ELSEVIER

Phytomedicine 11: 11–17, 2004
http://www.elsevier-deutschland.de/phymed

Phytomedicine

Flavonoid supplement improves leg health and reduces fluid retention in pre-menopausal women in a double-blind, placebo-controlled study

S. Christie[1], A. F. Walker[1], S. M. Hicks[1], and S. Abeyasekera[2]

[1] Hugh Sinclair Unit of Human Nutrition, The School of Biosciences, The University of Reading, Reading, UK
[2] School of Applied Statistics, The University of Reading, Reading, UK

Summary

Flavonoid extracts derived from plant foods have been shown to benefit certain types of fluid retention. However, no studies have investigated these compounds for use in premenstrual fluid retention, a complaint common among women with otherwise normal menstrual cycles. Therefore, we conducted a double-blind, placebo-controlled, pilot study into the effect of a daily flavonoid extract (Colladeen®, 320 mg oligomeric procyanidins) on premenstrual fluid retention. Fluid retention was assessed at baseline and throughout 4 menstrual cycles of the intervention using validated questionnaires. Leg girth was also measured at baseline and at the end of the study.
Thirty subjects completed the study (n = 18 active treatment; n = 12 placebo). Although no significant changes in leg girth measurements were noted, there was a significant improvement in subjective "leg health" scores after flavonoid treatment compared to placebo (p = 0.013). Furthermore, this was accompanied by an improvement in reported premenstrual fluid retention nearing significance (p = 0.066). We conclude that flavonoids supplements may provide a new therapeutic direction to counter premenstrual fluid retention and improve leg health. A larger study is now warranted.

Key words: flavonoids, fluid retention, leg health, premenstrual, grapeseed extract, dietary supplement

Introduction

Fluid retention, a common condition in women, is normally unaccompanied by evidence of cardiac, renal or hepatic disease. Various terms have been used to describe this benign form of fluid retention: idiopathic oedema, orthostatic oedema, cyclical oedema and fluid retention syndrome. Fluid retention of this type may present as a premenstrually-exacerbated occurrence (Abraham, 1981), or a non-cyclical event (Hechter et al. 1942). Sufferers frequently describe their legs as "heavy", "swollen" and "tight" on exertion (Anon, 1998). In addition, the abdomen, hands, breasts and area around the eye are frequently affected.

Several hypotheses have been postulated to explain the underlying aetiology of fluid retention (Christie et al. 2001). Research findings support the hypothesis that magnesium dysregulation may be involved in some subjects. Magnesium supplementation has been found to reduce symptoms of premenstrual fluid retention (Walker et al. 1998) as well as non-cyclical fluid retention (Barbeau et al. 1973). In the UK, the orthodox

0944-7113/04/11/01-011 $ 30.00/0

approach for the symptomatic relief of fluid retention is through the use of diuretics. Unfortunately, diuretic treatment with drugs is not without side effects (renal failure, gastrointestinal upset, cramps and decreased blood pressure have been reported: MIMS, 2002) and is frequently associated with poor clinical outcomes (MIMS, 2002).

Physicians in mainland Europe often prescribe flavonoid extracts for the relief of symptoms of fluid retention, following the dissemination of findings from German and Italian studies (Christie et al. 2001). Flavonoid extracts used in these studies are derived from grape seed *(Vitis vinifera)*, bilberry fruit *(Vaccinium myrtillus)* and cranberry fruit *(Vaccinium macrocarpon)*, and are standardised on their contents of oligomeric procyanidins (OPCs).

It has been suggested that flavonoids reduce symptoms of fluid retention through some or all of a number of mechanisms: (I) the protection and support of collagen-rich structures (Monboisse et al. 1984), (II) the reduction of metabolites known to increase vascular permeability (Kuhl et al. 1984), (III) the enhancement of intracellular vitamin C (Benstath et al. 1936; Gabe and Parrot, 1951; Rusenyak et al. 1961) and (IV) the protection of tissues from oxidative damage (Dewick, 1997).

Preclinical studies have shown flavonoid extracts to reduce experimentally-induced fluid retention and associated capillary permeability (Bonacina et al. 1973; Lietti et al. 1976; Lietti and Forni, 1976; Jonadet et al. 1983). Furthermore, clinical studies have shown a benefit of flavonoid extracts for certain microcirculatory disorders associated with oedema (reviewed in Christie et al. 2001), including tissue recovery following surgery. However, studies investigating the efficacy of these extracts to alleviate symptoms of premenstrual fluid retention are lacking. Therefore, the aim of this study was to investigate the effects of a commercially-available flavonoid extract on premenstrual fluid retention.

Materials and Methods

Subjects

Normally menstruating women suffering from fluid retention, were recruited from articles in women's magazines and national newspapers in the UK, following a press release. Women already using medication for fluid retention, (including flavonoid extracts), were excluded from the study. Further exclusion criteria were heart, kidney or liver disease, and malignancy. Written informed consent was obtained from each volunteer, and each volunteer's GP was notified of their patient's participation in the study. The study was started in the autumn and proceeded through the winter. It was allowed by the Ethics and Research Committee of The University of Reading and the West Berkshire Local Research and Ethics Committee.

Study design

The duration of the study was five menstrual cycles. The first cycle was used to collect baseline data, and treatment was given over the remaining four cycles. Prior to the study, subjects were randomised to receive a daily supplement of either a flavonoid extract containing 320 mg of OPCs (Colladeen®, manufactured by Lamberts Healthcare Limited, Century Place, Lamberts Road, Tunbridge Wells, Kent, TN2 3EQ, UK) or a physically-matched placebo. A randomisation list for two treatments was created using random number tables according to the method described by Pocock (1983). Block randomisation was used to obtain balanced groups with women of BMI (Body Mass Index) > or < 30. Numbered pill boxes containing the supplements were assigned consecutively to participants. Colladeen® capsules provided 80 mg of oligomeric procyanidins derived from a mixture of Grape seed (*Vitis vinifera; 66.4 mg OPCs; 85% OPC extract)*, Cranberry fruit *(Vaccinium macrocarpon; 12.6 mg OPCs; 16.4% OPC extract)* and Bilberry fruit *(Vaccinium myrtillus; 0.93 mg OPCs; 25% OPC extract)*. OPCs were determined according to the method of Gabetta et al. (2000). Dimeric OPCs were determined by HPLC thermospray mass spectrometry. Higher weight OPCs were determined by fractionation over Sephadex® LH-20 resin and analysis of the isolated fractions by gel permeation chromatography and electrospray mass spectroscopy. The placebo capsules contained equal parts (w/w) of micro-crystalline cellulose and calcium phosphate. Subjects were instructed to take 4 capsules per day before meals (either Colladeen® or placebo), 2 capsules in the morning and 2 in the evening. To maintain the double-blind nature of the study, treatment allocation was known only to the project director (AFW) who had no direct contact with the volunteers.

BMI and typical dietary nutrient intakes were assessed at baseline and the end of the study in order to record any changes or differences between groups which might influence outcome. Dietary intake was estimated using a validated 3-day diet diary, and analysed using CompEat (Lifeline, Nutritional Services, Pond House, 21 Craven Hill, London, W2 3EN). Apart from energy and alcohol intake, particular attention was paid to the intakes of nutrients that may play a role in the pathogenesis of fluid retention: sodium, calcium, magnesium, potassium, vitamin B6 and vitamin C.

All physical measurements were made during clinical visits at baseline, and during the last cycle of the study. These appointments were scheduled for the premenstrual phase of each cycle. Tolerance to supplementation was assessed in a post-study questionnaire.

Outcomes

Subjects prospectively recorded their symptoms throughout the study in a menstrual diary (MD). The MD was made up of items from two questionnaires; (1) a 19-item "premenstrual symptoms" questionnaire modified from Moos (1968) and used previously by our group (De Souza et al. 2000), and (2) a 7-item "leg health" questionnaire (Lagrue et al. 1993). Each symptom in the diary was scored on a 5-point scale (0 – very mild; 1 – mild; 2 – moderate; 3 – severe and 4 – very severe). Scored symptoms were categorised into subgroups as shown in Table 1. For each individual, "premenstrual" scores of each symptom subgroup were calculated. These "premenstrual" scores were calculated as the mean of daily subgroup scores over the 7 days prior to menstruation. "Postmenstrual" scores were calculated similarly using scores from the 7 days following menstruation (days 8–14 of the cycle).

Leg girth was monitored as an objective measure of fluid retention, using the Leg-O-Meter (Breard et al. 1998), a specially-designed instrument allowing an accurate, reproducible measure of leg girth. Blood pressure was monitored as a secondary outcome using an Omron 703CP automatic BP monitor (Omron Terminals Ltd, Chessington, UK). At each clinical visit, three readings of systolic and diastolic blood pressure (BP) were taken at rest. For each set of three blood pressure readings the first value was discarded and the mean of the last two used for the analyses.

Statistical analysis

In the absence of relevant data from similar studies on which to base a power calculation, the number of volunteers was limited by practical considerations. A two-sample t-test was used to confirm lack of baseline differences in outcome data between the two treatment groups. Final cycle premenstrual subgroup scores were compared between the two treatment groups using Analysis of Covariance (ANCOVA), using comparable baseline scores as covariate. Mean differences were deemed statistically significant if $p < 0.05$. Symptom subgroups "leg health" and "PS-Hydration" comprised the primary outcomes of the study. Similar analyses were used for other outcome data. Statistical analyses were performed using MINITAB (Minitab Inc., 3081 Enterprise Drive, State College, PA 16801-3008, USA).

Results

Study population characteristics

Thirty-six women were recruited onto the study, and 30 completed the protocol (n = 18 Colladeen®; n = 12 placebo). Reasons for dropout were personal in all cases, and so none were attributed to treatment. Table 2 compares the personal characteristics of subjects completing the study. Approximately 50% of subjects reported that their fluid retention was a premenstrually exacerbated condition, and the vast majority had been suffering from the problem for longer than 2 years. The oral contraceptive was currently used by 17% of subjects in the flavonoid group and 33% in the placebo group.

Mean (± s.e.m.) baseline BMI was 27.5 (±1.2) in the flavonoid treatment group, and 26.7 (±1.6) in the placebo group. BMI increased significantly in both

Table 1. Symptom subgroups scored in the menstrual diary.

Symptom subgroup	Maximum daily score	Symptoms (number of symptoms per category)
PS-Anxiety	16	Nervous tension, mood swings, irritability, anxiety (4)
PS-Craving	24	Headaches, craving for sweets, increased appetite, heart pounding, fatigue, dizziness or faintness (6)
PS-Depression	20	Depression, forgetfulness, confusion, insomnia, violent feeling (5)
PS-Hydration	16	Weight gain, swelling of extremities, breast tenderness, abdominal bloating (4)
Leg health	28	Swelling, heaviness, lack of agility, pain, varicosity, cramps, itchiness (7)

Table 2. Mean (± s.e.m.) baseline characteristics of subject groups completing the study (n = 18 flavonoid; n = 12 placebo).

Characteristic	Treatment group	
	Flavonoid	Placebo
Age (yrs)	40.7 (1.4)	36.5 (2.1)
Height (m)	1.60 (0.02)	1.70 (0.03)
Weight (kg)	71.6 (3.6)	71.6 (4.5)
Body Mass Index (BMI) (kg/m^2)	27.5 (1.2)	26.7 (1.6)
Regular menstrual bleeding (%)	100	100
Reported premenstrual exacerbation of fluid retention (%)	50	42
Duration of fluid retention:		
(i) 1–2 years, (%)	(i) 0	(i) 8
(iii) More than 2 years, (%)	(iii) 100	(iii) 92
Current oral contraceptive pill-users, (%)	17	33

treatment groups over the course of the study (+2.2% for Colladeen®; +1.5% for placebo), although these changes were not significantly different between the treatments. Interestingly, mean daily total energy intake (±s.e.m.) in both flavonoid and placebo groups were lower at baseline (1768 ± 126 versus 1688 ± 103 kcal, respectively) than in the last cycle of the study (1899 ± 83 versus 1914 ± 101 kcal, respectively). The treatment groups showed no significant differences in their intake of nutrients at baseline (data not shown), except that the flavonoid treatment group had a significantly lower mean daily sodium intake compared to the placebo group (2088 ± 133 versus 2551 ± 93 mg, respectively) and a significantly higher daily mean alcohol intake (8.5 ± 2.4 versus 2.9 ± 1.1 g, respectively). However, these differences are unlikely to have impacted on outcome, as no differences between intakes were found between the two groups at the end of the study. Mean potassium intakes were lower than the Reference Nutrient Intake (RNI) of 3500 mg per day (DH, 1991) in both supplement groups at baseline (2835 ± 211 versus 2596 ± 157 mg, respectively) and at the end of the study (3039 ± 188 versus 3183 ± 181, respectively).

Clinical outcomes

Mean premenstrual symptom scores over the 5 cycles of the study are shown in Table 3. There were no significant differences between the treatment groups at baseline. However, after 4 cycles of treatment the "leg health" scores of subjects receiving the flavonoid supplement were significantly lower than those receiving placebo ($p = 0.013$). Also at this time, subjects who had received the flavonoids had lower "PS-Hydration" scores, and this difference only just missed statistical significance ($p = 0.066$). There were no other significant differences between the treatments in any of the other premenstrual subgroup scores.

Fig. 1a and b show premenstrual and postmenstrual "leg health" scores for the two treatment groups. From cycle 2 onwards the daily flavonoid supplementation appeared to specifically and accumulatively reduce premenstrual fluid retention, without a sustained effect on postmenstrual fluid retention. By contrast, reduction in premenstrual leg scores induced by placebo treatment were not sustained by the end of the study.

Leg girth measurements were not significantly different between the treatment groups at baseline (e.g. mean [±s.e.m.] left thigh girth at baseline: 60.0 [±1.39] flavonoid group and 60.9 [±2.08] placebo group), nor after treatment. Similarly, no significantly different values in diastolic or systolic blood pressure readings were seen between the treatment groups at baseline (e.g. mean [±s.e.m.] systolic blood pressure at baseline: 122.4 [±3.13] flavonoid group and 123.4 [±3.14] placebo group), nor after treatment.

The post-study questionnaire revealed a high degree of tolerance to the test materials administered. One of the volunteers from the flavonoid treatment group reported a change in bowel habit, one subject mild headache and a further individual reported increased urinary output. Two volunteers from the

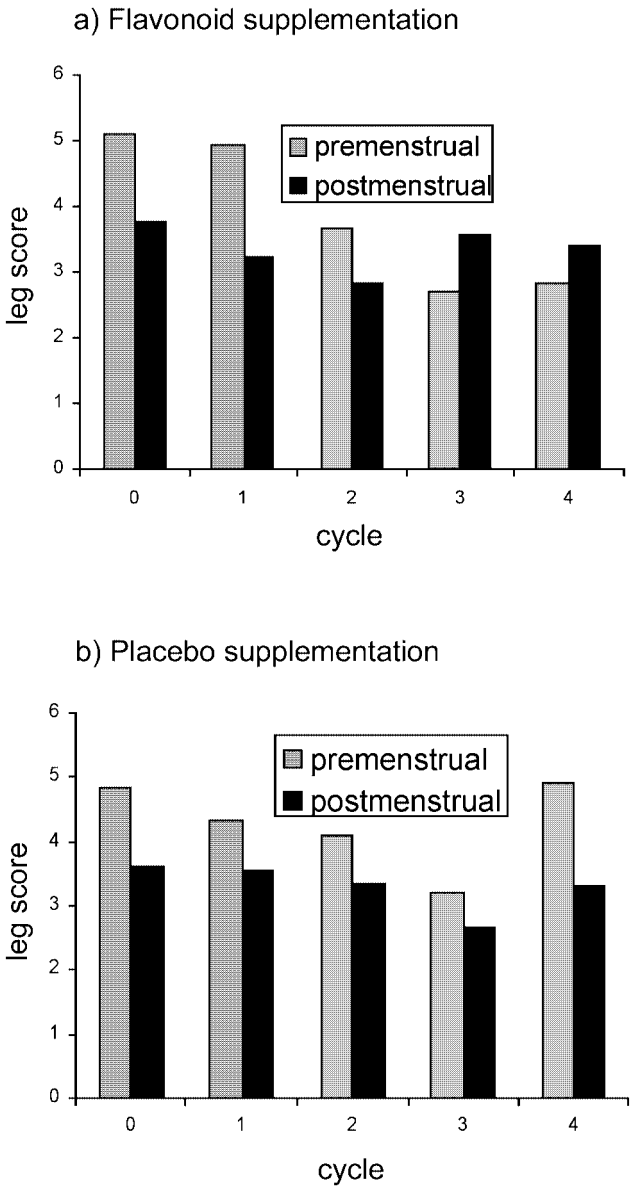

Fig. 1. Mean premenstrual and postpremenstrual leg scores at baseline (cycle 0) and after 4 months of a daily supplement of flavonoid extract or placebo. Premenstrual scores are an average of 7 days prior to menstruation and postmenstrual score are an average of days 8–14 of the cycle. Low leg scores indicate good leg health.

placebo group noticed mild gastrointestinal changes during the study. No serious side effects were recorded for either treatment.

Discussion

We conducted a randomised, placebo-controlled study to investigate the efficacy of a daily flavonoid supplement based on grapeseed extract for 4 months in the treatment of fluid retention. The results showed the flavonoid supplement to be superior to placebo for enhancing leg health and to hold promise for the alleviation of subjective symptoms of premenstrual fluid retention. To our knowledge this was the first such study examining the effects of a flavonoid extract for symptoms associated with premenstrual fluid retention.

Overall, subjects in the study exhibited a premenstrual exacerbation of symptoms of fluid retention (Fig. 1a and b), indicating that fluid retention was, at least in most of the women in the study, a cyclical phenomenon. Flavonoids were more effective in reducing premenstrually exacerbated fluid retention (Fig. 1a), than fluid retention at other times of the cycle. It has been suggested that all women are to some extent fluid retaining (Thorn, 1968), as there is a greater tendency for womens' compared with mens' ankles to swell in hot weather and on prolonged standing. Premenstrual fluid retention may represent an exaggeration of normal fluid-retaining mechanisms. The interesting observation from this study, that flavonoids specifically reduced premenstrual fluid retention, suggests that these compounds may help to normalise premenstrual fluid balance through their influence on these mechanisms, rather than acting as a diuretic via renal stimulation.

Subjects in this study had low intakes of potassium compared with the RNI (DH 1991). As fruit and vegetables are the main source of potassium in the diet,

Table 3. Mean premenstrual scores (±s.e.m.) before and at the end of 4 months of daily flavonoid or placebo supplementation. (Premenstrual scores are means of daily scores over 7 days).

Months of supplementation	Symptom Sub-group	Maximum daily score	Flavonoid treatment $n = 18$		Placebo treatment $n = 12$	
			Score	% max. score	Score	% max. score
0 (baseline)	PS –Anxiety	16	2.27 (0.57)	14.2	1.32 (0.59)	8.2
	PS – Craving	24	2.53 (0.44)	10.5	2.32 (0.63)	9.6
	PS – Hydration	16	2.97 (0.55)	18.6	3.30 (0.85)	20.6
	PS – Depression	20	0.98 (0.27)	4.9	1.09 (0.49)	5.4
	Leg score	28	5.09 (0.57)	18.2	4.82 (0.81)	17.2
1	PS –Anxiety	16	2.21 (0.58)	13.8	1.31 (0.59)	8.2
	PS – Craving	24	2.10 (0.56)	8.8	1.58 (0.79)	6.6
	PS – Hydration	16	2.69 (0.57)	16.8	2.88 (0.98)	18.0
	PS – Depression	20	1.37 (0.60)	6.8	1.05 (0.53)	5.2
	Leg score	28	4.94 (0.80)	17.6	4.33 (0.87)	15.5
2	PS –Anxiety	16	1.83 (0.49)	11.4	1.41 (1.00)	8.8
	PS – Craving	24	2.03 (0.54)	8.4	2.17 (0.96)	9.0
	PS – Hydration	16	2.18 (0.51)	13.6	3.36 (1.25)	21.0
	PS – Depression	20	1.01 (0.37)	5.0	1.14 (0.92)	5.7
	Leg score	28	3.66 (0.61)	13.7	4.08 (1.09)	14.6
3	PS –Anxiety	16	1.56 (0.45)	9.8	1.52 (0.70)	9.5
	PS – Craving	24	1.83 (0.39)	7.6	1.60 (0.70)	6.6
	PS – Hydration	16	1.91 (0.39)	11.9	3.06 (0.79)	19.1
	PS – Depression	20	0.96 (0.25)	4.8	0.76 (0.47)	3.8
	Leg score	28	2.69 (0.38)	9.6	3.20 (0.91)	11.4
4	PS –Anxiety	16	1.50 (0.37)	9.4	1.54 (0.72)	9.6
	PS – Craving	24	1.50 (0.32)	6.2	1.52 (0.78)	6.3
	PS – Hydration	16	1.69 (0.39) ◆	10.6	3.16 (0.96)	19.8
	PS – Depression	20	0.65 (0.23)	3.2	0.99 (0.61)	5.0
	Leg score	28	2.83 (0.47) Ψ	10.1	4.90 (1.31)	17.5

Ψ, significant difference between treatments; $p = 0.013$
◆, difference between treatments; $p = 0.066$

this finding suggests that the women had low intakes of fruit and vegetables and were, hence, unlikely to have met the recommended intake of 5 portions of fruit and vegetables per day. If this were so, they would also have had low intakes of flavonoids, which are mostly confined to this food group. Therefore, it might be that flavonoid supplementation is useful for normalising premenstrually exacerbated fluid retention in women whose intakes of fruit and vegetables are low.

The objective measures of leg girth showed no differences between flavonoid and placebo supplementation at the end of the study. Hence, these observations did not confirm the findings of a benefit in symptoms discussed above. The reason for this may be due to a lack of correlation between subjective complaints of fluid retention and objective signs of fluid retention, a phenomenon which has been observed previously (Denning et al. 1990). Furthermore there may have been flaws in the study protocol regarding the timing of leg girth measurement, which we were unaware of when the study was planned. A specific sign of fluid retention in women is a diurnal weight variation (Thorn, 1968), due to fluid accumulation during the day, as subjects adopt an upright posture. At night, when subjects adopt a recumbent position, enhanced diuresis ensues, with a return to normal weight by morning. In this present study, all leg measurements were taken in the morning rather than twice per day, so changes that may have occurred later in the day would not have been detected.

Although BMI increased in both treatment groups after 4 cycles of treatment, together with an increased energy intake, it is unlikely that this would have had substantial impact on outcome since both groups experienced the same effect. Our study commenced in the autumn and proceeded through the winter – a time when intake of food and body weight have been reported to increase (Shahar et al. 2001). The impact on outcome of the higher contraceptive use among the placebo group (33% versus 17% flavonoid group) is not possible to predict due to a lack of clear association between leg health and contraceptive use in the literature. However, fluid retention is a reported side-effect of the oral contraceptive pill (MIMS, 2002). Therefore it cannot be ruled out that the higher proportion of contraceptive pill users in the placebo group introduced some element of bias in our results. This is because the placebo group may have showed a diminished response to placebo due to the compounding effects of the oral contraceptive on fluid retention.

In conclusion, the findings of this randomised, double-blind pilot study, showed a beneficial effect on leg health and symptoms of premenstrual fluid retention of a daily supplementation of 320 mg of OPC flavonoids for 4 months compared with placebo. A larger-scale RCT is now warranted to confirm these findings.

Acknowledgements

Thanks are due to Lamberts Healthcare Ltd for the donation of the supplements and support of the post-graduate studies of SC. Thanks also to Helen Wolfenden and Elizabeth Head for technical assistance on clinic days, and to Julie Corke at Lamberts for administrative assistance.

References

Abraham GE (1981) Premenstrual Tension – Current Problems. Obstetrics and Gynaecology 3: 1–39

Anon (1998) Swollen ankles – Patient factsheet. General Practitioner. Haymarket Medical Ltd, UK, 1998

Barbeau A, Rojo-Ortega JM, Brecht NM (1973) Deficience en magnesium et dopamine cerebrale, cited in Durlach J. First Int. Symposium on Magnesium Deficit in Human Pathology. Paris Vittel F: 149–152

Bentsath A, Rusznyak ST, Szent-Gyorgyi A (1936) Vitamin nature of flavones. Nature 138: 798

Berard A, Kurz X, Zuccarelli F, Ducros J, Abenhaim L (1998) Reliability study of the Leg-O-Meter, an improved tape measure device, in patients with chronic venous insufficiency of the leg. Angiology 49: 169–173

Bonacina F, Galliani G, Pacchiano F (1973) Activity of anthocyanosides in acute inflammatory processes. Farmaco-Edprat 28: 428–434

Bonacina F, Pacchiano F (1974) Complementary activity of anthocyanosides in a preparation with anti-oedema and capillary-protective activity. Boll Chim Farm 113: 540–550

Christie S, Walker AF, Lewith GT (2001) Flavonoids – a new direction for the treatment of fluid retention? Phytother Res 15: 467–475

De Souza MC, Walker AF, Robinson PA, Bolland B (2000) A synergistic effect of a daily supplement for 1 month of 200 mg magnesium plus 50 mg vitamin B6 for the relief of anxiety-related premenstrual symptoms: a randomized, double-blind, crossover study. J Women's Health Gend Based Med 9: 131–139

Dewick PM (1997) Medicinal natural products – a biosynthetic approach. John Wiley and Sons, Chichester, UK

DH, Department of Health (1991) Dietary Reference Values for food energy and nutrients for the United Kingdom. Report on Health and Social Subjects No. 41. HMSO, London

Gabe M, Parrot JL (1951) Apport de l'histophysiologie à l'étude de la vitamine $C_{(2)}$ Prat Méd 59: 1740–1744

Gabetta B, Fuzzati N, Griffini A, Lolla E, Pace R, Ruffilli T, Peterlongo F (2000) Characterisation of proanthocyanidins from grape seeds. Fitoterapia 71: 162–175

Hechter OL, Krohn L, Harris J (1942) Effects of oestrogen and other steroids on capillary permeability. Endocrinology 30: 598–608

Jonadet M, Meunier MT, Bastide P (1983) Anthocyanosides from *Vitis vinifera*, *Vaccinium myrtillus* and *Pinus maritimus*. I. In vitro elastase inhibiting activities. II In vivo angioprotective activities. Pharm Belg 38: 41–46

Kuhl P, Shiloh R, Jha H, Murawski K, Zilliken F, (1984) 6,7,4′-trihydroxy isoflavan: a potent and selective inhibitor of 5-lipoxygenase in human and procine peripheral blood leukocytes. Prostaglandins 28: 783–804

Lagrue G, Behar A (1989) Oedematous syndromes caused by capillary hyperpermeability. Diffuse angioedema. J Mal Vasc 14: 231–235

Lietti A, Cristoni A, Picci M (1976) Studies on *Vaccinium myrtillus* anthocyanosides. Arzneim-Forsch 26: 829–832

Lietti A, Forni H (1976) Studies on *Vaccinium myrtillus* anthocyanosides II. Aspects of anthocyanidins pharmacokinetics in the rat. Arzneim-Forsch 26: 832–835

MIMS (1998) Monthly Index of Medical Specialities. Haymarket Medical Limited, UK

Monboisse JC, Braquet P, Borel P (1984) Oxygen-free radicals as mediators of collagen breakage. Agents and Actions 15: 49–50

Moos RH (1968) The development of a menstrual distress questionnaire. Psychosom Med 30: 853–67

Pocock SJ (1983) Clinical trials: a practical approach. John Wiley and Sons, Chichester, UK

Rusznyák I, Stark E, Fölfi Büki B (1961) Sull'azione della rutina e dell'acido ascorbico sulla resistenza dei capillari. Minerva Med 52: 2238

Shahar DR, Yerushalmi N, Lubin F, Froom P, Shahar A, Kristal-Boneh E (2001) Seasonal variations in dietary intake affect the consistency of dietary assessment. Eur J Epidemiol 17: 129–133

Thorn GW (1968) Approach to the patient with "idiopathic edema" or "periodic swelling". JAMA 206: 333–338

Walker AF, De Souza MC, Vickers MF, Abeyasekera S, Collins ML, Trinca LA (1998) Magnesium supplementation alleviates premenstrual symptoms of fluid retention. Journal of Women's Health 7: 1157–1165

Address

Ann F. Walker, Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, The University of Reading, Whiteknights, PO Box 226, Reading, RG6 6AP, UK
Tel.: 0118-931-8723; Fax: 0118-931-0800;
e-mail: a.f.walker@reading.ac.uk