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Effects of Annurca apple polyphenols on cholesterol excretion

Submission date	Recruitment status	[X] Prospectively registered
07/11/2017	No longer recruiting	[ ] Protocol
Registration date	**Overall study status**	[ ] Statistical analysis plan
09/11/2017	Completed	[ ] Results
Last Edited	**Condition category**	[ ] Individual participant data
10/11/2017	Nutritional, Metabolic, Endocrine	[ ] Record updated in last year

Plain English summary of protocol

Background and study aims

Metabolic syndrome is a serious risk factor for cardiovascular disease (heart disease) such as arteriosclerosis and is caused by hyperlipidemia (high levels of lipids in the blood), hyperglycemia (high blood sugar), and hypertension (high blood pressure). Arteriosclerosis (the thickening and hardening of the artery walls) is related to cardiovascular disease and cerebral infarction (when there is a blockage of the arteries that deliver blood to the brain), which are widely recognized as major public health problems. Reducing the risk of arteriosclerosis, especially dyslipidemia (abnormal amount of lipids in the blood), which was reported to be the strongest risk factor for arteriosclerosis, is extremely important. In some patients, the major abnormality in dyslipidemia is a postprandial serum triglyceride and cholesterol elevation (high levels of sugar and cholesterol after eating). In previous reports, administration of tea polyphenols (micronutrients) improved blood lipid levels and promoted lipid excretion in an animal study, while prevented postprandial serum triglyceride elevation in a clinical study. These reports suggest that tea polyphenols have a lipid excretion (removal) effect. Previous authors have demonstrated that the addition of tea catechins (a natural type of phenol and antioxidant) galloyl moiety to a bile salt micellar solution precipitated (separated) cholesterol and decreased the micellar solubility of cholesterol in a dose-dependent manner. In contrast, green tea catechins without a galloyl moiety did not precipitated cholesterol. Interestingly, apple procyanidins (apple polyphenols) are oligomeric (consisting of only a few) compounds consisting of catechin monomeric units. Specifically, dimeric procyanidins are the most structurally similar to the green tea catechins with a galloyl moiety. Therefore, it could be hypothesised that these may play a major role as regards our observed effects of apple extract samples on in vitro cholesterol micellar solubility. Specifically, the antioxidant composition and in vitro effects on cholesterol metabolism of polyphenolic extracts from Annurca apple, has been evaluated. By comparing experimental data with those from more common commercial apple cultivars, Annurca apple exhibited the highest polyphenolic concentration, while results obtained by incubating human hepatocellular liver carcinoma (HepG2) cell lines with apple extracts, indicated Annurca apple as the most effective in reducing cell cholesterol uptake, enhancing LDL-C receptor binding activity, and increasing cell medium Apo-AI concentration. The aim of the present study is to evaluate the effects of Annurca apple polyphenolic extract on intestinal cholesterol solubility in healthy subjects.

Who can participate?
Men and women between 18-83 years of age.

What does the study involve?
Participants are provided with a food frequency questionnaire at the beginning of the study. Participants are randomly divided into two groups (each one of 25 subjects, 15 men and 10 women).
They are followed by a cross-over design including 7-day washout periods and 10-day treatment periods. During the treatment periods, each group are given about 370 mg cholesterol a day from two eggs (about 50 g weight each) (185 mg cholesterol each within 30 min after lunch and dinner) and total 1000 mg Annurca apple polyphenolic extract in gastro-resistant capsules at two meals (one 500 mg capsule at each meal). Subjects are allowed to take water freely. All the participants are instructed to maintain their habitual patterns of physical activity throughout the entire study period. The physical characteristics are measured and 1 mL of blood is collected after 12 h fasting in the early morning. The blood samples are collected for biochemical examination at days 8, 18, 25 and 35 of the study period. Fecal samples are collected for the analysis of lipid excretion during the last three days of each treatment period.

What are the possible benefits and risks of participating?
In line with what already reported in literature as regards the effects of apple polyphenols, participants are expected to reveal a positive influence on the balance regarding the circulating lipoproteins. Conversely, no side effects are expected, since the maximum quantity of apple polyphenolic extracts (1000 mg) administered daily is in full accordance with the maximum polyphenolic extract daily intake, through food supplements and novel foods, indicated by the revised form (January 2015) of the Commission Regulation (EC) No. 258/1997, as the safe polyphenolic daily amount compatible with a good health state.

Where is the study run from?
The blood samples are collected by the Samnium Medical Cooperative (Italy) and analysed at the Department of Pharmacy, University of Naples “Federico II” (Italy).

When is the study starting and how long is it expected to run for?
October 2017 to January 2018

Who is funding the study?
1. Samnium Medical Cooperative (Italy)
2. University of Naples “Federico II” (Italy)

Who is the main contact?
Professor Gian Carlo Tenore

Contact information

Type(s)
Scientific

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Additional identifiers

EudraCT/CTIS number

IRAS number

ClinicalTrials.gov number

Secondary identifying numbers
123501

Study information

Scientific Title
Effects of Annurca apple polyphenolic extract on intestinal cholesterol solubility in healthy subjects: A monocentric, double-blind, randomised, placebo-controlled, cross-over study

Acronym
AMD

Study objectives
Annurca apple contains oligomeric procyanidins, mainly dimeric compounds, which are structurally similar to tea galloylated catechins for which the capacity to decrease intestinal cholesterol solubility has been demonstrated. Thus,
Aim 1: Evaluate the effects of Annurca apple polyphenolic extract on fecal cholesterol levels in the subjects.
Aim 2: Evaluate the effects of Annurca apple polyphenolic extract on plasma levels of TC, LDL-C, HDL-C, TG, in the subjects.

Ethics approval required
Old ethics approval format

Ethics approval(s)
Azienda Ospedaliera Gaetano Rummo Via dell'Angelo, 18/10/2017, ref: 123501

Study design
Dietary intervention trial parallel randomised cross-over open controlled study

Primary study design
Interventional

Secondary study design
Randomised cross over trial

Study setting(s)
Hospital

Study type(s)
Prevention

Participant information sheet
Not available in web format, please use contact details to request a participant information sheet: Gian Carlo Tenore giancarlo.tenore@unina.it

Health condition(s) or problem(s) studied
Metabolic Syndrome (MeTS)

Interventions
Participants are randomly allocated into two groups.

They are followed by a cross-over design including 7-day washout periods and 10-day treatment periods. During the treatment periods, each group are given about 370 mg cholesterol a day from two eggs (about 50 g weight each) (185 mg cholesterol each within 30 min after lunch and dinner) and total 1000 mg Annurca apple polyphenolic extract in gastro-resistant capsules at two meals (one 500 mg capsule at each meal).

Intervention Type
Supplement

Primary outcome measure
Fecal samples are collected for the analysis of lipid excretion during the last three days of each treatment period.

Secondary outcome measures
1. Clinical history is measured both by interviews and previous clinical data at baseline
2. Anthropometric measures are collected taking height and weight at baseline and at the end of the study period
3. Nutrient intake and dietary habits are measured using a seven day food record validated nutritional questionnaire at baseline and at the end of the study period
4. Blood pressure is measured using a blood pressure cuff at baseline and at the end of the study period
5. 24 hour ambulatory blood pressure is measured using blood pressure cuff at baseline and at the end of the study period
6. Plasma TC is measured using a Diacron International Free Carpe Diem spectrophotometer (Grosseto, Italy), and commercially available kits from Diacron International at days 8, 18, 25 and 35 of the study period
7. HDL-C is measured using a Diacron International Free Carpe Diem spectrophotometer (Grosseto, Italy), and commercially available kits from Diacron International at days 8, 18, 25 and 35 of the study period
8. LDL-C is measured using a Diacron International Free Carpe Diem spectrophotometer (Grosseto, Italy), and commercially available kits from Diacron International at days 8, 18, 25 and 35 of the study period

9. Triglyceride levels are measured using a Diacron International Free Carpe Diem spectrophotometer (Grosseto, Italy), and commercially available kits from Diacron International at days 8, 18, 25 and 35 of the study period
10. Blood analysis (AST, ALT, γ-GTP, ALP, LDH, Albumin, Total bilirubin, Creatinine) is measured using a Diacron International Free Carpe Diem spectrophotometer (Grosseto, Italy), and commercially available kits from Diacron International at days 8, 18, 25 and 35 of the study period

Overall study start date
18/10/2017

Completion date
04/01/2018

Eligibility

Key inclusion criteria
Men and women between 18-83 years of age, of white race, with the following range values of serum cholesterol parameters at baseline (week 0):
1. TC, 200-260 mg/dL
2. HDL-C, 30-45 mg/dL
3. LDL-C, 189-206 mg/dL
4. TG, 170-280 mg/dL
5. Body mass index (BMI) between 18 and 30 kg/m2

Participant type(s)
Healthy volunteer

Age group
Mixed

Lower age limit
18 Years

Upper age limit
83 Years

Sex
Both

Target number of participants
50

Key exclusion criteria
Healthy adults (males and females) with:
1. Smoking
2. Obesity (BMI >30 kg/m2)
3. Diabetes
4. Hepatic disease
5. Renal disease

6. Heart disease
7. Family history of chronic diseases
8. Drug therapy or supplement intake for hypercholesterolemia
9. Drug therapy or supplement intake containing apple polyphenols
10. Heavy physical exercise (>10 h/week)
11. Pregnant women, women suspected of being pregnant, women who hoped to become pregnant, breastfeeding
12. Birch pollen allergy
13. Use of vitamin/mineral supplements 2 weeks prior to entry into the study
14. Donation of blood less than 3 months before the study

Date of first enrolment
17/11/2017

Date of final enrolment
27/11/2017

Locations

Countries of recruitment
Italy

Study participating centre
Samnium Medical Cooperative
(Benevento, Italy) Viale C. Colombo, 18
Castelvenere
Italy
80131

Study participating centre
University of Naples "Federico II" (lead centre)
Department of Pharmacy
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Sponsor information

Organisation
Samnium Medical Cooperative

Sponsor details

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Sponsor type
Hospital/treatment centre

ROR
https://ror.org/02ww5xj89

Funder(s)

Funder type
Hospital/treatment centre

Funder Name
Samnium Medical Cooperative

Results and Publications

Publication and dissemination plan
Planned publication in a high-impact peer reviewed journal. Additional documents will be available at the Department of Pharmacy, University of Naples “Federico II”, Naples, Italy.

Intention to publish date
07/01/2019

Individual participant data (IPD) sharing plan
The datasets generated during and/or analysed during the current study will be stored in a non-publically available repository at the Department of Pharmacy, University of Naples “Federico II”.

IPD sharing plan summary
Stored in repository