Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 1527–1533
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Changes in plasma lipids and increased low-density lipoprotein susceptibility to oxidation in pregnancies complicated by gestational diabetes: consequences of obesity

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Received 18 January 2007; accepted 26 June 2007

Abstract

Dyslipidemia is associated with increased low-density lipoprotein (LDL) susceptibility to oxidation, a phenomenon associated with endothelial dysfunction, atherosclerosis, cell toxicity, and intrauterine growth retardation. The present study was designed to determine if women developing gestational diabetes mellitus (GDM) have both increased plasma lipids and LDL susceptibility to oxidation throughout pregnancy. We also wanted to study the effects of obesity upon these parameters. A nested case-control study was carried out in 45 women with uncomplicated pregnancies and 62 women diagnosed with GDM following the criteria of the American Diabetes Association. In all women, blood was drawn at 15, 24, and 32 weeks of gestation. Low-density lipoprotein oxidation was initiated by the addition of $CuCl_2$, and formation of conjugated dienes was monitored. Glucose, cholesterol, triglycerides, vitamin E, estradiol, and progesterone were determined. In GDM, elevated levels of glucose, cholesterol, and triglycerides were observed when compared with the control group even in the first trimester, before the detection of diabetes. In the control group, the lag phase in the LDL oxidation was 85.3, 84.4, and 95.6 minutes at 15, 24, and 32 weeks of pregnancy, compared with 63.3, 63.4, and 74.5 minutes in the GDM group ($P < .001$ in the 3 periods). These differences remained when adjusted for the body mass index. In a multiple linear regression analysis, a negative correlation was observed between the lag phase and the body mass index ($P < .001$) and cholesterol ($P < .001$), whereas a positive one appeared with vitamin E ($P < .05$) and time of gestation ($P < .001$). In pregnancy, GDM increases LDL susceptibility to oxidation. Obesity and hypercholesterolemia further exacerbate this effect.

1. Introduction

Gestational diabetes mellitus (GDM), one of the most common complications associated with pregnancy, has been associated with a higher risk of developing preeclampsia, fetal death, macrosomia, and other complications associated with this process, including shoulder dystocia, perinatal hypoglycemias, and respiratory distress [1-5]. Intrauterine growth retardation has also been linked to GDM [1-5]. To avoid these complications, major efforts have been conducted to normalize the plasma glucose levels in pregnancies complicated by GDM over the last few years. In contrast, less attention has been paid to dyslipidemia, frequently observed in women with GDM, and to low-density lipoprotein (LDL) oxidation, a process that could damage the placenta and could therefore lead to fetal death and intrauterine growth retardation. In fact, we have shown that increased LDL susceptibility to oxidation during pregnancy is associated with intrauterine growth retardation [6]. Low-density lipoprotein oxidation is modulated by different factors: some accelerate LDL oxidation, whereas others may retard the process. Hypercholesterolemia [7,8], androgens [9], progestogens [9], and high glucose concentration [10,11] are among the factors that may accelerate LDL oxidation. Estrogens [9,12] and vitamin E [13,14] retard the process.

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0026-0495/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2007.06.020

Therefore, because of dyslipidemia and hyperglycemia, pregnancies complicated by GDM may show accelerated LDL oxidation. Some authors have actually shown increased plasma levels of lipid peroxides in pregnancies complicated by GDM [15,16].

In view of the above considerations, in the present study, we wanted to determine throughout pregnancy the presence of lipid alterations in plasma and the LDL susceptibility to oxidation in women developing GDM. As obesity is associated with dyslipidemia, with a higher risk of developing GDM and increased oxidative stress [17], the effect of obesity upon these alterations was also studied.

2. Design

A nested case study was carried out from July 2001 to July 2004. During this time, all women attending the obstetrics clinic of the Foundation Hospital Alcorcón were asked to participate in the study. In our institution, we also follow immigrants, most of whom do not speak Spanish well; only white women who spoke Spanish fluently were included in the study. Under regular conditions, blood tests are performed here on all pregnant women at weeks 15, 24, and 32 of pregnancy, when, respectively, the triple screening test (β-human chorionic gonadotropin, α-fetoprotein, and determination of plasma estradiol levels to estimate the risk of neural tube defects and chromosomal alterations), the glucose challenge test, and different serologic determinations are obtained. After overnight fasting, a 15-mL sample of blood in EDTA (1 mg/mL) was obtained from each woman who volunteered to participate in the study. The blood was drawn at the time of regular laboratory tests, so the study did not cause extra clinical visits or vein punctures in participating women. Plasma was separated by centrifugation at 2000*g* for 10 minutes and divided into different aliquots. To preserve the LDL structure, one aliquot was stored in tubes containing sucrose, final concentration of 0.5%, and used for LDL isolation [18]. Another sample was stored in 1 mmol/L butylated hydroxytoluene and used for vitamin E measurement. The remaining plasma was stored without any further treatment until the different metabolic and hormonal parameters were analyzed. All samples were stored at −40°C.

After delivery, the medical records of all women participating in the study were reviewed. Pregnancy complications and outcomes that were documented in the electronic medical records were ascertained after delivery using specific search criteria. The prospective design of the study, in which all women were included before adverse pregnancy outcomes manifested, ensured that at weeks 15 and 24 of pregnancy, no subjects were known to have GDM.

During this period, 6082 women delivered in our institution; and one blood sample each from 1152 (18.9%) was obtained during one trimester. Of the 296 (4.8%) women who developed GDM during the time of the study, we obtained at least one blood sample during gestation in 62 of them (21%). Gestational diabetes mellitus was diagnosed following the criteria of the American Diabetes Association [19]. Briefly, a glucose challenge test was performed at week 24 of gestation on all pregnant women. All women with a glucose value greater than 140 mg/dL were submitted to an oral glucose tolerance test 1 hour after the administration of 50 g of glucose. Women with the diagnosis of GDM received dietary instructions, and insulin therapy was initiated following the criteria described by Metzger and Coustan [20]. Briefly, when in a 1- to 2-week interval, fasting glucose levels greater than 95 mg/dL or postprandial glucose levels greater than 140 or 120 mg/dL, respectively, 1 or 2 hours postprandial were observed more than twice, insulin therapy was started and doses changed to keep glucose under the above levels. Dietary instructions were given to all overweight women to limit the caloric intake to 2300 calories. Women with type 1 and type 2 diabetes mellitus were excluded from the study.

Among the women with GDM, 7 developed *hypertension*, defined as a rise in systolic blood pressure $\geq$150 mm Hg and diastolic blood pressure $\geq$90 mm Hg, in the third trimester. Urine proteins were not detected in a 24-hour urine collection in any of them. One had a newborn with a diaphragmatic hernia, one delivered a still newborn, and one underwent an induced abortion at week 20 because of a Down syndrome fetus.

As a control group, we used 45 pregnant women who also participated in the study during the 3 trimesters of gestation and delivered healthy newborn infants, with appropriate weight for gestational age, and who did not present any complications during pregnancy or delivery. Pregnancies with more than one fetus were excluded from the study.

The study was approved by the Foundation Hospital Alcorcón Ethics Committee, and all the women participating in the study gave informed written consent at the time of blood extraction.

3. Materials and methods

Low-density lipoprotein were isolated from EDTA-treated plasma obtained by ultracentrifugation following methods previously described in our laboratory [21] and passed through an Econo-Pac 10DG column (Biorad, Madrid, Spain) to remove EDTA and sucrose. The LDL protein concentration was determined immediately using the Lowry procedure [22]. Aliquots of 0.1 mg of LDL protein per milliliter were incubated with $CuCl_2$ (2.5 μmol/L). The formation of conjugated dienes was determined as described by Esterbauer et al [23]. In short, 1 mL of the LDL solution was incubated in a quartz cuvette at 37°C. Absorbance was read at 234 nm in a Beckman DU-640 spectrophotometer (Beckman Instruments, Fullerton, CA) every 10 minutes for a maximum of 4 hours or until the rapid phase of LDL oxidation reached a plateau. The lag phase was estimated as

Table 1
Characteristics of the subjects of the study

	Control group	Women with GDM
No. of women studied	45	62
Maternal age (y)	31.1 ± 0.5	32.2 ± 0.5
Gestational age (wk)	39.2 ± 0.2	38.7 ± 0.2
Birth weight observed (g)	3227.6 ± 38.8	3197.3 ± 7.5
Birth weight ratio	1 ± 0.01	1.01 ± 0.01
Sex (M/F)	25:25	27:33
Cigarette smoking	5 (10%)	17 (27%) *
Hemoglobin A_{1c} (%)	–	4.73

Values are expressed as the mean (SD). Birth weight ratio was determined by dividing the observed birth weight by the expected weight for gestational age using the tables of Delgado et al [24]. "*" denotes difference between control group and the group of women with GDM. M indicates male; F, female.

* $P < .05$.

the incubation time corresponding to the intersection of 2 lines drawn from the changes in optical density: one through the initial, slowly rising curve, which corresponds to the use of the endogenous antioxidants, and the other, a subsequent, rapidly rising curve, which corresponds to the propagation phase of the LDL oxidation [23]. The lag phase was expressed as minutes from the addition of $CuCl_2$. The slope was determined by linear regression and expressed as micromoles of conjugated dienes generated per minute.

Because most of the chemicals commonly used in the laboratory, including phosphate buffer saline, may contain metal ions as contaminants, all the solutions used in the experiments were treated with Chelex (iminodiacetic acid chelating resin; Sigma, Barcelona, Spain) to remove any metal traces.

Triacylglycerol and cholesterol in plasma were measured using a commercial kit (Triglycerides Enzymatic Trinder Method; Menarini Diagnostics, Florence, Italy). The inter- and intraassay coefficients of variation (CVs) for all lipid measures were less than 3%. Estradiol and progesterone were determined using the Vitros Auto Analyzer (Johnson & Johnson, Skillman, NJ). The inter- and intraassay CVs were less than 7% and less than 5%, respectively. Vitamin E in plasma was determined by high-performance liquid chromatography following methods previously described in our laboratory [21]. The interassay CV was less than 7.2% and the intraassay CV, less than 4.9%.

4. Statistical analysis

Results are expressed as the mean and the standard deviation (SD). The distribution of continuous variables was examined with the Shapiro-Wilks test. The χ^2 test was used to evaluate differences between categorical variables. Differences between the parameters obtained in normal pregnancies and those complicated by GDM were obtained using the Student t test. The difference among the 3 trimesters of the pregnancy was estimated in both groups by a general linear model of repeated measures. A multiple linear regression analysis was used to study the relationship between cholesterol, triglycerides, vitamin E, body mass index (BMI), time of gestation, estradiol, and progesterone with the lag phase. The statistical analysis was performed using the SPSS 12.0 program (SPSS Institute, Paris, France).

5. Results

Characteristics of the study subjects are shown in Table 1. No differences were observed regarding either maternal and gestational age between women with pregnancies complicated by GDM and the control group (Table 1). Birth weight and the birth weight ratio were similar in both groups (Table 1). Nine (14.3%) of the newborns from GDM mothers showed a birth weight above the 90th percentile, whereas

Table 2
Anthropometric parameters and treatment of GDM

	Total	Control group		Total	GDM group	
		BMI ≤25 kg/m^2	BMI >25 kg/m^2		BMI ≤25 kg/m^2	BMI >25 kg/m^2
No. of subjects studied	45	34	11	62	20	42
Height (m)[a]	1.6 ± 0.01	1.6 ± 0.05	1.62 ± 0.06	1.6 ± 0.01***	1.6 ± 0.01**	1.6 ± 0.01
Weight (kg)[a]	62.7 ± 1.38	58.4 ± 4.80^{+++}	75.2 ± 8.5	70.8 ± 1.72***	56.9 ± 6.6^{+++}	77.4 ± 10.61
BMI (kg/m^2)[a]	23.3 ± 0.53	21.6 ± 1.70^{+++}	28.5 ± 2.4	27.8 ± 0.68***	22.2 ± 2.1^{+++}	30.5 ± 4.22
Weight gain during pregnancy (kg)	12.6 ± 0.6	13.2 ± 3.30	12.2 ± 5.4	9.2 ± 1.12*	11.7 ± 4.10^{+++}	6.6 ± 4.05***
GDM treatment						
Diet alone	–	–	–	28 (46%)	11 (55%)	17 (40.5%)
Diet + insulin	–	–	–	34 (54%)	9 (45%)	25 (59.5%)

Values are expressed as the mean (SD). "*" denotes differences between control group and the group of women with GDM, belonging to the same group of BMI. "+" denotes differences between the same group (control or GDM), but different BMI.

[a] Data obtained before pregnancy.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

$^{+++}$ $P < .001$.

Table 3
Levels of fasting plasma glucose, cholesterol, and triglycerides in the subjects of the study

	Control group			GDM group		
	Total	BMI ≤25 kg/m^2	BMI >25 kg/m^2	Total	BMI ≤25 kg/m^2	BMI >25 kg/m^2
Glucose (mmol/L)						
15 wk of pregnancy	4.4 ± 0.42 (45)	4.5 ± 0.42 (34)	4.8 ± 0.33 (11)	5.2 ± 1.16 (47)	4.9 ± 0.44 (15)	5.3 ± 1.39 (32)
32 wk of pregnancy	4.4 ± 0.49 (45)	4.3 ± 0.40 (34)	4.7 ± 0.62 (11)	4.6 ± 0.50 (60)	4.5 ± 0.53 (19)	4.7 ± 0.48 (41)
Cholesterol (mmol/L)						
15 wk of pregnancy	4.3 ± 1.32 (45)	4.2 ± 1.40 (34)	4.1 ± 0.70 (11)	5.2 ± 1.27 *** (36)	5.0 ± 1.52 (12)	5.4 ± 1.13 **
24 wk of pregnancy	5.0 ± 1.55 (45)	5.2 ± 1.65 (34)	4.2 ± 0.88 (11)	5.8 ± 1.55 (33)	5.8 ± 1.40 (12)	5.8 ± 1.65 *** (21)
32 wk of pregnancy	5.9 ± 1.65 (45)	6.0 ± 1.58 (34)	5.4 ± 0.96 (11)	7.2 ± 1.65 *** (30)	7.9 ± 1.71 *** (14)	6.6 ± 1.40*,+ (16)
Triglycerides (mmol/L)						
15 wk of pregnancy	0.8 ± 0.33 (45)	0.8 ± 0.33 (34)	1.1 ± 0.33+ (11)	1.14 ± 0.46 *** (36)	1.1 ± 0.62 * (12)	1.2 ± 0.36 (24)
24 wk of pregnancy	1.2 ± 0.44 (45)	1.1 ± 0.45 (34)	1.3 ± 0.38 (11)	1.6 ± 1.64 ** (33)	1.8 ± 0.62 ** (12)	1.6 ± 0.68 (21)
32 wk of pregnancy	1.7 ± 0.65 (45)	1.7 ± 0.59 (34)	1.8 ± 0.10 (11)	2.1 ± 0.94 * (30)	2.4 ± 1.08 ** (14)	1.8 ± 0.70 (16)

Values are expressed as the mean (SD). "*" denotes differences between control group and the group of women with GDM, belonging to the same group of BMI. "+" denotes differences between the same group (control or GDM), but different BMI.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

$^+$ $P < .05$.

11 (17.5%) showed a birth weight below the 10th percentile for their gestational age and sex. As would be expected from the selection of subjects, in the control group, all the newborns showed a birth weight adequate to their gestational age. A higher number of current smokers (6-10 cigarettes per day) were observed in the GDM group when compared with the control group (Table 1). In each group, no differences were observed between smokers and nonsmokers upon these parameters (data not shown).

The initial body weight and the BMI in women with GDM were higher than those in the control group (Table 2). To determine the effects of overweight/obesity on the different parameters studied, the participating women were divided into 2 groups based on their BMI before pregnancy: BMI ≤25 kg/m^2 (lean group) and BMI >25 kg/m^2 (obese group). The weight gain in the GDM group was lower than that in the control group, although when GDM women were divided according to their BMI, the weight gain in the lean GDM women was similar to that in the control group; in contrast, in the obese GDM group, it was lower.

Forty-six percent of the diabetic mothers received dietary treatment during pregnancy and 54%, insulin. In the number of women receiving insulin therapy (Table 2), no statistically significant differences were observed between the lean and overweight group, although a trend toward a higher rate of insulin treatment was observed in the obese group.

At week 15 of pregnancy, the fasting plasma levels of glucose were higher in the GDM group than those in the control group, although they remained in the reference range, independent of the BMI (Table 3). In contrast, at week 32 of pregnancy, after the diagnosis and treatment of the GDM, no differences were observed in the plasma levels of glucose between women with GDM and the control group (Table 3). Plasma values of cholesterol and triglycerides at week 15 of

Table 4
Levels of vitamin E, estradiol, and progesterone in the subjects of the study

	Control group			GDM group		
	Total	BMI ≤25 kg/m^2	BMI >25 kg/m^2	Total	BMI ≤25 kg/m^2	BMI >25 kg/m^2
Vitamin E (μg/mg CHOL + TG)						
15 wk of pregnancy	4.49 ± 1.64 (45)	4.75 ± 1.76 (34)	3.84 ± 0.95 (11)	4.04 ± 2.16 (35)	4.37 ± 1.69 (11)	3.89 ± 2.36 (24)
24 wk of pregnancy	4.17 ± 1.63 (45)	4.34 ± 1.85 (34)	3.69 ± 0.77 (11)	3.54 ± 1.20 (29)	3.39 ± 0.54 (8)	3.59 ± 1.38 (21)
32 wk of pregnancy	3.78 ± 1.85 (45)	4.05 ± 2.11 (34)	3.21 ± 0.82 (11)	3.47 ± 0.96 (31)	3.39 ± 0.61 (15)	3.46 ± 1.20 (16)
Estradiol (ng/mL)						
15 wk of pregnancy	2.84 ± 1.34 (45)	2.95 ± 4.43 (34)	2.69 ± 1.34 (11)	3.28 ± 1.94 (37)	3.22 ± 1.97 (12)	3.31 ± 1.97 (25)
24 wk of pregnancy	8.69 ± 4.86 (45)	8.90 ± 4.96 (34)	8.79 ± 5.40 (11)	9.09 ± 5.36 (26)	9.70 ± 4.46 (7)	8.86 ± 5.75 (19)
32 wk of pregnancy	15.48 ± 6.98 (45)	16.07 ± 6.91 (34)	14.82 ± 8.07 (11)	14.74 ± 6.41 (25)	16.44 ± 6.61 (12)	3.17 ± 6.05 (13)
Progesterone (ng/mL)						
15 wk of pregnancy	31.51 ± 11.41 (45)	33.63 ± 10.95 (34)	28.82 ± 11.39 (11)	30.65 ± 13.69 (37)	30.12 ± 14.84 (12)	30.90 ± 13.41 (25)
24 wk of pregnancy	59.05 ± 29.34 (45)	59.58 ± 30.02 (34)	64.67 ± 30.91 (11)	52.74 ± 26.69 (25)	56.62 ± 25.50 (7)	51.23 ± 27.71 (18)
32 wk of pregnancy	155.34 ± 65.60 (45)	165.30 ± 72.28 (34)	133.61 ± 36.79 (11)	138.95 ± 61.02 (25)	157.78 ± 68.12 (12)	121.57 ± 50.12 (13)

Values are expressed as the mean ± SD.

pregnancy were higher in women with GDM than those in the control group, independent of their initial BMI (Table 3). In the 2 groups studied, the plasma levels of triglycerides and cholesterol increased during the course of pregnancy (Table 3), although to a lower extent in the GDM obese group (Table 3). During the third trimester of pregnancy in obese women, no differences were observed in either plasma triglycerides or cholesterol between the control and the GDM group.

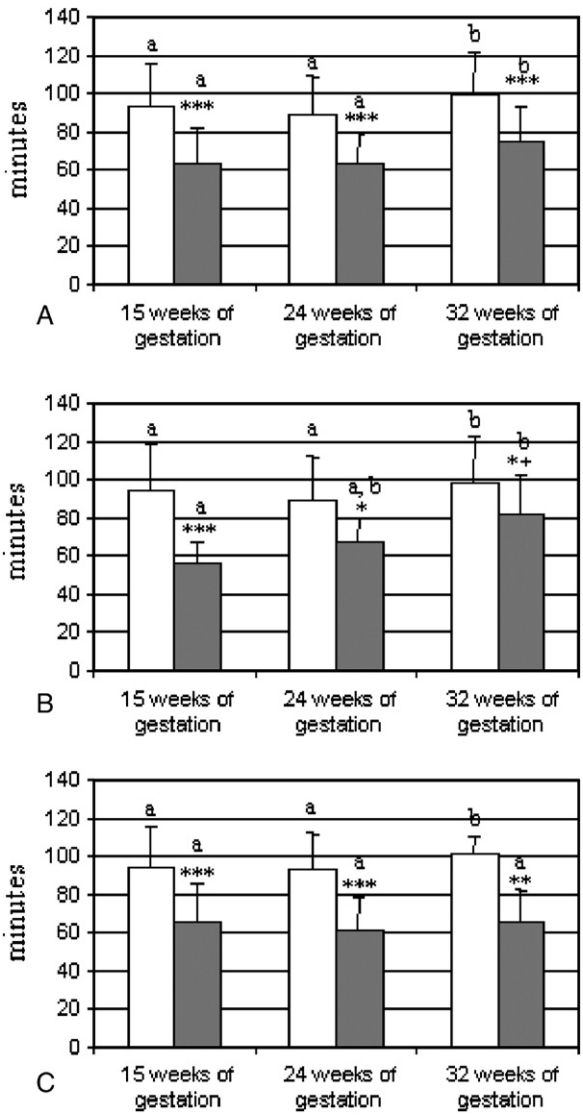

Fig. 1. Lag phase in the LDL oxidation in pregnant women with uncomplicated pregnancies (white column) and with pregnancies complicated by GDM (black column). A, All the women included in the study, independent of their BMI (43-45 women in the control group and 26-31 in the GDM group, depending on the trimester). B, Includes women with a BMI ≤25 kg/m^2 (28-30 in the control group and 8-14 in the GDM group). C, Women with a BMI >25 kg/m^2 (10 in the control group and 14-20 in the GDM group). Values are expressed as the mean (SD). "*" denotes the difference between the control group and pregnancies complicated by GDM (*P < .05, ***P < .001), with the same BMI. "+" denotes differences within the same group of either the GDM or control group, with different BMI (+P < .05). Different letters above the columns show statistically significant differences between trimesters in the same group.

No differences were observed along the pregnancy in the plasma levels of vitamin E, estradiol, and progesterone between the control and GDM groups (Table 4), even when both groups were divided into 2 groups based on their BMI.

When LDL from the different subjects was submitted to oxidation, the lag phase in the formation of conjugated dienes was shorter in the GDM group than that in the control group throughout pregnancy (Fig. 1). These differences remained when both groups were divided by their BMI into lean (BMI ≤25 kg/m^2) and obese (BMI >25 kg/m^2) groups before pregnancy (Fig. 1). Both in the control and GDM groups, the lag phase increased at week 32 when compared with the lag phase in LDL oxidation from weeks 15 and 24 (Fig. 1). However, when the subjects were divided by the BMI, we did not find an increased lag phase during the third trimester in obese women with GDM (Fig. 1).

Overall, no differences were observed in the lag phase between smokers and nonsmokers. When the lag phase in the GDM group was studied, there were a higher number of smokers in the GDM group; so we divided the women between nonsmokers and smokers. No differences were found regarding the lag phase in the LDL susceptibility to oxidation during the 3 trimesters of the study (62.2 ± 20.2 vs 67.8 ± 13.9, 66.0 ± 17.3 vs 58.3 ± 12, 76.0 ± 19.4 vs 71.2 ± 21.2 minutes, respectively, in nonsmokers vs smokers at weeks 15, 24, and 32 of gestation).

Because LDL susceptibility to oxidation may be affected by plasma levels of cholesterol, triglycerides, vitamin E, estradiol, progesterone, degree of obesity, and the time of gestation, a multiple linear regression analysis was performed including all these parameters. Using this model, the lag phase was negatively related to cholesterol (P < .001) and BMI (P < .001) and positively correlated to the gestational age (P < .001) and vitamin E (P < .05), with an R = 0.42.

6. Discussion

Present results show an increased LDL susceptibility to oxidation in pregnancies complicated by GDM, as well as increased plasma levels of cholesterol and triglycerides. These findings are already present at week 15 of pregnancy, even before the diagnosis of diabetes.

There are several metabolic alterations as well as increased incidence of obesity linked to obesity [3,5]. Among these metabolic alterations, there are several factors that could increase LDL susceptibility to oxidation. To elucidate factors involved in the higher LDL susceptibility to oxidation observed in LDL from women with GDM, 2 approaches were carried out. First, all the women included in the study were divided into 2 groups according to their BMI before pregnancy: one with the BMI ≤25 kg/m^2 (lean group) and the other group with the BMI >25 kg/m^2 (obese group). Under these conditions, women with GDM showed a higher LDL susceptibility to oxidation than the control group, independent of their BMI. Nevertheless, as gestational age

increases, a decreased LDL susceptibility to oxidation was observed in the control group and the GDM lean group. This finding is in agreement with the results showed by other authors in uncomplicated pregnancies [25]. In contrast, this result was not observed in the GDM obese group, where the lag phase in the LDL oxidation did not change throughout pregnancy, suggesting that in GDM women, obesity blunted the decreases in LDL susceptibility to oxidation observed at the end of gestation in normal pregnancies.

In a second approach, a multiple linear regression analysis was performed, including gestational age, BMI before pregnancy, cholesterol, triglycerides, estradiol, progesterone, and vitamin E. With this analysis, we found that both cholesterol and the BMI before pregnancy increased LDL susceptibility to oxidation. These findings were expected because in nonpregnant conditions, hypercholesterolemia has been associated with increased LDL susceptibility to oxidation [7,8]. Obesity has also been related to increased oxidative stress. Indeed, nonpregnant obese subjects have increased plasma levels of lipid peroxides [26,27] and increased oxidative stress in adipose tissue [17]. If these oxidized lipids were incorporated into the LDL, their susceptibility to oxidation might increase. In contrast, the gestational age and the plasma vitamin E acted, preventing the LDL oxidation, which is in agreement with the results presented by other authors: as pregnancy advances, there is a decreased LDL susceptibility to oxidation [25]. Higher plasma levels of vitamin E have also been related to decreased LDL susceptibility to oxidation. [23,28]. The absence of a relationship between the lag phase in LDL susceptibility to oxidation and the plasma levels of estradiol, a powerful antioxidant [9,12], was somewhat surprising. In the present analysis, the effect of estradiol upon LDL oxidation was blunted by the time of gestation. Nevertheless, if only samples from the third trimester, the time point when plasma estradiol levels reach the highest levels, were included in the study, estradiol acted as an LDL antioxidant, increasing the lag phase ($r = 0.239$; $P < .05$).

In general, the present results show that LDL susceptibility to oxidation increases in pregnancies complicated by GDM. This higher LDL susceptibility to oxidation is due to diabetes per se, as well as to the increased incidence of obesity and hypercholesterolemia found in these women.

How could increased LDL oxidation from pregnancies complicated by GDM affect fetal growth? Women with GDM have increased plasma levels of glucose and triglycerides, which could lead to an increased supply of nutrients to the fetus, increasing fetal growth and causing macrosomia [1-5,29] In contrast, the increased LDL susceptibility to oxidation may lead to placental damage and to alterations in the placental blood flow, affecting fetal growth. Low-density lipoprotein oxidation is cytotoxic for placental cells [30] and may affect endothelial relaxation [31,32]. In a previous study, we have shown an increased LDL susceptibility to oxidation in pregnancies with IUGR [6]. Other authors have shown that hydrogen peroxide enhances the contraction of the placental blood vessels in placentas from GDM when compared with placentas from normal pregnancies [33]. Therefore, an increased LDL oxidation, which can generate lipid peroxides, may decrease the placental blood flow and damage the placenta. This would compromise the supply of nutrients toward the fetus, leading to intrauterine growth retardation, as occurs in some pregnancies complicated by diabetes.

In the GDM group, we have observed as many newborns with low birth weight (17.5%) as with macrosomia (14.3%). This finding was also observed when the birth weights of all newborns from pregnancies complicated by GDM followed in our institution during the period of the study were analyzed. During this time, 296 women were diagnosed with GDM; and whereas 40 of them (13.5%) delivered newborns with a birth weigh below the 10th percentile, only 27 (9.1%) delivered newborns with a birth weight above the 90th percentile for their gestational age ($P < .001$). These results show that, at least in our population, GDM women following a fairly strict control of blood glucose may have a tendency to deliver small babies rather than large ones. Under these conditions, the deleterious effects of LDL susceptibility to oxidation upon fetal growth predominate over the growth-stimulating effects of glucose and triglycerides, mainly when major efforts are made to keep glucose in the reference range.

Pregnancies complicated by both GDM and obesity have been associated with an increased rate of congenital malformation [34,35], even during the first trimester of pregnancy when GDM is undiagnosed and when, in most cases, hyperglycemia is absent. It is attractive to speculate that increased LDL oxidation and the lipid peroxides generated during this process may play a role in the congenital malformations associated with GDM and obesity. In the experimental animal model, the oral administration of lipid peroxides generated from heated culinary oils increases the rate of congenital malformations [36]. Further studies need to be made to show the possible relationship between increased LDL susceptibility to oxidation and the higher incidence of congenital anomalies observed in GDM pregnancies.

In the present study, we were able to perform a longitudinal study only in the control group, with the analysis of the same women throughout pregnancy. However, in the GDM group, because of the recruitment problems, we were unable to follow the same women through pregnancy (only 8 women of the whole GDM population were studied during the 3 trimesters); and we could not perform a longitudinal study, rather a cross-sectional one. Therefore, in the GDM group, we were unable to analyze the changes in LDL susceptibility to oxidation throughout pregnancy. Nevertheless, as we found statistically significant differences in plasma glucose, cholesterol, triglycerides, and LDL susceptibility to oxidation between the GDM and control groups throughout pregnancy, it is clear that both populations have relevant metabolic

differences. What is more remarkable is that these differences are present even before the diagnosis of GDM.

In summary, during pregnancy, the development of GDM leads to increased LDL susceptibility to oxidation, as well as to increased plasma levels of triglycerides and cholesterol. These findings are already present at week 15 of gestation, before the diagnosis of gestational diabetes. Increased LDL oxidation may be involved in some of the complications associated with gestational diabetes, including intrauterine growth retardation, preeclampsia, and a higher risk of congenital malformations.

Acknowledgments

This work was supported by grants from the Spanish Ministry of Health (FIS 00/0304 and 02/1731) and from the University San Pablo-CEU (14/01). We would like to acknowledge the technical assistance of Mariano Lillo and the editorial help of Dr Boyd Metzger and Ms Linda Hamalainen.

References

[1] Persson B, Hanson U. Neonatal morbidities in gestational diabetes mellitus. Diabetes Care 1998;21(Suppl):B79-B84.
[2] Boyd EM, Usher RH, McLean FH. Fetal macrosomia: prediction, risks, proposed management. Obstet Gynecol 1983;144:768-73.
[3] Buchanan TA, Xiang AH. Gestational diabetes mellitus. J Clin Invest 2005;115:485-91.
[4] American Diabetes Association. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 2000; 26(Suppl):S5-S20.
[5] Kjos SL, Buchanan TA. Current concepts: gestational diabetes mellitus. N Engl J Med 1999;341:1749-56.
[6] Sánchez-Vera I, Bonet B, Viana M, Quintanar A, López-Salva A. Increased low-density lipoprotein susceptibility to oxidation in pregnancies and fetal growth restriction. Obstet Gynecol 2005;106:345-51.
[7] Lavy A, Brook GJ, Dankner G, Amotz AB, Aviram M. Enhanced in vitro oxidation of plasma lipoproteins derived from hypercholesterolemic patients. Metabolism 1991;40:794-9.
[8] Cominacini L, Garbin U, Pastorino AM, Davoli A, Campagnola M, De Santis A, et al. Predisposition to LDL oxidation in patients with and without angiographically established coronary artery disease. Atherosclerosis 1993;99:63-70.
[9] Zhu XC, Bonet B, Knopp RH. 17β-Estradiol, progesterone, and testosterone inversely modulate low-density lipoprotein oxidation and cytotoxicity in cultured placental trophoblasts and macrophages. Am J Obstet Gynecol 1997;177:16-209.
[10] Otero P, Herrera E, Bonet B. Dual effect of glucose on LDL oxidation. Dependence on vitamin E. Free Radic Biol Med 2002;33:1133-40.
[11] Kawamura M, Heinecke JW, Chait A. Pathophysiological concentrations of glucose promote oxidative modification of low density lipoprotein by a superoxide-dependent pathway. J Clin Invest 1994;94:771-8.
[12] Shwaery GT, Joseph A, Vita JA, Keaney JF. Antioxidant protection of LDL by physiological concentrations of 17b-estradiol. Requirement for estradiol modification. Circulation 1997;95:1378-85.
[13] Esterbauer H, Dieber-Rotheneder M, Striegl G, Waeg G. Role of vitamin E in preventing the oxidation of low-density lipoprotein. Am J Clin Nutr 1991;53:S314-21.
[14] Esterbauer H, Gebicki J, Puhl H, Jurgens G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. Free Radic Biol Med 1992;13:341-90.
[15] Toescu V, Nuttall SL, Martin U, Nightingale P, Kendall MJ, Brydon P, et al. Changes in plasma lipids and markers of oxidative stress in normal pregnancy and pregnancies complicated by diabetes. Clinical Science 2004;106:93-8.
[16] Toescu V, Nuttall SL, Kendall MJ, Martin U, Dunne F. Women with gestational diabetes should be targeted to reduce cardiovascular risk. BMJ 2002;325:359.
[17] Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004;114:1752-61.
[18] Rumsey SC, Galeano NF, Arad Y, Deckelbaum RJ. Cryopreservation with sucrose maintains normal physical and biological properties of human plasma low density lipoproteins. J Lipid Res 1992;33:1551-61.
[19] American Diabetes Association. Gestational diabetes mellitus. Diabetes Care 2002;25(Suppl 1):S94-6.
[20] Metzger BE, Coustan DR. Summary and recommendations of the Fourth International Workshop-Conference on Gestational Diabetes Mellitus. The organizing committee. Diabetes Care 1998;21: B161-7.
[21] Viana M, Barbas C, Bonet B, Bonet MV, Castro M, Fraile MV, et al. In vitro effects of a flavonoid-rich extract on LDL oxidation. Atherosclerosis 1996;123:83-91.
[22] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265-75.
[23] Esterbauer H, Striegl G, Puhl H, Rotheneder M. Continuous monitoring of in vitro oxidation of human low density lipoprotein. Free Radic Res Commun 1989;6:67-75.
[24] Delgado P, Melchor JC, Rodríguez-Alarcón J. Curvas de desarrollo fetal de los recién nacidos en el Hospital de Cruces (Vizcaya). An Esp Pediatr 1996;44:50-4.
[25] De Vriese SR, Dhont M, Christophe AB. Oxidative stability of low density lipoproteins and vitamin E levels increase in maternal blood during normal pregnancy. Lipids 2001;36:361-6.
[26] Mutlu-turkoglu U, Oztezcan S, Telci A, Orhan Y, Aykac-Toker G, Sivas A, et al. An increase in lipoprotein oxidation and endogenous lipid peroxides in serum of obese women. Clin Exp Med 2003;2:171-4.
[27] Myara I, Alamowitch C, Michel O, Heudes D, Bariety J, Guy-Grand B, et al. Lipoprotein oxidation and plasma vitamin E in nondiabetic normotensive obese patients. Obes Res 2003;11:112-20.
[28] Esterbauer H, Dieber-Rotheneder M, Striegl G, Waeg G. Role of vitamin E in preventing the oxidation of low-density lipoprotein. Am J Clin Nutr 1991;53:S314-21.
[29] Knopp RH, Magee MS, Walden CE, Bonet B, Benedetti TJ. Prediction of infant birthweight by gestational diabetes screening test: importance of plasma triglyceride. Diabetes Care 1992;15:1605-13.
[30] Bonet B, Hauge-Gillenwater H, Zhu XD, Knopp RH. LDL oxidation and human placental trophoblast and macrophage cytotoxicity. PSEBM 1998;217:203-11.
[31] Tanner FC, Noll G, Boulanger CM, Lusscher TF. Oxidized low density lipoproteins inhibit relaxations of porcine coronary arteries. Role of scavenger receptor and endothelium-derived nitric oxide. Circulation 1991;83:2012-20.
[32] Vergnani L. Effect of native and oxidized low-density lipoprotein on endothelial nitric oxide and superoxide production. Circulation 2000; 101:1261-6.
[33] Figueroa R, Omar HA, Tejani N, Wolin MS. Gestational diabetes alters human placental vascular responses to changes in oxygen tension. Am J Obstet Gynecol 1993;168:1616-22.
[34] García-Patterson A, Erdozain L, Ginovart G, Adelantado JM, Cubero JM, Gallo G, et al. In human gestational diabetes mellitus congenital malformations are related to pre-pregnancy body mass index and to severity of diabetes. Diabetologia 2004;47:509-14.
[35] Watkins ML, Rasmussen SA, Honein MA, Botto LD, Moore CA. Maternal obesity and risk for birth defects. Pediatrics 2003;111:1152-8.
[36] Indart A, Viana M, Grootveld M, Silwood CJ, Sánchez-Vera I, Bonet B. Teratogenic actions of thermally-stressed culinary oils in rats. Free Radic Res 2002;36:1051-8.