

# Effects of acute and one month ingestion of biologically aged Andalusian wines on endothelial function

<b>Submission date</b> 14/12/2012	<b>Recruitment status</b> No longer recruiting	<input type="checkbox"/> Prospectively registered <input type="checkbox"/> Protocol
<b>Registration date</b> 23/04/2013	<b>Overall study status</b> Completed	<input type="checkbox"/> Statistical analysis plan <input type="checkbox"/> Results
<b>Last Edited</b> 30/04/2013	<b>Condition category</b> Circulatory System	<input type="checkbox"/> Individual participant data <input type="checkbox"/> Record updated in last year

## Plain English summary of protocol

### Background and study aims

In epidemiological studies, moderate consumption of alcohol is consistently associated with a reduction in myocardial infarction as well as several cardiovascular risk factors. However, the effects of the different alcoholic beverages (fermented or distilled) are still debated, as are the subjects who show higher benefits in the cardiovascular system after moderate alcohol consumption. The biologically aged Andalusian wine could give cardioprotective effects because of its singular aging process.

The aim of this study is to understand the risk/benefit ratio of the consumption of 30 g a day (during 3 weeks) of ethanol in the form of gin or Andalusian biologically aged wine in different population subsets regarding several cardiovascular risk factors.

### Who can participate?

Males and females between 55 and 80 years old without documented cardiovascular disease (ischaemic heart disease, angina or recent or old myocardial infarction or previous or cerebral vascular accident, peripheral vascular disease) and who have diabetes mellitus or three or more of the following factors: current smoking, hypertension, hypercholesterolemia (LDL-cholesterol > 160 mg/dl), HDL-cholesterol < 40 mg/dl, overweight or obese (body mass index > 25 kg/m<sup>2</sup>) and/or family history of premature coronary heart disease.

Healthy males and females between 20 and 40 years.

### What does the study involve?

Participants will not consume alcohol for the first 15 days of the study. Then they will be randomly allocated to one of two groups: a wine intervention or a gin intervention.

Group 1 will be given 0.5g/Kg of aged wine for one day only and then 255ml of wine daily for 21 days.

Group 2 will be given 0.5g/Kg of gin for one day only and then 92ml of gin daily for 21 days. Then they will have 15 days without alcohol.

Then Group 1 will have the gin intervention and group 2 will have the wine intervention

What are the possible benefits and risks of participating?

It is expected that after the wine intervention the participants will show a decreased cardiovascular risk. There are no risks involved.

Where is the study run from?

Participants will be recruited in the Hospital Clinic of Barcelona and the Reina Sofia Hospital, Cordoba (Spain).

When is the study starting and how long is it expected to run for?

The study started in January 2013 and is expected to finish in December 2014.

Who is funding the study?

The Spanish Foundation for the Wine and Nutrition Research (Fundación para la Investigación del Vino y Nutrición FIVINN).

Who is the main contact?

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## Contact information

### Type(s)

Scientific

### Contact name

Dr Ramon Estruch

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## Additional identifiers

EudraCT/CTIS number

IRAS number

ClinicalTrials.gov number

Secondary identifying numbers

CP040970

## Study information

Scientific Title

Effects of acute and one month ingestion of biologically aged Andalusian wines on endothelial function: a randomized crossover trial

### **Study objectives**

The benefit of the main components of aged wine, namely ethanol and polyphenolic content is synergistic, but different between men and women and healthy or at high cardiovascular risk. No adverse events will be observed.

### **Ethics approval required**

Old ethics approval format

### **Ethics approval(s)**

Institutional Review Board of the Hospital Clínic of Barcelona (Spain). May 2011. Reference 2011 /6824

### **Study design**

Randomized crossover clinical trial

### **Primary study design**

Interventional

### **Secondary study design**

Randomised controlled trial

### **Study setting(s)**

Hospital

### **Study type(s)**

Screening

### **Participant information sheet**

Not available in web format, please use the contact details below to request a patient information sheet

### **Health condition(s) or problem(s) studied**

Arteriosclerosis and endothelial function

### **Interventions**

Run-in period (15d); intervention A - 21d; 15d wash-out and intervention B -21d. Before each intervention, 0.5g OH/Kg will be administered as gin or wine.

Intervention 1: 92 ml/day of gin

Intervention 2: 255 ml/day of aged wine

Acute phase before each intervention: 0.5g/Kg of gin or aged wine

### **Intervention Type**

Other

### **Phase**

Not Applicable

## Primary outcome measure

### 1. Leukocyte adhesion molecule expression

Lymphocyte and monocyte adhesion molecules on these cells will be marked with monoclonal antibodies (MAb) conjugated with fluorescein-isothiocyanate (FITC) and phycoerythrin (PE) by direct double immunofluorescence. The MAb of the adhesion molecules used will be: anti-CD11a (LFA-1), anti-CD40L, anti-CD11b (Mac-1) (Bender MedSystems Diagnostics, Vienna), anti-Sialyl Lewis (anti-CD15s) (Pharmingen, San Diego, CA), anti-CD49d (VLA-4) (Cytogmos) and CD31 (BD, NJ, USA). The monoclonal antibodies used to mark the T-lymphocytes will be anti-CD2 and monocytes, anti-CD14 (Caltag Laboratories, Burlingame, CA).

### 2. Soluble adhesion molecules

The following serum soluble adhesion molecules will be determined by ELISA kits:

2.1. sICAM-1, sVCAM-1, PECAM, hs-CRP, sE-selectin, and sP-selectin, as well as sMCP-1, TNF- $\alpha$ , IL-6 and IL-1B (Immunotech).

2.2. Nuclear Factor Kappa B by western blot of peripheral blood mononuclear cells.

2.3. Genes and proteins involved in inflammatory response will be determined by real time PCR and Western blot analysis (MCP-1, TF and TFPI, as markers of inflammation and LRP and the LDL receptor as lipoproteic receptors). Moreover, the expression metalloproteases and their activity will also be analyzed.

### 3. Oxidative stress

The following plasma molecules will be determined: superoxide dismutase (SOD) with the Superoxide Dismutase Assay SD125 (Randox Laboratories Ltd, UK), catalase (CAT) with the Catalase Assay (Cayman Chemical, Ann Arbor, MI, USA), glutathione reductase (GR) and reduced glutathione (GSH) with the Glutathione Reductase Assay GR2368 (Randox Laboratories Ltd, UK), glutation peroxidase with the RS 504 assay (Randox Laboratories Ltd, UK), el complejo GSH /GSSG (Bioxytech OxisResearch, Foster City, CA, USA), protein carbonyl groups with the Protein Carbonyl Assay (Cayman Chemical, Ann Arbor, MI, USA), lipid peroxides (LPO) with the LPO-CC Assay (Kamiya Biomedical Company, Seattle, USA), malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) with the Bioxytech LPO-586 (Oxys Research, Portland, OR, USA), total antioxidant capacity with the Total Antioxidant Powder (aqueous) Assay (Neogen Corporation, Lexington, KY, USA) and hidroxideoxiguanosina 8-(8-OHdG) with the 8-OHdG Check ELISA Kit (Genox Corporation, Baltimore, USA) in plasma in addition to the concentration of urinary isoprostanes with the Urinary Isoprostane Assay (Neogen Corporation, Lexington, KY, USA).

### 4. Nitric oxide

The concentration of nitrate / nitrite (NO (x)) in plasma will be determined through purifying the samples with Microcon YM-10 filters (Micon Bioseparations, Millipore, Billerica, MA, USA) and the commercial kit Nitrate / Nitrite Colorimetric Assay Kit (Cayman Chemical, Ann Arbor, MI, USA). In addition, the concentration of nitric oxide synthase (NOS) in plasma will be determined with the commercial kit Bioxytech ® Nitric Oxide Synthase Assay Kit <sup>TM</sup> (Oxys Research, Portland, OR, USA).

### 5. Wine biomarkers and urinary polyphenols

The urinary polyphenol levels will be also determined with the Folin-Ciocalteu assay (Sigma-Aldrich-Fluka, St. Louis, MO, USA). Furthermore, tartaric acid in urine as biomarker of wine intake will be also quantified by LC-MS/MS.

## Secondary outcome measures

### 1. Medical record

A complete medical record will be obtained from all participants, which included data on alcohol intake, smoking and dietary habits. Blood pressure and heart rate will be measured with an electronic apparatus Omron HEM-705CP (Netherlands).

### 2. Nutrition assessment and general analyses

All participants will complete a validated nutritional questionnaire at baseline to determine the total quantity of calories ingested in the previous month as well as the proportion corresponding to carbohydrates, lipids and proteins by using the Food Processor Nutrition & Fitness software (esha RESEARCH, Salem, OR, USA) . Overall nutrition will be determined by percentage of ideal weight, lean body mass and body mass index. Waist Perimeter will be measured. The proteic nutrition will be determined on the basis of the following parameters: hemoglobin, total lymphocyte count, total proteins, albumin, prealbumin, transferrin and retinol-binding protein. Serum and intraerythrocytary folic acid concentrations will be measured, as well as serum vitamin A, B1, B12, C, E, B-carotenes, Zn, Mg and Se concentrations.

Moreover, the following measurements will also be obtained: red blood cell count, hematocrit, mean corpuscular volume, leukocyte count, glucose, creatinine, electrolytes, uric acid, transaminases, lactate dehydrogenase, alkaline phosphatase, gammaglutamyl transpeptidase and bilirubin.

### 3. Coagulation tests

The following parameters will also be determined: platelet count, prothrombin time, and plasma fibrinogen.

### 4. Serum lipoproteins and others

Total cholesterol, triglycerides, cHDL, cLDL, Apo A1, Apo B, lipoprotein (a) and homocysteine will be determined.

### 5. Diet and exercise monitoring

All participants will follow an isocaloric diet prepared according to their personal preferences. The diet will be strictly monitored during the study. Diet compliance will be assessed from 7-days diet records administered before each evaluation. This assessment will be administered by trained personnel. The foods ingested will be converted into nutritional values with the aid of the Professional Diet Balancer software (Cardinal Health Systems, Inc., Edina, MN). Physical activity will also be evaluated with the Minnesota Leisure Time Physical Activity questionnaire which has also been validated in Spain. Control of the diet and physical exercise will be carried out before and after each intervention, the same day on which the clinical examinations are performed and blood is withdrawn for immunologic studies.

## Overall study start date

01/01/2013

## Completion date

31/12/2015

# Eligibility

## Key inclusion criteria

1. Males and females between 55 and 80 years old without documented cardiovascular disease (ischemic heart disease, angina or recent or old myocardial infarction or previous or cerebral vascular accident, peripheral vascular disease) and who have diabetes mellitus or three or more of the following factors:

1.1. Current smoking

1.2. Hypertension

1.3. Hypercholesterolemia [LDL-cholesterol > 160 mg/dl, HDL-cholesterol < 40 mg/dl]

1.4. Overweight or obese (body mass index > 25 kg/m<sup>2</sup>) and/or

1.5. Family history of premature coronary heart disease

2. Healthy males and females between 20 and 40 years

3. The participant should give signed informed consent

**Participant type(s)**

Patient

**Age group**

Adult

**Sex**

Both

**Target number of participants**

High cardiovascular risk subjects (males and females): 80, Healthy volunteers: 80

**Key exclusion criteria**

1. Subjects with a previous history of cardiovascular disease (ischaemic heart disease angina or recent or old myocardial infarction, cerebral vascular accident, or peripheral vascular disease)
2. Any severe chronic disease
3. Alcoholism or other toxic abuse

**Date of first enrolment**

01/01/2013

**Date of final enrolment**

31/12/2015

**Locations****Countries of recruitment**

Spain

**Study participating centre**

Hospital Clínic de Barcelona

Barcelona

Spain

08036

**Sponsor information****Organisation**

Spanish Foundation for the Wine and Nutrition Research (Fundación para la Investigación del Vino y Nutrición FIVINN) (Spain)

**Sponsor details**

Padilla, 38  
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28006

**Sponsor type**

Research organisation

**Website**

<http://www.fivin.com>

**ROR**

<https://ror.org/002jv8g15>

## **Funder(s)**

**Funder type**

Research organisation

**Funder Name**

Spanish Foundation for the Wine and Nutrition Research (Fundación para la Investigación del Vino y Nutrición FIVINN) (Spain)

## **Results and Publications**

**Publication and dissemination plan**

Not provided at time of registration

**Intention to publish date****Individual participant data (IPD) sharing plan****IPD sharing plan summary**

Not provided at time of registration