

Acute Effects of the Use of Estrogens in Association with Progestogens on Postprandial Triglyceridemia and Vascular Reactivity

Silvio C.M. Santos, Jaime Augusto Canashiro, Otavio C.E. Gebara José Mendes Aldrighi, Nubia Vieira, Amit Nussbacher, Humberto Pierri, João Serro-Azul, Maurício Wajngarten, Giuseppe Rosano, Jose A. F. Ramires
São Paulo, SP - Brazil

Objective

To assess whether hormone replacement therapy with estrogens in association with progestogens in postmenopausal hypertensive women alters postprandial triglyceridemia and vascular reactivity.

Methods

A double-blind, placebo-controlled, crossover study was carried out with 15 postmenopausal women (age range: 50 to 70 years, mean = 61.6 ± 6 years) randomly assigned to 2 weeks of placebo or oral ingestion of 0.625 mg of equine conjugated estrogens and 2.5 mg of medroxyprogesterone, fed a high-fat diet (897 calories; 50.1% fat). Vascular reactivity (VR - % of vessel diameter variation in the fasting period and 2 hours after meals) was measured by using the automated ultrasound method. Lipid profile and glycemia during the fasting period and 2 hours after a high-fat meal were measured.

Results

With placebo, vascular reactivity (VR) decreased from $3.20 \pm 17\%$ during the fasting period to $-2.1 \pm 30\%$ 2 hours after the meal ($P=0.041$). With the hormone replacement therapy, vascular reactivity decreased from $6.14 \pm 27\%$ during the fasting period to $-0.05 \pm 18\%$ 2 hours after the meal ($P=NS$). Postprandial triglyceridemia increased as follows: $35 \pm 25\%$ with placebo; and $12 \pm 10\%$ with hormone replacement therapy ($P < 0.05$).

Conclusion

In postmenopausal hypertensive women, 2 weeks of hormone replacement with an association of estrogens and progestogens decreased hypertriglyceridemia after a high-fat meal, an effect that may reduce the endothelial dysfunction occurring in the postprandial period.

Key words

hormone replacement therapy, triglyceridemia, vascular endothelium

Instituto do Coração of the Hospital das Clínicas - FMUSP and Instituto San Raffaele - Roma, Italy
Mailing address: Silvio C. M. Santos - InCor - Cardiogeriatrics
Av. Dr. Eneas C. Aguiar, 44 - Cep 05403-000 - São Paulo, SP - Brazil
E-mail: silviocmsantos@uol.com.br
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Endothelial dysfunction seems to be one of the earliest phenomena involved in atherogenesis. It is associated with different cardiovascular risk factors, such as age¹⁻³, postmenopausal hormone alterations^{4,5}, hypercholesterolemia⁶⁻⁸, hypertriglyceridemia⁹⁻¹¹, diabetes mellitus^{12,13}, smoking^{8,14}, hyperhomocysteinemia¹⁵, and arterial hypertension^{1,16,17}.

Recent studies^{9,11,18} have suggested that high-fat diets with triglyceride-rich lipoproteins can influence the initial mechanisms of atherosclerosis, because they cause acute alterations in endothelial function, beginning 2 hours after their ingestion. This effect can persist for several hours and can be due to the acute elevation in postprandial triglyceride levels rather than to triglyceride and cholesterol levels during the fasting period. Vogel et al¹¹ have suggested that high-fat diets could influence the mechanisms of atherogenesis through a direct (endothelium-dependent) and an indirect (cholesterol-dependent) pathway. They have also suggested that low-fat diets could be important in the prevention and treatment of coronary artery disease, even in individuals with cholesterol levels considered to be risk-free¹¹. The endothelial cells would play an important local regulator role through the secretion of several substances that control vascular tone and structure¹⁹. The direct relation between cardiovascular risk factors and endothelial dysfunction may be very significant, because both play an important role in the genesis of thrombosis and instability of the atherosclerotic plaque.

Postmenopausal women, in addition to age and decreased serum levels of estrogens, frequently have other factors that affect endothelial function, such as arterial hypertension and dyslipidemia²⁰.

These factors have shown that atherosclerotic disease has an epidemiological impact²¹.

Observational^{22,23} and experimental²⁴ studies have suggested the existence of cardiovascular benefits of hormone replacement therapy, with positive effects on lipoprotein levels, a beneficial effect on the metabolism of carbohydrates and insulin²⁵, in addition to direct vascular effects, depending^{26,27} or not depending^{28,29} on endothelial participation. However, recent clinical studies on long-term hormone replacement therapy failed to confirm this protective effect against cardiovascular events^{30,31}. Nevertheless, in clinical practice, hormone replacement therapy continues to be effective in controlling vasomotor reactions (in the short run) and osteoporosis (in the long run).

This study aimed at assessing whether the short-term (2

weeks) use of estrogens in association with progesterone in postmenopausal women with systemic arterial hypertension, as an associated risk factor for endothelial dysfunction, changes serum lipid levels and vascular reactivity before and after meal.

Methods

This study comprised 15 postmenopausal women ranging in age from 50 to 70 (mean, 61.4 ± 6.06) years with a mean menopause duration of 13 ± 7.5 years. All of them were using antihypertensive drugs, except for 2, who controlled blood pressure with just a low-sodium diet.

The inclusion criteria of the study were as follows: 1) no contraindication to the use of estrogens; 2) age between 50 and 70 years; 3) natural or surgical menopause; 4) primary arterial hypertension, independent of blood pressure levels; 5) recent (less than one year) measurement of total cholesterol level below 239 mg/dL and of triglycerides below 250 mg/dL, in a blood sample collected after a 12-hour absolute fast; 6) the patient should not smoke, or should have quit smoking at least 2 years before; and 7) the patient should not have diabetes. The characteristics of the population studied are listed in table I.

This study was approved by the committee on science and ethics of the institution. All patients signed the written informed consent.

The patients were maintained on antihypertensive therapy with no modifications during the entire study. They were randomly assigned to receive one capsule per day of placebo or conjugated estrogens (0.625 mg) in association with medroxyprogesterone acetate (2.5 mg) for 2 weeks. After this period, all patients returned to the institution after a 12-hour fast, in the morning between 7 AM and 9 AM, to undergo the following procedures: 1) collection of peripheral venous blood from the left arm for measuring glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride levels; 2) ultrasound study of the right brachial artery with the Philips CVI system - SD-800 device and a 7-MHz linear transducer, with the patient in the dorsal decubitus position at a constant room temperature of 22°C. After a 10-minute rest for stabilization of the parameters, the diameter of the vessel was measured with B-mode ultrasound, and then, the diameter and blood flow in the artery were measured on CVI-Q-mode (Cardio Vascular Imaging - M mode) ultrasound as follows: a) at baseline - 5 measurements,

and calculation of the mean; and b) after one minute of reactive hyperemia produced by interruption of blood flow with inflation of the cuff of the sphygmomanometer (whose width was appropriate for the size of the forearm) placed on the upper middle third of the right forearm. A sufficient space was kept for placing the transducer in the lower third of the forearm on the trajectory of the brachial artery. The cuff was inflated up to 300 mmHg and maintained at such for 5 minutes, being then deflated. Three measurements were taken in the first minute of hyperemia, and the mean was calculated. After that, the patients ingested a diet standardized by the Service of Nutrition of the Instituto do Coração, with a total caloric value of 897.62 Kcal and consisting of 37 g (16.5%) of proteins, 50 g (50.1%) of lipids, and 75 g (33.4%) of carbohydrates. After 2 hours, the same sequence performed during the fasting period was performed to assess the postprandial levels. Blood pressure was measured in the left forearm with an aneroid sphygmomanometer with the patient at rest, during the fasting period, and 2 hours after the meal. Heart rate was also measured on the same occasions.

After this first phase of the study, the patients remained for 2 weeks without medication (wash-out). Then, for 2 other weeks, they ingested one capsule per day of the second drug in the morning while they were fasting. After that, they returned to the institution after a 12-hour fast for the second phase of the study, when the same first-phase sequence was repeated, therefore, ending the random, double-blind, placebo-controlled, crossover study.

The measurements of serum glucose, total cholesterol, HDL-cholesterol, and triglycerides were performed by using the automated enzymatic method, as was LDL-cholesterol measurement in the floating material of the precipitate of beta-lipoproteins. The LDL-cholesterol levels were calculated with the Friedewald formula³² in the cases with high triglyceridemia.

The technique chosen to calculate the diameter and the flow in the brachial artery was based on the M-mode ultrasonography technique, which provides the mean instantaneous variation in the systolic and diastolic diameters, coupled with the time-domain ultrasonography (CVI - Q: Cardio Vascular Imaging - M mode). The latter automatically measures, through a specific program, blood flow in the vessel studied³³.

The following variables were directly measured or calculated in the different conditions studied: arterial diameter in millimeters (mm) (ADI); arterial flow in milliliters per minute (mL/min) (FL); flow-mediated vascular reactivity (VR) in % of diameter variation obtained with the formula: $VR = 100 \times (ADI \text{ after reactive hyperemia} - \text{baseline ADI}) / \text{baseline ADI}$; blood measurements of glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides in milligrams per deciliter (mg/dL); heart rate (HR) in beats per minute (bpm); systolic blood pressure (SBP) in mmHg; diastolic blood pressure (DBP) in mmHg; and mean blood pressure (MBP) in mmHg calculated using the formula $DBP + (SBP - DBP) / 3$.

Initially, all variables were analyzed descriptively. The quantitative variables (age, weight, height, duration of menopause, and body mass index - BMI) were analyzed through the observation of minimum and maximum values and calculation of the means and standard deviations. For the qualitative variables (treatment of arterial hypertension), the absolute and relative frequencies were calculated.

To assess the behavior of the groups in regard to the lipids,

Table I - Characteristics of the patients

N = 15	mean \pm standard deviation
Age	61.4 \pm 6 years
Menopause duration	13 \pm 7.5 years *
Weight	62 \pm 9.7 kg
Height	1.54 \pm 0.06 m
Body mass index	26.14 \pm 3.6 kg/m ²
Treatment of hypertension	
Isolated low-sodium diet	02 (13,3%)
Diuretic	10 (66%)
ACE inhibitor	08 (53%)
Beta-blocker	03 (20%)
Calcium channel antagonist	01 (6.6%)

* 2 patients underwent oophorectomy 22 years ago.



considering the conditions studied, analysis of variance was used with repeated measurements. Because the supposition of data normality was rejected, the nonparametric Wilcoxon test was used for comparing flow-mediated vascular reactivity, expressed as a percentage of the diameter variation during the fasting period and 2 hours after the meal in the placebo and hormone replacement phases. The significance level adopted for the tests was 5%.

Results

Table II shows the values of the means and standard deviations of the variables analyzed with the use of placebo or hormone replacement therapy in the different phases of the study. The analysis of the percentage of diameter variation (VR) between baseline and one minute after reactive hyperemia (RH) with placebo showed a statistically significant difference (VR = $3.2 \pm 17\%$ during the fasting period) when compared with the values obtained 2 hours after the meal (VR = $-2.1 \pm 30\%$; $P=0.041$). All the other parameters analyzed during the fasting period and 2 hours after the meal in the patients receiving placebo and in those receiving hormone replacement therapy showed no statistically significant differences. The hormone replacement therapy caused no significant difference in VR during the fasting period and after the meal ($6.14 \pm 26.94\%$ during the fasting period versus $-0.05 \pm 18\%$ after the meal).

Table III shows the means and standard deviations of the blood measurements of glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides during the fasting period and 2 hours after the meal in the placebo and hormone replacement therapy phases.

Analysis of the mean blood levels of glucose and cholesterol and its fractions in the placebo and hormone replacement therapy phases showed no statistically significant changes between the fasting period and 2 hours after the meal. In regard to triglyceride measurement, a statistically significant increase was observed in the placebo phase ($P = 0.03$), but not in the hormone replacement therapy phase ($P=0.34$).

Figure 1 shows the difference in lipid variations in each group. A significant variation in the triglyceride levels is observed in the placebo phase, but not in the hormone replacement therapy phase.

Table IV shows the means and standard deviations of systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP), and heart rate (HR) during the fasting period and 2 hours after the meal in the placebo and hormone replacement therapy phases. The analysis of these parameters showed no statistically significant hemodynamic changes at any phase of the study.

Body mass index showed no variation at any phase of the study ($26.14 \pm 3.6 \text{ kg/m}^2$).

Discussion

Our study showed that the association of estrogens and progestogens decreased hypertriglyceridemia after a high-fat meal, resulting in a tendency towards abolishing the inhibition of the vasodilation observed with a fatty diet, which is an acute effect potentially reducing cardiovascular risk.

The increase in life expectancy leads to a progressively more prolonged time of menopause, increasing the chance of systemic arterial hypertension occurring as a comorbidity, as its prevalence increases with aging^{1-5,20}. In addition to arterial hypertension, dyslipidemia is one of the major risk factors. More recently, the acute elevation in postprandial lipemia was recognized to cause alterations in endothelial function, influencing the initial mechanisms of atherosclerosis⁶⁻¹¹. Thus, the increase in triglyceridemia after a high-fat diet by hindering full vasodilation could act as an additional cardiovascular risk factor in these women. Recently, some studies have assessed the impact of triglyceridemia on endothelial function. One study¹⁰, using nonphysiological triglyceride emulsion, reported a reduction in vascular reactivity, both the flow-mediated one and the one that is independent of flow. Vogel et al¹¹ caused endothelial dysfunction by administering a high-fat diet with triglyceride-rich lipoproteins to young adults of both sexes with no risk factors. Those authors showed that the endothelial dysfunction was transient and had a significant correlation with postprandial triglyceridemia. Similar results were obtained in another study³⁴, confirming the correlation of the flow-mediated reduction in vasodilation with the postprandial triglyceride levels, but not with the fasting triglyceride or other lipid serum levels.

In the recent past, hormone replacement therapy was considered a therapeutic approach for reducing cardiovascular risk²²⁻²⁴. This concept was reviewed after the publication of long-term controlled clinical studies, which did not confirm the benefits and showed an increase in cardiovascular events^{30,31}. Future studies assessing the mechanisms of hormone replacement therapy and allowing adequacy of different doses and administration routes of the hormones may still be an alternative.

When comparing the results during the fasting period in the placebo and in the hormone replacement therapy phases, the following values were obtained: TC, a 9.8% reduction; HDL-C, a 4% increase; LDL-C, a 1.5% reduction; and TG, a 0.5% increase. These results obtained 2 weeks after using the association of estrogen and progesterone are similar to, but less expressive than,

Table II - Arterial diameter (ADI), blood flow (FL), percentage of diameter variation (VR) between baseline and after one minute of reactive hyperemia (RH) with placebo and hormone replacement therapy (HRT) during the fasting period and 2 hours after the ingestion of a high-fat diet (mean and standard deviation)

	Fasting period		2 hours after the meal	
	Placebo	HRT	Placebo	HRT
ADI - baseline (mm)	4.04 ± 0.70	4.10 ± 0.50	4.05 ± 0.59	4.21 ± 0.63
ADI - 1 min after RH (mm)	4.32 ± 1.09	4.41 ± 1.10	3.94 ± 1.30	4.21 ± 0.94
VR - 1 min	3.20 ± 17.04 *	6.14 ± 26.94	- 2.1 ± 30 *	- 0.05 ± 18
FL - baseline (mL/min)	27.18 ± 13.54	30.17 ± 17.46	27.72 ± 11.65	33.90 ± 17.01

* $P = 0.041$

those obtained in other large studies^{22,23,35}. This may be due to the relatively short-term use of hormones and the prolonged time of menopause. In regard to the variations in lipid profile observed between the fasting period and 2 hours after meals, the comparison of the effect of the placebo and the hormone replacement therapy revealed a $35 \pm 25\%$ increase in TG with the use of placebo versus a $12 \pm 10\%$ increase in TG with hormone replacement therapy. This result showing a smaller increase in TG levels after a high-fat diet in women receiving hormone replacement therapy may be interpreted as a beneficial effect of the hormones.

Analyzing the behavior of vascular reactivity and triglyceridemia in our study, the placebo provided the result expected, ie, smaller vasodilation concomitant with an elevation in triglycerides in the postprandial period. On the other hand, hormone replacement therapy provided greater vasodilation with no statistical significance, followed by a smaller elevation in triglycerides as compared with that caused by the placebo, a statistically significant difference. However, when analyzing the mean values, the diameters in the hormone replacement therapy phase showed a trend towards being greater than those in the placebo phase. The possible justifications

for the lack of statistical significance were as follows: the advanced age of the patients; relatively prolonged time of hypoestrogenism; presence of arterial hypertension, even if well controlled; and relatively short-term hormone replacement therapy. These factors together may cause endothelial dysfunction not effectively improved by the action of hormone replacement therapy. Therefore, other studies with the same objective are required to rule out the factors cited, to confirm the benefit of hormone replacement therapy on endothelial dysfunction caused by the elevated triglyceridemia in the absorptive period of the digestive process. This period may be considered a risk factor for atherosclerosis³⁶.

The methodology used in this study for assessing endothelial dysfunction gained importance in the last decade with the reports by Celermajer et al³⁷. Those authors used the following noninvasive method to study flow-mediated vasodilation in the brachial or femoral arteries: arterial occlusion performed for 5 minutes in the patient's forearm, causing reactive hyperemia; after that, the cuff used for flow interruption was deflated, and a significant and variable increase (3 to 7 times) was caused by shear stress; this way, a flow-mediated vasodilation was obtained. Vascular B-mode ultrasonography has been used for measuring the arterial diameters, and vascular Doppler ultrasound has been used for measuring flow velocity. The variability of the method is acceptable, and the measurements have been reproduced in several laboratories³⁸. Flow-mediated vasodilation in the brachial artery has shown a good correlation with the measurements of coronary endothelial function³⁹. The major advantage of this method is its noninvasive nature, allowing repetitions of the measurements in the same patient or studies with a large number of cases.

Although blood flow may theoretically be measured with ultrasound through calculations based on the spectral velocity obtained on Doppler and the diameter or cross-sectional area of the vessel obtained on B mode, the errors are very significant, limiting its practical use³³. Recently, a new ultrasound method using time domain for measuring blood flow, the CVI-Q technique, has been introduced and become a practical alternative of excellent accuracy, proving to be adaptable to clinical examination in routine vascular procedures^{40,41}. The vessel diameter and area are automatically determined based on the velocity measurements, reducing the errors associated when separated measurements are performed. Consequently, instantaneous flow measurements are obtained, which cannot be obtained with other techniques of ultrasound measurement. The CVI-Q method measures the pulsatile and the continuous component of the flow wave. In this way, unlike the Doppler technique, this new technique, by measuring the diameter with M mode and time domain to process the velocity data, overcomes the limitations of other techniques and improves the ac-

	Fasting period		2 hours after the meal	
	Placebo	HRT	Placebo	HRT
TC (mg/dL)	213 ± 25	192 ± 17	211 ± 26	196 ± 19
HDL-C (mg/dL)	50 ± 7	52 ± 8	50 ± 8	52 ± 8
LDL-C (mg/dL)	136 ± 24	115 ± 18	129 ± 20	119 ± 23
TG (mg/dL)	131 ± 65*	132 ± 53	177 ± 113*	148 ± 86
Glucose (mg/dL)	92 ± 13	96 ± 14	105 ± 11	104 ± 20

* P = 0.03

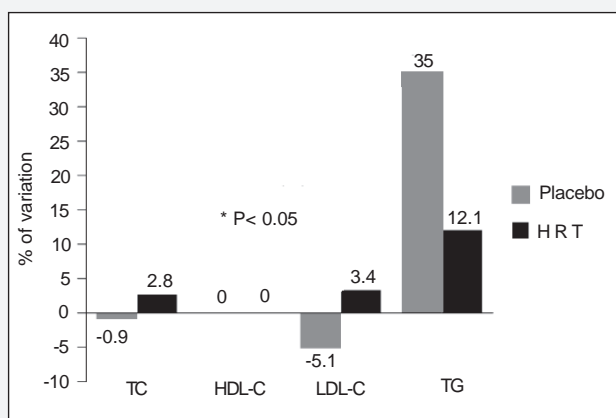


Fig. 1 – Lipid profile during the fasting period and 2 hours after the meal (percentage of variation) in the placebo and hormone replacement therapy phases.

	Fasting period		2 hours after the meal	
	Placebo	HRT	Placebo	HRT
SBP/DBP (mmHg)	147/92 ± 15/10	142/90 ± 15/8	141/87 ± 12/5	141/85 ± 13/4
MBP (mmHg)	110 ± 9	108 ± 10	105 ± 5	103 ± 6
HR (bpm)	67 ± 7	67 ± 9	69 ± 5	67 ± 7

P = NS



curacy of the blood flow data. Physiological variability remains a factor of error. This method allows the search for and choice of the best reading pattern based on an active search by the operator, and, in each monitor screening, a set of 7 to 10 flow waves are provided (depending on the heart rate on the occasion). In our study, this technique, still seldom used by us, proved to be interesting and very promising, although requiring an ultrasound device with more sophisticated and rather expensive technology.

In regard to limitations, despite the high prevalence of arterial hypertension among postmenopausal women, the population studied represents a special group, in which endothelial dysfunction may be more evident, and, therefore, not normalized by a relatively short-term (2 weeks) therapy, such as the one used in this study. Our findings cannot be extrapolated to other populations. Our study

used oral hormone replacement therapy, and, therefore, the effects of the transdermal therapy cannot be easily predicted. The transdermal route of administration has less evident effects on lipids, but still maintains vascular effects. Alternative routes should be studied in the future. Finally, the concomitant use of a progestogen in hormone replacement therapy may have attenuating effects on estrogen action. Only future studies comparing these formulations may answer these questions.

In conclusion, the short-term administration of conjugated estrogens in association with medroxyprogesterone in postmenopausal hypertensive women avoided elevation of triglyceride levels and showed a tendency towards abolishing the inhibition of vasodilating effects of a high-fat diet. These effects may represent an acute reduction in cardiovascular risk in this situation.

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