

Prevention of progression of early subclinical atherosclerosis in women: a pilot registry.

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Abstract

Aim: This registry study had the aim of evaluating the effect of ECOVITIS®, a new grape seed pharma-standard supplement on the progression of early subclinical atherosclerosis in asymptomatic, otherwise healthy women.

ECOVITIS® has been administered for 6 months in 32 women at the daily dosage of 300 mg in addition to Standard Management (SM) including diet and lifestyle changes. The 30 women who received SM only were considered as a control group.

Results: No side effects or drop out were observed. The tolerability was very good. Total cholesterol and triglycerides decreased significantly in the ECOVITIS® group; HDL cholesterol increased also significantly in the same group; blood pressure and oxidative stress were significantly decreased. All parameters connected with atherosclerosis progression were positively modulated in the ECOVITIS® group, in particular: IMT growth and subclinical intimal alterations.

Conclusion: In this pilot registry in healthy women the use of the new PS supplement ECOVITIS® is associated with slower growth of the IMT and slower progression or regression of early arterial wall alterations; the product needs more studies to be considered a possible form of control of very early atherosclerosis and of its progression in women. The study, performed in a period of time of 6 months, should be extended to a longer period of time and to a larger, more heterogenous sample of women to become clinically more valuable as a prevention.

Keywords: Grape Seeds, Atherosclerosis, Intima-media Thickness, Carotid Plaques, Atherosclerosis, Prevention.

Introduction

Preclinical atherosclerosis may last many years before becoming symptomatic. In women a possible progression – seen as intima-media thickening of the major arteries (carotids, femorals) - may be slower in comparison with males of the same age. A slower progression of atherosclerotic changes may be linked to the protective effects of estrogens.

Arterial wall progression appears to be faster when estrogen decline in the premenopausal period and during the menopausal transitions [1-5].

New methods allow to noninvasively evaluate by high-resolution ultrasound very early wall lesions (IMT thickening, arterial wall alterations in density) and to follow a possible progression in time.

High-resolution ultrasound at the carotid and femoral flow dividers, are reproducible measurements and can be made in a short period of time to follow up progression. The average value of IMT can be a definite marker of atherosclerosis progression in a defined population (i.e., younger women).

Statins, chitosan and other products may be used to lower

cholesterol and lipids with the final effect of controlling lipids and IMT in time [1,4-8].

PS supplements are ideal for long-term management as they do not expose patients (and preclinical subjects) to any unwanted effect and particularly to the side effects of statins [9].

ECOVITIS®, a new grape seed extract, on the basis of early observations, may control lipids, oxidative stress, endothelial dysfunction and the inflammatory response of the arterial wall to external injuries (shear stress), eventually leading to IMT thickening and IMT growth progression.

Aim of this study was the evaluation, in otherwise healthy women, of the arterial IMT considered as an early sign of atherosclerosis and its progression in an initial temporal window of 6 months to be eventually extended to a longer period of time.

Patients and Methods

Type of Study

Any PS supplements registry study [9] follows the field of activity of pharma-standard supplements of natural origin (PS) and their possible preventive, preclinical applications [7]. The best fields of

PS are preclinical conditions, to manage the risk conditions.

PS, unless there are specific claims, are not generally used for the treatment of clinical conditions. They are used for ‘minor’ medical problems. These studies produce supplementary data to be compared to “background” historical data (i.e., based on the best available management for comparable subjects).

In these studies, there is no defined group allocation and, usually, no randomization is organized by the investigators. Subjects decide, on the basis of an initial briefing which management group they want to join.

The subjects of the present study were part of the ongoing epidemiological study (San Valentino, Spoltore, Central Italy) aimed to screen and prevent subclinical cardiovascular risks. All subjects were followed according to an established Standard Management (SM).

The SM included suggestions about the diet and daily activity, control of salt (NaCl) and lipids/fats and caffeine, alcohol in the diet and an improved lifestyle, including regular exercise, regular sleep hours and stress management.

At inclusion an analysis of the food intake was considered, without imposing any strong caloric restriction, focusing those foods that may increase the rate of progression of subclinical atherosclerosis.

Subjects

Otherwise healthy women (in menopausal transition) not using any drug and without any condition were included. Their thyroid values were within normal values (T3,T4,TSH).

The inclusion criteria were: IMT of the carotids >1 mm and wall intimal granulation (in high resolution scans); age range between 40 and 55 years with mild-borderline hyperlipidemia and hypercholesterolemia (CH>220 mg/dL, triglycerides >200 mg/dL), BMI < 26.

After a preliminary briefing about the possible benefits of the mild hypolipemic management with ECOVITIS®, subjects agreed to enter the study. They were divided into 2 groups (SM and SM + GS).

Exclusion criteria included any chronic diseases causing symptoms or requiring drug treatment (diabetes, hypertension, cancer, allergy, intolerances, neurological and immunological disorders) and smoking.

In the SM plan, the diet was limited to the reduction of one portion of pasta/week, and one portion of cheese or processed meat/week. The increase of physical activity was also suggested in terms of at least 30 min of brisk walking/or physical activity each day [8].

According to long-term data in the San Valentino program, this SM was able to control the early phase of atherosclerosis (intimal granulation/bubbles). The subjects were separated into one group using the SM only and a comparable group using SM with the addition of ECOVITIS® (9).

Variables

The main target variable was carotid IMT measured by high-resolution ultrasound scanning. Secondary variables were considered including total cholesterol, triglycerides, HDL-cholesterol, oxidative stress (in Carr Units) and some physiological variables (blood pressure). All the variables were taken at baseline, at 3 and after 6 months.

Ultrasound Scanning

IMT was non-invasively determined with ultrasound scanning as previously described in detail [4,10,11].

Both carotids, femorals and aorta were scanned to exclude more advanced or severe disease with plaques using a PREIRUS Elastasonographer (Hitachi-Japan) with a high-resolution linear-array transducer (10-14 vMhZ). The carotids, femorals, and aorta arteries were imaged in transverse and longitudinal planes. Carotid and femoral artery examination included evaluation of the common carotid or femoral artery within 1.5 cm from the flow divider. The reported IMT is the average on 3 measurements at the femoral and 3 at the carotids.

IMT is caused by an increased thickening of the arterial wall: a ‘wafer’ arterial section including intima-media components was measured on the far-wall by ultrasound imaging with a specific Preirus software and a careful post-processing evaluation [12-14].

Plaques were defined as focal projections of at least 1.5 mm of the arterial wall into the lumen as defined according to previous work confirmed for reproducibility in post-processing on an Apple wide, high-resolution screen. IMT is caused by an increase of thickening of the inner wall components: the three main components, intima-media and adventitia are clearly visible by ultrasound. In plaques, even the smaller ones, this linear wafer structure is lost and there is no real layer structure to define the IMT structure.

IMT Specifications

The IMT expressed in this study is the average measurements of two carotids IMT, within a distance (proximal and distal) of 1.5 cm from the carotid flow divider. This IMT at the flow divider expresses the value of arterial thickening better than the simple evaluation in linear parts of the carotid/femoral arteries.

Most lesions and thickening of the arterial wall happen at the bifurcation levels where the combined effects of cardiovascular risk factors and the shear stress produce a faster and more significant thickening and the occurrence of thickening and plaques. The shear stress at the bifurcations multiplies the value of the effects of cardiovascular risk factors and make the genesis, occurrence and progression of arterial changes due to atherosclerosis faster.

Variation in wall density at the ‘near wall’ (Figure 1) as granulation at the IMT layer or as lower ‘density’ bubbles at the IMT layer at inclusion and end study - were considered a consequence of lipid accumulation (mainly triglycerides) into the wall structure. The bubbles appear to be more evident in subjects with high triglycerides and tend to disappear or to become less evident with lipid lowering management. Intimal granulation has been previously described in several studies and it is the basis of the ultrasound ‘biopsy’

classification (Figure 2) [10-14]. The use of elastosonography which produces a density color-coded map visualization of the arterial wall allows an easier and faster visualization on intimal-medial irregularities (Preirus, Hitachi).

Blood Sampling

Blood sampling was done at baseline and repeated at 6 months to determine the lipid profile and oxidative stress.

Oxidative stress (as plasma free radicals in Carr Units) was measured at inclusion and after 6 months using a Diacron Systems with a drop of blood from a finger. This test has been validated in previous studies [15,16]. It has been already shown that antioxidants [14] may favorably interfere with the vicious cycle between arterial inflammation and atherosclerosis [16].

Product

The subject supplemented with ECOVITIS® used 2 cp of 150 mg/day (8 am and 8 pm).

ECOVITIS® (Bonollo, Mestrino, PD, Italy) is a standardized grape seed extract obtained from Italian grapes with innovative extractive technology based on water extraction plus selective tangential filtration. ECOVITIS® is industrially reproducible and characterized by a very low amount of monomeric catechins and a higher and well-defined content of oligo-polymeric proanthocyanidins [17].

Compliance-Tolerability

The compliance was measured for any suggestion or indication and, for BP, The compliance was measured counting the capsules correctly used; ideal compliance was considered to be 95% of the theoretical doses to be taken. The tolerability was also evaluated by direct questioning or by internet contacts.

Statistical Analysis

For all the data the average and dispersion variable were calculated for each interdependent data to compare values in the supplement group vs the SM management group. The Anova (with Bonferroni correction) was used to compare the differences between baseline and end of treatments (data available, not reported in this report). The dimension of the samples (>25 cases/group) was considered sufficient to determine the differences between the two groups.

Results

The two main registry groups were comparable in term of age (Table 1).

No side effects were recorded. No subject had to stop the management.

The tolerability was very good. Compliance was >95% (>95 of tablets correctly used). Results are reported in Table 2.

At 3 and 6 months, total cholesterol and triglycerides decreased more ($p<0.05$) in the supplement group. HDL cholesterol was also improved more with the supplement ($p<0.05$).

Platelets and coagulation, and liver/kidney functional parameters were normal at inclusion (as inclusion items) and were unaffected

at the end of the registry.

The borderline increased blood pressure was marginally but significantly more improved (decreased) in the supplement group ($p<0.05$).

IMT growth at the carotid/femoral arteries (the main measurable demonstration of early atherosclerosis and the main registry target) did not increase as predictable in these subjects but decreased at 3 and 6 months (better than in the comparison group; $p<0.05$).

Intimal alterations 'bubbles' (as alterations in density of the wall) were present in all subjects at inclusion; the presence of these observations was decreased more ($p<0.05$) with the supplement at 6 months.

Oxidative stress was significantly decreased with ECOVITIS® ($p<0.5$) with no significant variations in controls (Table 2).

PREDICTIVE ANALITICS (SIEGEL) indicates that a group of some 60-80 selected women for a period of one year may be enough to detect a possible prolonged activity of the product on early IMT alterations and on their growth in time.

Conclusion

Statins, chitosan and other products controlling cholesterol and lipids together with an adequate change in lifestyle and an appropriate diet are generally used to lower lipids with the final target effect of controlling the progression of atherosclerosis characterized by the growth of IMT, the genesis of plaques and possible cardiovascular events [18-20]. Pharma-Standard supplements of natural origin are ideal for long-term management in asymptomatic subjects as they do not expose preclinical subjects to side effects (as observed in some 30% of subjects regularly using statins).

ECOVITIS®, a new PS grape seed extract, considering early evaluations and observations, may help controlling borderline increased lipids and by modulating the increase in oxidative stress in these subjects.

The local (arterial wall at the bifurcations with the higher dynamic stress) inflammatory response of the arterial wall to external injuries (shear stress) may be reduced. These alterations in flow dynamics eventually leads to IMT thickening and IMT growth in specific arterial sites [8-10].

Preclinical, subclinical atherosclerosis - only detectable by high resolution ultrasound- may last many years before becoming hemodynamically important and cause symptoms by stenosis or embolization.

New methods and technology allow the noninvasive quantitative assessment (by high-resolution ultrasound and elastosonography) of very early wall lesions (IMT thickening, arterial wall density inhomogeneity) and allow to follow a possible progression in time.

High-resolution ultrasound at the carotid and femoral flow dividers, are reproducible measurements and are made in a short period of time (in minutes).

The average value of IMT can be considered a significant marker of atherosclerosis progression in a defined population (i.e., younger women) [6,9,10].

In younger and healthy women, a possible atherosclerosis progression seen as intima-media thickening at the major arteries may be slower in comparison with comparable males of the same age also considering a different distribution of risk factors.

A slower progression of atherosclerotic changes may be possibly linked with the effects not being quantified-to the protective effects of estrogens.

In women, arterial wall progression appears to have a faster evolution when the level of estrogen declines (in the premenopausal period, after ovarian surgery and during and after the menopausal transitions [20]. Generally, (including men) [18, 20] atherosclerosis progression can be limited when the prevention measures are applied at the very early phases of the process [22-24]. Results are more evident in a large, non-selected population.

Table 2: Main data from the progression study.

Tests	Groups	INCL	3M	6M
Total Chol	SM + ECOVITIS®	100% (221;8)	-14.2%*	-16%*
	SM	100% (224;4)	-8.3%	-9%
Triglyc	SM + ECOVITIS®	244;11	-	195;9*
	SM	246;14	-	212;11
HDL CHOL	SM + ECOVITIS®	39;3	-	58;2*
	SM	37;2	-	55;4
Platelets+ Coagulation	SM + ECOVITIS®	Normal	-	Normal
	SM	Normal	-	Normal
Liver/Kidney parameters	All subjects	Normal	-	Normal
BL Pressure Decrease	SM + ECOVITIS®	139;3/87;3	-	SY-12;1.8%* DIA -8%*
	SM	13;47/89;3	-	SY-5.4;1% DIA -4.1%
IMT CAROTID/ FEMORALS mm	SM + ECOVITIS®	1.221;0.04	1.108;0.3*	1.082;0,03*
	SM	1.219;0.06	1.224;0.02	1.195;0.02
Intimal Irregularities Bubbles	SM + ECOVITIS®	32/32 (Cases)	-	16/32*
	SM	30/30	-	25/30
Oxstress	SM + ECOVITIS®	389;15	-	351;17*
Carr Units	SM	379;18	-	372;15

*= P<0.05 in comparison with controls.

In conclusion, in this pilot registry in healthy women the use of the new PS supplement ECOVITIS® is associated with a decrease in IMT progression and in early arterial wall alterations and may be considered a possible form of control of early atherosclerosis and of its progression. Also, altered endothelial dysfunction may be improved by ECOVITIS® [24].

The study, performed in a limited period of time (6 months), should be extended to a longer period and to a larger sample of women to become clinically valuable.

Table 1: Characteristics of the registry groups.

	Age	Drops
SM+ ECOVITIS® (32)	48.4;1.6	6
SM (30)	48.2;2.0	7

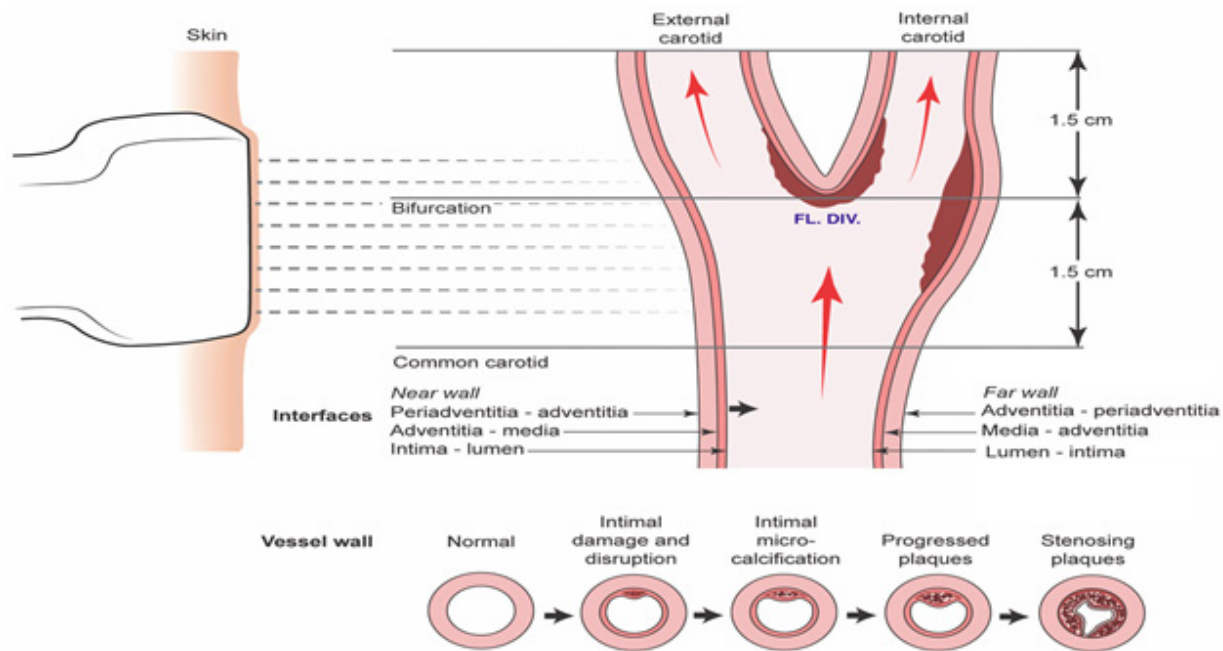


Figure 1: The summary diagram of the technology used in this study.

This supplement registry (open, nonclinical registry, including completely asymptomatic women, otherwise healthy) evaluates the effects of ECOVITIS® on IMT and arterial wall irregularities. Subjects in class 2 (intimal damage and initial disruption) are the target of this study.

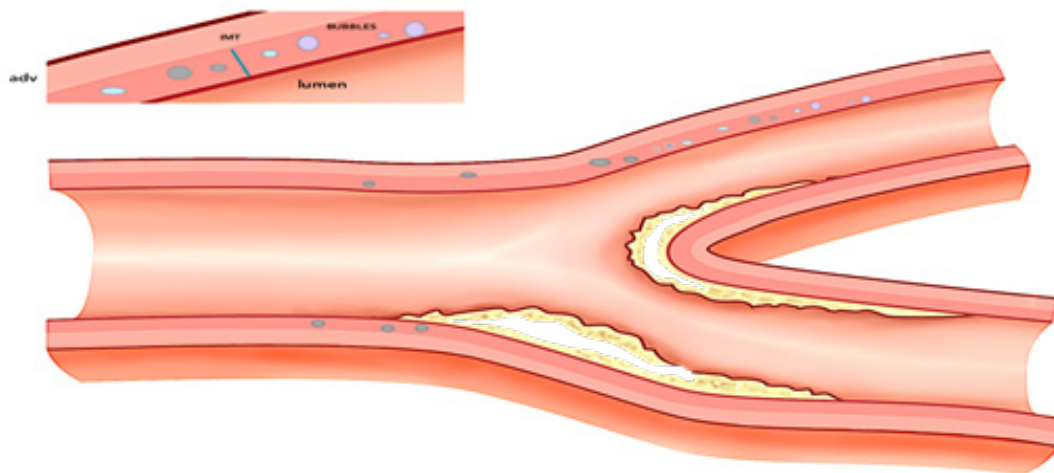


Figure 2: The high-resolution image of the wall shows irregularities (bubbles, alterations in density) at the 'near wall' as a very preliminary sign of atherosclerosis.

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