Effect of Pomegranate Extract Consumption on Cardiovascular Disease Risk Factors, Stress Hormones, and Quality of Life in Human Volunteers: An Exploratory Randomised, Double-Blind, Placebo-Controlled Trial

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Abstract

Background: Pomegranate extract (PE) provides a rich and varied source of biophenols, which can act as powerful antioxidants. The most abundant being ellagitannins, anthocyanins, and ellagic and gallic acid derivatives.. Evidence suggests that pomegranate juice consumption may alleviate cardiovascular disease (CVD) risk factors. This exploratory study investigates the effect of PEconsumption on blood pressure (BP), insulin resistance (HOMA-IR), stress hormone levels (cortisol/cortisone) and quality of life in healthy human volunteers.

Methods: Seven males and 22 females(n = 29) participated in a double-blind, randomised, placebo-controlled exploratory study (BMI: 25.05 ± 3.91 kg/m^2, age: 34.5 ± 13.7 years). All participants consumed either one PE (Pomanox, Pomegreat) or a placebo capsule daily, after a meal, for 4 weeks. Dietary history and habits and the health related Quality of Life questionnaire (Rand 36) were recorded pre- and post-intervention. BP, salivary cortisol and cortisone levels (am, noon, and pm) were assessed byELISAs, and fasting blood was obtained at baseline and after 4 weeks to compare glucose, insulin and insulin resistance parameters.

Results: All participants randomised in the study completed the intervention.Systolic BP was significantly reduced following PE from 120.3 ± 13.3 to 115.6 ± 13.1 mmHg (P = 0.012). There was a reduction in the HOMA-IR levels from2.22 ± 2.62 to 1.61 ± 1.88 (P = 0.045), and glucose, insulin and uric acid alldecreased from baseline. No significant changes were recorded in volunteerstaking the placebo. PEconsumption caused a significant drop of salivary cortisol levels (am; 39.5 ± 19.6%, p < 0.001 and noon; 43.1 ± 32.3%, p = 0.016). The salivary cortisol/cortisone ratio was also significantly reduced (am from 1.11 ± 0.51 to 0.55 ± 0.26, p < 0.001, noon 1.57 ± 0.85 to 0.75 ± 0.72, p < 0.001 and pm; 1.22 ± 0.90 to 0.74 ± 0.59, p = 0.011). Physical (p = 0.018) and social functioning (p = 0.021), pain (p = 0.003), general health (p = 0.008) and overall Quality of Life score (p = 0.007) were significantly improved in those taking the PE capsules. The intervention was delivered successfully with no withdrawals.

Conclusions: These results suggest that PE intake rich in biophenolsmay ameliorate cardiovascular risk factors, reduce stress levels and improve perceived health related quality of life. The reduction in salivary cortisol levels may prove beneficial for people suffering from chronic stress. This exploratory study provides useful information required to conduct a definitive trial.

Trial Registration: This trial was registered with The Clinical Trials.gov as NCT02005939.

Keywords: Pomegranate; Cardiovascular risk factors; Blood pressure; Insulin sensitivity; Cortisol; Quality of life

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Introduction

Cardiovascular disease (CVD) is the number one cause of mortality globally with an estimated 30% of all global deaths [1]. However, most CVDs can be prevented by addressing risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity, high blood pressure (BP), diabetes and raised lipids. Dietary factors represent a key component of the disease and choosing a diet rich in fruit and vegetables is considered to be important in reducing CVD risk [2].

Polyphenols are the most abundant antioxidants in the diet and are widespread in plants. Their total dietary intake could be as high as 1 g/day, which is much higher than that of all other classes of phytochemicals and known dietary antioxidants [3]. Current evidence strongly supports a contribution of polyphenols to the prevention of CVD. Much of the evidence on the prevention of diseases by polyphenols is derived from *in vitro* or animal experiments, which are often performed with doses much higher than those to which humans are exposed to through the diet. Biophenols can act as powerful antioxidants. The term biophenols provides a more comprehensive and chemically accurate expression to describe plant phenols. The prefix 'bio' demotes the biological origin as opposed to synthetic compound. Thus, 'biophenols' is an umbrella term that refers to all phenolic molecules derived from botanical origin [4].

Pomegranate (*Punicagranatum* L.) has been a medicinal food throughout history and interest in the potential health benefits of biophenol-rich pomegranate products has increased in recent years. Pomegranate provides a rich and varied source of biophenols. Current research indicates that the most therapeutically beneficial pomegranate constituents are the phenolic components ellagitannins (including punicalagins), ellagic acid derivatives and flavonoids and the polyunsaturated fatty acid, punicic acid [5]. *In vitro* testing has shown pomegranate juice and seed extracts to have 2-3 times the antioxidant capacity of either red wine or green tea [6]. Animal studies have shown PEs scavenge free radicals and decrease macrophage oxidative stress [7] and lipid peroxidation and studies conducted among elderly adults showed an increase in plasma antioxidant capacity [8] and increased function [9].

Promising results have been reported from human clinical trials due to pomegranates antioxidant, anti-diabetic, anti-hypolipidaemic, anti-carcinogenic and anti-inflammatory properties. These suggest the possible use of pomegranate juice and extracts as a therapy or adjunct for prevention and treatment of CVD, diabetes, and prostate cancer [5]. Numerous preclinical studies reveal that selected biophenols exhibit strong protective actions on many pathological conditions particularly those triggered by oxidative stress such as CVD and metabolic disorders [10-12].

Stowe [12] suggested that pomegranate juice may decrease systolic blood pressure, thus causing an overall positive effect on the progression of atherosclerosis and the ensuing potential development of coronary heart disease. Pomegranate juice has also been shown to reduce both systolic and diastolic blood pressure, fasting plasma insulin and insulin resistance calculated as HOMA-IR in a group of volunteers who were at high-risk of CVD [10]. Furthermore, the reduction of insulin resistance might have potential benefits in lowering the risk of developing type 2 diabetes mellitus, obesity and metabolic syndrome [10]. Chronic excessive activation of the Glucocorticoid receptor (GR) is known to induce obesity, insulin resistance, glucose intolerance and hypertension. Glucocorticoids have a direct effect on the heart and blood vessels mediated by GR and modified by local metabolism of 11β HSD enzymes which affect vascular function. Increased 11HSD-1 activity is implicated in the development of metabolic syndrome and identifying dietary constituents that influence 11HSD-1 activity could lead to novel methods of preventing CVD and associated risk factors. Tsang, Al-Dujaili., *et al*. [13] showed that consumption of pomegranate juice enhanced antioxidant status, reduced systolic blood pressure and waist circumference, with a reduction in the cortisol/cortisone ratio in both urine and saliva (possibly via inhibition of 11HSD-1 in the liver and adipose tissue).

A reduction in plasma NEFA in some participants was also found in a recent 4-week study (n = 28) investigating the effect of pomegranate juice in volunteers at high risk of CVD [13]. The role of NEFA appears to be important in human obesity. In particular, abdominal obesity is linked with increased NEFA levels, which are associated with an increase in blood pressure and resistance to suppression by insulin [14]. Furthermore, a pilot study including participants with type 2 diabetes with hyperlipidemia found concentrated pomegranate juice decreased cholesterol absorption, increased excretion of cholesterol, significantly reduced total and LDL cholesterol and improved total : HDL and LDL:HDL cholesterol ratios [15].

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Few studies, if any, have investigated the effect of pomegranate on healthy volunteers with normal blood pressure, their perceived quality of life, insulin resistance or salivary stress hormones which have a direct effect on the heart and blood vessels. Studies that have been conducted in this area have made use of pomegranate juice rather than extract [13]. PEs, which incorporate the major antioxidants found in pomegranates, have been developed as botanical dietary supplements to provide an alternative convenient form for consuming the biophenols found in pomegranate juice [16]. The extract provides a convenient alternative to juice and has little impact on energy intake. The aim of this exploratory study was to investigate the effect of PE intake on blood pressure, insulin resistance, stress hormone levels and quality of life in apparently healthy human volunteers.

Methods

Study design

Healthy volunteers aged 18-65 years, with a body mass index (BMI) between 18 and 35 were included in this parallel, randomized, double-blind, placebo-controlled study (refer to Figure 1). Participants were recruited by an email moderator advertisement at Queen Margaret University (QMU), Edinburgh, UK. Individuals with systemic disease, including heart disease and diabetes, allergic reactions, immunological conditions, or were pregnant or breastfeeding were excluded. All participants provided written informed consent and a lifestyle questionnaire to determine their eligibility. Participants were asked to complete a pre-intervention food frequency question-naire to examine the amount and type of polyphenolic compounds typically consumed. All participants were asked to maintain their usual diet and exercise regimes throughout the intervention. Following a 1-week run-in phase, eligible participants were randomly as-signed to receive either onePE (PE) (POMANOX®, Probeltebio) or placebo capsule daily, after a meal, for four weeks. Participants were asked to complete a 3-day food and alcohol record (completed for 2 weekdays and 1 weekend day) before the beginning of the interven-tion and for the same days in week 4. The study was conducted at QMU and the protocol was approved by QMU Research Ethics Commit-tee. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants were approved by the QMU Research Ethics Committee. The Clinical Trials.gov identifier was NCT02005939.

Supplements and placebo

The PE capsules and the placebo capsules appeared identical, each weighing 1.083g. The pomegranate capsule contained a 100% natural concentrated extract of the whole pomegranate (POMANOX®) and maltodextrin. The active ingredients, punicalagins, have beneficial powerful antioxidant and anti-inflammatory properties. Each capsule contained: 210 mg punicalagin (the recommended daily intake to provide the beneficial effects of these antioxidants), 328 mg other pomegranate polyphenols (e.g. flavonoids and ellagic acid) and 0.37 mg anthocyanins. The placebo capsule only contained maltodextrin to provide the same energy content as the PE capsules (6.52 kcal or27.28 kJ per capsule).

Pomegranate extract analysis

POMANOX® and placebo capsules were extracted in triplicate with 10 mL of 50% acetonitrile in ultrapure water containing 0.2% formic acid, vortex mixed to ensure dissolution and placed on a blood rotator at 45 rpm for 30 mins at 4ºC. After centrifugation (2780 X g, 5 mins, 5ºC), the supernatants were removed to fresh tubes and the extraction repeated on the pellet. The two extractions were com-bined (20 mL), mixed and 1 mL aliquots removed and dried in a centrifugal evaporator. The dried samples were re-dissolved in 500 µL of 5% acetonitrile in ultrapure water containing 0.1% formic acid.

For liquid chromatography mass spectrometry (LCMS), samples were analysed on an LCQ-Deca system, comprising Surveyor auto-sampler, pump and photodiode array detector (PDAD) and an ion-trap mass spectrometer (Thermo Fisher Scientific, Hemel Hempstead, UK). The PDAD scanned discrete channels at 280 nm, 365 nm and 520 nm. The samples were applied to a C18 column (Synergi Hydro C18 with polar end capping, 2 mm X 150 mm, Phenomenex Ltd.) and eluted using a gradient of 5% acetonitrile containing 0.1% formic acid to 40% acetonitrile containing 0.1% formic acid over 30 min at a rate of 200 µL/min. The LCQ-Deca LC-MS was fitted with an ESI (electrospray ionisation) interface and analysed the samples in positive and negative ion mode. All data shown is in negative mode. There

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were two scan events; full scan analysis followed by data-dependent MS/MS of the most intense ions using collision energies (source voltage) of 45%. The capillary temp was set at 250ºC, with sheath gas at 60 psi and auxiliary gas at 15 psi.

Measurements

All measurements were taken at baseline (week 0) and again on completion of the intervention period (week 4). Participants were asked to fast for 12 hours beforehand, except for water, and refrain from alcohol or extra physical activity on the previous day. The anthropometric measurements collected were body weight, measured on Salter scales; height is using a SECA Leicester stadiometer; and waist circumference using a steel tape (6 mm x 2 mm). Body mass index (BMI) was calculated using the following standard equation: BMI (Kg/m^2) = (weight Kg/ (height m)^2). Fat-mass and fat-free mass were measured using a Bodystat 1500 (2002) machine. The National Health and Nutrition Examination Survey (NHANES) handbook protocols and methods [17] were followed. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse rate were recorded on an A and D Medical UA-767 Plus Digital Blood Pressure Monitor (2005). Three readings of BP were taken while participants were seated at each visit and the mean was calculated.

Three fasting venous blood samples were taken from each participant and drawn into glucose, heparin and EDTA tubes, which were centrifuged at 3100 rpm for 10 minutes to extract plasma and then stored in a Sanyo commercial freezer at -75 to -85°C until analysis was conducted. Blood analysis was conducted at the Queen's Medical Research Institute (QMRI) at the Royal Infirmary of Edinburgh on a CobasFara automated chemistry analyser with Wako reagents. Tests were conducted for glucose, total cholesterol, HDL cholesterol, triglycerides, uric acid and LDL by equation (all mmol/L), and NEFA (mol/l). Insulin (mIU/L) was tested at QMU using the GenWay-EASIA immunoenzymetric assay kit. Calculations based on blood sample values included LDL cholesterol (mmol/l) via the Friedwald equation [18] and total cholesterol to HDL ratio. Insulin resistance was calculated by homeostasis assessment model (HOMA-IR) and calculated from fasting insulin and glucose concentration according to the formula: fasting insulin (μIU/mL) x fasting glucose (mmole/L)/22.5 [19].

Saliva samples were collected pre- and post-intervention at three time points during a single day (morning, noon and afternoon). Cortisol and cortisone levels in saliva samples were estimated by using highly specific and sensitive ELISA methods developed by Al-Dujaili., *et al*. [20,21] and 24-hour urine collections were made pre- and post intervention. Urine was tested for total phenolics (TP) by the Folin and Ciocalteau method using protocols developed by Singleton and Rossi (1965) [22]. Ferric-reducing antioxidant power (FRAP) capacity was also investigated. The FRAP assay was carried out according to the procedure of Benzie and Strain (1996) [23].

Self-rated health-related quality of life (HRQoL) was assessed at baseline and at 4 weeks via the Rand 36-item general health survey (RAND-36) [24]. This questionnaire covers HRQoL across both mental and physical domains. The 36-item questionnaire assesses eight health concepts: physical functioning, role limitations caused by physical health problems, role limitations caused by emotional problems, social functioning, emotional well-being, energy/fatigue, pain and general health perceptions as well as two summary scores: physical and mental health. Scores for the eight scales were calculated according to the summative method of calculating the mean of the items for each scale. Scores for the 8 subscales range from 0 to 100, where a higher score reflects a more positive health state.

Statistics and analysis

All statistical analyses were performed using SPSS for Windows, version 19 (SPSS Inc. and IBM 2010). P ≤ 0.05 was considered significant. All data were expressed as mean values and standard deviations unless otherwise stated. Differences in baseline characteristics were examined using independent *t* tests with PE and placebo groups as the independent variables and age, gender, SBP, DBP, pulse rate, waist circumference, upper arm circumference, BMI, fat mass, fat free mass, body weight, fasting insulin, glucose, uric acid, lipid profile, NEFA, salivary cortisol and cortisone, and antioxidant capacity (total polyphenols, TBARS and FRAP) as the dependent variables.

Paired t-tests were conducted to determine differences between the pre- and post-intervention biochemical markers and anthropometric measurements above. Relationships between the variables were assessed using Pearson's correlation co-efficient. ANOVAs were

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conducted to determine whether there were any differences between the independent groups as there is more than one continuous dependent variable. The models included the main effects of treatment and post-hoc Bonferroni adjustment was used to account for multiple testing. P values of ≤ 0.05 were considered significant.

Results

Participants

A total of 31 volunteers were assessed for eligibility for inclusion to this study. Twenty-nine participants (7 males and 22 females) were included and completed the study (Figure 1). The intervention was conducted between April to June 2012. Participants were aged between 19 and 62 years with a BMI between 18.5 and 32.7 kg/m². Tables 1 and 2 show baseline characteristics of the participants randomised to the PE capsules and the placebo (PL) capsules groups. The two groups were comparable on entry into the study with respect to age, anthropometric measures, blood pressure, and plasma cholesterol and lipid profile.
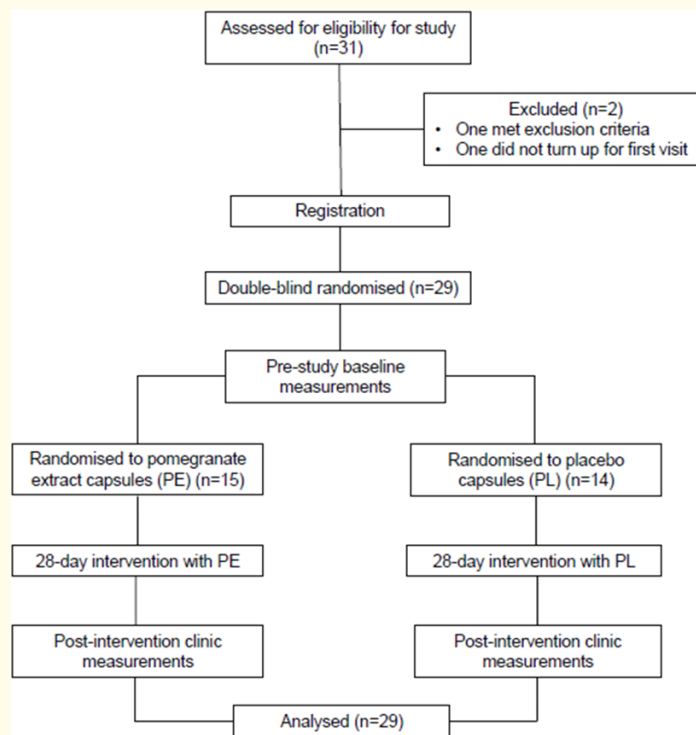

Figure 1: *Flow diadram of the enrolment, randomisation and follow up of study participants.*

Characterisation of *Pomanox* extract

LCMS analysis (See Figure 2 and Table1) confirmed that the study capsules were enriched in punicalagins as suggested by the manufacturers. The peak areas of the two main punicalagin peaks (α and β isomers) amount to ∼ 40% of the total area of peaks defined as phenolic components, which confirms the manufacturer's specifications. The placebo capsules effectively contained no phenolics.
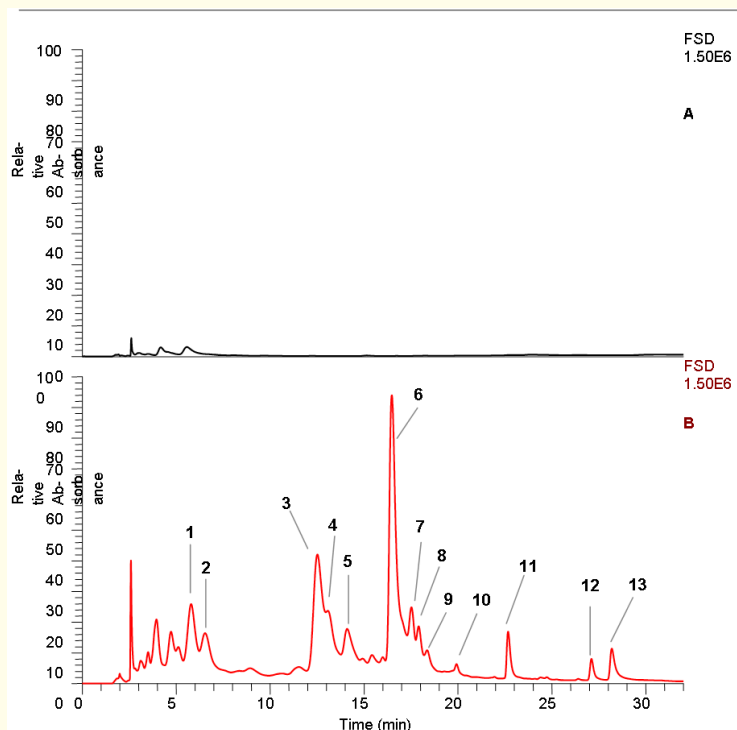
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Figure 2: *Phenolic components in pomegranate study capsules. UV traces at 280 nm, placebo and study capsules extracted as per methods. Full scale deflection (FSD) compared at 1.5e6 absorbance units to highlight the differences between the samples. A = placebo capsule and B = POMANOXcapsule.*

Peak	m/z [M-H]	MS^2	Identification
1	1415*	1113, 933, 783, 781, 633	Di (HHDP galloyl glucose) pentose
2	783	481, 301, 275	Pendunculagin isomer
3	1083	781, 721, 601, 575	α-Punicalagin
4	951	907, 783, 301	Granatin B isomer 1
5	951	907, 783, 301	Granatin B isomer 2
6	1083	781, 721, 601, 575	β-Punicalagin
7	1085	785, 631, 451	Digalloylgallagyl hexoside
8	935	633, 301	GalloyldiHHDP hexoside
9	1567	N/A	Sanguiin H10 isomer
10	633	463, 301	Galloyl HHDP hexoside
11	463	301, 275	EA hexoside
12	447	301	EA rhamnoside
13	301	301, 275	Ellagic acid (EA)

Table 1: *Putative Identifications of phenolic compounds based on liquid chromatography mass spectrometry (LCMS) data. *possible punicalagin isomer in this peak; underlined = major MS^2 fragments, N/A = MS^2 not available. HHDP = hexahydroxy-diphenoyl. unit*

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Effect on Anthropometric Measures

No significant changes in body weight or BMI were observed, however, waist circumference significantly decreased in both the PE group (p = 0.005) and placebo group (p = 0.004) after 4 weeks (Tables 2 and 3). No significant changes were seen in upper arm circumference in either group over the 4 week intervention. A slight decrease in fat mass and slight increase in fat-free mass was found in both groups after 4 weeks. A significant decrease in percentage fat mass (p = 0.038), and a significant increase in percentage fat-free mass (p = 0.038) was found in the placebo group at 4 weeks. In the PE group, the increase in fat-free mass was significant (p = 0.009), although the decrease in fat mass did not reach significance.

	Baseline (week 0)		Post intervention (week 4)		P value
	Mean	SD	Mean	SD	
Weight (kg)	70.07	13.70	70.39	13.78	0.233
BMI (kg/m^2)	25.33	4.45	25.41	4.50	0.455
Waist circumference (cm)	0.82	0.13	0.81	0.13	0.005
Upper arm circumference (cm)	0.31	0.40	0.31	0.40	0.172
Fat-mass (kg)	20.8	8.2	20.49	7.80	0.348
Fat-mass (%)	29.4	8.50	28.89	8.10	0.157
Fat-free mass (kg)	49.3	10.2	49.89	10.55	0.009
Fat-free mass (%)	70.62	8.50	71.11	8.10	0.157
Systolic Blood Pressure (mmHg)	120.33	13.26	115.58	13.05	0.012
Diastolic Blood Pressure (mmHg)	80.04	10.49	78.31	7.95	0.196
Plasma glucose (mmol/l)	5.37	0.51	5.31	0.49	0.688
Plasma insulin (mIU/l)	8.24	10.34	6.79	8.06	0.197
Total cholesterol (mmol/l)	4.45	0.73	4.49	0.77	0.654
HDL cholesterol (mmol/l)	1.72	0.29	1.65	0.30	0.085
LDL cholesterol (mmol/l)	2.34	0.72	2.34	0.77	0.940
Triglycerides (mmol/l)	0.90	0.43	1.09	0.60	0.126
HOMA-IR	2.22	2.62	1.61	1.88	0.045
Uric acid (mmol/l)	0.278	0.13	0.268	0.13	0.525
NEFA (mmol/l)	0.516	0.21	0.395	0.11	0.057

Table 2: Pomegranate extracts (PE) consumption. Effect on anthropometric, blood pressure, plasma cholesterol and lipid profile at baselines after 4 weeks. TC: Total cholesterol; HOMA-IR: Homeostasis model assessment of insulin resistance.

	Baseline (week 0)		Post intervention (week 4)		P value
	Mean	SD	Mean	SD	
Weight (kg)	72.01	12.05	71.79	12.29	0.616
BMI (kg/m^2)	24.77	3.43	24.64	3.54	0.359
Waist circumference (cm)	0.816	0.09	0.798	0.10	0.004
Upper arm circumference (cm)	0.312	0.028	0.312	0.032	0.939
Fat-mass (kg)	20.69	7.18	19.96	7.34	0.078
Fat-mass (%)	28.69	7.78	27.69	7.63	0.038
Fat-free mass (kg)	51.32	10.85	51.84	10.50	0.133
Fat-free mass (%)	71.31	7.77	72.31	7.63	0.038

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Systolic Blood Pressure (mmHg)	111.55	11.86	112.26	7.95	0.798
Diastolic Blood Pressure (mmHg)	79.98	5.71	77.64	5.49	0.114
Plasma glucose (mmol/l)	5.08	0.37	5.09	0.32	0.841
Plasma insulin (mIU/l)	10.92	8.26	7.65	5.79	0.078
Total cholesterol (mmol/l)	4.33	0.68	4.33	0.81	0.979
HDL cholesterol (mmol/l)	1.58	0.29	1.58	0.25	0.922
LDL cholesterol (mmol/l)	2.28	0.52	2.32	0.53	0.782
Triglycerides (mmol/l)	1.02	0.35	0.95	0.66	0.584
HOMA-IR	2.44	1.79	1.70	1.26	0.072
Uric acid (mmol/l)	0.24	0.05	0.27	0.05	0.067
NEFA (mmol/l)	0.37	0.14	0.45	0.17	0.048

Table 3: *Placebo capsule consumption. Effect on anthropometric, blood pressure, plasma cholesterol and lipid profile at baseline and following 4 weeks. TC: Total cholesterol; HOMA-IR: Homeostasis model assessment of insulin resistance.*

Effect on Stress Hormones

PE intake caused a significant drop in salivary cortisol levels (Figure 3a and 3b: am; $39.5 \pm 19.6\%$, $p < 0.001$ and noon; $43 \pm 32.3\%$, $p = 0.016$). Salivary cortisol/cortisone ratio was also significantly reduced (am from 1.11 ± 0.51 to 0.55 ± 0.26, $p < 0.001$, noon from 1.57 ± 0.85 to 0.75 ± 0.72, $p < 0.001$ and pm from 1.22 ± 0.9 to 0.74 ± 0.59, $p = 0.011$), suggesting a reduction in 11B-HSD1 activity. There was a slight increase in the salivary cortisol and cortisol/cortisone ratio in those taking the placebo (Figure 4a and Figure 4b) but this was not statistically significant.
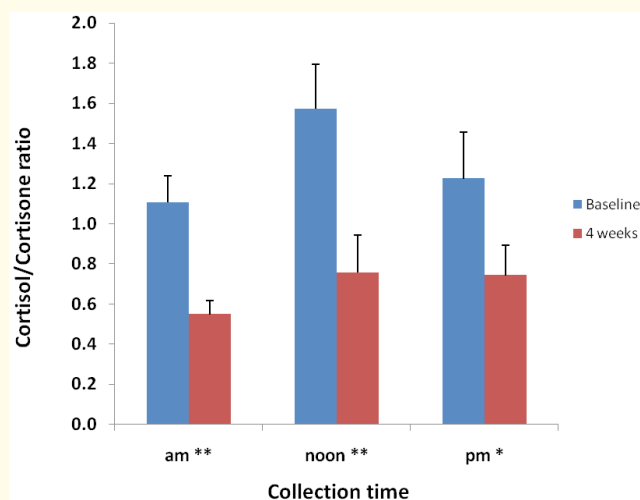

Figure 3a: *Effect of pomegranate extract on cortisol/cortisone ratio (mean and ± SEM).*
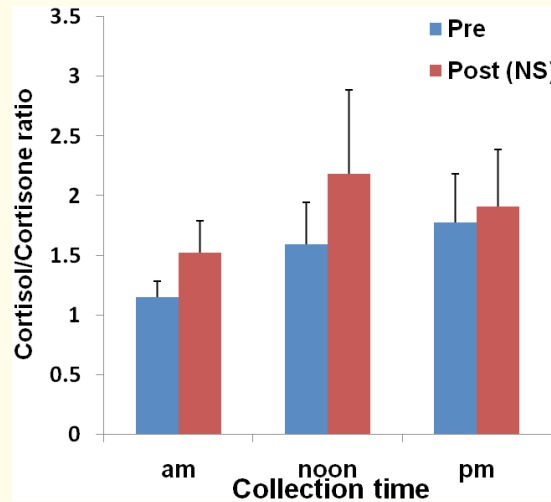***p = 0.001, * p = 0.01.*

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Figure 3b: *Effect of placebo on cortisol/cortisone ratio (mean and ± SEM).*
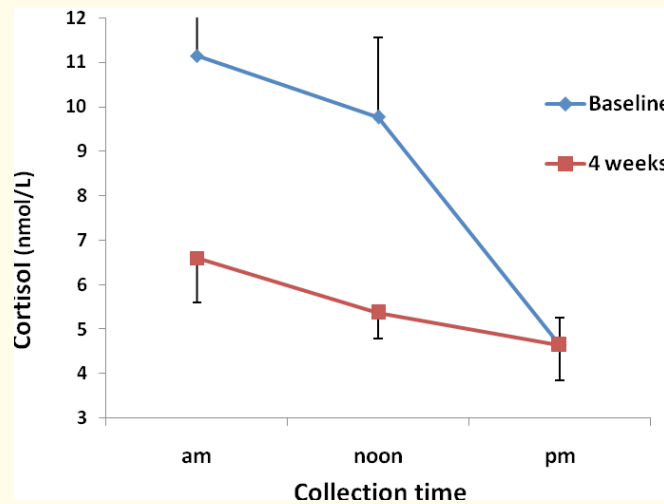
Figure 4a: *Effect of pomegranate extract on salivary cortisol (mean and ±SEM).*

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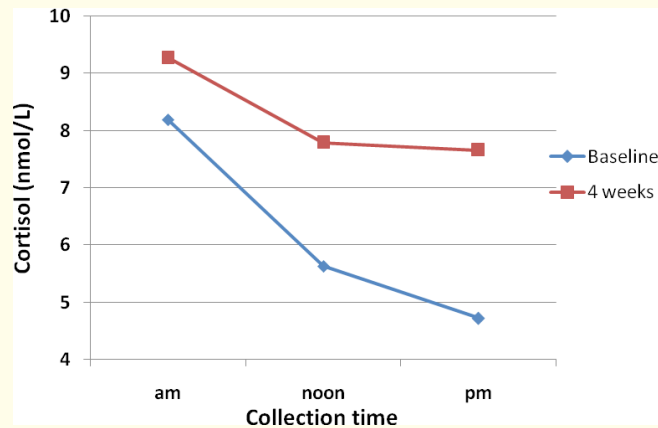
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Figure 4b: Effect of placebo on salivary cortisol (mean and ± SEM).

Effect on blood pressure, insulin and insulin resistance (HOMA-IR)

The PE group showed a significant decrease in SBP from 120.33 ± 13.26 mmHg to 115.58 ± 13.05 mmHg (p = 0.012) (Figure 5). Diastolic BP reduced from 80.04 ± 10.49 mmHg to 78.31 ± 7.95 mmHg, but this was not found to be significant (p = 0.196). There was a significant reduction in insulin resistance (HOMA-IR) from 2.22 ± 2.62 to 1.61 ± 1.88 (p = 0.045) (Table 3 and Figure 6) and although not significant, glucose, insulin and uric acid all decreased from baseline. No significant changes were recorded for the placebo arm of the study.
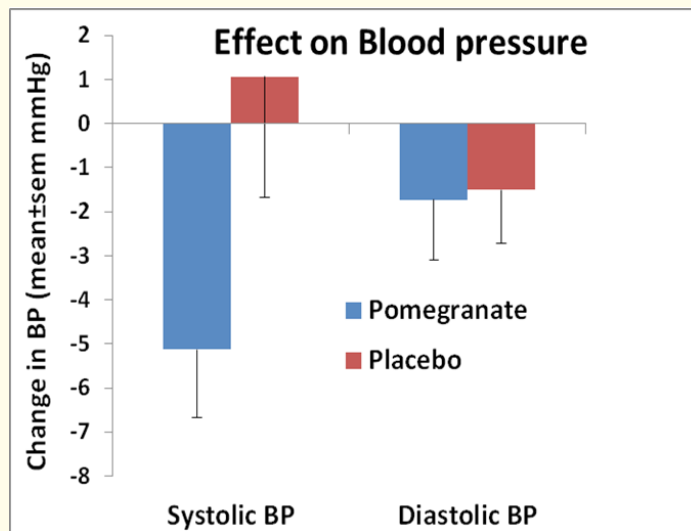

Figure 5: Effect of pomegranate capsule and placebo on systolic and diastolic blood pressure. There was a significant decrease (p = 0.012) in systolic blood pressure from pre-study to post-study in the PE group. Diastolic blood pressure decreased in both PE and placebo groups, but this did not reach significance.
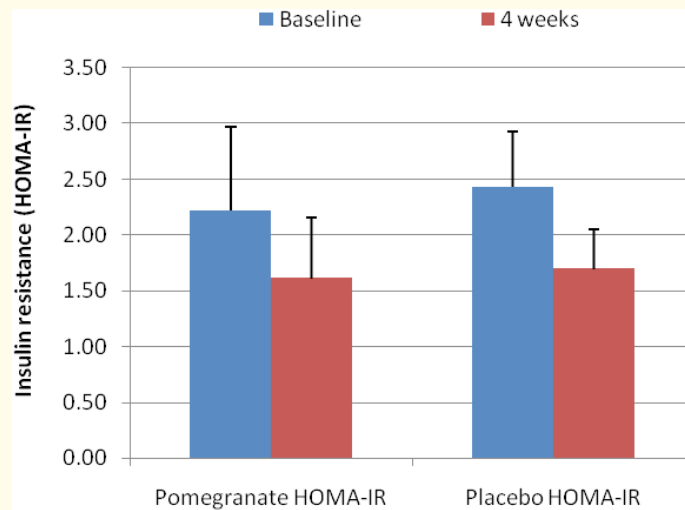
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Figure 6: *Effect of pomegranante capsules consumption on homeostasis model assessment of insulin-resistance (HOMA-IR). A significant reduction (p = 0.045) in insulin resistance (HOMA-IR) was observed in the PE group. A reduction was also observed in the placebo group, but this did not reach statistical significance (p = 0.072).*

Effect on blood lipids

No significant difference from baseline to 4 weeks was found in the placebo group with total, HDL and LDL cholesterol. In the PE group, total cholesterol and LDL cholesterol were similar at baseline and at 4 weeks and a slight decrease was found in HDL cholesterol from baseline to 28-days, although this was not statistically significant (p = 0.085). Triglycerides increased slightly in the PE group from baseline to 4 weeks, although this was not statistically significant (p = 0.126). In the placebo group a slight decrease in triglycerides was found over the same period, although again this was not significant (p = 0.584). A decrease in NEFA, although not significant, was seen in the PE group at 4 weeks. In contrast, a significant increase (p = 0.048) in NEFA was seen in the placebo group at 4 weeks.

Effect on health related quality of life

In the PE group, significant improvements were found in five of the quality of life parameters between baseline and 4 weeks: physical functioning (p = 0.018), social functioning (p = 0.021), pain (p = 0.003), general health (p = 0.008) and overall rand score (p = 0.007). Paired t-tests comparing scores at baseline and at 4 weeks in the placebo group found no significant differences except with emotional wellbeing, which was significantly improved (p = 0.016) (Table 4). Independent t-tests showed that there were no significant differences between the placebo and PE groups at baseline.

Discussion

The present study was conducted to investigate the cardio metabolic effects of consumption of PE, rich in biophenols, over a 4-week period in apparently healthy adults. Previous clinical studies have shown beneficial effects on CVD risk, for example, reduced SBP and improved insulin resistance [13]. These studies have mainly involved participants at elevated risk of CVD and have used pomegranate juice rather than extract. The present study suggests that PE consumption among healthy adults is beneficial in improving a variety of CVD risk factors.

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	Placebo			Pomegranate Extract (PE)		
	Basal	4 weeks	p-value	Basal	4 weeks	p-value
Physical functioning	94.29 (6.16)	96.07 (6.56)	0.292	92.33 (8.84)	98.00 (3.68)	0.018
Role limitations due to physical health	80.36 (36.92)	87.50 (32.15)	0.537	90.00 (28.03)	94.17 (14.07)	0.565
Role limitations due to emotional problems	76.19 (42.22)	88.10 (21.11)	0.239	70.01 (30.99)	77.23 (33.40)	0.216
Energy/Fatigue	58.57 (16.34)	58.57 (13.51)	1.000	53.33 (14.10)	60.00 (16.70)	0.131
Emotional well being	69.14 (16.93)	76.93 (10.72)	0.016	65.33 (13.91)	70.93 (14.46)	0.146
Social functioning	78.57 (23.22)	89.29 (12.84)	0.139	80.83 (14.07)	92.50 (12.32)	0.021
Pain	73.39 (23.24)	82.68 (15.30)	0.147	76.67 (16.92)	89.18 (8.57)	0.003
General health	71.43 (12.62)	73.21 (13.39)	0.572	64.33 (17.10)	71.00 (16.17)	0.008
Overall Rand	75.25 (14.61)	81.56 (7.93)	0.091	74.12 (10.70)	81.63 (11.08)	0.007

Table 4: Health related quality of life (RAND-36) scores (mean (SD)).
Paired t-test comparing scores pre-study and at 4 weeks in placebo and in PE groups.

We found that there was a slight increase in lean tissue from baseline to 4 weeks among participants in the PE group (p = 0.009). For the placebo capsules there was a significant decrease in the fat percentage (p = 0.038) from baseline to 4 weeks and a significant increase (p = 0.038) in percentage of lean tissue. This may reflect changes in participant behaviour (other than that prescribed by the trial protocol). This is known as the Hawthorn effect [25]. Previous clinical studies have found consumption of pomegranate juice to reduce SBP [26,27]. Our findings show a significant decrease in SBP from baseline to 4 weeks in the PE capsules groups, but no significant difference in the placebo capsule group. The mechanism by which PE consumption reduced SBP may relate to its ability to decrease angiotensin-converting-enzyme (ACE) activity (secondary to its antioxidant properties) or potentially due to a direct effect on serum ACE activity [26]. If PE consumption in healthy, normotensive volunteers reduces blood pressure, it may ameliorate recognised cardiovascular risk factors in overweight and obese populations. The concurrent ability to decrease insulin resistance could be of benefit to those who suffer from non-insulin dependent diabetes, metabolic syndrome or obesity.

The present study found a significant decrease in salivary cortisol levels in the PE group. A reduction in salivary cortisol levels may prove to be beneficial for people suffering from chronic stress. Cortisol is known to unfavourably influence BP and lipid profile as it can induce oxidative stress that has been linked to hyper insulinaemia and insulin resistance [28,29]. This might explain the findings that PE consumption has improved insulin resistance in our participants. Physiologically, cortisol is essential for the maintenance of blood glucose levels and its regulation. While it induces the liver to synthesise glucose by the process of gluconeogenesis if needed, it inhibits the effects of insulin. This action is absolutely vital to maintain ample amounts of glucose for the brain in times of increased activity as the latter relies primarily on glucose for fuel [30]. One of the main objectives of this study was to investigate whether the drop in blood pressure might be caused by inactivation of 11ß-HSD1 enzyme which converts cortisone to the active steroid, cortisol. Plasma cortisol is considered to be 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 inter conversion 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 [13]. This study has shown that PE intake has the potential to inhibit 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 previously reported, for example in the syndrome of apparent mineralocorticoid excess [31,32]. It has also been suggested that selective inhibitors of 11β-HSD1 may lower blood glucose and HOMA-IR in type 2 diabetic animal models [20].

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The findings showed a significant decrease in HOMA-IR in the PE group at 4 weeks. It has been found that insulin resistance is associated with increased cardiovascular risk [33]. Evidence from a number of studies has shown that impaired insulin action is accompanied by atherogenic lipid profile i.e. that is elevated plasma levels of triglycerides and LDL cholesterol and decreased HDL cholesterol [34]. The results of the present study do not support this, although a larger study with longer intervention period may find such an association. This study showed significant improvement in quality of life in the PE group from baseline to 4 weeks in overall RAND score and a variety of parameters indicating improvements in physical and mental health. The only significant improvement in HRQoL between baseline and 4 weeks in the placebo group could be due to the non-specific effects of trial participation [35].

The type of polyphenols in pomegranate extract and their combination might have impacted positively on CVD risk measurements. The potential for synergistic effects of combing various types of polyphenols in a supplement may be even more protective against CVD. This phenomenon has been reported in the Pomi-T trial for anti-cancer effect in prostate cancer patients [36]. All of the identified components in our study yield MS data that suggest that they are either ellagitannins or ellagic acid derivatives (see Table 1). Identifications are supported by data from Mena., *et al.* [37] and Borges., *et al.* [38].

Limitations and strengths of the study

The question of bioavailability of pomegranate polyphenols remains controversial. Some authors have concluded that phenolic compounds as those present in pomegranate are poorly absorbed and do not markedly contribute to antioxidant activity [37,38]. However, the majority of studies have shown demonstrable health benefits following the consumption of pomegranate juice or extract and attributed these to the biotransformation of pomegranate polyphenols [6,41-45]. There is also now good evidence that ellagitannin metabolites such as urolithins A, B and C and their glucuronides and sulphates are biologically active [46-48].

Data collection was by one investigator in one trial centre which assured a high quality of data collected and was likely to impact positively on the recruitment and retention of participants. The recruitment and retention rates for the study were excellent with one person assessed for eligibility meeting the exclusion criteria, and one person who did not attend the first visit. There were no withdrawals from the groups after the start of the intervention.

Conclusion

The primary aim of this study, which was to prepare the foundation of future trials, was to conduct initial testing of the effect of PE on CVD risk factors, stress and quality of life in healthy human volunteers. PE has the potential to provide a convenient, low cost and low-calorie intervention, compared to pomegranate juice, to benefit the population. The objective of this exploratory study was to recruit a large enough sample (approximately n = 30) to provide useful information about the aspects being assessed for feasibility and to identify any potential statistical trends [49]. This exploratory study has demonstrated that a larger double-blind, randomised, placebo-controlled trial investigating the effect of PE consumption on CVD risk factors, stress and quality of life in healthy human volunteers is justified.

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