M palmitate.

Results—*db/db* mice exhibited an increased monocyte binding to vessel, elevated blood pressure, and reduced endothelial-dependent vasorelaxation compared with *db/+* mice but each defect was attenuated in *db/db*+SB mice. The elevation of inflammatory molecules, NOX2 and inhibitor- κ B kinase observed in ECs from *db/db* vs. *db/+* mice was suppressed in *db/db*+SB mice. Glucose and palmitate increased endothelial inflammation in MAECs but were normalized by co-incubation with metabolites serum.

Conclusions—Dietary supplementation of strawberry attenuates indices of vascular inflammation and dysfunction in diabetic *db/db* mice. The effect of strawberry on vasculature is endothelial-dependent and possibly mediated through their circulating metabolites. Strawberry might complement conventional therapies to improve vascular complications in diabetics.

Keywords

strawberry; vascular inflammation; endothelial dysfunction; diabetes; metabolites

1. Introduction

Diabetes greatly increases the risk of cardiovascular disease, such as atherosclerosis, which accounts for the largest number of deaths among diabetic patients [1–3]. In diabetes, high glucose induced vascular inflammation and the subsequent endothelial dysfunction plays a major role in the development of vascular disease [2–4]. Hyperglycemia, dyslipidemia, pro-inflammatory cytokines, and vascular adhesion molecules contribute independently and synergistically to vascular dysfunction in diabetes by increasing endothelial cell (EC) inflammation [2–5]. Treating vascular complications associated with diabetes contributes to a heavy economic burden on patients and society. Due to the current epidemic of diabetes, there is a need to identify cost effective adjunct strategies for treating vascular complications associated with this disease.

Human studies support the vascular effects of anthocyanins, one class of flavonoids, widely found in berry fruits [6–12]. Anthocyanins are composed of anthocyanidin (aglycon component of anthocyanins such as pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin) and a sugar moiety. Strawberry is an excellent source of anthocyanins and the most commonly found anthocyanins in strawberries are the glycosidic derivatives of pelargonidin and cyanidin [10]. Recent study shows that consuming 2–3 servings of strawberries per week lowers the risk of myocardial infarction in humans [7]. Strawberry intake was shown to attenuate postprandial inflammation in overweight adults who ingested a high-carbohydrate, moderate-fat meal [13]. Further, intake of strawberries reduces vascular adhesion molecules in humans with cardiovascular risk factors [11, 12]. However, the effects of strawberry on endothelial dysfunction in general and diabetes in particular are unknown. Anthocyanins are extensively metabolized in humans by digestive enzymes and intestinal microbiota, suggesting the vascular effects could be mediated by their circulating metabolites [6, 14–17]. Indeed, data from our laboratory and others suggest strongly that

anthocyanin metabolites and not the parent anthocyanins might be the main cause of some of the benefits of the ingested parent compound [6, 18, 19].

In the present study, we investigated whether dietary supplementation of strawberries, at a nutritional dose, ameliorates vascular inflammation and dysfunction in diabetes. Further, we examined whether the effect of strawberry on vasculature is endothelial-dependent and the role of metabolites in exerting the vascular effects of strawberries. Specifically, we hypothesized that strawberry ameliorates vascular inflammation and dysfunction in diabetic mice and that the endothelial specific vascular beneficial effects of strawberry are mediated through their circulating metabolites.

2. Materials and methods

2.1. Materials

Materials are available in the online supporting material.

2.2. Pre-clinical model of type 2 diabetes

Six-week-old male diabetic *db/db* mice with a C57BLKS/J background (*db/db*; B6.Cg-m^{+/+}Lepr^{db}) and control *db/+* mice with the same background (Stock no. 000642) were obtained from the Jackson Laboratories (Bar Harbor, ME). *db/db* mice is a widely-used type 2 diabetic animal model that spontaneously develops endothelial dysfunction and vascular complications [4, 20–22]. We have previously demonstrated an enhanced endothelial inflammation in *db/db* mice [4, 20]. The mice were provided free access to a AIN 93 G rodent diet (Dyets Inc, Bethlehem, PA) with corn oil substituted for soy bean oil [4], and acclimated for a week before experiments were performed. Mice were exposed to experimental conditions beginning at seven weeks of age. Animals were maintained under artificial light in a 12-hour light/dark cycle, 23 ± 1° C, and 45 ± 5% humidity and were held under humane conditions. The Institutional Animal Care and Use Committee at the University of Utah approved animal experimental protocols.

2.3. Standard diet and strawberry supplemented diet

The freeze-dried strawberry powder was provided by the FutureCeuticals (Momence, IL). The customized pelleted diets prepared as shown in Supplementary Table 1 were supplied by Dyets Inc. (Bethlehem, PA). The strawberry supplemented diet was adjusted to compensate for the additional sugars and fiber provided by the freeze-dried strawberry. The amount of freeze-dried strawberry powder used in this study was calculated based on the extrapolation of doses from animals to humans by normalization to body surface area [23]. The nutritional dose of freeze-dried strawberry powder was based on average human consumption. 2.35% Freeze-dried strawberry powder in diet (w/w) is equivalent to two human servings of fresh strawberries (~160 g strawberries) per day [24].

2.4. Experimental groups

After 1 week of acclimation, *db/db* mice (seven-week-old) were divided into two groups and received standard diet (*db/db*) (n=20) or 2.35% freeze-dried strawberry supplemented diet (*db/db*+SB) (n=16) for 10 weeks. *db/+* mice (seven-week-old) received standard diet (*db/+*)

(n=20) for 10 weeks. The markers of inflammation and relevant endpoints were assessed at the organism level, organ level, and cellular level (Supplementary Fig. 1A).

2.5. Measurement of metabolic variables, blood pressure and collection of tissue samples

Blood glucose, serum lipids, glucose tolerance, insulin tolerance and blood pressure were measured as we have described [4, 20, 25] and detailed in the online supplement.

2.6. Measurement of vascular inflammation

The effect of strawberry supplementation on vascular inflammation in diabetic mice was assessed by measuring the binding of monocytes to the aortic vessel, determining the expression of inflammatory markers [chemokines such as monocyte chemoattractant protein-1 (MCP-1)/JE and KC, and adhesion molecules such as vascular cell adhesion molecule-1 (VCAM1), intercellular adhesion molecule-1 (ICAM1), and E-Selectin] in aortic vessels, and measuring circulating chemokines as we have described [4, 19, 26] and detailed in the online supplement.

2.7. Measurement of vascular function

Vascular function was assessed in two mesenteric artery segments using isometric tension techniques as we have described [25, 27–29] and detailed in the online supplement.

2.8. Isolation of ECs from carotid artery and assessment of endothelial effects of strawberry

To assess the influence of strawberry metabolites on endothelial cells, we further investigated vascular ECs for markers of inflammation. ECs were isolated from carotid arteries as we have described [30]. Briefly, carotid arteries were excised and perfused with QIAzol reagent. The vessel effluent containing the intimal fraction contains the carotid artery ECs (CAECs). The remaining components of the vessel contains media and adventitia. The purity of CAECs in the vessel effluent was confirmed by measuring platelet endothelial cell adhesion molecule-1 (PECAM-1) which is specific to ECs and smooth muscle actin (SMA) which is specific for smooth muscle cells. The mRNA expression of inflammatory and adhesion molecules, NADPH oxidases (NOXs), nuclear factor- κ B (NF κ B) signaling markers, endothelial nitric oxide synthase (eNOS), tumor necrosis factor- α (TNF- α), and nuclear factor erythroid 2 - related factor (Nrf2) were measured by qPCR [18].

2.9. Cell culture

Mouse aortic endothelial cells (MAECs) and mouse monocytic WEHI78/24 cells were cultured as described in the online supplement.

2.10. Measuring the effects of serum of strawberry-fed mice on endothelial inflammation

To assess whether the vascular effects of strawberry are mediated through their circulating metabolites we used serum derived from strawberry fed mice. Serum was collected at the fed state from non-diabetic control mice (*db/+* mice) supplemented with standard diet (control serum) or 2.35% freeze-dried strawberry diet (metabolites serum) for 10 weeks. MAECs

were grown to confluence in EC complete medium. After serum starvation for 12 h, MAECs (passage 4–5) were treated with or without 5% serum derived from strawberry fed mice (metabolites serum) or standard diet fed mice (control serum) and with or without 25 mM glucose (HG) and 100 μ M palmitate-BSA (Pal) (or) mannose and BSA (vehicle) for 3 days (Supplementary Fig. 1B). Palmitate (C16:0) (Sigma, MO) was coupled to fatty acid-free BSA in the ratio of 2 M palmitate to 1 M BSA [25]. HG+Pal is an established model to induce endothelial inflammation and the doses of glucose/palmitate were chosen based on a previous study indicating its ability to evoke endothelial inflammation in EC [31]. Upon completing the respective treatments, the following were assessed: WEHI78/24 mouse monocyte binding to MAEC and secretion of inflammatory chemokines in the medium.

2.11. Data Analysis

Comparison of one time point among groups was made using one-way ANOVA. All data points at all times in glucose and insulin tolerance tests were compared using repeated measures ANOVA. To analyze vascular function, comparison of multiple time points among groups was made using two-way repeated-measures ANOVA. Significant differences among means were identified by the Tukey post hoc test. Data were analyzed with the use of SPSS (Version 25; IBM) or Prism (Version 7.0; GraphPad). All data were expressed as mean $\pm$ SEM, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Strawberry supplementation does not alter the systemic metabolic milieu in db/db mice

Body weight, food intake, fasting and non-fasting blood glucose, and serum cholesterol and triglycerides were increased in *db/db* mice vs. *db/+* mice, but were not altered in *db/db*+SB vs. *db/db* mice (Supplementary Table 2). Likewise, strawberry supplementation did not alter the disruption of peripheral glucose homeostasis observed in *db/db* mice compared with *db/+* mice (Supplementary Fig. 2A, B).

3.2. Strawberry supplementation reduces vascular inflammation and modestly improves lipid peroxidation in db/db mice

There was an increase in binding of mouse monocytic WEHI 78/24 cells to the aortic vessel isolated from *db/db* mice compared with *db/+* mice (Fig. 1A). However, strawberry consumption reduced the binding of monocytes to aortic vessels in *db/db*+SB vs. *db/+* mice (Fig. 1A). The aortic vessels isolated from *db/db* mice exhibited a significant increase in the mRNA expression of MCP-1/JE, KC, and VCAM-1 compared with *db/+* mice (Fig. 1B, C). MCP-1/JE and KC are the mouse ortholog of MCP-1 and IL-8 respectively [4]. However, the expression of MCP-1/JE, KC, and VCAM-1 were significantly reduced in *db/db*+SB vs. *db/db* mice (Fig. 1B, C). Consistently, MCP-1/JE and KC were significantly increased in the serum of *db/db* mice compared with *db/+* mice but *db/db*+SB mice exhibited decreased levels of these serum chemokines (Fig. 1D, E). The lipid hydroperoxidation, as measured by the concentration of serum lipid hydroperoxides was increased in *db/db* vs. *db/+* mice and strawberry supplementation tended to decrease ($p = 0.06$) lipid hydroperoxides in *db/db*+SB mice (Fig. 1F).

3.3. Blood pressure was lower and endothelial vasorelaxation was improved in the strawberry supplemented db/db mice

Arterial pressure (systolic and diastolic) increased in *db/db* vs. *db/+* mice (Supplementary Table 2). However, the severity of hypertension was lower in *db/db*+SB vs. *db/db* mice. Relaxation to acetylcholine in mesenteric arteries precontracted with phenylephrine was impaired in *db/db* mice compared with *db/+* mice (Fig. 2A). Of note, the severity of impairment was lessened in *db/db*+SB vs. *db/db* mice (Fig. 2A). Importantly, endothelium-independent vasorelaxation was similar among groups (Fig. 2B), suggesting strongly that an endothelium-specific defect that existed in *db/db* mice was ameliorated by strawberry supplementation. The receptor and non-receptor mediated vasocontraction were similar among groups (Fig. 2C, D).

3.4. Strawberry supplementation reduces endothelial inflammatory molecules in db/db mice indicating the endothelial effects of strawberry bioactives

The endothelial effect of strawberry was assessed by measuring inflammatory molecules in CAECs isolated from experimental mice. The purity of CAECs in intimal fractions was confirmed by the presence of PECAM-1 and absence of SMA in the intimal fraction (Fig. 3A, B). CAECs isolated from *db/db* mice exhibited a significant increase in mRNA expression of MCP1/JE, KC, ICAM1 and VCAM1 as compared with the CAECs from *db/+* mice (Fig. 3C, D). However, the mRNA expression of MCP1/JE, KC, and VCAM1 was significantly reduced in CAECs from *db/db*+SB vs. *db/db* mice (Fig. 3C, D). This indicates an endothelial specific anti-inflammatory effect of strawberry bioactives.

3.5. Strawberry supplementation reduces the expression of inhibitor κ B kinase ($I\kappa K\beta$) and NOX2 in db/db mice

We further assessed whether the effect of strawberry on ECs is mediated through NF κ B, NOXs, eNOS or Nrf2. CAECs isolated from *db/db* mice exhibited an increased expression of $I\kappa K\beta$, $I\kappa B\alpha$, NOX2, and NOX4 compared with CAECs from *db/+* mice (Fig. 3E, F). Interestingly, the expression of $I\kappa K\beta$ and NOX2 were reduced in CAECs from *db/db*+SB vs. *db/db* mice (Fig. 3E, F). Further, the endothelial expression of $I\kappa B\alpha$ ($p = 0.06$) and NOX4 ($p = 0.06$) were modestly reduced in CAECs from *db/db*+SB vs. *db/db* mice. The expression of eNOS, TNF- α , and Nrf2 were similar among groups (Supplementary Fig. 3).

3.6. Strawberry metabolites suppress endothelial inflammation in MAECs exposed to high glucose and palmitate

HG+Pal treatment significantly increased the binding of mouse monocytic WEHI78/24 cells to MAEC which was associated with a significant increase in the secretion of MCP-1/JE and KC when compared with vehicle treated MAECs (Fig. 4A, B, C). However, co-treatment with serum obtained from strawberry fed mice significantly reduced monocyte binding to MAEC and reduced the secretion of the KC compared with control serum treated MAECs (Fig. 4A, C). This indicates the possible role of circulating metabolites in exerting the endothelial/vascular effects of strawberry.

4. Discussion

Epidemiological and clinical studies support the cardiovascular benefits of strawberry consumption [7, 10]. We investigated the hypothesis that dietary strawberry at a nutritional dose ameliorates vascular complications in *db/db* mice. Our results indicate that dietary supplementation of strawberry reduces vascular inflammation and endothelial dysfunction that otherwise was displayed by *db/db* mice. Further, the vascular effects of strawberry appeared to be endothelial specific and likely mediated through their circulating metabolites.

The influence of strawberry supplementation on endothelial inflammation and dysfunction was assessed because both contribute importantly to the pathogenesis of atherosclerosis. The binding of monocytes to the aortic vessel followed by their transmigration into the subendothelial space play a major role in the development of atherosclerosis [9].

Inflammatory chemokines and adhesion molecules are involved in monocyte rolling, and their interaction with endothelium [9, 18]. In the present study, mouse monocytic WEHI 78/24 cells had significantly higher binding to the aortic vessel isolated from *db/db* mice *vs.* *db/+* mice. This was accompanied by the increased expression of MCP1/JE, KC, ICAM1, and VCAM1 in the aortic vessels of *db/db* mice compared with control mice as reported in previous studies [32, 33]. Strawberry supplementation suppressed MCP-1/JE, KC, and VCAM1 in *db/db*+SB mice which is associated with reduced binding of monocytic WEHI78/24 cells to the diabetic vasculature. This is consistent with a previous study that showed strawberry supplementation reduces circulating ICAM1 and VCAM1 in humans with cardiovascular risk factors [11, 12]. Further, strawberry supplementation reduced the serum MCP-1/JE and KC in *db/db*+SB compared with *db/db* mice. Our data indicate that the effects of strawberry on inflammatory molecules are regulated both at the transcriptional and translational levels.

Endothelial dysfunction plays a pivotal role in the development of vascular complications and atherosclerosis in type 2 diabetes [22, 33]. Evidence shows that endothelial dysfunction associated with impaired endothelium-dependent vasodilation in resistance arteries contributes to increased blood pressure in *db/db* mice [34, 35]. In our study, *db/db* mice exhibited an increase in blood pressure compared with control *db/+* mice as we have reported previously [4, 20]. However, few studies reported normal or mild elevation of blood pressure values in *db/db* mice as compared with control C57BLKS/6J mice [36, 37]. This discrepancy could be due to the different control animals used in these studies. In the present study, arterial hypertension observed in *db/db* mice was lowered by dietary supplementation of strawberry. Our experimental design included parallel assessment of vascular function in mesenteric arteries from all groups, because these resistance-sized vessels are known to contribute importantly to peripheral resistance and blood pressure [38]. Consistent with previous studies, vasorelaxation evoked by acetylcholine (i.e., endothelium-dependent) but not sodium nitroprusside (endothelium-independent) was impaired in *db/db* mice compared with control mice [34, 35]. As hypothesized, the severity of dysfunction observed in *db/db* mice was attenuated in *db/db*+SB mice. Hence, it is not unreasonable to speculate that dietary strawberry might have improved arterial pressure in *db/db* mice secondary to improved endothelial function. However, we cannot exclude the possibility that the

bioactives of strawberry may act on multiple targets to exert the vascular beneficial effects of strawberry.

Next we sought to determine a possible cause for the endothelial-specific effect of strawberry. In this regard, elevated mRNA expression of MCP1/JE, KC, ICAM1, and VCAM1 observed in CAECs from *db/db* mice was attenuated in *db/db*+SB mice. We interpret these data to indicate that strawberry supplementation has a direct effect on endothelial cells. Taken together, our study is the first to provide the evidence for the benefits of strawberry on vascular inflammation at the organism level (mice), tissue level (vessel), and cellular level (ECs).

Dietary supplementation of strawberry did not affect metabolic parameters such as body weight, food intake, blood glucose, serum lipids, glucose or insulin tolerance in *db/db*+SB mice. This indicates that the observed vascular effects of strawberry are not mediated through the improvement in these metabolic parameters and are not likely to be due to any secondary effect.

Anthocyanins are extensively metabolized in humans suggesting the vascular benefits of strawberry might be mediated by their circulating metabolites [6, 14–17]. Indeed, data from our laboratory and others suggest that anthocyanin metabolites might be the principle actors in some of the benefits of the ingested parent compound [6, 18, 19]. To further explore this, we assessed whether the vascular and endothelial effects are mediated through the metabolites of strawberry. In our study, serum containing strawberry metabolites suppressed HG+Pal-induced monocyte binding to MAEC and reduced the secretion of inflammatory KC by MAECs. A recent study showed that 21 polyphenolic metabolites appear in the plasma following the consumption of strawberries in humans [17]. Pelargonidin-3-O-glucuronide (P3G) is the major metabolite of the most abundant anthocyanin pelargonidin-3-O-glucoside [16, 17]. We speculate that the endothelial benefits could be due to these strawberry metabolites present in the serum. Further, we used 5% serum in our *in vitro* study in which strawberry metabolites were diluted 20x their normal concentration and thus the results might have been even more significant *in vivo*.

We also determined the possible mechanisms involved in the vascular effects of strawberry. NOXs are a family of reactive oxygen species (ROS) generating enzymes and the effects of NOX1, NOX2 and NOX4 on vasculature are well documented [39, 40]. NOX-derived ROS play a major role in the maintenance of normal physiological vascular function as well as vascular pathology such as the development of endothelial dysfunction, inflammation, and atherosclerosis [40]. Indeed, an increased mRNA expression of NOX2, gp91phox and p22phox subunits of NOX was reported in diabetic vasculature [32, 33, 39]. Consistent with these studies, in our study the mRNA expression of NOX2 and NOX4 were greatly increased in CAECs isolated from *db/db* mice *vs.* *db/+* mice. Strawberry supplementation significantly reduced NOX2 and modestly reduced NOX4 in *db/db* mice indicating the effect of strawberry could be mediated through NOXs. In addition, strawberry supplementation modestly reduced serum lipid hydroperoxides in *db/db* mice. NFκB is one of the major targets of NOX derived ROS and NOXs activate NFκB signaling in diabetic vasculature [20, 41, 42]. p50/p65 is the most abundant form of NFκB. IKKβ activates the nuclear

translocation of p50/p65 by degrading the inhibitor I κ B α [20]. In the nucleus, p50/p65 binds to the promoters of NF κ B-dependent inflammatory genes and upregulates the genes involved in atherosclerosis [20]. High glucose increases ROS which activates p65 nuclear translocation in ECs and aortic vessels that participate in diabetes-caused vascular complications [20, 43]. In our study, CAECs from *db/db* mice exhibited an increased expression of IKK β and I κ B α . Increased IKK β expression indicates the activation of NF κ B signaling and increased I κ B α expression could be an adaptive response to this activation. Strawberry supplementation significantly reduced the expression of IKK β and modestly reduced I κ B α in CAECs of *db/db*+SB mice. These data indicate that strawberry likely attenuates NF κ B signaling by suppressing NOX in *db/db*+SB mice.

4.1. Limitations

In our study, we assessed the mRNA expression in CAECs and aortic vessels, determined the vascular inflammation in aortic vessels, and measured the vascular function in segments of mesenteric artery. Studies indicate that the gene expression is differentially regulated throughout the vasculature because of the heterogeneity in the structure and function of the endothelium [44, 45]. Hence, the effect of strawberry on vasculature could depend on their anatomical location and we acknowledge this limitation. However, it is important to note that the comparisons between *db/+*, *db/db*, and *db/db*+SB mice in this study were performed in the same segment of the arterial tree. Further, our study indicates that the vascular benefits of strawberries could be due to strawberry metabolites present in the serum. However, we do not know with certainty as we did not measure the metabolites in the serum. Future research is warranted to better understand and validate the bioactivities of strawberries.

4.2. Conclusions

Our findings indicate that dietary supplementation of strawberry ameliorates endothelial dysfunction, improves vascular inflammation, and reduces endothelial inflammatory and adhesion molecules in *db/db* mice. Further, we provide robust evidence that the effect of strawberry on diabetic vasculature is endothelial specific and is possibly mediated via circulating metabolites of anthocyanins. Of note, the vascular benefits of strawberry were achieved at a nutritional dose, i.e., one that is equivalent to 160 g of strawberries. This is an important consideration when contemplating the translational relevance of findings from diabetic mice to patients with debilitating disease. Further, our results promote further exploration into the effect of strawberry on inflammatory and atherogenic pathways and these studies are ongoing. In conclusion, our study provides strong proof of concept for further considering strawberry as an adjunct therapy to preventing, delaying the onset, or reversing vascular complications associated with diabetes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

C.P., and P.V.A.B. designed research; C.P., D.B., B.R.C., S.G., C.D., J.E.M., J.C., J.K., J.D.S., and P.V.A.B. conducted research; C.P., J.D.S., and P.V.A.B. analyzed data; and C.P., J.D.S. and P.V.A.B. wrote the paper. P.V.A.B. had primary responsibility for final content. All authors read and approved the final manuscript.

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Highlights

- Cardiovascular disease is 2–4-fold more prevalent in patients with diabetes
- Dietary strawberry attenuates vascular inflammation and dysfunction in diabetic mice
- Effect of strawberry on diabetic vasculature is endothelial-dependent
- Endothelial effects of strawberry are mediated through circulating metabolites
- Strawberry might complement existing therapies to improve vascular complications

Fig. 1.

Monocyte binding (A), inflammatory chemokines (B) and adhesion molecules (C) in aortic vessels, serum MCP1/JE (D), serum KC (E) and serum hydroperoxides (F) of *db/+*, *db/db* and *db/db+SB* mice treated for 10 wk. Values are mean $\pm$ SEM, $n=5-6$. Comparison among groups was made using one-way ANOVA and Tukey post hoc tests were performed when significant main effects were obtained. Labelled means without a common letter differ, $P < 0.05$. *db/+*, Standard diet fed control mice; *db/db*, standard diet fed diabetic mice; *db/db+SB*, strawberry fed diabetic mice; E-sel, E-selectin; ICAM-1, intercellular adhesion molecule-1; MCP1, monocyte chemoattractant protein-1; VCAM-1, vascular cell adhesion molecule-1.

Fig. 2.

Endothelium-dependent vasorelaxation (A), endothelium-independent vasorelaxation (B), receptor-mediated vasoconstriction (C), and non-receptor mediated vasoconstriction (D) in *db/+*, *db/db* and *db/db+SB* mice treated for 10 wk. Values are mean $\pm$ SEM, $n=5-6$.

Comparison of multiple time points among groups was made using two-way repeated-measures ANOVA and Tukey post hoc tests were performed when significant main effects were obtained. Labelled means without a common letter differ, $P < 0.05$. *db/+*, Standard diet fed control mice; *db/db*, standard diet fed diabetic mice; *db/db+SB*, strawberry fed diabetic mice.

Fig. 3.

(A) PECAM-1 and SMA in intimal fraction. (B) PECAM-1 in intimal fraction and media/adventitia. Expression of inflammatory chemokines (C), adhesion molecules (D), NFκB signaling markers (E), and NADPH oxidases (F) in CAECs isolated from *db/+*, *db/db* and *db/db+SB* mice treated for 10 wk. Values are mean ± SEM, $n=5-6$. Comparison among groups was made using one-way ANOVA and Tukey post hoc tests were performed when significant main effects were obtained. Labelled means without a common letter differ, $P < 0.05$. CAECs, carotid artery endothelial cells; *db/+*, Standard diet fed control mice; *db/db*, standard diet fed diabetic mice; *db/db+SB*, strawberry fed diabetic mice; E-Sel, E-selectin; ICAM-1, intercellular adhesion molecule-1; IKKβ, inhibitor κB kinase, PECAM-1, platelet endothelial cell adhesion molecule-1; SMA, smooth muscle actin; VCAM-1, vascular adhesion molecule-1.

Fig. 4.

Monocytes binding to MAECs (A), and secretion of MCP1/JE (B) and KC (C) in MAECs treated with $\pm$ HG/Pal or Man/BSA $\pm$ metabolites serum or control serum for 3 days. Values are mean $\pm$ SEM, $n=5$. Comparison among groups was made using one-way ANOVA and Tukey post hoc tests were performed when significant main effects were obtained. Labelled means without a common letter differ, $P < 0.05$. BSA: bovine serum albumin; HG, High glucose; MAECs, mouse aortic endothelial cells; Man, Mannose; MCP1 – monocyte chemoattractant protein-1; Pal, palmitate-BSA.