British Journal of Nutrition (2012), **108**, 1789–1799
doi:10.1017/S0007114512000037

Dietary strawberry powder reduces blood glucose concentrations in obese and lean C57BL/6 mice, and selectively lowers plasma C-reactive protein in lean mice

Mardi A. Parelman[1], David H. Storms[2], Catherine P. Kirschke[2], Liping Huang[2] and Susan J. Zunino[2]*

[1]*Department of Nutrition, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA*
[2]*United States Department of Agriculture, Agricultural Research Service, Western Human Nutrition Research Center, 430 West Health Sciences Drive, Davis, CA 95616, USA*

(Submitted 30 August 2011 – Final revision received 23 November 2011 – Accepted 16 December 2011 – First published online 1 February 2012)

Abstract

The purpose of the present study was to test the anti-inflammatory and blood glucose (BG)-regulating capacity of strawberries in a mouse model of diet-induced obesity. A total of thirty-six male C57BL/6J mice were randomly divided into four groups (nine mice per group). Mice were fed a low-fat diet (LF, 13 % fat), the LF supplemented with 2·6 % freeze-dried strawberry powder (LFSB), a high-fat diet (HF, 44 % fat) or the HF supplemented with 2·6 % strawberry powder (HFSB). Blood samples were collected to measure BG, inflammation and systemic markers for endocrine function of pancreas and adipose tissue. Splenocytes were harvested at the end of the study and activated with either anti-cluster of differentiation (CD) 3/anti-CD28 antibodies or lipopolysaccharide to test immune responsiveness. The HF increased non-fasted BG, insulin, soluble intracellular adhesion molecule-1, E-selectin, leptin, resistin and plasminogen activator protein-1 ($P<0·05$). High dietary fat decreased IL-4 production from activated splenocytes ($P<0·05$). BG concentrations were lower in the mice supplemented with SB (10·64 mmol/l) compared to the non-supplemented mice (11·37 mmol/l; $P=0·0022$). BG values were approximately 6·5 % lower in the supplemented mice. Additionally, SB lowered plasma C-reactive protein in the LFSB group compared to the other three groups ($P<0·05$). The dietary intake of SB approximated one human serving of strawberries. These results, although modest, support a promising role for dietary strawberries in reducing the risks associated with obesity and diabetes, and regulating the levels of inflammatory markers in non-obese individuals.

Key words: Diet-induced obesity: Inflammation: Strawberry powder: Blood glucose

Obesity is associated with chronic, low-grade systemic inflammation and increases the risks of developing insulin resistance, type 2 diabetes and CVD[1–3]. Insulin resistance, a major metabolic factor in obesity and type 2 diabetes, is mediated by attenuation or desensitisation of insulin receptor (IR) signalling by two basic mechanisms: (1) serine phosphorylation of the IR and IR substrate (IRS) proteins and/or (2) dephosphorylation of the activating tyrosine residues of the IR or IRS[4]. Enzymes responsible for the IR-desensitising process include protein kinase B, phosphoinositide-3 kinase, glycogen synthase kinase-3, extracellular signal-regulated kinase, c-Jun N-terminal kinase, IκB kinase and protein tyrosine phosphatase 1B[4,5]. IκB kinase is notable for its role in downstream signalling by inflammatory cytokines through the activation of the transcription factor NF-κB, since chronic inflammation promotes insulin resistance.

Dysfunction of the vascular endothelium is involved in the progression of insulin resistance to type 2 diabetes, as well as the development of atherosclerosis[6]. Soluble forms of adhesion molecules such as soluble intracellular adhesion molecule-1 (sICAM-1), E-selectin and soluble vascular adhesion molecule-1 (sVCAM-1) secreted by the vascular endothelium circulate in the blood and the levels of these molecules in the circulation are used as secondary measures of endothelial dysfunction. In both mice and humans, obesity is associated with increased concentrations of these cellular adhesion molecules[7–11]. Elevated levels of these molecules predict future risk for type 2 diabetes in previously healthy

Abbreviations: BG, blood glucose; CD, cluster of differentiation; CRP, C-reactive protein; GTT, glucose tolerance test; HF, high-fat diet; HFSB, high-fat diet supplemented with 2·6 % strawberry powder; i.p., intraperitoneal; IFN-γ, interferon-γ; IR, insulin receptor; IRS, insulin receptor substrate; LF, low-fat diet; LFSB, low-fat diet supplemented with 2·6 % freeze-dried strawberry powder; LPS, lipopolysaccharide; PAI-1, plasminogen activator inhibitor-1; SB, strawberry powder; sICAM-1, soluble intracellular adhesion molecule-1; sVCAM-1, soluble vascular adhesion molecule-1; Th, T helper.

* **Corresponding author:** Dr S. J. Zunino, fax +1 530 752 5271, email susan.zunino@ars.usda.gov

women[10], and are positively correlated with type 2 diabetes in older adults[9].

In addition to the increased markers of endothelial dysfunction, obesity is associated with elevated circulating levels of acute-phase proteins, pro-inflammatory cytokines and decreased levels of anti-inflammatory cytokines. C-reactive protein (CRP) is an acute-phase protein expressed in the liver and adipose tissue[12–14]. Elevated levels of circulating CRP are positively associated with increased adiposity[15], and increased risk for developing diabetes[16–18], CVD[13,18] and leptin resistance[19]. Additionally, an inverse relationship exists between CRP and adiponectin levels in obesity and the metabolic syndrome[13,20]. Adiponectin is expressed exclusively by adipocytes[21,22]. Recently, it was demonstrated in cell culture models that adiponectin inhibits CRP production by preventing NF-κB-mediated transcription of CRP[23]. Most evidence indicates that adiponectin becomes dysregulated in obesity and that the circulating levels of adiponectin decline with increasing adiposity[24,25]. Reduced levels of adiponectin are associated with undesirable health consequences such as impaired insulin sensitivity, type 2 diabetes and/or CHD[26,27].

IL-6[12,16,28], TNF-α[15,29], plasminogen activator inhibitor-1 (PAI-1)[30–32], resistin[33,34] and leptin[35,36] are elevated in the blood of obese humans and rodents. IL-6 is a cytokine produced by macrophages and T lymphocytes, and approximately one-third of circulating IL-6 originates from adipose tissue[37]. In primary cultures of human adipocytes, IL-1 and IL-6 induced CRP production from adipocytes into the medium[38]. TNF-α, a cytokine produced by activated macrophages, activates endothelial cells and T cells. It contributes to the progression of insulin resistance and type 2 diabetes by promoting the phosphorylation of serine residues instead of tyrosine residues on IRS-1[39]. Therefore, phosphoinositide 3-kinase is prevented from docking with IRS-1 and the insulin- signalling cascade is blocked. TNF-α was shown to induce the production of PAI-1, an inhibitor of serine proteases, in renal cell culture systems[40,41]. PAI-1 is produced in the endothelium, liver, adipose tissue and macrophages. PAI-1 deficiency protected mice from the negative consequences of diet-induced obesity[30].

Consuming a high-fat diet contributes to elevated concentrations of resistin[33], leptin[42] and CRP[15], and has been shown to alter the immune response[43,44]. In rodents, resistin is produced exclusively by adipocytes, circulating levels of resistin increase with increasing adiposity, and elevated levels are associated with decreased insulin sensitivity[33,45]. In humans, however, resistin is produced by macrophages rather than adipocytes[46–48]. Treatment of human vascular endothelial and smooth muscle cells with resistin suggests that resistin may play a role in the progression of atherosclerosis[49]. Leptin is produced by adipocytes and acts in the hypothalamus to suppress food intake. In rodents, leptin levels increase with increasing adiposity[35,42]. CRP may be involved in facilitating leptin insensitivity by preventing leptin from properly binding to the leptin receptor[19]. Lymphocytes have leptin receptors and are modulated by leptin[50]. The role of leptin in the activation of immune cells leading to the production of cytokines such as TNF-α and IL-6 was recently reviewed[51]. A diet high in fat alters the cluster of differentiation (CD) 4^+ T helper (Th) 1 and 2 responses in mice[24]. Interferon-γ (IFN-γ) is considered the hallmark pro-inflammatory Th1 cytokine[52]. It primes macrophages for phagocytosis, induces $CD4^+$ T cells towards the Th1 profile and suppresses differentiation to the Th2 profile. Th2 cells are considered less inflammatory and instead influence the B cell antibody response by producing cytokines such as IL-4 and IL-5, TNF-α and IL-10[52].

Strawberries are a fibre-rich fruit that contain numerous bioactive compounds including polyphenols with potential anti-inflammatory activities[53–57]. The main objective of the present study was to evaluate the ability of dietary strawberry powder (SB) prepared from whole strawberries to prevent inflammation as well as glucose intolerance associated with diet-induced obesity. The C57BL/6 mouse is a well-characterised and widely used model of diet-induced obesity[24,41,58,59]. These mice mimic the human characteristics of the metabolic syndrome. When fed a diet high in fat, C57BL/6 mice gain weight as well as display the propensity for inflammation, hyperglycaemia, hyperinsulinaemia and an increased tendency to develop type 2 diabetes. The specific hypothesis for this study was that a diet supplemented with 2·6 % SB, the amount comparable to two servings per d in humans, would reduce inflammation and inflammatory-mediated dysfunction associated with obesity in C57BL/6 mice.

Materials and methods

Mice

A total of thirty-six male C57BL/6J mice (approximately 5 weeks of age) were obtained from Jackson Laboratory. The mice were housed, three per cage, in a pathogen-free facility on the campus of the University of California, Davis. All mice had access to food and water *ad libitum*. Body weight was measured weekly. The mice were euthanised after 24 weeks of dietary intervention by CO_2 asphyxiation. Of these, one animal died during the course of the study. Institutional and national guidelines for the care and use of animals were followed, and experimental procedures involving animals were approved by the University of California, Davis Institutional Animal Care and Use Committee.

Diets

The California Strawberry Commission provided the freeze-dried SB. The SB was produced from Individually Quick Frozen kosher, conventional (non-organic) whole strawberries supplied by Anacapa Foods and Frozsun Foods. The powder was prepared and packaged by Van Drunen Farms by a commercial food freeze-drying process. The mixture of strawberries used to generate the powder contained University of California public cultivars Camarosa (37 %), Ventana (13 %) and Diamante (13 %), and two proprietary varieties (37 %) in production in 2004. The polyphenolic composition of the powder was previously defined by others and the main polyphenolic components were anthocyanidin derivatives of cyanidin and pelargonidin, quercetin, kaempferol and ellagic

acid[55,60]. For the purpose of this study, four customised, defined pelleted diets were prepared by Harlan Teklad (Table 1)[61]. The control diets were adjusted to compensate for the additional sugars provided by the SB. The high-fat diets were approximately 20 % higher in energy density compared to the low-fat diets and vitamins, minerals, L-cystine and protein in the HF were adjusted accordingly. *Tert*-butylhydroquinone, an antioxidant, was increased in the high-fat diets to reduce oxidation of the extra fat. All diets were sterilised by γ-irradiation and vacuum packaged. The diets were stored at − 20°C and thawed immediately before use. The mice were fed a low-fat control diet without SB for 1 week of acclimatisation. After 1 week, the mice were randomly assigned to four dietary treatment groups (nine mice per group): low-fat control diet without SB (LF), LF supplemented with 2·6 % freeze-dried SB (LFSB), high-fat control diet without SB (HF) and HF supplemented with 2·6 % SB (HFSB). The Food and Drug Administration recommends extrapolation of doses from animals to humans by normalisation to body surface area[62,63]. We estimated that the average weight of the mice would reach 30 g overall during the study and predicted that they would consume an overall average of 5 g of food/d. In this study, one human serving of frozen strawberries (80 g) was used to calculate the amount of powder needed per serving. And here, two human servings of strawberries in the form of the freeze-dried powder weighed 22 g and for the purposes of the calculations we used a 70 kg adult as a reference point (22 g SB/70 kg body weight = 314 mg/kg) for the calculations described next. Based on these assumptions, the animal equivalent dose for the human servings was derived using the following formula: animal equivalent dose (mg/kg) = 314 mg/kg × mouse K_m factor 37/human K_m factor 3[62,63]. We then determined that 2·6 % SB/kg of mouse diet would be comparable to two servings per d in humans. Estimates of daily food intake were determined by dividing the loss of food mass from the wire food trough by the number of mice per cage and the number of days between feedings.

Table 1. Composition of diets*

Ingredients	LF (g/kg)	LFSB (g/kg)	HF (g/kg)	HFSB (g/kg)
Casein	180	180	220	220
L-Cys	2·7	2·7	3·2	3·2
Sucrose	200	194·8	200	194·8
Maize starch	341·5	341·5	89·6	89·6
Maltodextrin, 10	100	100	120	120
Maize oil	10	10	10	10
Anhydrous milkfat	40	40	210	210
Cellulose	50	50	59·6	59·6
AIN-93G mineral mix†	35	35	42·6	42·6
Calcium phosphate dibasic	2·5	2·5	3	3
AIN-93 vitamin mix†	15	15	18·2	18·2
Choline bitartrate	2·5	2·5	3	3
Tert-butylhydroquinone	0·01	0·01	0·04	0·04
Fructose	10·4	0	10·4	0
Dextrose	10·4	0	10·4	0
Strawberry powder	0	26	0	26
Energy density (kJ/g)	15·48	15·48	19·25	19·25

LF, low-fat diet; LFSB, LF supplemented with 2·6 % freeze-dried strawberry powder; HF, high-fat diet; HFSB, HF supplemented with 2·6 % strawberry powder; AIN, American Institute of Nutrition.

* LF and LFSB contained 70 % kJ from carbohydrate, 17 % kJ from protein and 13 % kJ from fat. HF and HFSB contained 39 % kJ from carbohydrate, 17 % kJ from protein and 44 % kJ from fat.

† The mineral and vitamin concentrations for these diets come from the AIN-93G mixes[58].

Blood glucose measurements

Blood was obtained from the ventral tail artery of non-fasted mice to monitor blood glucose (BG) using an Accu-Check glucose monitor (Roche Diagnostics)[64–66]. The glucose meter was used to reduce the total amount of blood taken per month and remain within our maximum safe bleed volumes designated by the University of California, Davis Institutional Animal Care and Use Committee. BG tests were conducted at the end of the 1-week acclimatisation period (baseline), and subsequently at 4, 6, 8, 14, 16, 20, 22 and 24 weeks on the diets. An intraperitoneal (i.p.) glucose tolerance test (GTT) was performed at week 12. Mice were fasted overnight before receiving an i.p. injection of 2 g D-glucose/kg body weight (50 % dextrose, injectable; Hospira, Inc.). BG levels were monitored at 0 min (before the i.p. injection), and at 15, 30, 60 and 120 min following the glucose injection.

Immunohistochemistry

Harvested pancreases were fixed in 4 % paraformaldehyde overnight, paraffin embedded and serially sectioned at a thickness of 5 μm. For immunohistochemistry, the sections were deparaffinised, rehydrated and the antigen unmasked for a subset of mice (four mice per group). Antigen retrieval was done by boiling the slides for 20 min in 0·1 M-Tris buffer (pH 10·0). The guinea pig polyclonal insulin antibody (1 mg/ml) was purchased from Abcam and diluted in a 1:100 ratio. Preimmune guinea pig serum (Jackson ImmunoResearch) was concentration matched and used as a negative control. The VECTASTAIN ABC system (Vector Laboratories) was used for secondary antibody staining and 3,3′-diaminobenzidine substrate kit for peroxidase (Vector Laboratories) was used to stain the tissue. Digital images of the pancreatic tissue sections were taken using a Nikon Eclipse 800 (Diagnostic Instrument, Inc.). Insulin-positive stained areas were counted as islets (four mice per group, one section per mouse, seven to thirty islets per section). Estimated measures of islet area were captured with SPOT Advanced Imaging Software, version 4.6 (Diagnostic Instruments, Inc.) using the calibrated 'region' tool.

Systemic markers of inflammation

Plasma was isolated from mice at baseline, and at 10, 18 and 24 weeks after dietary interventions to determine the levels of systemic cytokines. Approximately 50–300 μl of blood were collected into lithium heparin microvette tubes (Sarstedt). The blood was centrifuged (900 ***g***, 4°C) for 10 min and plasma was recovered and stored in aliquots at − 80°C until the time of use. Plasma was assayed using Milliplex immunoassay kits (Millipore) as per the manufacturer's protocol. All samples were measured in duplicate. Briefly, neat or diluted plasma samples, standards and quality controls were incubated with antibody

beads overnight at 4°C. Neat plasma was used in the adipokine panel (leptin, resistin, PAI-1, insulin, IL-6, TNF-α and monocyte chemoattractant protein-1), whereas the remaining assays that required the plasma to be diluted in assay buffer were as follows: CRP was diluted 1:200, sICAM-1, sVCAM-1 and E-selectin were diluted 1:100 and adiponectin was diluted 1:5000. Assays were run on a Bio-Rad Bioplex instrument. Plasma isolated at baseline, and at 10, 18 and 24 weeks after dietary interventions was used to determine the levels of CRP, sICAM-1, E-selectin, sVCAM-1 and adiponectin. The concentration of leptin, resistin, PAI-1, insulin, IL-6 and TNF-α was examined at baseline, and at 10 and 24 weeks on the modified diets.

Primary splenocyte culture

Spleens were dissected under sterile conditions, placed in Roswell Park Memorial Institute (RPMI). 1640 supplemented with 1 mM-sodium pyruvate, 2 mM-L-glutamine, 60 mg/l penicillin, 100 mg/l streptomycin (Invitrogen) and 10 % fetal bovine serum (Sigma). Immediately after dissection, each spleen was perfused with the medium and the splenocytes were dispersed by shredding the tissue. Erythrocytes were removed from the splenocyte mixture by washing the cells one or two times in an erythrocyte lysis buffer as previously described[67]. Cells were counted and diluted to 2×10^6 cells/ml in modified RPMI-1640 with β-mercaptoethanol (Invitrogen) supplemented with 10 % fetal bovine serum, and then plated in ninety-six-well plates at a final concentration of 2×10^5 cells/well. The stimulatory treatments were plates pre-coated with anti-CD3/anti-CD28 antibodies (BD Pharmingen) overnight (4°C) at a final concentration of 1 mg/l each or lipopolysaccharide (LPS; Sigma) was added to the splenocytes at a final concentration of 10 μg/l and incubated at 37°C in 5 % CO_2. After 24 or 72 h, the cells were harvested and centrifuged at 200 ***g*** for 10 min at 4°C. The controls for the CD3/CD28 stimulation were wells coated with isotype antibody and for the LPS treatment control, the cells were treated with endotoxin-free water. Supernatants were collected and stored in aliquots at −70°C until the time of analysis. The supernatant from cells challenged with LPS was collected after 24 h and IL-6, IL-1β and TNF-α were measured to test innate immune response by monocytes/macrophages. The concentration of IFN-γ, TNF-α, IL-4 and IL-10 was measured in the supernatant collected from the cells that were incubated with anti-CD3/anti-CD28 for 72 h to test the Th1/Th2 response. All samples were run in duplicate using multiplex kits from Millipore according to the manufacturer's protocol. The assays were run on a Bio-Rad Bioplex instrument using multiplex kits from Millipore according to the manufacturer's protocol.

Oxygen radical absorbance capacity assay

The overall antioxidant capacity of plasma for mice from each dietary group was determined using the oxygen radical absorbance capacity assay as previously described[68]. Trolox (6-hydroxy-2,5,7,8-tetramethylchoman-2-carboxylic acid) was used to generate the standard curve. The loss of the fluorescein signal due to oxidation by peroxyl radicals from the breakdown of 2,2′-azobis(2-methylpropionamidine) dihydrochloride was measured over time. Calculations of plasma Trolox equivalents were determined by comparing the areas under the curves of fluorescence intensity *v.* time. The excitation wavelength was 490 nm, the emission wavelength was 515 nm, and the assay was run for 2 h at 37°C on a SpectraMAX GeminiXS spectrofluorometer using SoftMAX PRO v4.0 software (Molecular Devices).

Statistical analyses

Statistical analyses were performed with SAS Software version 9.2 for Windows. Outcome variables were assessed for conformance to the normal distribution, and transformed, if needed, using Box–Cox power transformations (logarithm was used for E-selectin, adiponectin, leptin, resistin, BG, 24 h IL-6, 72 h IL-6 and insulin-positive area; reciprocal square root was used for body weight, insulin; IL-6 and 72 h TNF-α). All data were analysed using PROC MIXED; fixed effects were dietary fat, SB and time (if appropriate), as well as the interactions of the fixed effects. Outcome variables were compared with the repeated-measures ANCOVA where appropriate. Subject was included as a random effect for repeated data in the analysis of body weight, BG, GTT, CRP, sICAM-1, E-selectin, sVCAM-1 and adiponectin. Baseline values were used as covariates in the analysis of CRP, sICAM-1, E-selectin, sVCAM-1, adiponectin, resistin, leptin, PAI-1, insulin and systemic IL-6. When significance was detected among the fixed effects, Tukey's test was used for *post hoc* analysis. Graphs were generated using GraphPad Prism software version 5.00 for Windows. All data are presented as means with their standard errors. Differences were considered significant at $P<0{\cdot}05$.

Results

Body weight

As expected, the mice fed a diet high in fat gained more weight than the mice fed a diet low in fat ($P<0{\cdot}001$; Fig. 1), beginning at week 3 until the end of the study. There was also a significant dietary fat × SB interaction ($P<0{\cdot}001$). *Post hoc* analysis of the repeated-measures analysis of body weights revealed that the HF and HFSB mice did not differ in body weights. The LF mice weighed more than the LFSB mice, noticeably during 6–12 weeks on the diet, but not at the end of the study. To determine if the differences in overall food intake may have contributed to the differences between the LF and LFSB groups, estimated feed intake records (by cage) were analysed. Mice in the high dietary fat groups ate less feed per day compared to mice in the low dietary fat groups (low-fat groups 2·88 (SEM 0·02) g/d *v.* high-fat groups 2·64 (SEM 0·03) g/d, $P=0{\cdot}0005$). However, SB supplementation did not affect the estimated food intake and no interactions were observed.

Strawberry supplementation lowered non-fasted blood glucose levels in mice, but did not alter response during intraperitoneal glucose challenge

Non-fasted BG levels were monitored throughout the course of the study. Mice fed the LF had lower BG levels than mice

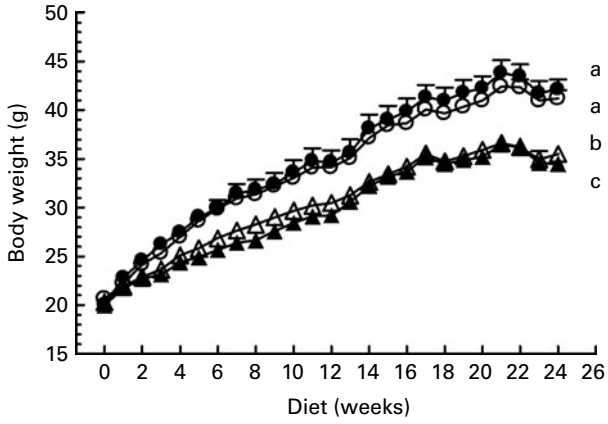

Fig. 1. Growth curves over 24 weeks during the dietary intervention. Weekly body weights were obtained for all mice throughout the study. Dietary groups: high-fat (–○–), high-fat strawberry (–●–), low-fat (–Δ–), low-fat strawberry (–▲–). Values are means, with their standard errors represented by vertical bars (n 8–9). [a,b,c] Mean values with unlike letters were significantly different. Mice in the high dietary fat groups gained more weight than mice in the low dietary fat groups ($P<0·001$). A *post hoc* analysis of a dietary fat × strawberry interaction revealed that the mice receiving the low-fat diet weighed more than the mice receiving the low-fat strawberry diet at 6–12 weeks ($P<0·001$).

fed the HF (LF and LFSB groups *v.* HF and HFSB groups, $P=0·0426$; Fig. 2(A)). There was a significant main effect of SB on the BG concentration. BG concentrations were significantly lower in the SB-supplemented mice (10·64 mmol/l for LFSB + HFSB) compared to the non-SB-supplemented mice (11·37 mmol/l for LF + HF, $P=0·0022$; Fig. 2(B)). BG values were approximately 6·5 % lower in mice supplemented with SB. There was no dietary fat × SB interaction. BG concentrations also changed over time across all four dietary groups ($P<0·0001$). During the 12th week of the study, all mice underwent a GTT. Mice in the high dietary fat groups had higher maximum BG levels in response to the i.p. glucose challenge ($P = 0·0003$; Fig. 3). Mice fed HF also experienced marginally delayed glucose clearance compared to the mice fed either of the LF ($P=0·0686$ for dietary fat × time interaction). Supplementing the diet with SB did not make an impact on the response to the i.p. glucose challenge. To determine if long-term consumption of dietary SB made an impact on islet morphology and insulin production in the pancreas under 'normal' conditions (LF) and in combination with a persistent HF, immunohistochemical analysis of pancreases from a subset of mice was performed. No differences were detected in insulin-positive staining, islet morphology or the number of islets per tissue section (data not shown).

Systemic markers of inflammation

Systemic inflammation was monitored by measuring inflammatory cytokines in plasma of the mice. Plasma samples were collected at baseline (study week 0), and then again at predetermined intervals. An interaction between dietary fat and strawberry made an impact on circulating CRP concentrations ($P=0·034$). The *post hoc* analysis revealed that the LF group supplemented with SB (LFSB group) had lower overall CRP levels compared to the other three groups (Fig. 4). No effect of time, or any interaction between SB and time occurred in relation to CRP.

Mice fed the HF had increased circulating sICAM-1 and E-selectin compared to mice fed the LF ($P=0·047$, $P=0·038$, respectively; Table 2). E-selectin levels were also modified by time, regardless of the dietary group ($P=0·001$; Table 2). Neither dietary fat nor time affected sVCAM-1 levels in these mice. Strawberry supplementation did not affect sICAM-1, E-selectin or sVCAM-1. As expected, consumption of the HF resulted in elevated leptin, PAI-1, resistin and insulin levels ($P<0·05$; Table 2) and marginally increased circulating IL-6 levels compared to the mice fed the LF ($P=0·051$; Table 2). The interaction of the HF and time resulted in elevated PAI-1 levels ($P=0·031$), whereas time contributed independently to increased concentrations of leptin, resistin and IL-6 ($P<0·025$; Table 2). Supplementation with SB did not ameliorate the dietary fat or time effect. There were no differences in

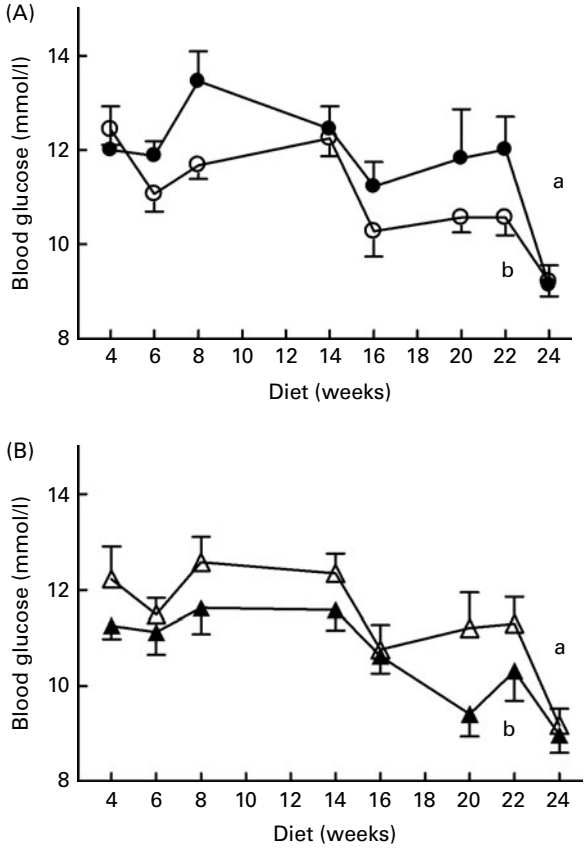

Fig. 2. Dietary fat and strawberry (SB) supplementation influenced non-fasted blood glucose (BG) in mice. A small puncture was made in the ventral tail artery and 5 μl of blood were collected on a test strip to measure non-fasted BG levels using an Accu-Check blood glucose monitor. Values are means, with their standard errors represented by vertical bars (n 8–9). [a,b] Mean values with unlike letters, as analysed by repeated-measures ANCOVA, were significantly different. (A) Mice in the high dietary fat groups (–●–, high-fat and high-fat strawberry) had higher overall BG levels compared to mice in the low dietary fat groups (–○–, low-fat and low-fat strawberry), $P=0·0426$. (B) SB supplementation lowered overall BG concentrations regardless of the level of dietary fat. Mice not receiving the strawberry powder (–Δ–, low dietary fat + high dietary fat groups) had greater overall BG than mice receiving SB supplementation (–▲–, low dietary fat strawberry + high dietary fat strawberry), $P=0·0022$.

British Journal of Nutrition

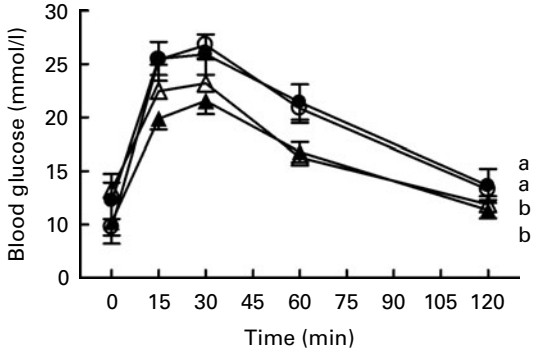

Fig. 3. Consumption of a high-fat diet impaired glucose tolerance in mice after 12 weeks on the diet and this effect was not ameliorated by strawberry (SB) supplementation. Mice were fasted overnight and then injected intraperitoneally with 2 g glucose/kg body weight. A small puncture was made in the ventral tail artery and 5 μl of blood were collected onto a test strip and read using an Accu-Check blood glucose monitor. Blood glucose concentrations were measured at baseline, and at 15, 30, 60 and 120 min after glucose administration. Values are means, with their standard errors represented by vertical bars (n 8–9). [a,b] Mean values with unlike letters were significantly different. Mice fed high-fat diets (–○–, high-fat and –●–, high-fat SB) had delayed glucose clearance compared to mice fed low-fat diets (–Δ–, low-fat and –▲–, low-fat SB); $P<0{\cdot}05$. No effects of SB or interactions were observed.

adiponectin levels among the dietary groups. TNF-α and monocyte chemoattractant protein-1 levels fell below the detectable limits of the assay.

Effect of diet on immune response in supernatants of primary cultured splenocytes

To test the ability of dietary SB to prevent diet-induced perturbation of the immune response, we cultured primary splenocytes with LPS for 24 h or with anti-CD3/CD28 for 72 h and analysed cytokine production in the cell supernatants. The concentrations of secreted TNF-α, IL-1β and IL-6 were analysed to determine the innate immune response to pathogens. There was a trend towards an elevated IL-6 response in the supernatant from the mice fed the HF compared to those in the low dietary fat groups, but this difference did not reach the level of significance ($P=0{\cdot}079$; Table 3). There was no effect of SB or interaction between dietary fat and SB on IL-6 production. The levels of TNF-α and IL-1β secreted in response to the LPS challenge did not differ among the groups at 24 h. We analysed the Th1 and Th2 cytokine profiles of the adaptive response by measuring the concentration of IFN-γ, TNF-α, IL-4 and IL-10 in the supernatant after 72 h. The diets did not affect the concentrations of IFN-γ, TNF-α or IL-10 after 72 h activation with anti-CD3/CD28 (Table 3). However, 72 h T cell stimulation resulted in reduced IL-4 concentration in the supernatant from the mice fed HF compared to the mice fed LF ($P=0{\cdot}009$; Table 3). SB supplementation did not attenuate this effect.

Strawberry powder supplementation did not increase the antioxidant capacity of plasma in mice

The antioxidant capacity of the plasma was tested after 24 weeks of dietary intervention using the oxygen radical absorbance capacity assay. The level of fat in the diet did not influence the antioxidant capacity of the plasma ($P=0{\cdot}8885$). Strawberry supplementation, regardless of the level of fat in the diet, did not have any effect on the antioxidant capacity of the plasma ($P=0{\cdot}6488$), and there was no interaction between dietary fat and SB ($P=0{\cdot}1017$).

Discussion

In the present study, we investigated the ability of dietary strawberries, in the form of freeze-dried powder, to prevent inflammation and glucose intolerance associated with diet-induced obesity in male C57BL/6J mice. We found that the estimated intake of SB per mouse was equivalent to approximately one human serving of strawberries per d rather than the target of two servings. However, even with this low level of intake, our analysis revealed a novel and unexpected interaction between the LF and strawberry supplementation, such that the LFSB group had lower CRP concentrations than the other groups. The absence of an effect on CRP in the HFSB group does concur with what others have recently reported. In one study, no change in CRP levels occurred after a 4-week intervention during which women with the metabolic syndrome drank beverages made from freeze-dried SB[69]. There is evidence, however, that supports a potentially protective role of dietary strawberries in terms of CRP levels and cardiovascular risk. A moderate but significant reduction in overall risk for developing elevated CRP levels was observed among women from the Women's Health Study that reported consuming at least three servings of strawberries weekly *v.* women who consumed no servings[70]. Recently, strawberries were reported to decrease the plasma levels of CRP in response to a high-fat meal in human volunteers[71]. Multiple polyphenols found in the strawberries were detected in the plasma of these subjects. Analysis of National Health and Nutrition Examination Survey (NHANES) data showed an inverse association between CRP levels and

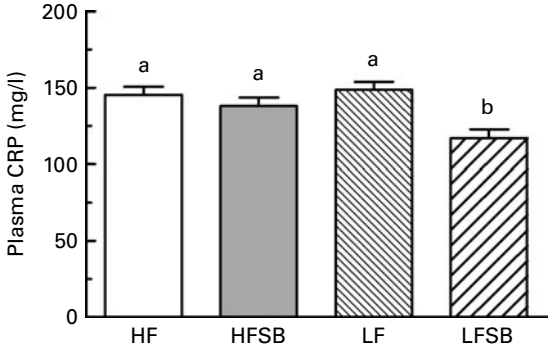

Fig. 4. Strawberry (SB) supplementation reduced plasma C-reactive protein (CRP) in mice fed a low-fat diet (LF), but not in mice fed a high-fat diet (HF). Blood was collected from mice at baseline, and at 10, 18 and 24 weeks during dietary intervention. Milliplex immunoassay kits were used to assay plasma CRP on a Bio-Rad Bioplex instrument. Repeated-measures ANCOVA analysis revealed an overall dietary fat × SB interaction after 24 weeks of feeding ($P<0{\cdot}05$). The data represent the combined means for weeks 10, 18 and 24 for each dietary group. [a,b] Mean values with unlike letters were significantly different. HFSB, high-fat strawberry powder; LFSB, low-fat strawberry powder.

Table 2. Circulating cytokines and insulin levels in mice by time and dietary fat group

(Mean values with their standard errors, *n* 4–9 per dietary group)

	Low dietary fat groups (LF + LFSB)						High dietary fat groups (HF + HFSB)						PROC MIXED*		
	10 weeks		18 weeks		24 weeks		10 weeks		18 weeks		24 weeks		*P*		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Fat	Time	Fat × time
sICAM-1 (pg/ml)	38·9	1·6	35·9	1·8	37·3	2·3	41·7	2·8	43·6	1·8	39·7	1·6	0·047	0·534	0·924
E-selectin (pg/ml)	88·7	6·1	74·8	8·0	74·4	5·3	120·6	14·3	80·1	5·2	73·8	6·0	0·038	0·001	0·317
sVCAM-1 (pg/ml)	1782	106	1622	236	1273	229	1297	197·0	1741	215	1388	166	0·996	0·143	0·138
Leptin (pg/ml)	7037	530·3	–	–	11 743	590	13 601	1978	–	–	23 163	1591	<0·001	<0·001	0·300
PAI-1 (pg/ml)	1292	318	–	–	1496	137	2253	212	–	–	3079	339	<0·001	0·071	0·031
Resistin (pg/ml)	1462	88	–	–	3735	210	2065	164	–	–	5400	385	<0·001	<0·001	0·956
IL-6 (pg/ml)	6·0	1·4	–	–	14·3	2·3	23·4	7·3	–	–	20·5	4·6	0·051	0·021	0·120
Insulin (pg/ml)	712	99	–	–	1565	193	1090	163	–	–	2174	348	0·024	<0·001	0·222
CRP (ng/ml)	130·52	6·65	127·93	7·86	139·77	8·29	136·51	5·78	147·28	7·56	147·11	5·60	0·084	0·237	0·482
Adiponectin (ng/ml)	16 367	1611	16 285	1542	17 748	1504	18 705	1142	15 864	1326	14 752	1176	0·472	0·217	0·261

LF, low-fat diet; LFSB, LF supplemented with 2·6 % freeze-dried strawberry powder; HF, high-fat diet; HFSB, HF supplemented with 2·6 % strawberry powder; sICAM-1, soluble intracellular adhesion molecule-1; sVCAM-1, soluble vascular adhesion molecule-1; PAI-1, plasminogen activator inhibitor-1; CRP, C-reactive protein.

* Data were analysed using PROC MIXED repeated-measures ANCOVA with baseline values as the covariate (baseline data not shown). *P* value was significant when $P<0{\cdot}05$. All plasma samples were measured in duplicate. No significance was found for the main effect of strawberry or strawberry × time interactions (data not shown). The 18-week time point was not assessed for leptin, resistin, PAI-1, resistin, IL-6 and insulin.

Table 3. Cytokine production in supernatants from primary splenocyte cultures after 24 or 72 h stimulation with lipopolysaccharide or anti-CD3/anti-CD28

(Mean values with their standard errors)

	LF		LFSB		HF		HFSB		*P**		
Cytokines	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Dietary fat	SB	Fat × SB
24 h											
n	8–9		8–9		9		7–8				
IL-6 (pg/ml)	22·88	8·64	11·78	2·09	137·11	88·38	124·49	72·53	0·079	0·498	0·842
IL-1β (pg/ml)	10·78	2·21	7·08	1·51	8·22	1·15	8·11	2·36	0·673	0·299	0·327
TNF-α (pg/ml)	14·54	2·48	13·03	1·78	20·44	3·10	17·97	5·24	0·108	0·548	0·885
72 h											
n	9		9		9		7–8				
IL-4 (pg/ml)	342·20	61·04	425·25	44·61	308·69†	18·07	229·88†	27·66	0·009	0·914	0·072
IFN-γ (pg/ml)	243 753	24 122	217 521	15 857	242 369	17 418	229 266	16 482	0·786	0·307	0·731
TNF-α (pg/ml)	297·04	29·01	290·89	21·78	315·20	29·23	265·46	30·01	0·768	0·407	0·353
IL-10 (pg/ml)	1681·34	298·28	1669·77	189·14	1767·89	301·84	1450·84	222·05	0·801	0·532	0·561

LF, low-fat diet; LFSB, LF supplemented with 2·6 % freeze-dried strawberry powder; HF, high-fat diet; HFSB, HF supplemented with 2·6 % strawberry powder; SB, strawberry; IFN-γ, interferon-γ.

* Data were analysed using ≥ PROC MIXED repeated-measures ANCOVA. *P* value was significant when $P<0{\cdot}05$. All supernatant samples were measured in duplicate.

† Mean values of the high dietary fat groups were significantly different from the mean values of the low dietary fat groups in the same row.

intake of quercetin, kaempferol and anthocyanins in humans[72]. Taken together, these data suggest a role for dietary polyphenols in the reduction of CRP observed in the present study.

Obesity-related perturbations to the immune system have been described[24,43] and we further demonstrated this in our study. The immune response from mice in the high dietary fat groups was skewed as detected by the exaggerated reduction of IL-4 production in response to the T-cell activation stimuli and a trend towards increased IL-6 production after LPS stimulation. The small sample size used in this study may explain why the 24 h IL-6 data did not reach the level of significance despite large differences in mean values. We selected a low level of dietary SB that would be easily attainable by the human population. Starting with a low level of SB and somewhat over-estimating how much feed per d the mice would consume, the daily level of SB intake in this study was lower than expected. Therefore, it is certainly possible that the actual level of dietary SB used in this study was inadequate to ameliorate the negative consequences associated with long-term consumption of a HF on immune function.

To our knowledge, we are the first to report that long-term consumption of SB reduces non-fasted BG levels in mice, regardless of the level of fat in the diet. These data suggest that this pattern of consumption may play a role in maintaining or lowering non-fasted BG levels. Recently, Torronen *et al.*[73] reported that a single dose of a mixed berry purée containing strawberries along with other berries improved postprandial glucose response in healthy human subjects during GTT. In other studies[69,74], however, BG levels remained unchanged after SB supplementation trials. The human BG values were obtained on fasted subjects, whereas we obtained non-fasted BG concentrations. Our BG tests were conducted at the same time each week during the non-active time of day. Torronen *et al.*[73] specifically examined postprandial responses to glucose challenges in metabolically healthy subjects, whereas the other two studies looked at longer-term effects of strawberry consumption on glucose parameters. The participants in the Basu *et al.*[69] study were obese and had the metabolic syndrome. The Jenkins *et al.*[74] study was part of a 5-year ongoing cholesterol-lowering dietary intervention in which the hyperlipidaemic participants had already been involved in the study for approximately 2·5 years before the start of the strawberry supplementation. The participants were encouraged to eat 1 lb/d (about 450 g/d) of fresh strawberries for 1 month while they continued with the low-cholesterol diet. In our study, mice started SB supplementation at the same time they were placed on the LF or HF. The timing of supplementation in relation to exposure to the other dietary components may contribute to the overall effect of SB supplementation on lowering BG values in our mice. Glucose tolerance as assessed by GTT was also affected by the HF. The observation that high-fat feeding delays clearance of glucose from the blood and contributes to insulin resistance is well documented in the literature[36,42,75]. Similar to our findings, another group observed that SB supplementation did not prevent diet-induced glucose intolerance in mice during GTT[76].

Adiponectin most often decreases with increasing obesity and the resulting hypoadiponectinaemia is associated with decreased anti-inflammatory and insulin-sensitising potential[21,22,25,59]. Although we expected to detect a decrease in adiponectin in the HF groups, the lack of change in adiponectin levels between the dietary groups does agree with at least one other study that used C57BL/6 mice fed a HF[77]. Other systemic inflammatory markers we examined in this study did change as we expected and the outcomes do concur with the existing body of evidence regarding the positive relationship between obesity and leptin[35,42], PAI-1[30,78], resistin[33,34], and IL-6[15,79], sICAM-1[10,11] and E-selectin[9,80]. The actual level of dietary SB supplementation employed in this study did not ameliorate these effects.

Obesity increases the risk for the development of type 2 diabetes, CVD and risks for complications during infection. In the present study, we found that the obese hyperglycaemic mice that consumed a diet supplemented with SB powder for 24 weeks had improved BG. Strawberries have a low glycaemic index[81,82], which makes this fruit an acceptable and desirable part of the diet of hyperglycaemic or diabetic individuals. Interestingly, anthocyanin-rich whole cherries and anthocyanin-enriched extracts from bilberries have shown glucose-lowering effects in other animal studies[83,84], supporting a promising role for strawberries as a dietary means for reducing hyperglycaemia in humans. The obese mice in the present study also showed a unique dietary fat × SB interaction, resulting in lower CRP concentration in the LFSB group. These findings suggest that regular consumption of strawberries equivalent to at least one serving per d may contribute to the maintenance of BG in obesity, and may be beneficial in regulating aspects of systemic inflammation in non-obese individuals.

Acknowledgements

The present study was supported by the United States Department of Agriculture (USDA) Current Research Information System project nos. 5306-51530-013-00D and 5306-51530-018-00D. S. J. Z. is a member of the scientific advisory board for the California Strawberry Commission. The authors report no conflict of interest. M. A. P. and S. J. Z. designed the research; M. A. P. and D. H. S. conducted the research; C. P. K. and L. H. provided training for the immunohistochemistry and provided comments on the manuscript; M. A. P. analysed the data; M. A. P. and S. J. Z. wrote the paper; M. A. P. and S. J. Z. had primary responsibility for the final content. All authors read and approved the final manuscript. USDA is an equal-opportunity provider and employer.

References

1. Pradhan AD, Cook NR, Buring JE, *et al.* (2003) C-reactive protein is independently associated with fasting insulin in nondiabetic women. *Arterioscler Thomb Vasc Biol* **23**, 650–655.

2. Temelkova-Kurktschiev T, Siegert G, Bergmann S, *et al.* (2002) Subclinical inflammation is strongly related to insulin resistance but not to impaired insulin secretion in a high risk population for diabetes. *Metabolism* **51**, 743–749.
3. Festa A, D'Agostino R Jr, Tracy RP, *et al.* (2002) Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes* **51**, 1131–1137.
4. Watson RT, Kanzaki M & Pessin JE (2004) Regulated membrane trafficking of the insulin-responsive glucose transporter 4 in adipocytes. *Endocr Rev* **25**, 177–204.
5. Tanti J-F & Jager J (2009) Cellular mechanisms of insulin resistance: role of stress-regulated serine kinases and insulin receptor substrates (IRS) serine phosphorylation. *Curr Opin Pharmacol* **9**, 753–762.
6. Hadi HA & Suwaidi JA (2007) Endothelial dysfunction in diabetes mellitus. *Vasc Health Risk Manag* **3**, 853–876.
7. Ouchi N, Kihara S, Arita Y, *et al.* (1999) Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* **100**, 2473–2476.
8. Ferri C, Desideri G, Valenti M, *et al.* (1999) Early upregulation of endothelial adhesion molecules in obese hypertensive men. *Hypertension* **34**, 568–573.
9. Leinonen E, Hurt-Camejo E, Wiklund O, *et al.* (2003) Insulin resistance and adiposity correlate with acute-phase reaction and soluble cell adhesion molecules in type 2 diabetes. *Atherosclerosis* **166**, 387–394.
10. Meigs JB, Hu FB, Rifai N, *et al.* (2004) Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. *JAMA* **291**, 1978–1986.
11. Brake DK, Smith EO, Mersmann H, *et al.* (2006) ICAM-1 expression in adipose tissue: effects of diet-induced obesity in mice. *Am J Physiol Cell Physiol* **291**, C1232–C1239.
12. Memoli B, Procino A, Calabro P, *et al.* (2007) Inflammation may modulate IL-6 and C-reactive protein gene expression in the adipose tissue: the role of IL-6 cell membrane receptor. *Am J Physiol Endocrinol Metab* **293**, E1030–E1035.
13. Ouchi N, Kihara S, Funahashi T, *et al.* (2003) Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation* **107**, 671–674.
14. Anty R, Bekri S, Luciani N, *et al.* (2006) The inflammatory C-reactive protein is increased in both liver and adipose tissue in severely obese patients independently from metabolic syndrome, type 2 diabetes, and NASH. *Am J Gastroenterol* **101**, 1824–1833.
15. Park HS, Park JY & Yu R (2005) Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-alpha and IL-6. *Diabetes Res Clin Pract* **69**, 29–35.
16. Pradhan AD, Manson JE, Rifai N, *et al.* (2001) C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* **286**, 327–334.
17. Dehghan A, van Hoek M, Sijbrands EJ, *et al.* (2007) Risk of type 2 diabetes attributable to C-reactive protein and other risk factors. *Diabetes Care* **30**, 2695–2699.
18. Gonzalez AS, Guerrero DB, Soto MB, *et al.* (2006) Metabolic syndrome, insulin resistance and the inflammation markers C-reactive protein and ferritin. *Eur J Clin Nutr* **60**, 802–809.
19. Chen K, Li F, Li J, *et al.* (2006) Induction of leptin resistance though direct interaction of C-reactive protein with leptin. *Nat Med* **12**, 425–432.
20. Tsatsanis C, Zacharioudaki V, Androulidaki A, *et al.* (2006) Peripheral factors in the metabolic syndrome: the pivotal role of adiponectin. *Ann N Y Acad Sci* **1083**, 185–195.
21. Maeda K, Okubo K, Shimomura I, *et al.* (1996) cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochem Biophys Res Commun* **221**, 286–289.
22. Scherer PE, Williams S, Fogliano M, *et al.* (1995) A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* **270**, 26746–26749.
23. Devaraj S, Torok N, Dasu MR, *et al.* (2008) Adiponectin decreases C-reactive protein synthesis and secretion from endothelial cells: evidence for an adipose tissue-vascular loop. *Arterioscler Thomb Vasc Biol* **28**, 1368–1374.
24. Mito N, Hosoda T, Kato C, *et al.* (2000) Change of cytokine balance in diet-induced obese mice. *Metabolism* **49**, 1295–1300.
25. Hu E, Liang P & Spiegelman BM (1996) AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* **271**, 10697–10703.
26. Hotta K, Funahashi T, Arita Y, *et al.* (2000) Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thomb Vasc Biol* **20**, 1595–1599.
27. Weyer C, Funahashi T, Tanaka S, *et al.* (2001) Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* **86**, 1930–1935.
28. Vozarova B, Weyer C, Hanson K, *et al.* (2001) Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. *Obes Res* **9**, 414–417.
29. Park HT, Cho SH, Cho GJ, *et al.* (2009) Relationship between serum adipocytokine levels and metabolic syndrome in menopausal women. *Gynecol Endocrinol* **25**, 27–31.
30. De Taeye BM, Novitskaya T, Gleaves L, *et al.* (2006) Bone marrow plasminogen activator inhibitor-1 influences the development of obesity. *J Biol Chem* **281**, 32796–32805.
31. Hoffler U, Hobbie K, Wilson R, *et al.* (2009) Diet-induced obesity is associated with hyperleptinemia, hyperinsulinemia, hepatic steatosis, and glomerulopathy in C57Bl/6J mice. *Endocrine* **36**, 311–325.
32. Lijnen H, Maquoi E, Demeulemeester D, *et al.* (2002) Modulation of fibrinolytic and gelatinolytic activity during adipose tissue development in a mouse model of nutritionally induced obesity. *Thomb Haemost* **88**, 345–353.
33. Steppan CM, Bailey ST, Bhat S, *et al.* (2001) The hormone resistin links obesity to diabetes. *Nature* **409**, 307–312.
34. Steppan CM, Brown EJ, Wright CM, *et al.* (2001) A family of tissue-specific resistin-like molecules. *Proc Natl Acad Sci U S A* **98**, 502–506.
35. Lin S, Thomas TC, Storlien LH, *et al.* (2000) Development of high fat diet-induced obesity and leptin resistance in C57Bl/6J mice. *Int J Obes Relat Metab Disord* **24**, 639–646.
36. Harte RA, Kirk EA, Rosenfeld ME, *et al.* (1999) Initiation of hyperinsulinemia and hyperleptinemia is diet dependent in C57BL/6 mice. *Horm Metab Res* **31**, 570–575.
37. Weisberg SP, McCann D, Desai M, *et al.* (2003) Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* **112**, 1796–1808.
38. Calabro P, Chang DW, Willerson JT, *et al.* (2005) Release of C-reactive protein in response to inflammatory cytokines by human adipocytes: linking obesity to vascular inflammation. *J Am Coll Cardiol* **46**, 1112–1113.
39. Gao Z, Hwang D, Bataille F, *et al.* (2002) Serine phosphorylation of insulin receptor substrate 1 by inhibitor kappa B kinase complex. *J Biol Chem* **277**, 48115–48121.
40. Li X, Kimura H, Hirota K, *et al.* (2005) Synergistic effect of hypoxia and TNF-alpha on production of PAI-1 in human proximal renal tubular cells. *Kidney Int* **68**, 569–583.
41. Kishida M, Urakaze M, Takata M, *et al.* (2005) PGE1 inhibits the expression of PAI-1 mRNA induced by TNF-alpha in

British Journal of Nutrition

human mesangial cells. *Exp Clin Endocrinol Diabetes* **113**, 365–371.
42. Gallou-Kabani C, Vige A, Gross MS, *et al.* (2007) C57BL/6J and A/J mice fed a high-fat diet delineate components of metabolic syndrome. *Obesity (Silver Spring)* **15**, 1996–2005.
43. Smith AG, Sheridan PA, Harp JB, *et al.* (2007) Diet-induced obese mice have increased mortality and altered immune responses when infected with influenza virus. *J Nutr* **137**, 1236–1243.
44. Amar S, Zhou Q, Shaik-Dasthagirisaheb Y, *et al.* (2007) Diet-induced obesity in mice causes changes in immune responses and bone loss manifested by bacterial challenge. *Proc Natl Acad Sci U S A* **104**, 20466–20471.
45. Steppan CM, Wang J, Whiteman EL, *et al.* (2005) Activation of SOCS-3 by resistin. *Mol Cell Biol* **25**, 1569–1575.
46. Yang RZ, Huang Q, Xu A, *et al.* (2003) Comparative studies of resistin expression and phylogenomics in human and mouse. *Biochem Biophys Res Commun* **310**, 927–935.
47. Savage DB, Sewter CP, Klenk ES, *et al.* (2001) Resistin/Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-gamma action in humans. *Diabetes* **50**, 2199–2202.
48. Patel L, Buckels AC, Kinghorn IJ, *et al.* (2003) Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. *Biochem Biophys Res Commun* **300**, 472–476.
49. Jung HS, Park KH, Cho YM, *et al.* (2006) Resistin is secreted from macrophages in atheromas and promotes atherosclerosis. *Cardiovasc Res* **69**, 76–85.
50. Lord GM, Matarese G, Howard JK, *et al.* (1998) Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* **394**, 897–901.
51. Fernandez-Riejos P, Najib S, Santos-Alvarez J, *et al.* (2010) Role of leptin in the activation of immune cells. *Mediators Inflamm* **2010**, 568343.
52. Romagnani S (2000) T-cell subsets (Th1 versus Th2). *Ann Allergy Asthma Immunol* **85**, 9–18, quiz 18, 21.
53. Cho SY, Park SJ, Kwon MJ, *et al.* (2003) Quercetin suppresses proinflammatory cytokines production though MAP kinases and NF-kappaB pathway in lipopolysaccharide-stimulated macrophage. *Mol Cell Biochem* **243**, 153–160.
54. Olsson ME, Ekvall J, Gustavsson KE, *et al.* (2004) Antioxidants, low molecular weight carbohydrates, and total antioxidant capacity in strawberries (Fragaria × Ananassa): effects of cultivar, ripening, and storage. *J Agric Food Chem* **52**, 2490–2498.
55. Zhang Y, Seeram NP, Lee R, *et al.* (2008) Isolation and identification of strawberry phenolics with antioxidant and human cancer cell antiproliferative properties. *J Agric Food Chem* **56**, 670–675.
56. Hernanz D, Recamales AF, Melendez-Martinez AJ, *et al.* (2007) Assessment of the differences in the phenolic composition of five strawberry cultivars (Fragaria × Ananassa Duch.) grown in two different soilless systems. *J Agric Food Chem* **55**, 1846–1852.
57. Chen CC, Chow MP, Huang WC, *et al.* (2004) Flavonoids inhibit tumor necrosis factor-alpha-induced up-regulation of intercellular adhesion molecule-1 (ICAM-1) in respiratory epithelial cells though activator protein-1 and nuclear factor-kappaB: structure-activity relationships. *Mol Pharmacol* **66**, 683–693.
58. Collins S, Martin TL, Surwit RS, *et al.* (2004) Genetic vulnerability to diet-induced obesity in the C57BL/6J mouse: physiological and molecular characteristics. *Physiol Behav* **81**, 243–248.
59. Bullen JW Jr, Bluher S, Kelesidis T, *et al.* (2007) Regulation of adiponectin and its receptors in response to development of diet-induced obesity in mice. *Am J Physiol Endocrinol Metab* **292**, E1079–E1086.
60. Seeram NP, Adams LS, Zhang Y, *et al.* (2006) Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells *in vitro*. *J Agric Food Chem* **54**, 9329–9339.
61. Reeves PG, Nielsen FH & Fahey GC Jr (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition *ad hoc* writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr* **123**, 1939–1951.
62. Contrera JF, Matthews EJ, Kruhlak NL, *et al.* (2004) Estimating the safe starting dose in phase I clinical trials and no observed effect level based on QSAR modeling of the human maximum recommended daily dose. *Regul Toxicol Pharmacol* **40**, 185–206.
63. Reagan-Shaw S, Nihal M & Ahmad N (2008) Dose translation from animal to human studies revisited. *FASEB J* **22**, 659–661.
64. Ayala JE, Samuel VT, Morton GJ, *et al.* (2010) Standard operating procedures for describing and performing metabolic tests of glucose homeostasis in mice. *Dis Mod Mech* **3**, 525–534.
65. Chan CB, Liu X, Jung DY, *et al.* (2010) Deficiency of phosphoinositide 3-kinase enhancer protects mice from diet-induced obesity and insulin resistance. *Diabetes* **59**, 883–893.
66. Ranieri SC, Fusco S, Panieri E, *et al.* (2010) Mammalian lifespan determinant p66shcA mediates obesity-induced insulin resistance. *Proc Natl Acad Sci U S A* **107**, 13420–13425.
67. Stephensen CB, Rasooly R, Jiang X, *et al.* (2002) Vitamin A enhances *in vitro* Th2 development via retinoid X receptor pathway. *J Immunol* **168**, 4495–4503.
68. Prior RL, Hoang H, Gu L, *et al.* (2003) Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(FL))) of plasma and other biological and food samples. *J Agric Food Chem* **51**, 3273–3279.
69. Basu A, Wilkinson M, Penugonda K, *et al.* (2009) Freeze-dried strawberry powder improves lipid profile and lipid peroxidation in women with metabolic syndrome: baseline and post intervention effects. *Nutr J* **8**, 43.
70. Sesso HD, Gaziano JM, Jenkins DJ, *et al.* (2007) Strawberry intake, lipids, C-reactive protein, and the risk of cardiovascular disease in women. *J Am Coll Nutr* **26**, 303–310.
71. Edirisinghe I, Banaszewski K, Cappozzo J, *et al.* (2011) Strawberry anthocyanin and its association with postprandial inflammation and insulin. *Br J Nutr* **106**, 913–922.
72. Chun OK, Chung S-J, Claycombe KJ, *et al.* (2008) Serum C-reactive protein concentrations are inversely associated with dietary flavonoid intake in U.S. adults. *J Nutr* **138**, 753–760.
73. Torronen R, Sarkkinen E, Tapola N, *et al.* (2010) Berries modify the postprandial plasma glucose response to sucrose in healthy subjects. *Br J Nutr* **103**, 1094–1097.
74. Jenkins DJ, Nguyen TH, Kendall CW, *et al.* (2008) The effect of strawberries in a cholesterol-lowering dietary portfolio. *Metabolism* **57**, 1636–1644.
75. Scheyer SA, Wilson DL & LeBoeuf RC (1998) C57BL/6 mice fed high fat diets as models for diabetes-accelerated atherosclerosis. *Atherosclerosis* **136**, 17–24.
76. Prior RL, Wu X, Gu L, *et al.* (2008) Whole berries versus berry anthocyanins: interactions with dietary fat levels in the

C57BL/6J mouse model of obesity. *J Agric Food Chem* **56**, 647–653.
77. Sumiyoshi M, Sakanaka M & Kimura Y (2006) Chonic intake of high-fat and high-sucrose diets differentially affects glucose intolerance in mice. *J Nutr* **136**, 582–587.
78. Lijnen H (2005) Effect of plasminogen activator inhibitor-1 deficiency on nutritionally-induced obesity in mice. *Thomb Haemost* **93**, 816–819.
79. Klover PJ, Clementi AH & Mooney RA (2005) Interleukin-6 depletion selectively improves hepatic insulin action in obesity. *Endocrinology* **146**, 3417–3427.
80. Wu H, Gower RM, Wang H, *et al.* (2009) Functional role of CD11c + monocytes in atherogenesis associated with hypercholesterolemia. *Circulation* **119**, 2708–2717.
81. Foster-Powell K, Holt SHA & Brand-Miller JC (2002) International table of glycemic index and glycemic load values: 2002. *Am J Clin Nutr* **76**, 5–56.
82. Jenkins DJA, Srichaikul K, Kendall CWC, *et al.* (2011) The relation of low glycaemic index fruit consumption to glycaemic control and risk factors for coronary heart disease in type 2 diabetes. *Diabetologia* **54**, 271–270.
83. Seymour EM, Singer AAM, Kirakosyan A, *et al.* (2008) Altered hyperlipidemia, hepatic steatosis, and hepatic peroxisome proliferator-activated receptors in rats with intake of tart cherry. *J Med Food* **11**, 252–259.
84. Takikawa M, Inoue S, Horio F, *et al.* (2010) Dietary anthocyanin-rich bilberry extract ameliorates hyperglycemia and insulin sensitivity via activation of AMP-activated protein kinase in diabetic mice. *J Nutr* **140**, 527–533.

British Journal of Nutrition