Clinical Research: Sponsored Studies

SRD Health & Wellness Support Programs – Clinical Research & Sponsored Studies

Citrus Health and Wellness benefits derive from the presence of a complex mixture of nutritional and other phytochemical components (natural plant chemicals) of citrus products. The SRD has the responsibility to provide and sponsor the necessary research to verify the continued nutritional and other health benefits associated with citrus consumption.

Current and Recent Studies:

INRA: OJ and Cardiovascular Protection – "Effects of the consumption of orange juice on vascular protection and immune function. A human study on the specific contribution of citrus flavanones". (Completed)
Morand C. and Milenkovich D.

The main objectives of this project were:
(1) To characterize the effects of single dose and made repeated consumption of orange juice on vascular protection and the immune response.
2) To assess the specific role of polyphenols present in citrus fruits in this protection. The study involved a clinical study, randomized and crossover, among healthy men 50 to 65 years that are slightly overweight. The volunteers, who continued to follow their usual diet, consumed daily for 4 weeks

1) Orange juice (500ml)
2) An isocaloric control beverage (placebo)
3) The same drink spiked with a dose of polyphenols similar to that given by orange juice.

The main effects of chronic intake of orange juice or hesperidin were:

- 4 wk consumption of orange juice or purified hesperidin, the major orange phenolic, significantly decreased DBP in healthy subjects.
- A significant improvement in endothelium-dependent microvascular reactivity (blood vessel plasticity) was also observed following both orange juice and hesperidin ingestion. The improvement of endothelial function was reflected by positive trends in plasma nitric oxide and sVCAM-1 levels, both markers of blood vessels health.
- These results appear to orange juice may induce an improvement of blood circulation.
- It was also reported that no weight increase was noted with the various regimens.

Global gene expression profiles were determined by using whole human genome DNA microarrays. Both orange juice and hesperidin consumption induced significant modulation of gene expression; orange juice changed expression of 3422 genes while hesperidin intake modulated expression of 1819 genes. Many of these differentially expressed genes are implicated in the onset of atherosclerosis.
It was also noted that changes in leukocyte gene expression mediated by were observed in fasted subjects while hesperidin metabolites were no longer present in blood circulation These results appear to indicate that longer term consumption of orange juice alters gene expression to a potentially protective cardiovascular effect.

Publications

- **Identification of molecular targets of hesperidin, the major flavonoid of orange juice, in relation with its beneficial vascular action in healthy men**. Dragan Milenkovic, Christiane Deval, André

Mazur, Augustin Scalbert, Christine Morand American Heart Association Basic Cardiovascular Sciences Annual Conference 2009 - Molecular Mechanisms of Cardiovascular Disease.

- **How does orange juice consumption affect vascular protection in human – what role for hesperidin?** Christine Morand, Dragan Milenkovic, Delphine Lioger, Jean François Martin, Claude Dubray, Augustin Scalbert, André Mazur. ICPH 2009 4th International conference on polyphenols & health.

- **Hesperidin contributes to the vascular protective effects of orange juice: a randomized crossover study in healthy volunteers**. Christine Morand, Claude Dubray , Dragan Milenkovic, Delphine Lioger , Jean François Martin, Augustin Scalbert, André Mazur. Am J Clin Nutr. 2011, 93:73-80.

- **Nutrigenomic effects of hesperidin, the major polyphenol of orange, related to its cardiovascular protective effects.** Christine Morand, Dragan Milenkovic, Audrey Chanet, Christiane Deval, Andrzej Mazur. Experimental Biology 2011, Conference Washington, D.C.

- **Orange juice, as well as hesperidin, modify expression of pro-atherogenic genes in leukocyte of human volunteers.** Dragan Milenkovic, Christiane Deval, André Mazur, Christine Morand A. Am J Clin Nutr , 2011(Submitted)

Two smaller projects were derived from the main orange juice study (in progress) --

INRA: OJ and Cardiovascular Protection: metabolomics analysis of samples collected in the main study.
The study, not completely finished, revealed differences in the blood levels of orange juice metabolites among the volunteers and also that drinking orange juice does not appear to increase the fructose blood level

INRA: OJ and Cardiovascular Protection: Determination of cellular and molecular mechanisms of orange juice polyphenols in human monocytes.
Several metabolized forms of hesperidin are found in the bloodstream after OJ digestion, and have been synthesized as purified hesperidin metabolites. They will be used in an *in vitro* study to determine if one or more of these metabolites has the capacity to stimulate the gene expressions associated with several early steps of atherosclerosis,

Institut National de la Recherche Agronomique

***INRA: GJ, Cardiovascular and Bone Health* –Morand C., Horcadjada MN, and Milenkovich D**
"Effects of grapefruit juice consumption on vascular and bone functions. A human cross-over study on the specific contribution of citrus flavanones (Part I CVD) (In progress)
Cross-over, placebo-treatment, randomized, 14-month intervention trial is being performed on 52 post-menopausal healthy women; 50-65 years-old. Participants consumed 350 ml of grapefruit juice or an isocaloric placebo.

Goal: Obtain clinical proof, in healthy postmenopausal women, that regular consumption of grapefruit juice may improve vascular function and particularly

1) To characterize the medium (3 months) and long term (6 months) effects of grapefruit juice consumption on vascular protection in humans.
2) To evaluate the differentials in genetic expression associated with these characterizations/evaluations of the effects of grapefruit juice and grapefruit polyphenols

"Effects of grapefruit juice consumption on vascular and bone functions. A human cross-over study on the specific contribution of citrus flavanones (Part II Bone)" (in Progress)

This study is being conducted on the same group of participants.

Goal: Obtain clinical proof, in healthy postmenopausal women, that regular consumption of grapefruit juice may contribute to the maintenance of bone mineral density (BMD).

1) To characterize the medium (3 months) and long term (6 months) effects of grapefruit juice consumption on bone health and particularly bone density in humans.
2) To evaluate the specific roles of grapefruit polyphenols on these protective effects.

University of Nottingham: OJ and Metabolic Syndrome – "Study to assess the effects of orange juice consumption of insulin sensitivity and plasma lipids in overweight women"; **McDonald I. and Simpson L. (In Progress)**

The trial is a 12 weeks, parallel group clinical dietary intervention study, plus 4 week washout. It includes 32 overweight or mildly obese women who are otherwise healthy as the participants. Participants consume 250ml of orange juice or a placebo daily for 12 weeks.

The primary aim of the present proposal is to determine the effect of daily consumption of 250ml of orange juice compared to placebo, on insulin sensitivity in overweight women who have mild insulin resistance but who are otherwise healthy. Secondary aims are to assess the effects of Orange Juice on;

1) Plasma lipid markers of cardiovascular risk and hormones which contribute towards carbohydrate metabolism and insulin sensitivity
2) Gene expression of insulin sensitivity markers and inflammatory molecules in subcutaneous adipose tissue and white blood cells
3) Plasma concentrations of inflammatory cytokines (e.g. IL6, CRP)
4) Resting BP and heart rate

"Pilot study in overweight men to assess the effects of orange juice on plasma lipids"

The study is similar to the clinical dietary intervention with women trial but participants will be 32 overweight men.

The primary aim of the study is to determine the effect of daily consumption of 250ml of orange juice compared to placebo, on insulin sensitivity in overweight men who have mild insulin resistance but who are otherwise healthy.

Secondary aims are to assess the effects of orange juice on;

1) Plasma lipid markers of cardiovascular risk and hormones which contribute towards carbohydrate metabolism and insulin sensitivity
2) Plasma concentrations of inflammatory cytokines (eg IL6, CRP)
3) Resting BP and heart rate.

***University of Reading: OJ, Brain Health and Cognition* –- Spencer J P E, Butler L.**
"The impact of long-term hesperidin-rich orange juice consumption on neuro-cognitive performance (Part I)" (In progress)
A 12-week human dietary intervention cross-over study is evaluating the potential impact of hesperidin-rich orange juice ingestion on cognitive performance in 45 healthy adults (50-60 years old) individuals consuming 500ml of orange juice daily.

The effects of the juice consumption are followed with a battery of cognitive tests aimed at:

(1) Memory: working memory, long-term memory, spatial memory, visual memory and recognition

(2) Executive Function (involving processes such as planning, cognitive flexibility, abstract thinking, rule acquisition, initiating appropriate actions and inhibiting inappropriate actions, and selecting relevant sensory information).

The levels of a specific brain activity marker, the serum brain-derived neurotrophic factor (BDNF). This marker is active in the hippocampus, cortex, and basal forebrain—areas vital to learning, memory and higher thinking and BDNF itself is important for long-term memory.

Changes in neural activity (brain blood flow) are being assessed by measuring brain blood flow using state-of-the-art functional magnetic resonance imaging (fMRI), comparing baseline to completion of the intervention study.

"The impact of hesperidin-rich orange juice on recovery from neuro-cognitive fatigue (Part II)" (In progress)
Twenty healthy adults (50-60 years old) will be evaluated to determine the potential impact of hesperidin-rich orange juice on recovery from neuro-cognitive fatigue, with and without consumption of 250 ml of orange juice following induced fatigue.

Fatigue will be induced by subjecting participants to four separate batteries of cognitive tasks separated by 90 min. They will then consume either the orange juice (Test day 1) or control (Test day 2) prior to performing two more batteries of cognitive tests. Participants will also undergo fMRI scanning and have a blood sample collected for BDNF measurements.

State University of New York at Buffalo - A Randomized Controlled Study of the Potential Anti-oxidative and Anti-Inflammatory Effect of Orange Juice* ; Dandona P., Ghanim H.; *(Completed)
In this study 48 normal weight subjects were divided into 4 groups of 12. Subjects from 2 groups were given a single 300 kcal challenge of either Dexicola (glucose) drink (300 mL) or orange juice (634 mL); each group was challenged one week with one of the two treatments, then challenged the following week with the other treatment.

The other 2 groups were challenged with a single meal of either 300 kcal of Dexicola drink + 900 kcal of a 'high fat- high carbohydrate (HFHC) breakfast meal (egg muffin and sausage muffin sandwiches and two hash brown servings, which contained 81g carbohydrates, 51g fat and 32 g protein), or 300 kcal of orange juice + 900 kcal

of HFHC; each group was challenged one week with one of the two treatments, then challenged the following week with the other treatment.

Blood samples were collected prior to and at 1, 3 and 5 hr following each challenge. Spot urine samples were collected from all the subjects during both challenges before and at 3 and 5 hours after the challenge.
The authors reported that:

- The combination of glucose or water and the HFHC meal induced oxidative and inflammatory stress and increased plasma endotoxin concentrations.
- In contrast, orange juice intake with the HFHC meal prevented meal-induced oxidative and inflammatory stress, including the increase in endotoxin concentrations.
- These observations were reported to indicate that orange juice could play a role in preventing postprandial oxidative stress, inflammation, insulin resistance, and development of atherosclerosis.

Publications:

- Ghanim H, Sia CL, Upadhyay M, Korzeniewski K, Viswanathan P, Abuaysheh S, Mohanty P, Dandona P "Orange juice neutralizes the proinflammatory effect of a high-fat, high-carbohydrate meal and prevents endotoxin increase and Toll-like receptor expression". Am J Clin Nutr. (2010) 91:940-9.
- Dandona P. "Orange juice or fructose intake does not induce oxidative and inflammatory response." Diabetes Care (2007)30:1406-11

Vanderbilt University: Weight Management and Grapefruit — "Effects of Grapefruit or Grapefruit Juice on Anthropometry, Dietary Intakes, Appetite and Metabolic Profile in Overweight and Obese Young and Middle-Aged Adults"; **Jensen G. And Silver H.D.** ***(Completed)***

- Phase 1. To compare the effects of a 3-month intervention of adding an equicaloric serving of grapefruit or grapefruit juice before meals to a comprehensive monitored weight loss program on
    - Anthropometric profile
    - Dietary intakes
    - Metabolic profile of community-dwelling overweight and obese adults aged 21 to 50 years.
    - Phase 2. To determine whether an equicaloric preload of grapefruit or grapefruit juice in combination with low fat or low glycemic index diet alters perceived and biological indicators of
        - Appetite
        - Hunger
        - Satiety and /or satiation

      to influence total 24-hour dietary intakes in the overweight and obese adult aged 21 to 50 years who is following a comprehensive monitored weight loss intervention.

Eighty-five obese adults (BMI 30-39.9) were randomly assigned to (127g) of

- Grapefruit (GF)
- Grapefruit juice (GFJ)
- Or water preload

for 12 weeks after completing a 2-week caloric restriction phase. Preloads were matched for weight, calories, water content, and energy density. Weekly measures included blood pressure, weight, anthropometry and 24-hour dietary intakes.

Results reported indicated that the total amount of food consumed did not change over time. However, after preloads were combined with caloric restriction, total energy intakes decreased by 20-29% from baseline

values. Subjects experienced 7.1% weight loss overall. However, differences were not statistically significant among groups. Nevertheless, the amount and direction of change in serum HDL-cholesterol levels in GF (+6.2%) and GFJ (+8.2%) preload groups was significantly greater than water preload group (-3.7%).

Publication

Silver HJ, Dietrich MS, Niswender KD. Effects of grapefruit, grapefruit juice and water preloads on energy balance, weight loss, body composition, and cardiometabolic risk in free-living obese adults. Nutr Metab (Lond). (2011)2:8-11

WATSON CLINIC LLP
Quality Healthcare for Every Generation

Watson Research Clinic: GF and Atorvastain (Lipitor) – Serum Concentrations and Clinical Effects of Atorvastatin in Patients taking Grapefruit Juice Daily **Reddy P.J.; *(Completed)***

The primary objectives of the study were to evaluate the lipid effects and the safety of grapefruit consumption in patients receiving Atorvastatin therapy. The secondary objectives were to evaluate the effects on quality of life when grapefruit juice is added to Atorvastatin therapy.

This study used a prospective, single center, open labeled, non-randomized, parallel design to investigate two groups of hyperlipidemic patients on existing stable 90 day Atorvastatin therapy.). Group A maintained a steady 10mg to 40mg (10mg, 20mg, or 40mg) daily dose of Atorvastatin and Group B maintained one-half of their usual run-in dose of Atorvastatin. Both Group A and Group B drank one 1 O-ounce bottle of grapefruit juice every morning .

Conclusions reported: The patients on extended stable ATOR treatment, addition of daily GFJ in typical quantities slightly elevates serum ATOR levels but has no meaningful effect on the serum lipid profile, and causes no detectable adverse liver or muscle effects. Reduction of ATOR dosage when moderate amounts of GFJ are co-ingested does not appear to be necessary.

Publications

The safety of Grapefruit juice in patients taking Atorvastin. Reddy P., Ellington D., Zhu Y., Browne, K.Jr. (Poster,AHA Annual Conference on Cardiovascular Disease, 2007)

Serum concentrations and clinical effects of Avorstatin in patients taking grapefruit juice daily.(2011) Reddy P., Ellington D., Zhu Y., Zdrojewski Y. , Parent S.J., . Harmatz J.S., Derendorf H., Greenblatt D.J., Browne, K.Jr. (2011 British Journal of Clinical Pharmacology (in press)