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HUMAN AND CLINICAL NUTRITION

Intake of polyphenol-rich pomegranate pure juice influences urinary glucocorticoids, blood pressure and homeostasis model assessment of insulin resistance in human volunteers

Catherine Tsang, Nacer F. Smail, S. Almoosawi, I. Davidson and Emad A. S. Al-Dujaili*

Department of Dietetics, Nutrition and Biological Sciences, Queen Margaret University, Queen Margaret University Drive, Musselburgh, East Lothian EH21 6UU, UK

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Abstract

Pomegranate juice (PJ; also known as pomegreat pure juice) provides a rich and varied source of polyphenolic compounds that may offer cardioprotective, anti-atherogenic and antihypertensive effects. The aim of this study was to investigate the effect of PJ consumption on glucocorticoids levels, blood pressure (BP) and insulin resistance in volunteers at high CVD risk. Subjects (twelve males and sixteen females) participated in a randomised, placebo-controlled cross-over study (BMI: 26·77 (SD 3·36) kg/m^2; mean age: 50·4 (SD 6·1) years). Volunteers were assessed at baseline, and at weeks 2 and 4 for anthropometry, BP and pulse wave velocity. Cortisol and cortisone levels in urine and saliva were determined by specific ELISA methods, and the cortisol/cortisone ratio was calculated. Fasting blood samples were obtained to assess plasma lipids, glucose, insulin and insulin resistance (homeostasis model assessment of insulin resistance). Volunteers consumed 500 ml of PJ or 500 ml of a placebo drink containing a similar amount of energy. Cortisol urinary output was reduced but not significant. However, cortisol/cortisone ratios in urine ($P = 0·009$) and saliva ($P = 0·024$) were significantly decreased. Systolic BP decreased from 136·4 (SD 6·3) to 128·9 (SD 5·1) mmHg ($P = 0·034$), and diastolic BP from 80·3 (SD 4·29) to 75·5 (SD 5·17) mmHg ($P = 0·031$) after 4 weeks of fruit juice consumption. Pulse wave velocity decreased from 7·5 (SD 0·86) to 7·44 (SD 0·94) m/s ($P = 0·035$). There was also a significant reduction in fasting plasma insulin from 9·36 (SD 5·8) to 7·53 (SD 4·12) mIU/l ($P = 0·025$) and of homeostasis model assessment of insulin resistance (from 2·216 (SD 1·43) to 1·82 (SD 1·12), $P = 0·028$). No significant changes were seen in the placebo arm of the study. These results suggest that PJ consumption can alleviate key cardiovascular risk factors in overweight and obese subjects that might be due to a reduction in both systolic and diastolic BP, possibly through the inhibition of 11β-hydroxysteroid dehydrogenase type 1 enzyme activity as evidenced by the reduction in the cortisol/cortisone ratio. The reduction in insulin resistance might have therapeutic benefits for patients with non-insulin-dependent diabetes, obesity and the metabolic syndrome.

Key words: **Pomegranate juice: Blood pressure: Glucocorticoids: Obesity: 11β-Hydroxysteroid dehydrogenase: Homeostasis model assessment of insulin resistance**

CVD is a major cause of mortality in Western countries, particularly in Scotland where death rates are among the highest in the world. Dietary factors represent a key component of the disease and specific dietary constituents are considered to be important to health. Polyphenols are secondary metabolites widespread in the plant kingdom and form an integral part of the human diet where it has been estimated that their total dietary intake may be as high as 1 g/d[1]. Polyphenols have perceived health benefits in their ability to neutralise free radicals and reactive oxygen species, and thus cardiovascular health may be improved, as LDL-cholesterol has a reduced capacity to be oxidised and therefore to reduce

Abbreviations: 11β-HSD, 11β-hydroxysteroid dehydrogenase; BP, blood pressure; DBP, diastolic blood pressure; FRAP, ferric-reducing antioxidant power; HOMA-IR, homeostasis model assessment of insulin resistance; PJ, pomegranate juice; SBP, systolic blood pressure.

* **Corresponding author:** Dr E. A. S. Al-Dujaili, fax +44 131 474 0001, email ealdujaili@qmu.ac.uk

atherogenicity and platelet aggregation[2–4]. The pomegranate (*Punica granatum* L.) has been a medicinal food throughout history. Pomegranate juice (PJ; also known as pomegreat pure juice) might have potent antioxidant and anti-atherosclerotic properties attributed to its abundance of polyphenols including ellagic acids, gallotannins and anthocyanins (cyanidin, delphinidin and pelargonidin glycosides) and other flavonoids. The most abundant of these polyphenols is punicalagin, an ellagitannin implicated as the bioactive constituent responsible for >50 % of the juice's antioxidant activity[5,6]. Numerous studies have been carried out for the identification and quantification of phenolic compounds and their effects on antioxidant activity of PJ in human subjects and animals[7–9], and have concluded that most pomegranate flavonoids and ellagitannins such as punicalagin and ellagic acid can be detected in the plasma and urine. However, the levels were found to be lower than expected, which could be due to the fact that the bioavailability process is complicated with large individual variations and requires further investigation using a large number of volunteers. In addition, it was also discovered that even ellagic acid metabolites such as urolithins A and B and their glucuronide and sulphate derivatives are biologically active[10–12].

Several studies have demonstrated that consumption of PJ can exert positive effects on cardiovascular risk, hypertension, atherosclerosis, endothelial dysfunction and inflammation[13–15]. Clinical studies have shown a beneficial effect on vascular health following PJ consumption, with reduced systolic blood pressure (SBP), enhanced antioxidant status and improved insulin resistance in type 2 diabetic and hypertensive patients[13,15,16]. In addition, some researchers have shown that PJ consumption reduces carotid intima-media thickness and blood pressure (BP)[14,17]. As powerful antioxidants, phenolics may protect the body from damaging oxidation reactions[18,19]; however, other mechanisms may also operate which are currently under investigation. In a limited study of hypertensive patients, consumption of PJ for 2 weeks was shown to reduce SBP by inhibiting serum angiotensin-converting enzyme[15]. Obesity and diabetes are other underlying factors linked to metabolic and cardiovascular risk[14] as well as abnormal cortisol levels and metabolism[20].

The importance of cortisol in regulating BP has been highlighted in several conditions, and chronic excessive activation of the glucocorticoid receptor is known to induce obesity, insulin resistance, glucose intolerance and hypertension[21]. Glucocorticoids exert a direct effect on the heart and blood vessels via 11β-hydroxysteroid dehydrogenase type 1 enzyme (11β-HSD1) and 11β-HSD2. Increased 11β-HSD1 activity is implicated in the development of the metabolic syndrome and identifying dietary constituents that influence 11β-HSD1 activity could lead to novel methods of preventing CVD and associated risk factors. The association between excess cortisol and various parameters of the metabolic syndrome, including hypertension and insulin resistance, is now increasingly recognised[22,23]. The aim of the present study was to examine the influence of a 4-week consumption of PJ (Pomegreat Pure®) containing 1685 mg/l polyphenols on salivary and urinary cortisol and cortisone levels, and their ratio as indicative of the activity of 11β-HSD enzymes. In addition, the assessment of cardiometabolic risk factors: BP, arterial compliance, plasma lipid profile, glucose, insulin and insulin resistance, in a high-risk group (age 40–65 years) of apparently healthy male and female participants will be made.

Experimental methods

Study design

The study used a randomised, placebo-controlled, cross-over design (Fig. 1). Following a 1-week run-in phase, eligible subjects were randomly assigned to receive 500 ml PJ containing 1685 mg/l polyphenols or 500 ml placebo (water placed in a dark container plus the addition of equivalent carbohydrates to match the energy content of the juice). Participants followed each intervention for 4 weeks, after which they were crossed over to the next intervention separated by a 1-week washout period. Participants were provided with a list of phenolic-rich foods to avoid including green tea, red wine, dark chocolate and berries. Compliance with the dietary restrictions was monitored with a 3-d food diary record. Compliance was also measured by assessing the total phenolic content of 24 h urine samples collected at baseline, the mid-point and at the end of the study period. All participants provided written informed consent and a lifestyle questionnaire to determine their eligibility. The study was conducted at Queen Margaret University and the protocol was approved by Queen Margaret University Research Ethics Committee. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Queen Margaret University Research Ethics Central Committee. Written informed consent was obtained from all subjects and has been recorded and stored according to the university guidelines.

Inclusion and exclusion criteria

Participants were recruited by an email moderator advertisement at Queen Margaret University, Edinburgh, UK. Volunteers were aged between 40 and 65 years with a BMI between 25 and 35 kg/m^2. Only those sedentary or moderately active (defined as

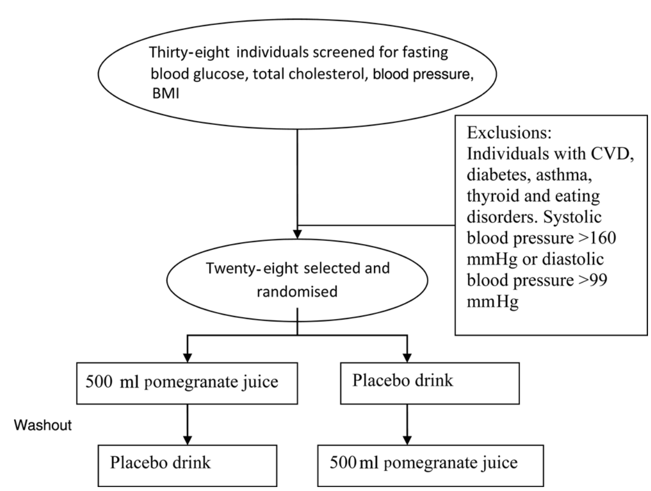

Fig. 1. Flow diagram of the study design protocol.

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less than two aerobic sessions per week) were included. Participants were excluded if they presented with signs of the metabolic syndrome, i.e. fasting plasma glucose >6·1 mmol/l, TAG level >1·7 mmol/l, low HDL-cholesterol (<1·01 mmol/l for men and <1·25 mmol/l for women), hypertension, central obesity (waist circumference >108 cm for men and >88 cm for women) or if they had moderate hypercholesterolaemia. Individuals were excluded also if they reported to have CVD, diabetes, asthma, SBP >160 mmHg or diastolic blood pressure (DBP) >99 mmHg. Individuals with thyroid gland disorders or eating disorders, as well as those taking regular medication or nutritional supplements (such as antioxidants or fish oil) known to affect any dependent variable measured, were also excluded.

Materials and chemicals

Gallic acid and Folin–Ciocalteau's phenol regent were purchased from Sigma. Methanol (HPLC grade) and HCl were purchased from Rathburn Chemicals. Acetone, acetic acid (glacial) and Na_2HPO_4 were obtained from Fisher Scientific. Na_2CO_3, disodium hydrogen orthophosphate and sodium dihydrogen orthophosphate dehydrate were purchased from BDH Chemicals Ltd. All other chemicals and reagents were obtained from Sigma unless otherwise stated.

Measurements

Fasted blood was collected at baseline and at the end of the 4-week intervention period. Plasma was obtained by centrifugation (at 1500 ***g*** for 10 min) within 30 min of blood collection and stored frozen at −80°C prior to analysis. Plasma lipids and glucose analyses were undertaken at the Clinical Biochemistry Laboratory, Western General Hospital, Edinburgh, Scotland, UK using an automated platform (Olympus). Fasting plasma insulin was estimated using a sensitive human Insulin ELISA kit (GenWay), and insulin resistance (homeostasis model assessment of insulin resistance (HOMA-IR)) was calculated by the method of Turner and co-workers[24] using the formula HOMA-IR = fasting glucose × fasting insulin/22·5. Unstimulated saliva samples were collected (08.00 hours, noon and 18.00 hours) from volunteers in sterile plastic containers at baseline and after 4 weeks of the juice or placebo intake.

Serum NEFA were quantified using an automated enzymatic colorimetric method (Wako) at the Rowett Institute of Nutrition and Health, Aberdeen, UK. Urinary and plasma levels of total polyphenols[25] and ferric-reducing antioxidant power (FRAP)[26] were measured by established methods. Plasma levels of total phenolics were assessed by a modification of the Folin–Ciocalteau method as described by Serafini *et al.*[27]. Cortisol and cortisone levels in saliva and urine samples were estimated by using highly specific and sensitive ELISA methods published previously[28]. The ratio of cortisol to cortisone was calculated to give an indication of the activity of 11β-HSD1. All samples from the same subject were tested on the same day to prevent any inter-day variation. A validated automated A&D Medical UA-767 BP monitor (A&D Medical) was used to measure BP according to Grassi *et al.*[29]. Three readings of BP were usually taken at each visit and the average was calculated. Arterial compliance was assessed by pulse wave velocity using a Vicorder™, (Skidmore Medical), and again three measurements were taken at each visit.

Statistical analysis

All statistical analyses were performed using SPSS for Windows, version 16.10 (SPSS Inc.). $P \leq 0·05$ was considered significant. Continuous normally distributed data are expressed as mean values and standard deviations unless otherwise stated. Differences in baseline characteristics were examined using an independent-samples paired Student's t test with BMI, age, waist circumference, fasting insulin, glucose, lipid profile, BP, urinary and plasma total polyphenols and FRAP as the dependent variables. ANOVA were conducted using the general linear model procedure to assess the differences in the effect of PJ and placebo on outcome measures. The models included the main effects of treatment, time and treatment–time interaction. Bonferroni adjustment was used to account for multiple testing.

To examine whether the effect of PJ on BP, insulin and HOMA-IR was dependent on BMI, we also ran a series of linear regression models. Changes in BP, insulin and HOMA-IR were modelled as dependent variables. BMI, treatment and BMI–treatment interaction were fitted as predictors, as shown in the following equation:

$$y = (a_1x_1) + (a_2x_2) + (a_3x_3) + a_4,$$

where y is the change in outcome from the baseline per person per treatment group; x_1 is the dummy variable, 0 for placebo and 1 for PJ; x_2 is the BMI expressed as a continuous variable; x_3 is the product term for dummy variable and BMI; and a_{1-4} is the β coefficients.

Results

Subjects

Eligible participants were aged between 40 and 65 years with a BMI between 25 and 35 kg/m^2 (Table 1). In this randomised, placebo-controlled, cross-over study, twenty-eight volunteers (twelve males and sixteen females) completed the study and each consumed 500 ml/d of PJ for a period of 4 weeks. Of the total number recruited, eight were excluded for one of the reasons mentioned in Fig. 1 and two subjects dropped out of the study due to the inconvenience of travelling to the university campus following baseline assessment.

Anthropometric and metabolic results

The 4-week intervention did not significantly change plasma levels of lipids (total cholesterol, TAG, LDL-cholesterol or HDL-cholesterol) or glucose concentrations, which were all within the anticipated physiological range (Table 1). There was a significant reduction in pulse wave velocity after 2

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Table 1. Anthropometric, pulse wave velocity (PWV), ferric-reducing antioxidant power (FRAP), total phenolics (TP), plasma cholesterol and lipid profile measurements at baseline and following 2 and 4 weeks of pomegranate juice consumption
(Mean values and standard deviations)

	Basal		2 weeks			4 weeks		
Parameter	Mean	SD	Mean	SD	*P*	Mean	SD	*P*
Weight (kg)	79·02	13·1	78·71	13·9	0·057	78·9	13·3	0·337
BMI (kg/m^2)	26·77	3·25	26·71	3·46	0·095	26·72	3·27	0·298
Systolic blood pressure (mmHg)	136·2	6·3	131·1	7·6	0·033	128·9	5·13	0·034
Diastolic blood pressure (mmHg)	80·27	4·29	75·5	5·17	0·031	76·2	4·89	0·039
PWV (m/s)	7·748	0·86	7·49	0·92	0·035	7·44	0·94	0·049
Urinary FRAP (mmol Fe^{2+}/d)	3·46	0·92	4·58	1·11	0·012	4·32	0·91	0·031
Urinary TP (mmol gallic acid equivalents/d)	2·59	0·71	3·33	0·81	0·001	2·99	0·65	0·002
Plasma FRAP (mmol Fe^{2+}/l)	1·1	0·17				1·24	0·17	0·001
Plasma TP (mmol gallic acid equivalents/l)	3·23	0·41				3·48	0·46	0·015
Plasma glucose (mmol/l)	4·89	0·37				4·81	0·53	0·437
Plasma insulin (mIU/l)	9·364	5·81				7·53	4·12	0·025
Total cholesterol (mmol/l)	5·34	0·77				5·45	1·0	0·52
HDL-cholesterol (mmol/l)	1·52	0·41				1·52	0·44	0·87
LDL-cholesterol (mmol/l)	3·28	0·71				3·31	0·73	0·23
TAG (mmol/l)	1·175	0·67				1·147	0·39	0·86
HOMA-IR	2·216	1·43				1·825	1·12	0·028

HOMA-IR, homeostasis model assessment of insulin resistance.

weeks ($P = 0·035$) and 4 weeks of PJ consumption. There was also a significant reduction in both SBP and DBP in the treatment group compared with the control group (Table 1), SBP from 136·2 (SD 6·3) to 131·1 (SD 7·6) ($P = 0·033$) to 128·9 (SD 5·1) mmHg ($P = 0·034$) after 2 and 4 weeks, respectively. DBP decreased from 80·27 (SD 4·29) to 75·5 (SD 5·17) mmHg ($P = 0·031$) after 2 weeks of fruit juice consumption.

No significant changes in body weight or BMI were observed; however, eleven of the twenty-one participants showed a decrease in plasma NEFA levels following fruit juice consumption for 4 weeks (from 0·483 (SD 0·16) to 0·396 (SD 0·14) mmol/l, $P = 0·017$), and this was not observed in the placebo group. Plasma antioxidant capacity was significantly increased following 4 weeks of PJ intake; FRAP increased from 1098 (SD 170·5) to 1241 (SD 173·7) μmol Fe (II)/l ($P = 0·001$) following fruit juice consumption. Plasma total phenols were also significantly increased from 3·23 (SD 0·41) to 3·48 (SD 0·46) mmol gallic acid equivalents/l ($P = 0·015$). Urinary antioxidant capacity was significantly increased following 2 and 4 weeks of PJ intake. After 2 weeks, the FRAP level went up from 3·46 (SD 0·92) to 4·58 (SD 1·11) mmol Fe (II)/d ($P = 0·012$). The urinary total phenolics level was also significantly increased from 2·59 (SD 0·71) to 3·33 (SD 0·81) mmol gallic acid equivalents/d ($P = 0·001$). There was no significant change in the plasma lipid profile, BP, fasting glucose and insulin, plasma and urinary FRAP and total phenolics following the placebo arm of the study (Table 2).

Hormonal results

The results of cortisol and cortisone levels are shown in Fig. 2. Urinary free cortisol levels were reduced following PJ consumption compared to basal values, but not significantly. No significant difference was obtained in urinary free cortisone output. When the free cortisol/cortisone ratio was calculated, there was a significant reduction in urinary ($P = 0·009$) and salivary ratios ($P = 0·024$), indicating a possible

Table 2. Blood pressure (BP), pulse wave velocity (PWV), plasma cholesterol and lipid profile measurements at baseline and following 4 weeks of placebo drink consumption
(Mean values and standard deviations)

	Basal		4 weeks		
Parameter	Mean	SD	Mean	SD	*P*
Diastolic BP (mmHg)	78·9	11·1	80·9	10·9	0·397
Systolic BP (mmHg)	132·6	18·2	133·8	16·3	0·498
PWV (m/s)	7·62	0·78	7·54	0·94	0·249
Plasma TC (mmol/l)	4·58	0·47	4·51	0·51	0·742
Plasma HDL-C (mmol/l)	1·52	0·59	1·46	0·56	0·339
Plasma LDL-C (mmol/l)	2·52	0·69	2·54	0·79	0·993
Plasma TAG (mmol/l)	1·21	0·53	1·14	0·51	0·433
Plasma glucose (mmol/l)	4·65	0·48	4·74	0·27	0·537
Plasma insulin (mIU/l)	10·65	9·12	10·54	7·72	0·737
HOMA-IR	2·245	0·23	2·226	0·12	0·937

TC, total cholesterol; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance.

effect on 11β-HSD1 activity. Placebo data showed no significant difference (the urinary basal ratio was 1·029 (SD 0·28) and post-placebo 1·042 (SD 0·34)). There was a significant reduction of fasting plasma insulin levels from 9·364 (SD 5·81) to 7·53 (SD 4·12) mIU/l ($P = 0·025$) and of insulin resistance (HOMA-IR) from 2·216 (SD 1·43) to 1·825 (SD 1·12) ($P = 0·028$) (Table 1). There was no significant change in all the parameters tested following the placebo arm of the study (Table 2).

Blood pressure, insulin and homeostasis model of insulin resistance results

The effect on SBP, DBP, insulin and HOMA-IR was also studied using ANOVA. Here the changes in response between the basal and the intervention (pomegranate or placebo) were calculated, and the results are shown in Figs. 3 and 4. For BP scores, the data were analysed using the differences between the basal values and those following PJ or placebo intake.

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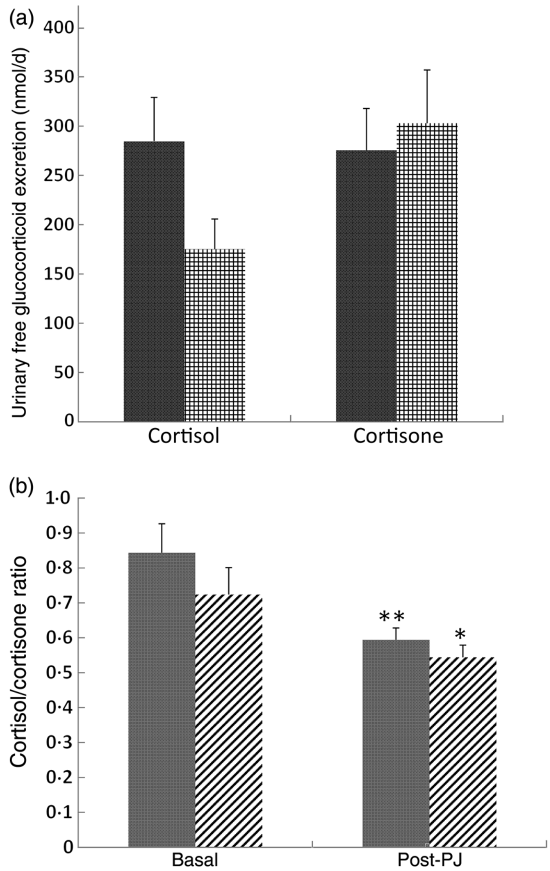

Fig. 2. Glucocorticoid results. (a) Urinary free cortisol and cortisone outputs at baseline (■) and post-pomegranate juice (PJ; ▩) and (b) urinary (▩) and salivary (///) free cortisol/cortisone ratios. Values are means, with standard errors represented by vertical bars. Mean value was significantly reduced following pomegranate juice intake compared with baseline: * $P = 0{\cdot}024$, ** $P = 0{\cdot}009$.

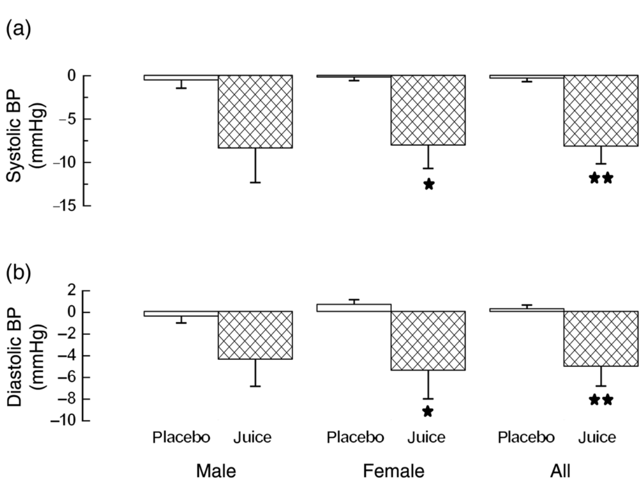

Fig. 3. Effect of pomegranate juice consumption on blood pressure (BP) reduction. There were significant reductions in systolic BP (a) and diastolic BP (b) differences from basal values when both male and female data were pooled. Separate effects in males were not always significant, but those in females were. Values are means, with standard errors represented by vertical bars. * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

The effects of PJ intake compared with the placebo were significant for all the variables when both males and females were pooled (Fig. 3). Separately, the effects in males were not always significant, but in females, the differences were. For the effect

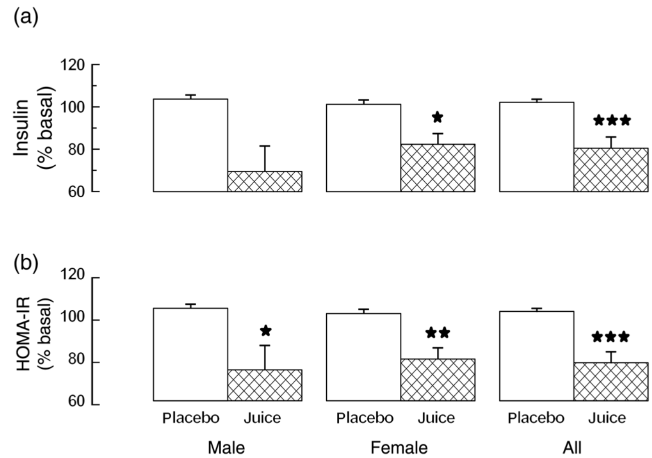

Fig. 4. Effect of pomegranate juice consumption on insulin (a) and homeostasis model assessment of insulin-resistance (HOMA-IR) (b) calculated as percentage difference from basal values. Values are means, with standard errors represented by vertical bars. There were significant reductions in percentage fasting plasma insulin differences in females and pooled data but not in males. However, there were significant reductions in percentage of HOMA-IR differences for males, females and pooled data. * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

on plasma insulin and HOMA-IR (Fig. 4), the percentage differences from basal values were analysed (e.g. 100 × basal insulin/insulin PJ and 100 × basal insulin/insulin placebo). Again, the results indicate that a significance effect was more apparent when all the data were pooled together.

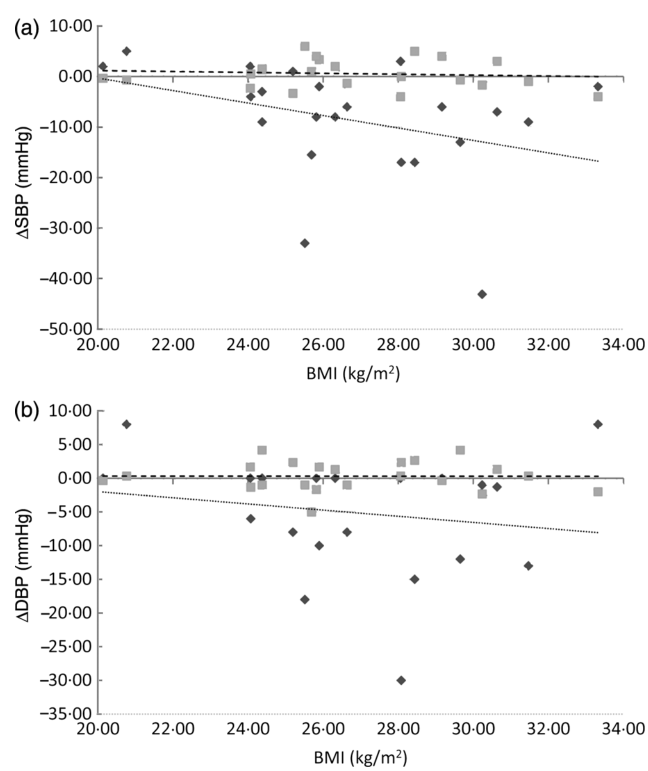

Fig. 5. Association between BMI and changes in systolic blood pressure (SBP) (a) or diastolic blood pressure (DBP) (b) following intake of pomegranate juice (♦) or placebo (■). The graph shows trend lines for pomegranate juice (. . . .) and placebo (- - -). There was a significant interaction between BMI and treatment for SBP ($P = 0{\cdot}013$) and DBP ($P = 0{\cdot}022$).

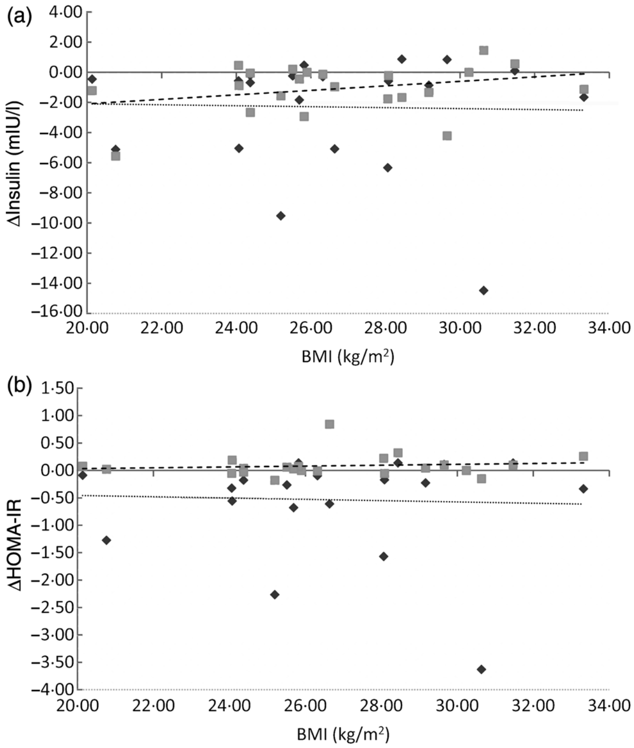

Fig. 6. Association between BMI and changes in insulin (a) and homeostasis model assessment of insulin resistance (HOMA-IR) (b) following intake of pomegranate juice (♦) or placebo (■). The graph shows trend lines for pomegranate juice (. . . .) and placebo (- - -). There was no significant interaction between BMI and treatment for insulin ($P = 0·469$) or HOMA-IR ($P = 0·431$).

For the results of linear regression models investigating whether the effects of PJ on BP, insulin and HOMA-IR were dependent on BMI, we also ran a series of regression analyses between the changes in BP, insulin and HOMA-IR and BMI. The association between BMI and changes in SBP or DBP following intake of PJ or placebo (Fig. 5) showed a significant interaction between BMI and treatment for SBP ($P = 0·013$) and DBP ($P = 0·022$). On the other hand, the association between BMI and changes in insulin and HOMA-IR following the intake of PJ or placebo (Fig. 6) showed that there was no significant interaction between BMI and treatment for insulin ($P = 0·469$) or HOMA-IR ($P = 0·431$).

Discussion

The present study was conducted to investigate the cardio-metabolic effects of PJ consumption over a 4-week period in apparently healthy male and female participants at high CVD risk. Previous clinical studies have shown beneficial effects on cardiovascular risk: reduced SBP, improved insulin resistance in non-insulin-dependent diabetes mellitus and hypertensive patients following the consumption of PJ[15,16,30,31]. Similarly, we also observed a significant reduction in SBP and DBP following 2 and 4 weeks of PJ consumption. The drop in SBP was from 0·5 to 22 mmHg and the drop in DBP was from 0 to 12 mmHg. Those with higher baseline BP had the most marked drop in their BP following PJ consumption (e.g. for subject 16, BP decreased from 160/91 to 141/74 compared with subject 1 from 135/64 to 130/61). One of the main objectives of this study was to investigate whether the drop in BP might be caused by inactivation of 11β-HSD1, which converts cortisone to the active steroid, cortisol. The circulating level of cortisol is an important factor in BP regulation and can also exert negative effects on the cardiovascular system at an autocrine level. There are two isozymes of 11β-HSD that catalyse the interconversion of active cortisol and inactive cortisone. 11β-HSD1 is mostly abundant in liver and adipose human tissue. It functions mainly as an oxoreductase converting cortisone to cortisol. We have shown that PJ intake has the potential for inhibiting 11β-HSD1 activity as is evident from the reduction in the cortisol/cortisone ratio in both urine and saliva. The role of this enzyme in hypertension has been reported previously, for example, in the syndrome of apparent mineralocorticoid excess[32]. A combination of assaying urinary free cortisone and free cortisol is a powerful method of assessing 11β-HSD1 activity that proved to be very advantageous in this regard[33]. However, we could not directly assess the enzyme activity in this study (by PCR expression for example) due to protocol limitations. In addition, it has been postulated that selective inhibitors of 11β-HSD1 may lower blood glucose and HOMA-IR in type 2 diabetic animal models[34]. Cortisol is known to be essential for the long-term maintenance of blood glucose and could also unfavourably influence BP and lipid profile as it can induce oxidative stress that has been linked to hyperinsulinaemia and insulin resistance[22,23]. This might explain our findings that PJ consumption has improved insulin resistance in our cohort. In this study, there was a reduction in free cortisol levels but it was not significant. Further investigation to establish whether tissue-specific alteration in 11β-HSD1 occurs following PJ consumption, in particular the effects on liver and adipose tissues, would be warranted. It is widely accepted that obesity is an inflammatory condition that may play an important role in hypertension[35,36]. Linear regression analysis of our results has shown that there was a significant interaction between BMI and the reduction in SBP and DBP following PJ intake. The drop in BP might also be partly explained by the inhibition of angiotensin-converting enzyme activity following PJ intake as reported by Aviram & Dornfield[15], a reduction in carotid intima-media thickness[14,17], an improvement of arterial elasticity (as we have observed in this study) or a combination of all these factors.

We did not find any significant changes in plasma total cholesterol, HDL, LDL or TAG following PJ intake, which was similar to the placebo results. This could be due to the small number of participants and their age group or the short washout period (1 week) that perhaps was not enough for levels to go to baseline. We accept that this can be regarded as one limitation of this study. However, studies conducted in both human subjects and animals have shown conflicting results. While some studies demonstrated a reduction in TAG and total cholesterol levels following PJ consumption (3–10 %), other studies have found no effects on circulating lipids[37]. However, several studies have consistently found a reduction in oxidised LDL levels and plaque formation associated with atherosclerosis following PJ consumption[3,38,39].

As powerful antioxidants polyphenols present in fruits may help protect the body from damaging oxidation reactions; however, the mechanism by which antioxidants exert their effects is not fully understood. Certainly, antioxidants play a key role in the scavenging of free radicals generated through oxidative metabolism and may offer protection to biomolecules (DNA, lipids and proteins) against damage. However, there is increasing evidence that individual phenolics or certain classes of phenolics may exert additional beneficial effects independent of their antioxidant properties. Among these are the deceleration of prostate cancer[40], anticancer effect in human breast cancer[41], enhancement of NO biological actions[42] and Alzheimer's disease prevention[43]. In addition, phenolic extracts from plants were suggested to cause insulin-like effects in glucose utilisation, and effectively inhibit intestinal α-glucosidase activity. The inhibitory effectiveness of the extracts were thought to be related to their anthocyanin content, in particular diacylated anthocyanins from these sources that were most effective[44]. Moreover, certain phenolics also inhibit α-amylase activity, which could prove synergistic to their potential therapeutic effect on post-meal blood glucose levels. Tannins and ellagic acid in particular are potent inhibitors of α-amylase activity. Tannic acid and tannin-rich non-alcoholic components of red wine have been shown to reduce serum glucose levels following a starch-rich meal in patients with non-insulin-dependent diabetes mellitus[45]. A recent review of the role of antioxidants in abnormal glucose metabolism[46] has concluded that experimental data are in favour of a beneficial role, but clinical data in human subjects remain controversial.

The question of bioavailability of pomegranate polyphenols remains controversial. Some authors have concluded that phenolic compounds such as those present in pomegranate are poorly absorbed and do not markedly contribute to antioxidant activity[47,48]. However, an overwhelming number of studies have shown demonstrable health benefits following the consumption of PJ or extract mainly due to the biotransformation of pomegranate polyphenols[3–8,13,37–39,41–44]. There is also now good evidence that even ellagitannin metabolites such as urolithins A, B and C and their glucuronides and sulphates are biologically active[10–12]. In the present study, we observed significant reductions in both insulin and insulin resistance (HOMA-IR) following PJ consumption. Diabetic patients may be more prone to oxidative stress because as hyperglycaemia facilitates the production of free radicals[49], dietary antioxidants may be important therapeutically in the control of non-insulin-dependent diabetes mellitus. There have been reports that naturally occurring polyphenols can inhibit pancreatic lipase and thereby influence fat digestion and affect energy intake. A recent study investigated the effects of different berry phenolic extracts on their ability to inhibit pancreatic lipase *in vitro*[50]. The most effective were from the *Rubus* family (i.e. raspberry, cloudberry and bramble) and strawberry. There is also a suggestion that ellagitannins present in berries and pomegranate could be important for lipase inhibition. Commercial PJ is known to contain significant levels of both ellagitannins and anthocyanins which may influence fat digestion and excretion. Although we did not investigate the influence of PJ on pancreatic lipase activity *in vitro,* future studies will focus on this important aspect. In the present study, we did not find a significant reduction in overall body weight; however, we did observe a trend towards a reduction in waist circumference and plasma NEFA in some of our subjects. The role of NEFA in human obesity appears to be important, and experimental evidence links the NEFA-induced presser response to sympathetic activation[51]. Abdominal obesity is linked to increased NEFA levels, which are associated with an increase in BP and seem to be resistant to suppression by insulin[52–54].

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