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**Abstracts and Poster**

Guest Editors

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## Reference:

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### Stability Evaluation to Formulate a Nutraceutical with Polyunsaturated Fatty Acids (PUFAs) and Oxidation-catalyzing Iron and Zinc

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**Introduction:** For 'mental and cognitive health performance' EFSA (European Food Safety Authority) accepts RDA (recommended daily allowances) of Fe (15 mg/d) and Zn (10 mg/d).  $\Omega$ -3 PUFA are essential for brain development and recommended upon deficiency, during pregnancy and early childhood. Equazen<sup>®</sup>, a marketed PUFA nutraceutical, demonstrated positive effects on working memory (learning skills) (1, 2). To check redox interactions of Fe, Zn and a vegetable antioxidant (AO) with PUFA a simple liquid formulation test was evaluated.

**Methods:** Tocopherol-stabilized PUFA triglycerides (DHA (C<sub>22:5</sub>):EPA(C<sub>20:5</sub>):GLA=9:3:1 [rel.conc.]) was assayed for peroxides [PV] and p-anisidine [AV] for primary and secondary oxidation products (Ph.Eur.). The oil-mineral suspension was filled in brown snap cap vials. Different Fe(II)salts, a Fe(III)-hydroxy-polymaltose complex (Maltofer<sup>®</sup>), inorganic and organic Zn salts in RDA were tested at 40°C and 75% rel. humidity over 4 weeks. Three samples per time point were centrifuged (10 min 4000 rpm) and the supernatant analyzed. A plant-derived AO, rich in phenols and rosmarinic acid, was checked for stabilizing. Saturated medium chain triglycerides (Miglyol<sup>®</sup>) served as blank.

**Results:** PV/AV [mean $\pm$ SD] at 40°C/75% humidity; \*exceeding Ph. Eur. limits.

Blank: 0/0

Equazen<sup>®</sup> (t=0): 1.0 $\pm$ 0.0/6.9 $\pm$ 0.9; (t=4 weeks): 38.7 $\pm$ 0.2\*/24.1 $\pm$ 2.5

Equazen<sup>®</sup>+Fe sulfate or fumarate or Maltofer<sup>®</sup> (t=4w): 18.2\*/143\* or 25.6\*/330\* or 27.5\*/150\*

Equazen<sup>®</sup>+Zn sulfate or Zn lactate (t=4weeks): 19.0\*/140.0\* or 11.0\*/31.3\*

Equazen<sup>®</sup>+Maltofer<sup>®</sup>+Zn lactate:

Without AO (t=0): 1.2 $\pm$ 0.0/2.1 $\pm$ 0.3; (t=4 weeks): 20.8 $\pm$ 0.1\*/49.8 $\pm$ 0.2\*

With AO (t=0): 0.0 $\pm$ 0.0/0.6 $\pm$ 0.1; (t=4 weeks): 16.3 $\pm$ 0.1\*/14.1 $\pm$ 0.4

#### Conclusions:

1. Ambient air and elevated temperatures oxidize liquid PUFA readily despite tocopherol content (Ph.Eur. limits for PV (<10) and/or AV (<30) exceeded within 4 weeks).

2. Transformation of peroxides into secondary products is not conc.-correlated, but catalyzed by: Fe(III)<Fe(II); Zn lactate<Zn sulfate. The AO significantly increased the stability (PV and AV within the limits after 4 weeks stress test).

3. The proposed oxidation test in a liquid formulation is sensitive, reliable, non-expensive and assists a rational evaluation of PUFA formulations with potentially interacting pro-oxidatives.

#### References:

1. Richardson AJ, Montgomery P: The Oxford Durham study: a randomized controlled trial of dietary supplementation with fatty acids in children with developmental coordination disorder. *Pediatrics*. 2005; 115 (5):1360-1366.
2. Portwood M.M.: The role of dietary fatty acids in children's behaviour and learning. *Nutrition and Health* 2006; 18: 219-232.

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### Natural Vegetarian Meal Replacement: Metabolic Modulation and Effects on Oxidative Stress

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Changes in lifestyle with short lunch breaks contributed to ever more frequently prescribed meal replacements in medical practice. GoJuvo<sup>®</sup>, a new commercial plant-based vegetarian meal, is composed by low glycemic index food. As very few data are available on GoJuvo metabolic effects, our aim was to verify if nutritional GoJuvo claims agreed with scientific tests by assessing nutritional parameters. Serum glucose, insulin and triglycerides concentrations were evaluated 3 times in 20 patients (4M/16F, mean BMI 31.4 kg/m<sup>2</sup>, aged 50.4 $\pm$ 11.5, enrolled at the Obesity and Work outpatients Clinic of the Milan Policlinico Hospital: T0 after overnight fasting; T1a 2 hours after GoJuvo administration (40 g in 300 ml of plain water); T1b 2 hours after a standard Mediterranean meal (60% carbohydrates, 25% lipids, 15% proteins). In 10 women (aged 54 $\pm$ 8.4) glycemic status and lipid panel were also evaluated three months after taking GoJuvo as meal replacement (T2).

**Results:** expressed as mean $\pm$ SD and, in brackets, mean delta values percentage of parameters' changes.

Glucose (mg/dL): T0=91.6 $\pm$ 9.2, T1a=87.0 $\pm$ 10.7(-4.3%), T1b=94.9 $\pm$ 23.1(+4.4%)

Triglycerides (mg/dL): T0=96.8 $\pm$ 8.1, T1a=89.9 $\pm$ 36.1(-5.8%), T1b=119 $\pm$ 48.8(+37.6%)

Insulin (mg/dL): T0=10.6±7.1, T1a=11.0±13.4(+2.1%), T1b=49.0±60.9(+306%)

The increase in glycaemia, insulin and triglycerides mean delta values percentage was lower after GoJuvo administration than after the Mediterranean meal. At T2 no significant changes were found in glycaemic status, but HDL-cholesterol concentrations were significantly higher than at T0 (58.3±13.6 vs 50.0±13.5 mg/dL; p=0.015) and triglycerides concentrations were lower at T2 than at T0, though not significant (76.3±16.1 vs 94.1±38.0 mg/dL; p=0.09). Our data suggest that GoJuvo metabolic modulations are better than post-prandium ones due to 60% of integral rice contained in 40 g of GoJuvo, mixed with other vegetables and fruit. GoJuvo prolonged use seems to improve the lipid panel.

#### Reference:

1. König D et al. *Ann Nutr Metab* (2008); 52:74–8.

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### Protective Effect of Propill, a Quercetin/ Zinc-Based Food Supplement, on Oral Contraception-Induced Oxidative Stress

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We previously showed that oral contraception (OC) with ethinylestradiol and drospirenone is associated with an oxidative stress (OS) status characterized by high levels of lipid peroxides (LOOH) and oxidized LDLs (oxLDL), and by an unfavourable copper to zinc ratio (1). Because such OS profile could be associated with increased cardiovascular risk, we prepared a specific dietary supplement (ProPill™) susceptible to reduce the levels of OS in women under OC.

A double blind randomized placebo controlled study was conducted to validate the effect of ProPill™ on oxidative stress. 50 women under OC with ethinylestradiol and drospirenone were randomized to receive ProPill™ (n=15), placebo (n=15), quercetin alone (n=10) or zinc alone (n=10). The different investigational products were taken during four consecutive menstrual cycles. The levels of LOOH, oxLDL, Cu and Zn, and the expression of 200 genes involved in oxidative stress pathways were assessed at different time points. No significant adverse effects were recorded during the study.

Results showed a significant reduction of the Cu/Zn ratio during the ProPill™ treatments. The reduction was statistically significant compared to placebo and to quercetin alone. A significant decrease of oxLDL was only observed during the ProPill™ treatment and the decrease was statistically significant compared to placebo. In addition, the microarray analysis revealed 44 genes to be significantly differentially expressed in the group treated with ProPill™ compared to the placebo group. The observed gene profile suggests that the treatment with ProPill™ regulates the expression of genes involved in different biological pathways.

In conclusion, the synergistically and complementary biological effects of quercetin and zinc in the ProPill™ formulae and the absence of significant adverse events observed during the study, suggest that ProPill™ could efficiently protect women from some secondary effects of oral contraception.

#### Reference:

- D. De Groote et al. *Contraception* 80 (2009) 187–193.

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### Supplementation with Encapsulated Vegetable and Fruit Juice Powder Concentrate Improves Microcirculation and Ultrastructure in Skin

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The objective of the study was to determine changes in skin physiological parameters during the intake of vegetable and fruit juice powder concentrate (JPC) vs. placebo capsules. The detection of microcirculation, hydration, transepidermal water loss, ultrastructure, i.e. skin thickness and density were determined during the study.

Microcirculation measurements were performed by the O2C device (Lea Instruments, Giessen, Germany), hydration measurements with the Corneometer CM 825 prior to and during the study. Transepidermal water loss was measured with the Tewameter (Courage & Khazaka Electronics, Cologne, Germany). Analyses of skin thickness and density were made by ultrasound measurements (Dermascan C, Cortex Technology).

52 female volunteers took part in the study, from 40 to 65 years in age, complying with all inclusion criteria. The study was performed as a randomized, placebo-controlled, double-blind study. All tests were performed prior to first intake of the described capsules and after 6 and 12 weeks.

All test results were statistically analyzed. Descriptive statistics and pre-post differences were calculated and each combination was compared. Percentage changes and p-values were determined at all measuring points. The following results were obtained in this study:

1. Significant improvement of microcirculation by 36 % (flow 1 mm depth) could be shown after 12 week supplementation with the JPC capsules.
2. Significant improvement of the skin thickness by 6% and skin density by 17%, measured by ultrasound (B-Scan) was detected in the JPC group
3. Significant increase of skin hydration by 9% as well as a decrease of TEWL by 4% by the treatment after 12 weeks could be shown. This constitutes an improvement of the barrier function of the skin.

Microcirculation and ultrastructure of the skin could be improved. Further skin physiological parameters like skin hydration, barrier function were positively influenced. The placebo group showed only minor or no positive effects.