Lack of predictive power of plasma lipids or lipoproteins for gestational diabetes mellitus in Japanese women

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ABSTRACT
Aims/Introduction: To determine the diagnostic potential of plasma lipids and apolipoproteins in gestational diabetes mellitus (GDM), we carried out a retrospective cohort study of 1,161 Japanese women at 20–28 weeks of gestation who underwent a glucose challenge test (GCT).

Materials and Methods: A total of 1,161 Japanese women at 20–28 weeks of gestation underwent a GCT. Participants with a positive test (GCT+) underwent a subsequent oral glucose tolerance test. Clinical and biochemical parameters were determined and quantification of apolipoproteins (Apo), including ApoB, ApoB48, ApoA-I and ApoC-III, was carried out.

Results: The prevalence of GCT(+) with a 130 mg/dL glucose cut-off and GDM was 20% and 4%, respectively. There was a trend for increased triglycerides and ApoC-III in GDM(+) participants. However, the difference in plasma triglycerides, ApoC-III or ApoB48 did not reach statistical significance between GDM(+) and GDM(−) women. Values of 1-h glucose (P < 0.001) and fasting glucose (P = 0.002) were significant risk factors for GDM.

Conclusions: Prediction of GDM using only the ApoC-III value is not easy, although triglycerides and ApoC-III were higher in the GDM(+) group. The present findings show no significant difference in plasma lipid levels between women diagnosed with GDM and those with normal glucose tolerance.

INTRODUCTION
Unmanaged hyperglycemia during pregnancy, or gestational diabetes mellitus (GDM), is closely associated with three clinical implications. First, GDM increases the risk of complications during the perinatal period1. Second, GDM poses a high risk for type 2 diabetes development in the maternal body in the future2. Third, GDM predisposes the offspring to a high incidence of diabetes and metabolic dysfunction3. Hence, GDM represents one of the crucial pathologies of pregnancy, and if it occurs it needs to be closely monitored and managed. Clinically, factors taken into consideration to assess the risk for GDM include a positive urine glucose test (glucose above a designated threshold), a familial history of diabetes mellitus, obesity, excess weight gain, history of having macrosomia and age. A routine glucose challenge test (GCT) is carried out routinely for all pregnant women, and patients with a positive test (GCT+) undergo an oral glucose tolerance test (OGTT) as further confirmation.

Pregnancy is accompanied by profound changes in lipid metabolism. The early phase of pregnancy can result in increased triglycerides (TG) as a consequence of increased lipogenesis and suppressed lipolysis, whereas the mid-phase of pregnancy can enhance lipolysis and elevate fatty acid concentrations. This change in lipid metabolism represents a physiological adaptation in the mother’s body that involves switching...
from glucose metabolism to an increased preference for lipid metabolism in order to preserve glucose for fetal growth\textsuperscript{4}. Although increased TG during pregnancy might be a natural phenomenon, unmanaged GDM is recognized as an increased risk for type 2 diabetes. Glucose intolerance in women with GDM, as a consequence of diminished insulin action, is associated with reduced lipoprotein lipase (LPL) activity and overproduction of TG-rich very low-density lipoproteins. The hallmarks of diabetic dyslipidemia mainly include hypertriglyceridemia, elevated low-density lipoprotein-cholesterol (LDL-C) and decreased high-density lipoprotein-cholesterol (HDL-C). Dyslipidemia during pregnancy can exacerbate insulin resistance in GDM, leading to a worse clinical outcome\textsuperscript{5}. It has been shown that hypertriglyceridemia in obese pregnant women not only exerts a negative effect on maternal clinical outcomes, but can also have a long-term impact on the development of metabolic syndrome in her offspring.

The present study examines the interrelationship between GDM and diabetic dyslipidemia in Japanese women. Specifically, we attempted to determine whether or not the plasma lipid and/or lipoprotein parameters are predictive in the diagnosis of GDM. In addition to determining the concentrations of plasma apolipoprotein (Apo)B and ApoA-I, which are well-established markers for plasma LDL-C and HDL-C, respectively, we also determined the concentration of ApoB-48 and ApoC-III. The level of ApoB-48, a protein constituent of chylomicrons, reflects the postprandial absorptive ability for dietary lipids\textsuperscript{6}. In contrast, the level of ApoC-III can be correlated to hepatic production of endogenous lipids containing very low-density lipoproteins. Plasma concentrations of ApoC-III are positively associated with the risk of coronary artery disease or atherosclerosis, presumably because of the ApoC-III inhibitory effect on LPL-mediated TG hydrolysis\textsuperscript{7}. The plasma concentration of ApoC-III is elevated in pre-eclamptic women\textsuperscript{8}. However, it remains unknown whether or not ApoC-III concentration is associated with GDM. A recent study using a proteomic approach suggested that plasma ApoC-III concentration could serve as a biomarker for predicting GDM\textsuperscript{9}.

**MATERIALS AND METHODS**

**Participants**

A total of 1,183 pregnant women with no previous diabetes were enrolled in the study during December 2010 through July 2011 at the Japanese Red Cross Medical Center. Exclusion criteria included: a fasting blood glucose $>$126 mg/dL, glycated hemoglobin (HbA\textsubscript{1c}) $>$6.5%, a blood glucose measurement of $>$200 mg/dL 2-h after an OGTT. The present study was carried out in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

**GCT and OGTT**

Standard 1-h 50-g GCTs were carried out between weeks 20 and 28 of gestation. Participants with a 1-h blood glucose...
>130 mg/dL were considered GCT(+) in this cohort. The blood glucose threshold of 130 mg/dL, instead of 140 mg/dL set by the Japan Assessment of GDM Screening Trial Group, was used for this cohort because of the relatively older age (average 34 years) of the participants. Informed consent was obtained at 16–20 weeks of gestation. The GCT(+) women also underwent a 10–12-h fasting 75-g OGTT. Venous blood samples were drawn at 0, 0.5, 1 and 2 h for biochemical analyses. As specified by the International Association of Diabetes and Pregnancy Study Groups1 and the American Diabetes Association criteria10 for GDM diagnostic threshold, GDM was defined as a plasma glucose concentration equal or higher than one of the following measurements at screening: baseline 92 mg/dL, 1-h post OGTT 180 mg/dL and 2-h post OGTT 153 mg/dL.

Biochemical Analyses
Height and weight were routinely recorded, and body mass index was calculated to estimate overall adiposity. Blood samples collected for the GCT and OGTT were centrifuged at 800 g for 6 min. Specimens were aliquoted and stored at −80°C before being assayed. Glucose was quantified using a glucose oxidase kit with a GA 1170 auto-analyzer (ARKRAY Inc., Kyoto, Japan). Total cholesterol (TC), TG, HDL-C and LDL-C were quantified using enzymatic colorimetric methods according to manufacturers’ protocols (Cholestest CHO; Sekisui Medical Co. Ltd., Tokyo, Japan). Insulin and ApoB-48 were measured by using a chemiluminescent enzyme-immunoassay system (Lumipulse Presto-II, Lumipulse f system, respectively; Fujirebio Inc., Tokyo, Japan)11, and ApoA-I, ApoB and ApoC-III were determined by turbidometric immunoassay kits according to the manufacturer’s recommendations (Sekisui Medical Inc., Tokyo, Japan). The C-reactive protein (CRP) was measured using the Nanopia CRP kit provided by Sekisui Medical Co. Ltd. The various lipid and lipoprotein concentrations were measured in fasting samples. Insulin sensitivity as a measure of basal insulin sensitivity during an OGTT was estimated using the homeostasis model assessment of insulin resistance, calculated as (fasting glucose [mg/dL] × (fasting insulin [μU/mL]) / 40512. The early insulin response during an OGTT was estimated as the insulinogenic index: (Δinsulin [30–0 min] / Δglucose [30–0 min]).13

Statistical Analysis
In the univariate analyses, t-tests with unequal variances were used to examine different means between the two groups compared. All statistical tests were two-sided. P-values < 0.05 were considered statistically significant. For the multivariate analysis, logistic regression analyses were carried out between the data for GDM(+) and GDM(−). The dependent variable was denoted as ‘1’ for GDM(+) and ‘0’ for GDM(−). Significant factors from the univariate analyses with a large enough sample size were used for multivariate analyses. For the model selection, we used Akaike’s Information Criteria14. The statistical procedures were carried out using open source free software of Statistical Language R (University of Auckland, Auckland, New Zealand)15. All the data are presented as mean ± standard error (SE), unless otherwise indicated.

The statistical analysis was extended to define receiver operating characteristic curves for the lipid markers asking their predictive value. We examined the receiver operating characteristic curves for the seven lipid markers, respectively. Then, we calculated the area under the curve for the receiver operating characteristic of each lipid marker.

RESULTS
Participants and Prevalence of GDM
Of the total 1,183 pregnant women enrolled at the Japanese Red Cross Medical Center, physicians ordered an OGTT for 22 of them directly (without a prior GCT) on the basis of having a previous history of GDM or a large size fetus. Five of these 22 women were GDM(+) (data not shown). The remaining 1,161 women underwent a GCT; 842 women were GCT(−) and 319 women were (GCT+; the latter representing 27.5% of the total women tested; Figure 1). The majority of GCT(+) patients (n = 266) were asked to complete an OGTT, whereas the remainder (n = 53) were not, because they had relocated to other hospitals. The 266 GCT(+) women carried out the

Table 1 | Baseline characteristics of the participants who completed an oral glucose tolerance test

| Characteristics | n  | Mean (±SD) |
|-----------------|----|------------|
| Anthropomorphic parameters | Age (years) | 265 | 34.7 (5.18) |
|                  | Height (cm)   | 264 | 1595 (5.10) |
|                  | Weight (kg)   | 264 | 52.4 (7.90) |
|                  | BMI (kg/m²)   | 263 | 20.6 (2.97) |
| Inflammation and glycemic indices | 1-h glucose† (mg/dL) | 266 | 1502 (18.8) |
|                  | 1-h insulin† (mU/L) | 231 | 65.3 (39.6) |
|                  | CRP (mg/dL) | 175 | 0.25 (0.44) |
|                  | HOMA-IR | 258 | 1.27 (1.64) |
|                  | Insulinogenic index, ΔIADG | 258 | 104 (0.66) |

Lipid apolipoprotein concentration, mg/dL

| TG | 176 | 1752 (799) |
| TC | 175 | 2554 (43.7) |
| LDL-C | 176 | 1308 (37.2) |
| HDL-C | 176 | 824 (15.0) |
| ApoA-I | 176 | 2169 (30.8) |
| ApoB | 175 | 1262 (31.0) |
| ApoB48 | 176 | 263 (1.78) |
| ApoC-III | 176 | 145 (3.95) |

Δ, change; Apo, apolipoprotein; BMI, body mass index; CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance; OGTT, oral glucose tolerance test; SD, standard deviation; TC, total cholesterol; TG, triglycerides. Based on a glucose challenge test.
OGTT, and 45 of them tested GDM(+). Normally, GCT(−) subjects are not given an OGTT for ethical reasons. However, 16 out of the 842 GCT(−) women had an OGTT ordered for them on the basis of a history of GDM or fetus size, and three of the women tested were GDM(+). All together, 53 of 1,183 participants were diagnosed GDM(+) showing that the prevalence of GDM in the present study was 4.1% in the Japanese Red Cross Medical Center cohort.

The baseline characteristics of participants who completed an OGTT are shown in Table 1. Table 2 shows the data comparing the GDM(+) and GDM(−) groups on the basis of clinical characteristics and biochemical findings. Significant differences (P < 0.05) were recorded for height, bodyweight, and BMI between GDM(+) and GDM(−) (Table 2), strongly suggesting that GDM is influenced by anthropometric characteristics, such as adiposity, in this cohort. Glucose concentrations were significantly higher during the OGTT in GDM(+) participants (n = 45) as compared with those in GDM(−) participants (n = 238; Figure 2). However, as compared with the threshold GDM value specified by the International Association of Diabetes and Pregnancy Study Groups, the glucose concentrations of the GDM(+) women at baseline and 60-min post-test were below the International Association of Diabetes and Pregnancy Study Groups threshold, and only the 120-min post-test glucose concentrations were significantly higher than the threshold.

Figure 2 | Changes in plasma glucose and insulin levels of oral glucose tolerance test for glucose challenge test positive participants. (a) Changes in mean plasma glucose concentrations. (b) Changes in mean plasma insulin concentrations. The errors bars represent standard error. (a) The International Association of Diabetes and Pregnancy Study Group’s threshold at each time-point. The International Association of Diabetes and Pregnancy Study Group’s definition of gestational diabetes mellitus (GDM) uses a cut-off threshold of glucose 60 min after oral glucose tolerance test of >180 mg/dL. The mean (±standard deviation) concentration of 167.1 ± 45.0 mg/dL in the current study is below this level. **P < 0.001.
concentrations were higher than the threshold value (Figure 2a). Likewise, the insulin level was also significantly higher in the GDM(+) group only 120 min after the OGTT (Figure 2b).

There was a trend for an increase in homeostasis model assessment of insulin resistance in the GDM(+) group, although this did not reach statistical significance (P = 0.104; Table 2). However, the insulinogenic index was significantly lower in the GDM(+) group (Table 2), indicating an impaired insulin sensitivity and/or insulin secretion (presumably as a result of β-cell dysfunction) in GDM(+) women, as reported by others previously16. The CRP inflammation marker was comparable between GDM(+) and GDM(−) groups (Table 2).

Although lipid parameters, such as TG and ApoC-III, were higher in the GDM(+) group, the difference was not statistically significant. TC, LDL-C and HDL-C did not show any significant differences between the two groups (Table 2).

The average plasma ApoC-III concentration in normal pregnant women was 14.3 ± 0.31 mg/dL (Table 2). Plasma concentrations of ApoC-III between GDM(−) and GDM(+) groups were not statistically different (P = 0.197).

The mean (±SE) plasma ApoB-48 concentration in normal pregnant women was 2.58 ± 0.14 mg/dL (Table 2), which is higher than that in non-pregnant healthy Japanese women (0.21 mg/dL)17. However, there was no significant difference in ApoB-48 between GDM(−) and GDM(+) (P = 0.412; Table 2). Likewise, plasma concentrations of ApoB (126.0 ± 2.56 mg/dL) and ApoA-I (215.0 ± 2.50 mg/dL) in normal pregnant women of this cohort were also higher than those in the non-pregnant 30-39-year-old women. Plasma ApoB and ApoA-I levels were not statistically different (P = 0.709; Table 2). Plasma ApoC-III and ApoB-48 were not statistically different (P = 0.412; Table 2). The difference in plasma ApoC-III did not reach statistical significance between GDM(−) (15.6 mg/dL) and GDM(+) (14.5 mg/dL) women. It has been reported that ApoC-III could be a potential biomarker in women at 16–20 weeks of gestation who subsequently develop GDM7. However, our data do not suggest that lipid or lipoprotein parameters have sufficient predictive power for GDM. It is known that during the mid-phase of pregnancy, maternal energy metabolism switches to enhanced lipolysis, a change that leads to increased levels of circulating fatty acids. This functional metabolic adjustment appears to be a general phenomenon during pregnancy, and is unrelated to the mild glucose abnormality observed between GDM(+) and GDM(−) subjects. Insulin resistance does increase.

### Table 3 | Results of the logistic regression analysis for pregnant women with or without gestational diabetes mellitus

| Covariates                  | Regression coefficients | Standard error | P-value | Relative risk | 95% Confidence interval |
|-----------------------------|-------------------------|----------------|---------|---------------|-------------------------|
| Intercept                   | -17.79                  | 3.085          | <0.001  |               |                         |
| Age (≥30 years vs <30 years)| 1.386                   | 0.709          | 0.050   | 3.999         | 1.00–16.04              |
| BMI                         | 0.122                   | 0.059          | 0.039   | 1.130         | 1.00–1.27               |
| 1-h glucose                 | 0.031                   | 0.009          | <0.001  | 1.031         | 1.01–1.05               |
| 1-h insulin                 | 0.029                   | 0.047          | 0.533   | 1.030         | 0.95–1.13               |
| Fasting glucose             | 0.094                   | 0.030          | 0.002   | 1.098         | 1.04–1.16               |

BMI, body mass index. Participants who completed an oral glucose tolerance test (n = 254). The dependent variable for pregnant women with or without gestational diabetes mellitus was ‘1’ and ‘0’, respectively.
during pregnancy, and we found that insulin secretion was increased in the GDM(+) group compared with the GDM(−) group, apparently in response to high glucose concentrations at 2 h. The hyperinsulinemia in the GDM(+) group might contribute to mild hypertriglyceridemia and hyper-ApoC-III (Table 2).

Compared with Western populations, obesity is far less prevalent in Japan. However, the number of Japanese patients with diabetes has continued to increase. Genetic factors in Japanese or Asian people are thought to explain this phenomenon, which has its origins in an inherently strong resistance to starvation. First, the peroxisome proliferator-activated receptor gamma gene, a β3 adrenergic receptor genetic mutation, and the carpaine-10 gene have been linked to an ability to accumulate lipids and increase the risk for diabetic mellitus18. Second, adiponectin, which can improve the insulin resistance of adipocytes in those with diabetes, is more prone to genetic mutations in Japanese vs Western populations. In fact, half of all Japanese people seem to have an adiponectin genetic mutation19. Third, the ability to secrete insulin from the pancreatic β-cells in Japanese people is less than that of Europeans19. Combinations of these factors might also affect the likelihood of developing diabetes mellitus. Therefore, the lack of predictive power of plasma lipid or lipoprotein parameters for GDM in Japanese women might be attributable to genetic factors.

The prevalence of GDM in the present study, based on the diagnostic guidelines recommended by the World Health Organization, was 4.1%. There was a tendency for a significantly higher BMI, higher insulin concentrations at 2 h during the OGTT and a low insulinogenic index in the GDM(+) group compared with the GDM(−) group. It is therefore suggested that a woman with a high BMI before pregnancy appears to develop GDM more easily as a result of oversecretion of insulin and lower insulin action during the early stage of pregnancy.

In Japan, a GCT during the second trimester of pregnancy is recommended by the Japan Society of Gynecology and Obstetrics. Currently, the screening method for GDM in Japan is to measure plasma glucose concentrations in the first trimester of pregnancy or GCT in the second trimester. Japan Assessment of GDM Screening reported that the GCT provides higher sensitivity and specificity at mid-term, and is better from a cost performance perspective. A recent systematic review concluded that the GCT is acceptable to screen for GDM20. The meta-analysis that also shows that the GCT has limited sensitivity and specificity (pooled sensitivity of the studies including all pregnant women was 0.74, with a pooled specificity of 0.85). However, the GCT also has drawbacks as a screening method; a GCT carried out in the afternoon tends to give false positive results21, and the most recent meal can affect the GCT result22. The extent of these disadvantages remains an issue deserving further discussion, and necessitates the accumulation of more evidence before it can be confirmed.

It should be noted that it is not reasonable to carry out an OGTT on every pregnant woman from an economic perspective because of the associated costs. In the present study of pregnant women, we were not permitted ethically to overload glucose during the tolerance test, as this could potentially add excess stress to the participants. Therefore, GCT(−) participants were not allowed to undergo an OGTT.

The original threshold for an elevated blood glucose test (≥140 mg/dL) was arbitrary and validated by the ability to predict a positive 3-h OGTT in the mother. However, the sensitivity of the GCT is improved if a lower plasma glucose threshold (>130 mg/dL) is used.

Because previous studies have reported that age is an important risk factor for GDM, our medical center uses >130 mg/dL as the cut-off value for blood glucose concentration. We chose this value because pregnant women attending our center have an older mean age of 34 years.

In order to determine the factors that contribute to GDM, we examined the factors that had the strongest influence on this outcome. The most influential factors were plasma glucose concentration 1 h after GCT and fasting glucose (Table 3). These factors were identified by comparing GDM with clinical and biochemical parameters. It has been reported that age is the main risk factor for GDM. In the present study, we showed that being older than 30 y was a major risk factor for GDM.

The present study had some limitations. For example, some patients did not have a complete dataset. Increasing the sample size would add to analytical power, and we will seek to recruit a greater number of patients in any future study.

In conclusion, during pregnancy, women with dysfunctional glucose metabolism have an associated abnormal lipid metabolism that results in a lipoprotein metabolism unlike that experienced when they are not pregnant. Prediction of GDM using only the ApoC-III value is not easy; however, pregnant women with higher concentrations of ApoC-III might require more medical supervision, and the same was true for ApoB-48 with respect to diet (meal) absorptive ability. Anthropometric characteristics, such as age and BMI, are contributing factors to GDM. The GCT remains an effective screening tool to diagnose GDM in Japanese women.

DISCLOSURE
Dr Ito is a consultant for Fujirebio, Co., Inc. and receives a consultancy fee from Fujirebio, Co., Inc. The other authors declare no conflict of interest.

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