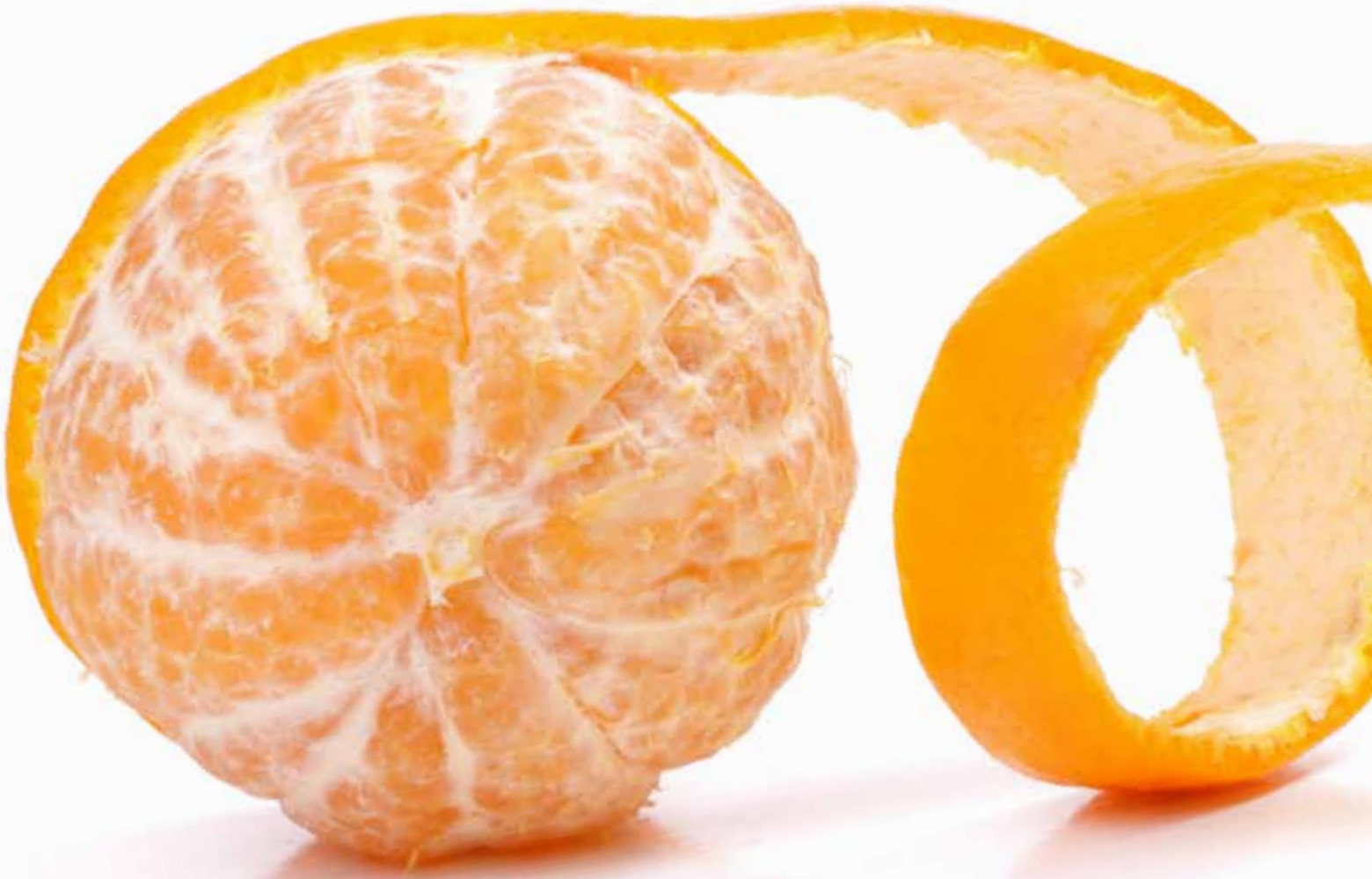


THE RISTOMED STUDY: GENDER DIFFERENCES IN RESPONSE TO DIETARY SUPPLEMENTATION

Patrizia d'Alessio, Rita Ostan, Luzia Valentini, Isabelle Bourdel-Marchasson, Alessandro Pinto, Fabio Buccolini, Claudio Franceschi, Marie C Bené

present results of a study examining the impact of diet on quality of life

> “Within the RISTOMED study, orange peel extract supplementation contributed to anti-inflammation and mood improvement, as documented by two different pre-clinical studies in rodents.”

PATRIZIA D’ALESSIO, University Paris Sud-11 and Biopark Cancer Campus 1, France; **RITA OSTAN,** Department of Experimental Pathology, University of Bologna, Italy; **LUZIA VALENTINI,** Charité—Universitätsmedizin Berlin, Section of Nutritional Medicine of the Department of Gastroenterology, Berlin, Germany; **ISABELLE BOURDEL-MARCHASSON,** CHU de Bordeaux, University of Bordeaux, France; **ALESSANDRO PINTO,** Sapienza University of Rome, Experimental Medicine Department—Medical Physiopathology, Food Science and Endocrinology Section—Food Science and Human Nutrition Research Unit, Italy; **FABIO BUCCOLINI** Voxnet, Rome Italy; **CLAUDIO FRANCESCHI,** Department of Experimental Pathology, University of Bologna, Italy; **MARIE C BENE.** CHU Nancy and University of Lorraine, France.

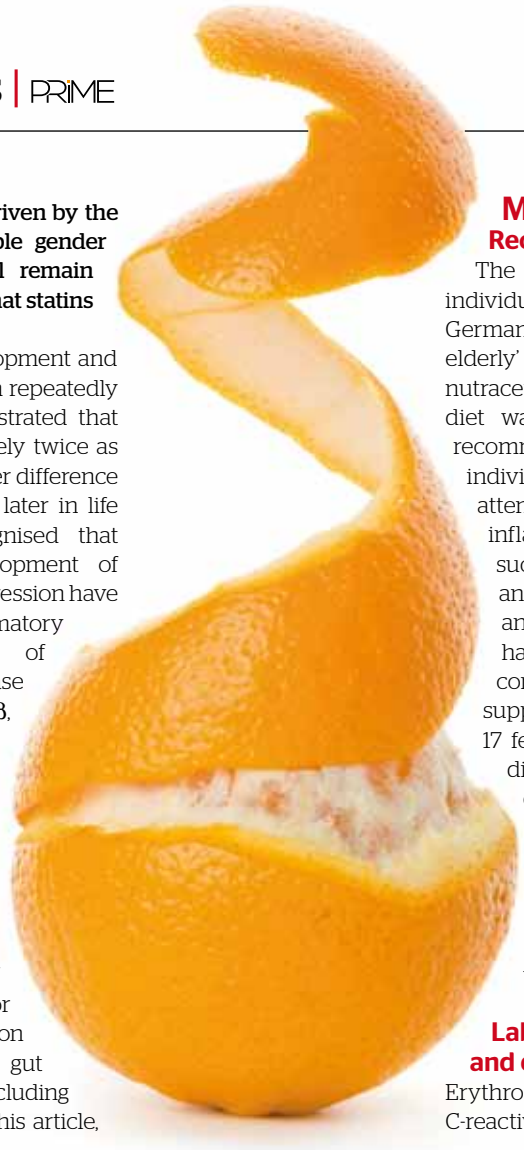
email: patriz.dalessio@gmail.com

ABSTRACT
Full title
Gender differences in response to dietary supplementation by orange peel extract in elderly people in the RISTOMED study: impact on quality of life and inflammation.

A number of health issues have shown to be questionable when considered from the perspective of gender differences. Quality of life parameters in particular, seem to be experienced differently by men and women. Depression—one of the hallmarks of the ageing population, particularly affecting women because of greater longevity—has a causal role in the development and course of cardiovascular diseases. Epidemiological data demonstrate that depression and anxiety are approximately twice as common in women as in men. This gender difference emerges during adolescence and persists later in life, and into old age. Furthermore, it is well recognised that inflammation contributes to the development of depression. In this article, the authors report a study performed in elderly healthy men and women, examining biological and behavioural responses after 56 days of a specifically developed dietary programme, with or without anti-inflammatory dietary supplementation, with an orange peel extract (OPE) containing d-Limonene; the European study RISTOMED. The authors show an impact of both gender and initial inflammation status on the effects of dietary control. OPE was more efficient in subjects with an initially high inflammatory status and improved both interleukin 6 (IL-6) levels and depression scores in women.

TWENTY-FIVE YEARS OF GENDER issue analysis have contributed to the reclassification of diseases. Among cardiovascular diseases, myocardial infarction is indeed the number one cause of death in women, but as its symptoms are different to those seen in men, it has not been recognised as such for a long time. Although age-specific stroke incidence is higher in men[1], women are generally more affected by strokes at an older age owing to their increased longevity. Consequently, strokes in women have been reported to be of a greater severity and to result in a worsened outcome[2]. Even if there are still conflicting data, these findings may be partly explained by the fact that women are often older and have a greater number of comorbidities at the time of stroke onset and diagnosis[2]. The effectiveness of primary prevention of stroke and other cardiovascular events with aspirin has been tested in a number of trials, but three of these did not include women[3]. Data seem to suggest that aspirin is able to prevent myocardial infarction in men and stroke in women. ▷

KEYWORDS
gender, quality of life, nutraceutical, depression, disease, orange peel extract



▷ **However, the findings in women were driven by the results of a single study, and the possible gender differences for aspirin effectiveness still remain unclear[3]. Furthermore, Dale et al[4] showed that statins reduce the risk of stroke, but only in men.**

A causal role of depression in the development and course of cardiovascular diseases has been repeatedly established[5]. Epidemiological data demonstrated that depression and/or anxiety are approximately twice as common in women as in men[6-8]. This gender difference emerges during adolescence and persists later in life and into old age[5,9,10]. It is well recognised that inflammation contributes to the development of depression[11]. In fact, patients with major depression have been found to have activated inflammatory pathways, including the elevation of pro-inflammatory cytokines and acute phase proteins, such as interleukin 6 (IL-6), IL-1β, tumour necrosis factor-α (TNF-α), and C-reactive protein[12].

The European study RISTOMED (www.ristomed.eu), providing a new e-service for a dietary approach to older patients, is a multi-centre 'open-label' randomised study (CORDIS FP7), using a personalised approach to provide e-health/telemedicine services[13]. The study focuses on the effect of a specific dietary programme alone, or associated nutraceutical interventions on inflammatory status, oxidative stress and gut microbiota in a healthy elderly population, including women and men in equal distribution. In this article, the authors relate the observed differences between women and men according to baseline inflammatory status for their response to the diet alone, and that associated with the supplementation of an orange peel extract (OPE) containing d-Limonene. OPE has known anti-inflammatory properties active on inflammatory markers, depression and anxiety[14-20].

> **"Patients with major depression have been found to have activated inflammatory pathways, including the elevation of pro-inflammatory cytokines and acute phase proteins such as IL-6, IL-1β, TNF-α, and C-reactive protein."**

Materials and methods

Recruitment, diet and supplementation

The RISTOMED study enrolled 125 healthy individuals from three different countries (Italy, France, Germany). They all received an 'optimal diet for the elderly' either supplemented or not with a range of nutraceutical compounds for a period of 56 days. The diet was developed on the basis of the current recommendations for elderly people and personalised individual dietary requirements, with particular attention given to food compounds that can affect inflammation, oxidative stress and gut microbiota, such as polyunsaturated fatty acids (PUFAs), antioxidant vitamins, polyphenols, flavonoids, and fibres. The diet was adapted to the dietary habits for each country. In this report, the authors compared the group receiving the diet without supplementation (14 males, mean age 69.6±4.1 years; 17 females, 71.3±3.8 years) to that which received diet supplemented with daily soft gel capsules containing OPE (14 males, mean age 70.6±4.4 years; 16 females, 69.6±3.3 years). OPE containing 95% d-Limonene purchased from Golgemma (Espéraza, France) was added to sunflower oil (Olvea) and were packaged in soft gel capsules for oral administration to patients.

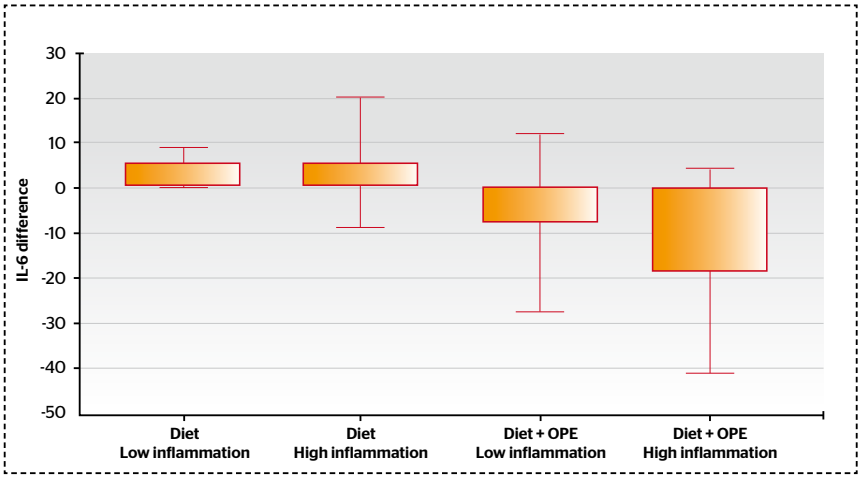
Laboratory measurements and questionnaires

Erythrocyte sedimentation rate (ESR), high-sensitivity C-reactive protein (CRP), white blood cell count (WBC) and fibrinogen were measured using standard haematology methods. Plasma levels of IL-6 and TNF-α were determined using multiplex sandwich-ELISA (SearchLight, Aushon Biosystem, Billerica, MA) according to the manufacturer's instructions.

An inflammation score was calculated at baseline using the values of ESR, CRP, WBC, fibrinogen, IL-6 and TNF-α. This enabled the separation of the patients into two groups of respectively low and high inflammation, so that inflammatory status could be evaluated according to the scores of these markers.

The SF-36v2 Health Survey was used to evaluate what each subject felt about his/her health using 36 items covering functional status, wellbeing, and an overall evaluation of health, that together are referred to as quality of life (QoL). Two summary scores – physical component summary (PCS) and mental component summary (MCS) – were calculated to distinguish an eventual physical dysfunction and bodily pain from psychological distress and emotional problems. The State-Trait Anxiety Inventory-X (STAI-X) questionnaire was used to assess the anxiety state and trait, and to describe each subject's feelings at a particular point. The Center for Epidemiologic Studies Depression ▷

Figure 1 Inflammation sub-groups and IL-6 variation in diet *versus* diet plus OPE



DEALER REQUIRED

AQUA *filling*

hydrophilic gel

LONG LASTING
LOW COST
DERMAL FILLER

Aquafilling is the cutting edge dermal filler for face and body plastic which exceeds women's and doctors' expectations. Aquafilling makes each woman irresistible in her most natural beauty. The amazing effect after the procedure lasts up to **36 months in face** and up to **5 years in body.**

Aquafilling has broken all stereotypes in plastic and reconstructive surgery. It is a non-invasive method for face and body plastic procedures. Thanks to its structure (98 % – physiologic solution of sodium chloride) the filler fully corresponds to the inner substance of human body providing highly safe and natural effect.

Learn more at **www.aquafilling.com**
Contact us: info@aquafilling.com

Visit our booth at 21st ISAPS Congress, Geneva 04-07.09.2012 and at EMAA, Paris 12-14.10.2012

biomatrix

ReFit

VIORA

Real Patients. Real Results. Reaction.

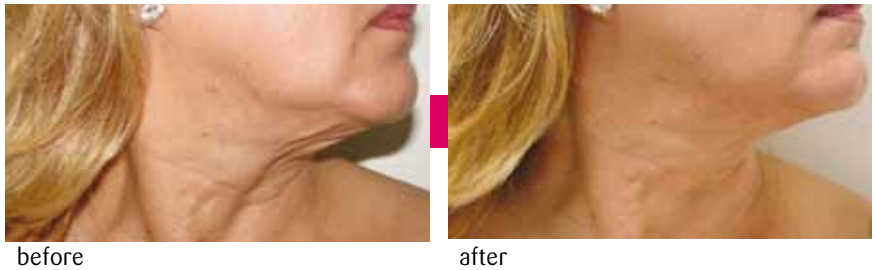
ReFit - the ultimate solution for loose skin after excessive weight loss combining tightening & contouring capabilities, available exclusively with the Reaction™ system!

Viora's unique, advanced CORE™ technology enables ReFit treatments to be performed no matter how weight loss has occurred: childbirth, diet, exercise, or surgical procedure.

- Safe
- Non-invasive
- No anesthesia needed
- No down-time
- Effective

before after

before after



before after

For more information: www.vioramed.com | info@vioramed.com

Aesthetic trends come and go
but your patients' needs remain the same:
Effectiveness · Safety · Fast Results!

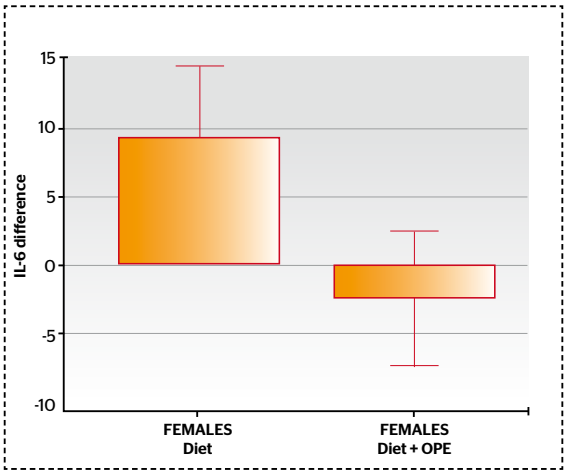


Figure 2 IL-6 variation in diet *versus* diet plus OPE in women

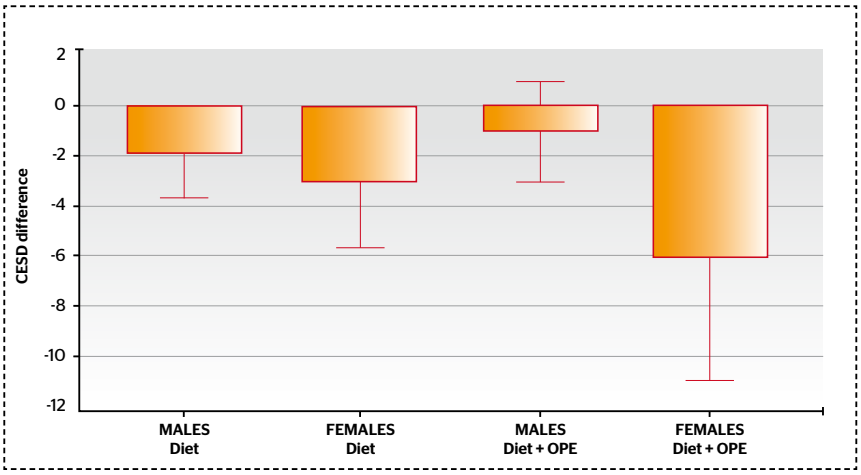


Figure 3 Gender partition and CES-D variation in diet *versus* diet plus OPE

▷ Scale (CES-D) questionnaire was used to assess how the subject felt and behaved during the previous week. For example, the CES-D scores the mood with a possible value of 0-60, with higher scores indicating more depressive symptoms, while scores between 0 and 15 indicate no depressive symptoms. All results were analysed comparing day 1 to day 56.

Statistical analysis

All data are presented as mean ± standard error of the mean (SEM). Statistical analyses were carried out using the Statview 5 statistical package (SAS Institute Inc., USA) and MedCalc Software (Mariakerke, Belgium). Data at day 1 and day 56 were compared using paired Student's t-Tests, ANOVA or Mann-Whitney U-tests. For all comparisons, differences were considered to be significant at the level of $P<0.05$.

Results

Biological parameters

When analysing the biological parameters of males and females it appeared that only ESR was significantly improved at day 56 in females receiving diet only ($P=0.02$) and in males receiving diet plus OPE ($P=0.03$). No difference was seen for CRP, fibrinogen, WBC or TNF-α.

> "When subjects were divided according to their inflammatory status, low or high, at baseline, no significant difference was seen in patients receiving diet only."

When subjects were divided according to their inflammatory status, low or high, at baseline (as described in the Materials and Methods section), no significant difference was seen in patients receiving diet only. A significant improvement was, however, observed in patients with high inflammatory status receiving diet plus OPE for ESR ($P=0.02$), WBC ($P=0.05$) and fibrinogen ($P=0.02$). Furthermore, IL-6 levels increased in patients with diet only, while it decreased in both inflammatory subgroups for patients supplemented with OPE (*Figure 1*). IL-6 levels also increased in females receiving diet only, while it decreased in those receiving OPE supplementation (*Figure 2*).

Effects on QoL and mood parameters

With regard to the CES-D scale, most individuals in the RISTOMED study were within the absence of symptoms range, with a mean value of 8.05±7.77. No statistically significant differences were observed between centres ($P=0.06$). A significantly higher score was observed at baseline in females (9.96±8.71) compared with males (5.84±5.86; $P=0.003$). Although scores improved (i.e. decreased) in both genders, at the end of the study they diverged (F=6.67±6.39 *vs* M=3.96±5.22; $P=0.012$). This gender-significant difference was confirmed only in the French sub-group before ($P=0.033$) and in the German sub-group after the diet period ($P=0.026$), but not in the other groups (data not shown). When the analysis was made, stratifying by both centre and gender, it was observed that the significant differences between day 1 and day 56 were confirmed in the German males ($P=0.002$) and French females ($P<0.001$), but not in the other groups (data not shown).

When comparing QoL and mood parameters with regard to gender status, it appeared that only SF 36-v2 MCS improved in males receiving diet only ($P=0.009$) or OPE ▷

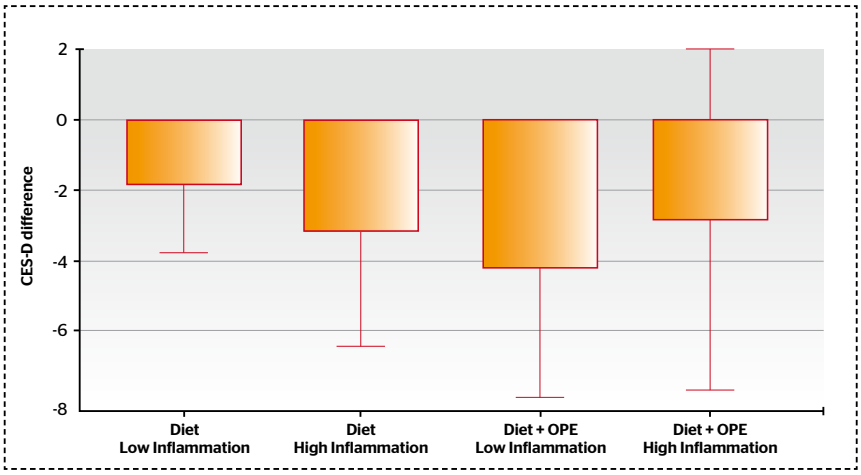


Figure 4 Inflammation subgroups and CES-D variation in diet *versus* diet plus OPE

▷ supplementation (*P*=0.02). CES-D improved globally in both genders with diet only (*P*=0.04 and 0.02) and in females supplemented with OPE (*P*=0.01). However, CES-D scores improved greater in females in both groups (*Figure 3*).

When considering the inflammatory status of the subjects, both SF-36v2 PCS and CES-D improved more in subjects with an initially high inflammatory status receiving diet only (*P*=0.03 and 0.04), while SF 36-v2 MCS improved in subjects supplemented with OPE (*P*=0.007). However, *Figure 4* shows that although both regimens improved CES-D whatever the inflammatory status, the best result was observed with OPE supplementation for subjects with low inflammation.

Discussion

Inflammation is a universal mechanism of defence harnessed by all living species. Stressful situations are known to be able to induce a low-grade inflammation status, independent of lifestyle. Ongoing stress, associated with systemic inflammation, enhances the propensity to develop disease, accelerating frailty, and thus disability. Furthermore, chronic inflammation is associated with depressive symptoms and mood alterations. OPE, containing d-Limonene, has been shown to display a consistent anti-inflammatory activity in animal models and human studies, when administered orally[14-18].

The RISTOMED study[13] revealed that globally, OPE supplementation to diet was more effective in highly inflamed *versus* less inflamed participants. Also, the RISTOMED sample shows a mean value indicating a normal mood without significant statistical differences between the centres. With regard to the possible biological basis sustaining this effect, it must be noted that peripheral IL-6 levels are mostly decreased by OPE treatment in highly inflamed participants.

In summary, diet could induce specific molecular effects when supplemented with specific food components. Within the RISTOMED study, OPE

> “Personalised medicine is opening new perspectives to gender and diversity issues. The study revealed some significant differences between women and men in their capacity to respond to an anti-inflammatory agent for its mood modulation capacity.”

supplementation contributed to anti-inflammation and mood improvement, as documented by two different pre-clinical studies in rodents[17, 18]. The present study shows that a natural anti-inflammatory compound taken orally also acts on mood. Indeed, it has been shown that d-Limonene displays anxiolytic-like effects in animal rodent models[21, 22]. For its design and its results, it is coherent with tailored approaches that are now considered crucial in the elaboration of pharmacologic strategies to face the treatment of interconnected inflammatory and degenerative diseases. Personalised medicine is opening new perspectives to gender and diversity issues. The study, which originally intended to be an equalised study between female and male distribution of participants, revealed some significant differences between women and men in their capacity to respond to an anti-inflammatory agent for its mood-modulation capacity[19, 20].

The year 2012 has been dedicated and promoted by the EU Commission to active ageing and intergenerational solidarity. In order to expand the experimental evidences drawn from the study – as well as giving the opportunity to the concepts, partners and teams involved to futher develop – one of the aims of the RISTOMED project is to promote tools for the realisation of 'beauty from within'. New projects have been elaborated by members of the consortium and calls for proposals will be submitted (e.g. ICT topics for healthy ageing and ambient assisted-living).

Key points

- 2012 has been dedicated and promoted by the EU Commission to active ageing and intergenerational solidarity
- The European study RISTOMED is a multi-centre 'open-label' randomised study using a personalised approach to provide telemedicine services
- A causal role of depression in the development and course of cardiovascular diseases has been repeatedly established
- Diet could induce specific molecular effects when supplemented with specific food components
- There is a need to evaluate the gender differences in future studies on inflammation, quality of life, mood and nutrition

Conclusions

Although the results are not statistically significant – as a result of the small sample size – it appears quite evident that the response to diet or diet supplemented with OPE, is affected by gender differences. In particular, the gender differences appear to affect inflammatory status, QoL and mood changes after an anti-inflammatory dietary intervention associated or not with the OPE supplement. This highlights the need to evaluate the gender differences in future studies on inflammation, QoL, mood and nutrition.

▶ ***Declaration of interest*** *none*

References

1. Michel P, Odier C, Rutgers M et al. The Acute STroke Registry and Analysis of Lausanne (ASTRAL): design and baseline analysis of an ischemic strokeregistry including acute multimodal imaging. Stroke 2010; 41(11): 2491–8
2. Katsiki N, Ntaios G, Vemmos K. Stroke, obesity and gender: a review of the literature. Maturitas 2011; 69(3): 239–43
3. Adelman EE, Lisabeth L, Brown DL. Gender differences in the primary prevention of stroke with aspirin. Womens Health (Lond Engl) 2011; 7(3): 341–52
4. Dale KM, Coleman CI, Shah SA, Patel AA, Kluger J, White CM. Impact of gender on statin efficacy. Curr Med Res Opin 2007; 23(3): 565–74
5. Steiner M. Serotonin, depression, and cardiovascular disease: sex-specific issues. Acta Physiol (Oxf) 2011; 203(1): 253–8
6. Nolen-Hoeksema S, Girgus JS. The emergence of gender differences in depression during adolescence. Psychol Bull 1994; 115(3): 424–43
7. Frackiewicz EJ, Sramek JJ, Cutler NR. Gender differences in depression and antidepressant pharmacokinetics and adverse events. Ann Pharmacother 2000; 34(1): 80–8
8. Solomon MB, Herman JP. Sex differences in psychopathology: of gonads, adrenals and mental illness. Physiol Behav 2009; 97(2): 250–8
9. Naninck EF, Lucassen PJ, Bakker J. Sex differences in adolescent depression: do sex hormones determine vulnerability? J Neuroendocrinol 2011; 23(5): 383–92
10. Streiner DL, Cairney J, Veldhuizen S. The epidemiology of psychological problems in the elderly. Can J Psychiatry 2006; 51(3): 185–91. Erratum in: Can J Psychiatry 2006; 51(6): 404
11. Lotrich FE, El-Gabalawy H, Guenther LC, Ware CF. The role of inflammation in the pathophysiology of depression: different treatments and their effects. J Rheumatol Suppl 2011; 88: 48–54
12. Howren MB, Lamkin DM, Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. Psychosom Med 2009; 71(2): 171–86
13. RISTOMED. European FP7 Capacities study n° 222230. www.ristomed.eu (accessed 11 June 2012)
14. D'Alessio P. Endothelium as pharmacological target. Curr Op Invest Drugs 2002; 2: 1720–4
15. D'Alessio P. Aging and the endothelium. Exp Gerontol 2004; 39(2): 165–71
16. D'Alessio P .Composition for prevention or treatment of cell degeneration using a molecule able to maintain the reversibility of expression of adhesion molecules and the polymerization of actin fibers. 2005. (PCT; WO 2005/105074)
17. Bisson JF, Menut C, d'Alessio P. Anti-inflammatory senescence actives 5203-L molecule to promote healthy aging and prolongation of lifespan. Rejuvenation Res 2008; 11(2): 399–407
18. D'Alessio P. Use of a monoterpene to increase tissue repair. 2009. (WO 2009/040420)
19. D'Alessio P. New anti-inflammatory molecule AISA 5203-L promotes adaptive strategies in cell aging. In: Wang P et al. eds, Adaptation Biology and Medicine. Narosa Publishing House Ltd 2011; 6: 369–78
20. D'Alessio P, Bennaceur A, Ostan R, Franceschi C. New targets for the identification of an anti-inflammatory anti-senescence activity. In: Nagata T. ed, Senescence. http://tinyurl.com/cpfn8o3 (accessed 11 June 2012)
21. D'Alessio P. Use of a monoterpene to treat or prevent stress. 2008. (WO 2008/138905)
22. de Almeida AAC, Pereira Costa J, de Carvalho RBF, de Sousa DP, de Freitas RM. Evaluation of acute toxicity of a natural compound (+)-limonene epoxide and its anxiolytic-like action. Brain Res 2012; 1448: 56–62