Effects of Estrogen/Medrogestone Therapy on the Apoprotein B-Containing Lipoproteins in Postmenopausal Women with Type 2 Diabetes Mellitus under Satisfactory and Non-satisfactory Glycemic Control

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Abstract

Background: Information is lacking on the effects of hormone replacement therapy in women with diabetes, especially during moderate chronic hyperglycemia.

Objectives: To study the effects of HRT on the lipid profile and the low density lipoprotein subclass distribution in women with type 2 diabetes under satisfactory and non-satisfactory glycemic control.

Methods: Fifty-four postmenopausal women after a 6 week run-in diet were randomized to receive either placebo (HbA1c < 8%, n = 13; HbA1c > 8%, n = 17) or HRT (HbA1c < 8%, n = 11; HbA1c > 8%, n = 13) for 12 weeks. HRT consisted of cyclical conjugated estrogens 0.625 mg/day plus medrogestone 5 mg/day. At the beginning and at the end of each treatment period the LDL subclass distribution was estimated by density gradient ultracentrifugation.

Results: At the baseline and during the study, the HbA1c level was significantly higher in hyperglycemic patients than in the near-normoglycemic controls (baseline 10.2 ± 2.9 vs. 6.5 ± 0.7%, $P < 0.01$). They showed a trend for higher total and LDL cholesterol, triglycerides and lower high density lipoprotein-cholesterol compared to near-normoglycemic controls, as well as significantly higher triglyceride concentrations in very low density lipoprotein, intermediate density lipoprotein and LDL-1 particles and cholesterol content in LDL-1 and -2 particles. HRT decreased LDL-cholesterol in both groups. In the normoglycemic patients a small increase in HbA1c was observed (6.5 ± 0.7 vs. 7.4 ± 1%, $P = 0.04$). In all cases, HRT did not modify the proportion of LDL represented by denser LDLs.

Conclusions: HRT did not modify the LDL subclass distribution, even in the presence of moderate chronic hyperglycemia in women with type 2 diabetes.

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Numerous studies have demonstrated that patients with type 2 diabetes have an increased cardiovascular risk. Moreover, cardiovascular events are the most frequent cause of mortality in this disorder [1]. There are several explanations for the development of atherosclerosis in type 2 diabetes; some could be modified with proper diagnosis and treatment. The lipid abnormalities fall into this category [2,3]. More than 75% of cases with type 2 diabetes have either hypercholesterolemia, hypertriglyceridemia or low concentrations of HDL-cholesterol [4]. The abnormal lipid levels could be due to chronic hyperglycemia, obesity/insulin resistance or the co-existence of other secondary (e.g., albuminuria, hypothyroidism, etc.) or primary dyslipidemias. Some lipid disturbances cannot be assessed with a simple lipid profile, since severe changes in the composition and subclass distribution of several classes of lipoprotein have been observed in patients, even in those with normal cholesterol and triglyceride concentrations. The accumulation of the smaller and denser subclasses of LDL is one of those changes. This defect has been proven to be an independent predictor for cardiovascular disease in diabetic and non-diabetic populations [5]. As a mean, cases with type 2 diabetes have a peak diameter significantly smaller than do non-diabetic subjects (26.2 ± 0.08 vs. 25.8 ± 0.1 nm, $P < 0.001$). Also, the proportion of LDL particles represented by the small dense subclass is higher in this condition (67.7 ± 1.7 vs. 58.6 ± 2.2%, $P < 0.005$). The appearance of small dense LDLs has been related to changes in the activity of the hepatic lipase and cholesterol ester transfer protein induced by the insulin resistance. Also, the diameter of LDL particles has a close relationship with glycemic control. Furthermore, the correction of hyperglycemia is associated with significant changes in LDL diameter and substantial modifications in the LDL particle distribution. The effects of diabetes on the concentrations of small dense LDLs seem to be greater in women than in men [6]. The recent demonstration that statins or fibrates could decrease cardiovascular mortality in diabetics reinforces the importance

HRT = hormone replacement therapy
LDL = low density lipoprotein
HDL = high density lipoprotein

for studying and treating the lipid disturbances in this entity [7,8].

The relative risk of having a cardiovascular event is greater in women than in men with type 2 diabetes [9]. The event rate sharply increases in the first few years after menopause, and estrogen deficiency is the most likely reason for the progression of atherosclerosis observed in postmenopausal women [10]. Several reviews and meta-analyses have concluded that estrogen replacement therapy decreases the risk of coronary heart disease by 35 to 50% [11–13]. However, the only published prospective trial failed to demonstrate a benefit in women with coronary heart disease [14]. Only about 10% of postmenopausal women in Britain use hormone replacement therapy [15], while a greater and growing percent of women receive this treatment in the USA [16]. However, information is lacking regarding the effect of different HRT modalities on cardiovascular mortality and coronary risk factors in patients with conditions such as diabetes, which is an increased cardiovascular risk [15].

Multiple mechanisms could contribute to reduce the progression of vascular lesions during estrogen therapy. Some of the possible protection may be attributed to reduced LDL-cholesterol concentrations, due to increased LDL receptor concentration. Increased HDL2-cholesterol and decreased lipoprotein(a) concentrations are other potentially protective actions of estrogen [17]. However, some of its effects on the lipid profile may be deleterious [18]. Estrogen decreases LDL particle size, while the hepatic production of triglycerides and the apoprotein B-containing lipoproteins are increased. At least two different groups have shown that estrogen therapy decreases the mean peak diameter of LDL particles [19–22]. This action is greater in women with an LDL pattern A (predominance in the LDL range of the large LDL subclass). The modification in LDL particle distribution could be explained either by a lower concentration of the larger LDLs or an increased amount of the smaller denser LDLs. Both mechanisms are possibly based on the estrogen-induced increased expression of the LDL receptor and the triglyceride enrichment of the LDL particles that occur during estrogen use. The first mechanism explains the lower amount of large LDLs, while the second one could contribute to the generation of small dense LDL particles. Campos and co-workers [23], using endogenous labeling of apoprotein B, suggest that the shift in relative distribution of LDL particles is caused by preferential lowering of the concentration of light LDL-1 particles and not to increased concentration of denser LDL particles. However, there is scant information about these effects for other forms of HRT. These data are urgently needed since only a minority of cases are treated with estrogen alone. In most women with a uterus a progestin is added to the treatment; these drugs had opposite effects to those described above.

Studies on the effects of estrogen on lipid metabolism have been performed in healthy postmenopausal women using only estrogen therapy. However, in clinical practice this is not the most common situation. Indeed, many postmenopausal women suffer from diabetes or other disorders, which could play the role of confounder when the effects of estrogens on the lipid metabolism are studied. Chronic hyperglycemia, glycosylation of lipoproteins, and reduced LDL receptor concentrations are some of the factors present in women with type 2 diabetes that can contribute to the accumulation of smaller denser LDLs. Also, insulin or glibenclamide may have different effects on LDL particle distribution [24]. In this study the effects on the LDL particle distribution of the most frequently used form of hormone replacement therapy were assessed against placebo in women with type 2 diabetes who had the same degree of glucose control during the entire study period. Subjects were studied under satisfactory glucose control (HbA1c $<8\%$) and after chronic moderate hyperglycemia (HbA1c 8–12%)

Material and Methods

Patients

The study group comprised women who were previously diagnosed with type 2 diabetes according to the ADA criteria and were 50–65 years of age. Participation in the study required that they had not had menses for at least one year, had not taken HRT during the previous 6 months, and that their body mass index was 28–35 kg/m^2. Patients were classified into two groups based on their HbA1c concentrations. The "satisfactory control group" consisted of women whose HbA1c was below 8% both at baseline and at least once during the previous year. The "non-satisfactory control group" included women whose HbA1c was between 8 and 12% both at baseline and at least once during the previous year before. Candidates were excluded if they had type 1 diabetes mellitus, uncontrolled hypertension, fasting triglycerides above 500 mg/dl, severe renal dysfunction, nephrotic syndrome, alcoholism (>10 drinks per week), active liver disease or hepatic enzyme elevation (serum aspartate or alanine aminotransferase levels >2.5 times the upper limit of normal), severe venous insufficiency in the lower limbs, history of venous thromboembolism, symptomatic angina pectoris or cardiac insufficiency, occurrence of a major vascular event within 3 months prior to screening, or diagnosis of a serious or chronic disease that would threaten the patient's safety or life expectancy. Concomitant use of anti-obesity medication, bile acid sequestrants, cyclosporin, insulin or any drug with potential effects on lipid metabolism were prohibited during the study and the preceding 3 months. After enrolment, patients were excluded if their fasting plasma glucose level was above 350 mg/dl on two different days or if their fasting triglyceride level was above 500 mg/dl in any measurement.

The Ethics Committee of Instituto Nacional de Ciencias Medicas y Nutricion approved the protocol and every patient provided witnessed, written informed consent prior to entering the study.

Study design

This was a double-blind placebo-controlled parallel-group study. It included a run-in 4 week diet period and a 12 week randomized treatment with placebo or hormone replacement therapy. Patients attended an initial screening visit at which an isocaloric diet was prescribed by a registered dietitian. This visit

was followed by a qualifying visit 2 weeks later. Within 2 weeks patients returned for a baseline visit when blood samples were collected and drug treatment was allocated if the HbA1c met the inclusion criteria. Patients who had qualified were randomized to receive matched placebo or conjugated equine estrogen 0.625 mg for 21 days plus medrogestone 5 mg for 10 days every month. The study medication was taken with breakfast. All patients were scheduled for a return visit every 3 weeks until week 12 (visits 3 to 7). Throughout the trial patients were required to comply with an isocaloric diet consisting of 50% carbohydrates, 10% protein, 30% fat and 30 g/day fiber. Dietary advice was given at the initial visit and compliance with the diet was assessed at every subsequent visit using a 3 day food record. Drug compliance was also measured at every visit.

Efficacy parameters

The primary efficacy evaluation was based on change in LDL particle distribution observed between baseline and the end of active treatment. The baseline value was obtained after the 4 week lead during the diet period. Secondary efficacy evaluations were based on the change from baseline to week 12 for total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol and HbA1c concentrations.

Safety evaluation

Before entering the baseline phase, a complete physical examination and clinical laboratory evaluation were performed. The laboratory evaluation included a blood count, pregnancy test, urine examination, creatine kinase levels, liver function tests and a glycemic profile (fasting plasma glucose and HbA1c). These tests were repeated at the end of the study. At each visit the liver function tests and glycemic profile were measured. Patients were excluded from the study if they developed severe hyperglycemia (>350 mg/dl) or any other significant deviation from safety tests. An ALT or AST concentration three times above the upper limit of normal on two consecutive measurements 1 week apart (+3 days) was considered as indication for exclusion from the study. Other reasons for dismissal were lack of compliance to the drug or diet.

Laboratory analyses

The laboratory of the Department of Endocrinology and Metabolism at our institute performed all lipid and clinical laboratory measurements using standardized procedures. This laboratory is certified for standardization of tests by the External Comparative Evaluation of Laboratories Program of the College of American Pathologists. Blood samples were taken after an overnight fast (9–12 hours). All laboratory analyses were performed with commercially available standardized methods. Glucose was measured using the glucose oxidase method, HbA1c using latex immunoagglutination inhibition (Bayer Laboratories). Total serum cholesterol and triglycerides were measured using an enzymatic method (SERA-PAK CV 3.3%). HDL-cholesterol was precipitated with phosphotungstic acid and Mg^{++} (CV 2.5%). LDL-cholesterol concentration was estimated by the Friedewald formula. Direct LDL-cholesterol was determined by ultracentrifugation (quantification) at baseline and at the end of the treatment and in every patient whose triglyceride levels were above 4.5 mmol/L. Apoprotein B concentration was measured by an immunonephelometric method. LDL subclass distribution was assessed with a density gradient ultracentrifugation method using a Beckman SW40 Ti rotor [25]. According to its density LDL particles were classified as light (d = 1.019–1.035 g/L) or dense LDL (d = 1.036–1.063 g/L). Twenty aliquots (0.5 ml) were collected in every gradient. Cholesterol, triglycerides and apoprotein B concentrations were measured in each aliquot [26].

Statistical analysis

Statistical analysis was performed with the Statgraphics program version 7.1. Differences between groups were evaluated using the two-tailed paired *t* test. All testing was two sided and conducted at a 5% level of significance. The sample size was based on the assumption that a change of 20% or greater in the proportion represented by the small dense LDLs would be considered as clinically significant.

Results

The study group comprised 54 postmenopausal women with type 2 diabetes. Twenty-four patients had a HbA1c <8% during and at the beginning of the study; the remaining 30 had a HbA1c >8%. After a 4 week diet run-in period the patients were randomized to receive either placebo (HbA1c <8, n = 13; HbA1c >8, n = 17) or HRT (HbA1c <8, n = 11; HbA1c >8, n = 13) for 12 weeks. No significant change in BMI, diet or physical activity was observed in any of the groups during the study.

The clinical characteristics of the women included in the study are shown in Table 1. As expected, hyperglycemic patients had higher levels of plasma triglycerides and lower concentrations of HDL-cholesterol, but these difference did not achieve statistical significance. These differences were greater in the results from the density gradient ultracentifugation. Hyperglycemic cases had significantly greater concentrations of cholesterol and triglycerides in the VLDL/IDL, light and dense LDL particles.

The effects of placebo and hormone replacement therapy in subjects with HbA1c <8% are shown in Table 2. As expected, no change in weight, glucose, HbA1c or lipid levels resulted from placebo treatment. HRT caused a small but statistically significant increase in HbA1c levels (6.6±1.1 vs. 7.5±1.1%, $P < 0.05$), but no change in plasma glucose was detected in the

ALT = alanine aminotransferase
AST = aspartate aminotransferase
BMI = body mass index
VLDL/IDL = very low/intermediate density lipoprotein

Table 1. Characteristics and lipid profile of the subjects at baseline

Variable	HbA1c <8% (n=25)	HbA1c >8% (n=30)	*P*
Age (yr)	56±2.9	54±5.8	NS
Diabetes duration (yr)	4.3±0.91	5.9±3.7	NS
BMI (kg/m^2)	29.6±4.5	30.4±4.1	NS
HbA1c (%)	10.3±2.5	6.1±1.2	<0.001
Fasting plasma glucose (mg/dl)	116±44	191±66	<0.001
Cholesterol (mg/dl)	222±39	226±38	NS
LDL-C (mg/dl)	142±37	146±31	NS
HDL-C (mg/dl)	51±13	46±10	NS
Triglycerides (mg/dl)	148±64	181±117	NS
Density gradient ultracentrifugation			
VLDL/IDL cholesterol (mg/dl)	65±27	99±21	<0.05
LDL1-cholesterol (mg/dl)	156±37	227±30	<0.01
LDL2-cholesterol (mg/dl)	95±34	112±26	<0.05
VLDL/IDL triglycerides (mg/dl)	105±55	162±72	<0.05
LDL-1 triglycerides (mg/dl)	42±22	73±21	<0.05
LDL-2 triglycerides (mg/dl)	24±20	27±12	<0.05

Data are expressed as mean±SD

The concentrations obtained from the density gradient ultracentrifugation correspond to the area under the curve in the corresponding density range. The gradient used in this report is not useful to separate the VLDL and IDL particles; these results are presented as a single density range.

Table 2. Effects of the estrogen/progestin therapy in women with HbA1c<8%

Variable	Placebo (n=13)	Estrogen/progestin (n=11)	*P*
HbA1c basal (%)	6.1±1.2	6.6±1.1	NS
HbA1c final (%)	6.3±0.9	7.5±1*	NS
LDL-cholesterol basal (mg/dl)	136±33	150±41	NS
LDL-cholesterol final (mg/dl)	137±27	124±38*	NS
Triglycerides basal (mg/dl)	157±73	137±51	NS
Triglycerides final (mg/dl)	145±50	156±64	NS
HDL-cholesterol basal (mg/dl)	50.7±13	51.1±13	NS
HDL-cholesterol final (mg/dl)	49.9±10	54.8±11	NS
Density gradient ultracentrifugation	**Baseline**	**Final**	
Estrogen/progestin group			
VLDL-IDL cholesterol	65±27	89±50	NS
LDL-1 cholesterol	170±84	111±51	0.05
LDL-2 cholesterol	91±34	65±19	NS
VLDL-IDL triglycerides	117±50	126±44	NS
LDL-1 triglycerides	38±17	76±37	<0.01
LDL-2 triglycerides	19±14	39±20	0.02
% LDL particles represented by light LD	66±29	63±18	NS
Placebo group			
VLDL-IDL cholesterol	71±45	81±36	NS
LDL-1 cholesterol	136±73	195±84	NS
LDL-2 cholesterol	102±22	89±11	NS
VLDL-IDL triglycerides	99±79	116±67	NS
LDL-1 triglycerides	54±32	54±18	NS
LDL-2 triglycerides	31±29	31±17	NS
% LDL particles represented by light LDL	59±24	67±16	NS

Data are expressed as mean±SD.

* $P < 0.05$ between baseline and final measurements.

The concentrations obtained from the density gradient ultracentrifugation correspond to the area under the curve in the corresponding density range. The gradient used in this report is not useful to separate the VLDL and IDL particles; these results are presented as a single density range.

fasting samples. Estrogen/progestin therapy significantly reduced LDL-cholesterol concentrations (150±41 vs. 124±38, $P<0.01$). Slightly higher concentrations of triglycerides and HDL-cholesterol concentrations were observed at the end of treatment, but these differences were not statistically significant. The mean percent of change in triglyceride concentration was 17% (range 0–41%). At the end of treatment only one of the 11 HRT-treated patients had fasting triglycerides above 200 mg/dl. These lipid changes were also observed in the results from the density gradient ultracentrifugation. An increased triglyceride content was found in all apoprotein B-containing lipoproteins. This change was statistically significant for both LDL subclasses. In addition, a decreased cholesterol content in the LDL subclasses was observed. This change was markedly greater for the light LDLs. The proportion of LDL (assessed by the area beyond the curve of the apoprotein B concentration on the LDL range) represented by the light and dense LDLs was not modified by the treatment.

The effects of placebo and hormone replacement therapy in patients with HbA1c >8% are shown in Table 3. Patients remained hyperglycemic during the whole study, as shown by the lack of modification between the baseline and final HbA1c concentrations. No change in weight, glucose, HbA1c or lipid levels resulted from placebo treatment. Unlike the former group, HRT had no impact on either the HbA1c or fasting plasma glucose concentrations. Regarding the lipid responses to the treatment, the changes followed the same trend but were significantly greater. Significantly higher concentrations of triglyceride concentrations were observed at the end of treatment; this change was markedly greater than in the cases with HbA1c <8% ($P<0.05$). One woman had fasting triglycerides above 1,000 mg/dl after 12 weeks of treatment, but no symptoms were related to this abnormality. Her baseline value was 427 mg/dl (the highest value of the study group). At the end of the treatment, 5 of the 13 women who received HRT had triglycerides above 200 mg/dl. The mean percent change in triglyceride concentration was 44% (range 0–112%). Slightly higher HDL-cholesterol compared to baseline was found at the end of the treatment, while estrogen/progestin therapy significantly reduced LDL-cholesterol concentrations (150±41 vs. 124±38, $P<0.01$). These changes were also observed in the results of the density gradient ultracentrifugation. An increased

Table 3. Effects of the estrogen/progestin therapy in the hyperglycemic patients

Variable	Placebo (n = 17)	Estrogen/ progestin (n = 13)	*P*
HbA1c basal (%)	10.2 ± 2.4	10.5 ± 2.7	NS
HbA1c final (%)	10 ± 2.5	0.6 ± 3.5	NS
LDL-cholesterol basal (mg/dl)	145 ± 29	150 ± 36	NS
LDL-cholesterol final (mg/dl)	140 ± 38	125 ± 45*	NS
Triglycerides basal (mg/dl)	176 ± 89	187 ± 151	NS
Triglycerides final (mg/dl)	162 ± 83	251 ± 244*	NS
HDL-cholesterol basal (mg/dl)	47 ± 9	45.1 ± 11	NS
HDL-cholesterol final (mg/dl)	51 ± 8	48 ± 9	NS
Density gradient ultracentrifugation	**Baseline**	**Final**	
Estrogen/progestin group			
VLDL-IDL cholesterol	99 ± 59	81 ± 54	NS
LDL-1 cholesterol	227 ± 87	184 ± 78	0.05
LDL-2 cholesterol	112 ± 69	104 ± 61	NS
VLDL-IDL triglycerides	162 ± 98	236 ± 192	0.01
LDL-1 triglycerides	73 ± 43	75 ± 46	NS
LDL-2 triglycerides	27 ± 15	42 ± 22	0.05
% LDL particles represented by light LDL	66.8 ± 16	63 ± 17	NS
Placebo group			
VLDL-IDL cholesterol	94 ± 47	75 ± 41	NS
LDL-1 cholesterol	221 ± 65	211 ± 70	NS
LDL-2 cholesterol	114 ± 60	119 ± 55	NS
VLDL-IDL triglycerides	192 ± 117	168 ± 120	NS
LDL-1 triglycerides	53 ± 31	61 ± 38	NS
LDL-2 triglycerides	3 ± 21	31 ± 20	NS
% LDL particles represented by light LDL	66 ± 16	64 ± 16	NS

Data are expressed as mean ± SD.

* $P < 0.05$ between baseline and final measurements.

The concentrations obtained from the density gradient ultracentrifugation correspond to the area under the curve in the corresponding density range. The gradient used in this report is not useful to separate the VLDL and IDL particles; these results are presented as a single density range.

triglyceride content of all apoprotein B-containing lipoproteins was found, which was statistically significant for the dense LDL subclass. Also, there was a decreased cholesterol content of the LDL subclasses, which was markedly greater for the light LDLs. The proportion of LDL (assessed by the area beyond the curve of the apoprotein B concentration on the LDL range) represented by the light and dense LDLs was not modified by the treatment.

Discussion

The data reported here clearly show that the effects of HRT on the lipid profile are modulated by the presence of chronic hyperglycemia in women with type 2 diabetes. Hypertriglyceridemia, the most troublesome adverse effect of estrogen therapy, was more common and severe in the presence of a HbA1c above 8%. Mean triglyceride concentration at the end of HRT was significantly higher in the hyperglycemic group (251 ± 244 vs. 156 ± 64 mg/dl, $P < 0.01$). Furthermore, in the group of women with HbA1c above 8%, chylomicronemia was precipitated in one patient and 38% had fasting triglycerides above 200 mg/dl after 12 weeks of HRT. In contrast, only one woman with HbA1c below 8% had abnormally high concentrations of triglycerides at the end of the treatment. Other effects on the lipid profile usually observed during HRT (e.g., lower LDL-cholesterol and higher HDL-cholesterol) seem not to have been affected by the HbA1c concentrations. The higher triglyceride concentrations observed at the end of HRT in the hyperglycemic women could be explained by either a higher baseline value or a greater hypertriglyceridemic response to estrogens during chronic hyperglycemia. Both mechanisms seem to have played a role in women with HbA1c > 8%, based on the higher baseline values and the greater percent change (17 vs. 44%, $P < 0.05$) during HRT. These data suggest that glucose control must be assessed before prescribing HRT to women with type 2 diabetes. In the presence of HbA1c above 8% and triglycerides above 200 mg/dl, the risk of having chylomicronemia and possible related complications (pancreatitis) might be higher than in women under satisfactory glucose control. To the best of our knowledge, no prospective study has reported this observation. The higher triglyceride response to HRT was reported in a cross-sectional study assessing the effects of HRT in women with and without diabetes, but the interaction with glucose control was not measured [27]. Additional studies assessing the safety of HRT in hyperglycemic women are required.

Our data are in accordance with the complex effects of estrogen and progestins on the lipoprotein metabolism. HRT resulted in triglyceride enrichment of almost every apoprotein B-containing lipoprotein, as shown in Tables 2 and 3. These results concur with the reports by Campos [23,28] who demonstrated that estrogen therapy increases the hepatic production of triglycerides and apoprotein B by 50%. These changes are counterbalanced by an increased expression of the LDL receptor, as this mechanism blunts any accumulation of apoprotein B-containing particles caused by the hepatic overproduction of apoprotein B. In fact, the overexpression of the LDL receptor decreased the LDL-cholesterol concentrations observed in this study. However, as discussed in the following paragraphs, the overproduction of triglycerides may change the composition and metabolism of the VLDL, IDL and LDL particles.

HRT did not modify the LDL particle distribution, even in the presence of chronic hyperglycemia. This may be the result of the combination of metabolic abnormalities with opposite consequences. Triglyceride enrichment of the LDL particles induced by HRT suggests that the production of the small dense LDL particles is increased, based on previous reports that triglyceride enrichment of the LDL particle is the main determinant for the formation of the small dense LDL [29].

However, this abnormality may be counterbalanced by the overexpression of the LDL receptor, which reduces the concentration of all LDL particles. The clearance of the light LDL by the LDL receptor is significantly greater, but this change indirectly reduces the amount of the dense LDL since the light LDL is the precursor for the production of the small dense particle [30]. The combination of these phenomena results in decreased concentration of both LDL subclasses and a similar proportion of the light and dense subclasses. These postulated mechanisms are in accordance with the significant reduction of LDL-1 cholesterol and the smaller change in LDL-2 cholesterol found in the density gradient ultracentrifugation, and the lower LDL cholesterol observed during HRT. If these conclusions are true, we postulate that the co-existence of any other disorder that down-regulates the LDL receptor (i.e., hypothyroidism, nephrotic syndrome) may disrupt this equilibrium [31], resulting in the accumulation of all apoprotein B-containing particles during HRT. This sequence of events may explain the higher triglyceride response observed during HRT in hyperglycemic women, which is due to the adverse effects of hyperglycemia on the function of the LDL receptor. As a result, triglyceride-rich particles are accumulated in plasma. However, this explanation does not fit the lack of modification of the LDL pattern during HRT and chronic hyperglycemia. Additional research, including kinetic studies using endogenous labeling of apoprotein B, is necessary to understand the complex interaction between the effects of HRT and chronic hyperglycemia.

The data presented here confirm that significant abnormalities in the composition of the apolipoprotein B-containing lipoprotein may exist in women with type 2 diabetes, even those with a "normal" lipid profile. The baseline lipid profile of the women with HbA1c above or below 8% were not statistically different, although higher triglycerides and lower HDL-cholesterol were found in the hyperglycemic group. However, when the results of the density gradient ultracentrifugation were compared large differences were found between the two groups. These data suggest that the fasting lipid profile may underestimate the lipoprotein abnormalities in subjects with type 2 diabetes [32].

A small but statistically significant increase in HbA1c concentrations was found after 12 weeks of HRT in women with baseline levels below 8%. This deleterious change may be explained by the known effects of the progestins on the insulin action [33]. This modification on insulin sensitivity does not seem to have a clinical effect in subjects with HbA1c above 8%. Unfortunately, the vast majority of studies evaluating the effects of HRT on glucose control have been done in non-diabetic women. Brussard et al. [34] reported that short-term estrogen therapy improves insulin resistance and has neutral effects on glucose control in women with type 2 diabetes. No study has previously evaluated the effect of the estrogen/progestin combination on insulin sensitivity and glucose control in women with type 2 diabetes.

HRT yields a mixture of possible beneficial and deleterious effects on lipoprotein metabolism. The triglyceride enrichment of apoprotein B-containing lipoprotein plus the prothrombotic and pro-inflamatory actions [35] of HRT may help to explain the lack of benefit observed in the Heart and Estrogen/Progestin Replacement Study (HERS). Our data demonstrate that some of the adverse effects of HRT may interact with chronic hyperglycemia, making it more frequent and severe. This report reinforces the importance of assessing the safety and efficacy of HRT in women with type 2 diabetes and the urgent need for additional studies in this subset of postmenopausal women.

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