Natural Pomegranate juice improves mood, wellbeing and enhances Salivary Testosterone levels in healthy volunteers

Emad A S Al-Dujaili* and Nacer F Smail

School of Health Sciences, Dietetics, Nutrition and Biological Sciences, Queen Margaret University, Edinburgh, UK

* Corresponding Author Present address: Cardiovascular Centre, Queens Medical Research Institute, Edinburgh University, Edinburgh, UK
Email: ealdujai@staffmail.ed.ac.uk
BACKGROUND

Throughout history, pomegranate symbolizes fertility and wellbeing, and has been postulated to enhance sexual drive. Pomegranate (*Punica granatum* L) provides a rich and varied source of polyphenols. Ellagitannins, tannins, anthocyanins, ellagic and gallic acids are the most abundant antioxidants found in the fruit. An animal study has shown that pomegranate juice intake increases testosterone level and sperm quality (Turk *et al*, 2008). Recently, we have shown that pomegranate juice consumption may alleviate stress and cardiovascular risk factors by reducing systolic and diastolic blood pressure and inhibiting 11β-HSD1 activity (Al-Dujaili, 2011).
AIM OF PROJECT
To investigate whether daily intake of natural pomegranate juice can influence mood and salivary Testosterone levels in healthy volunteers.
METHODS

A Placebo-controlled, repeated measure intervention trial in healthy subjects was conducted (figure 1) to assess whether consumption of pomegranate fruit juice (RJ Foods, UK: 100% fruit juice, 500mL/day) affects work-related stress levels as measured by a stress and mood questionnaire (simplified PANAS-X) and salivary Testosterone and Cortisol levels (using highly sensitive and specific ELISA methods). Participants consuming their normal diet were asked to provide three saliva samples (morning, noon and evening) on one random day during the run in-phase before the intervention and following one week and 2 weeks of pomegranate pure juice intake (figure 2). Age of subjects ranged from 21-64y, mean =39.1±13.8y, and overall BMI mean was 25.6± 4.5kg/m². The project was approved by QMU Ethical Committee. At each visit, 3 readings of Blood pressure and PWV (Vicorder™) were taken after participants had been at rest for approximately 10 minutes. As a control, 12 subjects (matched for age and BMI) were given sugared water of same amount of kcal in dark containers for 2 weeks.
Figure 1: Schematic diagram of study protocol design.

Subjects recruitment (n=60)

5-day run-in phase

Males (n=22)
- Anthropometric and BP measurements
- Pulse Wave Velocity assessment
- Saliva samples collection
- PANAS-X Stress and Mood questionnaire
- Two day diet diary

Females (n=38)

14 days Intervention
(500mL Pomegranate Pure juice per day)
Placebo=Water+Sugar

Repeat all above measurements
Statistics and Analysis of data
**Figure 2: Nutritional content per 100mL of natural Pomegranate Pure juice.**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Polyphenols</td>
<td>158mg</td>
</tr>
<tr>
<td>Energy</td>
<td>48kcal</td>
</tr>
<tr>
<td>Protein</td>
<td>0.3g</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>11.6g</td>
</tr>
<tr>
<td>Of which sugars</td>
<td>11.6g</td>
</tr>
<tr>
<td>Fat</td>
<td>Trace</td>
</tr>
<tr>
<td>Of which saturates</td>
<td>Trace</td>
</tr>
<tr>
<td>Fibre</td>
<td>Trace</td>
</tr>
<tr>
<td>Sodium</td>
<td>trace</td>
</tr>
</tbody>
</table>
RESULTS 1

There was a significant reduction in SBP, DBP and arterial elasticity (PWV) after 2 weeks of the juice consumption (figures 3-5). Table 1 shows that the average overall PANAS-X score increased from 73 to 77 (p=0.0008), positive score increased from 29 to 31 (p=0.017) and negative score decreased from 16 to 14 (p=0.029).

In addition, analysis of some individual attributes showed that (a) All subjects felt that they were more enthusiastic (p=0.025), inspired (p=0.0007), Proud (p=0.022) and Active (p=0.046), and (b) They felt less distressed (p=0.05), nervous (p=0.046), guilty (p=0.006) and ashamed (p=0.01).

Subjects who consumed the placebo juice did not show any significant changes in anthropometry, physiological, mood attributes and hormonal parameters.
Figure 3: Effect of Pomegranate juice intake on systolic blood pressure in all subjects ($p<0.0001$).

Figure 4: Effect of Pomegranate juice intake on diastolic blood pressure in all subjects ($p<0.001$).
<table>
<thead>
<tr>
<th>Code</th>
<th>Positive /50</th>
<th>Negative /50</th>
<th>Overall score (20-100)</th>
<th>Basal</th>
<th>Intervention</th>
<th>Overall score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Basal</td>
<td>Intervention</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>29</td>
<td>16</td>
<td>73</td>
<td>31</td>
<td>14</td>
<td>77</td>
</tr>
<tr>
<td>SD</td>
<td>6.130</td>
<td>6.484</td>
<td>8.952</td>
<td>7.732</td>
<td>4.359</td>
<td>8.597</td>
</tr>
<tr>
<td>Student Ttest overall score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P=0.0008</td>
</tr>
<tr>
<td>Student Ttest positives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P=0.017</td>
</tr>
<tr>
<td>Student Ttest negatives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P=0.029</td>
</tr>
</tbody>
</table>
RESULTS 2

Overall salivary Testosterone levels has significantly increased after Pomegranate pure juice consumption (p<0.001) at all time points tested (figure 6). However, circadian rhythm of the hormone secretion was maintained throughout the study. Salivary testosterone levels were increased in both males and females (figures 7 and 8). There was also a significant reduction in salivary levels of Cortisol following the juice consumption (p<0.001) at all times of the day (figure 9). The ratio of cortisol to cortisone at all time points was also significantly decreased (figure 10) suggesting that the juice intake might inhibit the activity of the enzyme 11βHSD1 which is responsible for the activation of the inactive hormone, Cortisone to the active form, Cortisol.
Figure 5: Effect of Pomegranate juice intake on pulse wave velocity (assessing arterial health) in all subjects (p=0.003).

Figure 6: Overall Salivary Testosterone (pmole) levels before, week1 and week2 of pomegranate juice intake in healthy volunteers. Significance after week 1 (am p<0.0001; noon, p=0.001; pm p<0.0001).
Figure 7: Male daily salivary Testosterone (pmole) levels before, week1 and week2 of pomegranate juice intake in healthy volunteers. Significance after week 1 (am, p<0.0001; noon, p=0.001; pm, <0.0001).

Figure 8: Female daily salivary Testosterone (pmole) levels before, week1 and week2 of pomegranate juice intake in healthy volunteers. Significance after week 1 (am, p<0.0001; noon, p=0.001; pm, <0.0001).
Figure 9: Comparison of daily salivary Cortisol levels before, one week and 2 weeks of pomegranate juice intake in healthy volunteers (mean ±SD). Significance after week 1.
Figure 10: Effect of pomegranate juice intake on the ratio of Cortisol to Cortisone, an indicator of 11 βHSD1 enzyme activity. Significance after week 1 (am p<0.0001; noon p=0.001; pm= 0.003).
The study shows that consumption of natural pomegranate pure juice for 2 weeks has a slight but significant increase in salivary testosterone in both males and females. Feeling of stress and nervousness was also reduced with improved wellbeing in a large group of workers from different disciplines.

These findings could be due to the fact that pomegranate pure juice intake causes a significant reduction in both systolic and diastolic blood pressure and salivary levels of the stress hormone, cortisol and the ratio of cortisol/cortisone (figures 9 and 10). There was also an improvement in arterial health as measured by pulse wave velocity.

In addition, the known effect of polyphenols-rich pomegranate juice in reducing oxidative stress by neutralising the damaging effects of free radicals might also have an important impact.
Discussion and Conclusion 2

The slight increase in testosterone may also explain the improvement in mood as also shown by PANAS-X score. We hypothesise that the small increase in salivary testosterone (mostly free fraction) might possibly be due to the inhibition of the enzyme UDP-glucuronosyltransferases (2B7, 2B15, and 2B17) by pomegranate flavonoids.

Conjugation of anabolic steroids during phase II metabolism such as glucuronidation is mainly driven by UDP-glucuronosyltransferase (UGT), which has been shown to be inhibited in vitro by a range of compounds including flavonoids (Jenkinson et al, 2013).

We suggest that future studies should investigate the effect of daily intake of pomegranate juice on large cohorts of Medical and AHP workers and try to elucidate possible mechanisms of pomegranate flavonoids action in modulating the levels of androgens and stress.
hormones.
References
Al-Dujaili, Nacer Smail (2011) Pomegranate juice consumption reduces salivary cortisol levels and improves mood and positive affects at work in healthy volunteers. 5th International Conference on Polyphenols and Health, Barcelona, 17-20 Oct. page 145.