Discussion

Raanan Shamir, MD

Dr Shamir: Dr Catalano, you looked at insulin influence on gene expression, showing that insulin in the placenta causes upregulation of the cholesterol pathway. We are not used to seeing this in medication cultures. I mean fibroblasts or macrophages. Did you follow this throughout pregnancy?

Dr Catalano: The trouble is getting placenta samples at different times during the pregnancy. That is why we started with delivery—the sample was easy to get. We went back to the 1st trimester, which was hard to get, but we were still able. I think the best way is to use a chorionic villus sampling. Do it in early pregnancy so we can have a baseline, look at the environment the woman is exposed to, and then repeat it again in late pregnancy. Whether the study is a randomized controlled trial or just observational, we can see the changes in the mother that did not affect gene expression.

Dr Shamir: Did you have a chance to look at both premature and full-term infants?

Dr Catalano: No, just full-term infants.

Dr Shamir: This is a very noble finding; usually insulin does not enhance cholesterol synthesis just by induction of the enzymes that are responsible for cholesterol.

Dr Catalano: Right. I think it may have to do with the lipid that is already in the placenta, and then not just the placenta tissue but the macrophages that also may be in there. So it may be coming from the cytokines.

Dr Pan: Dr Catalano, you mentioned using anti-inflammatory supplements to decrease low-grade inflammation. Did you get results from that?

Dr Catalano: No. It is ongoing so we have no results yet. It is interesting that these data about looking at aspirin as an anti-inflammatory relative to the issue of diabetes are in the adult literature but not related to pregnancy. We want to use something that we think is safe in pregnancy.

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Dr Pan: We used an obese rat model to look at whether macronutrients can modify the inflammatory markers but we did not see any changes whether we increased fat, decreased fat, increased carbohydrate, or increased protein. As long as the weight stayed the same, the fat mass always produced those markers in our system.

Dr Catalano: We use a 3-day dietary recall to get dietary histories. We are trying to measure three concentrations so that we were blind to the results and the randomization. The thought is that we will be able to look at the results objectively and see if there is a dose response because I am not sure everyone took all the pills all the time. We also can look at macronutrients to see whether there is a relationship.

Dr Poston: Dr Catalano, you commented about the use of metformin in gestational diabetes mellitus (GDM). It has been used successfully to reduce macrosomia in women with GDM [Ijäs H et al. *BJOG*. 2011;118:880–885]. I share your concerns about the long-term influence on the child. We do not know anything about that. But that aside, two studies are going on in the United Kingdom in which obese pregnant women are being randomized to receive metformin or not. Do you think that is a sensible approach to improve pregnancy outcomes?

Dr Catalano: I can give my personal opinion only, and I do not think there is an official opinion. I think one study shows that metformin is equivalent to insulin, but not better than insulin. I have seen only some preliminary data, but no follow-up data. I know that insulin is not fun to take, but I know that it works and that it does not cross the placenta. It has been used for 60 years without any problems.

Dr Poston: A lot of obese pregnant women have normal glucose tolerance and do not have insulin resistance. Do you think giving insulin to sensitize women who have normal glucose tolerance, which is what happened in that study, is safe? Will that not induce a hypoglycemic response when they have normal insulin sensitivity?

Dr Catalano: The studies have been done only in women with GDM, but insulin resistance increases even in women who do not have GDM. I think induction of a hypoglycemic response is possible but unlikely because insulin resistance would have to be overcome.

Dr Poston: We should do a trial to find out.

Dr Shamir: But when we look at the data, we see it is not GDM. The lower the blood glucose level, the fewer the complications. It is not only an abnormal glucose tolerance test, if I understand you correctly.

Dr Catalano: But if we lower blood glucose levels too much, we can get into problems with fetal growth restriction. I think one researcher reported that when we push so hard that a woman's glucose level is 87 mg/dL, we increase the risk of her baby being born small for gestational age.

Dr Poston: That is my concern.

Dr Catalano: But I do not know whether we can do that with metformin.

Dr Yajnik: Just by treating GDM we introduce an element of growth restriction?

Dr Catalano: That is a hard question to answer. We may be. We talked about insulin resistance relating to carbohydrate and lipid, but it also relates to protein metabolism. There is no question that amino acid turnover changes in pregnancy, and if we give the pregnant woman insulin, we probably will suppress that to a certain degree. Could this have an adverse effect? I think the answer is yes, but I will not speculate.

Dr Yajnik: The reason I ask is that a follow up of children of the ACHOS study was published. These children were on average 150 g lighter than the control group at birth, and had a lower incidence of macrosomia and associated perinatal adverse outcomes [Crowther CA et al. *N Engl J Med*. 2005;352:2477-2486]. However, at 5 years of age, they had caught up with and were similar to the control group [Gillman MW et al. *Diabetes Care*. 2010;33:964-968].

So, I think an element of growth restriction was introduced in utero, which promoted rapid catch-up. It would prove interesting to see whether they have an earlier adiposity rebound, which is a risk factor for future diabetes. Long-term follow up of children in both of the GDM intervention studies is critical to answer the question of long-term benefits.

Dr Godfrey: Data about the effects of GDM treatment at age 7 years are quite reassuring in terms of beneficial effects without apparent detrimental effects.

Dr Catalano: I think we are talking about treating someone who does not have gestational diabetes.

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Dr Poston: But the follow-up group was a small subgroup of the main trial, so it was not necessarily representative of the whole trial population.

Dr Yajnik: Agreed. Follow-up was only in South Australia?

Dr. Poston: Yes. About 200 subjects.

Dr Catalano: In our trial, we did not find any difference at age 3 or 4 years.

Dr Shamir: But the study has no nutrition data after birth. That is one of the difficulties.

Dr Catalano: Not for the whole cohort, but nutritional data were produced for some of the sub-cohorts. As you probably know, there is a grant to fund a follow-up study. I am sure we will get nutritional data then.

Dr Rueda: Dr Catalano, you described an interesting positive role of the microbiome reaching the placenta, modifying the inflammatory response and influencing different target outcomes, so this is perhaps a potential road to modifying the grade of inflammation at the maternal-fetal interface.

A recent publication showed that the microbiota colonizing the placenta is different in low-birth-weight infants than in normal weight infants [Fichorova RN et al. *mBio*. 2011;2:e00280-10]. Do you think a role might exist for the colonizing microbiota of the placenta, modifying the proinflammatory to anti-inflammatory profile there and, as a consequence, modifying functional outcomes in the infant? Also how about modifying obesity prevalence in the infant through this mechanism?

Dr Catalano: I have heard of that paper but have not read it. I think that this is an interesting issue in light of our discussion about genes or the environment expressing the placenta. Just as epigenetic changes occur, I would think that microbiome changes could occur, depending on what a person eats. A study out of Finland showed that a probiotic diet can decrease the risk of GDM and change the bacteria in the microbiome. What is the mechanism? I do not know anyone who is looking at this yet.

Dr Shamir: Jeffrey Gordon came out with the theory relating obesity and probiotics, but he changed the way he looks at this relationship. Now it is more the effect of nutrition on the microbiota than the cause and effect between the microbiota and obesity.

Dr Godfrey, you were talking about your randomized trial in which you supplement women with vitamin D. What is the supplementation dose you are using?

Dr Godfrey: We have trialed with two different doses of vitamin D. In an initial phase, we looked for a dose that produced a decent increment in vitamin D levels. We ended up using 1000 units.

Dr Shamir: Now people are talking about 2000 units as being the maximum dose.

Dr Godfrey: That is the reason why it was done on an ethics committee. We had to go through an initial phase of assessing the effects that we saw.

Dr Shamir: I like your comment about starting supplementation much earlier during pregnancy. We are conducting a study in which we are giving 400 units vs 2000 units during the 3rd trimester. Our institutional review board gave us trouble but finally cleared the 2000 units, but perhaps if we started earlier, we could have used smaller amounts of vitamin D.

Dr Catalano: What is the reference value for a normal vitamin D level?

Dr Shamir: There are no studies showing this. The only question is what is a safe dose?

Dr Catalano: Is there a biomarker that one could use such as bone density?

Dr Shamir: There is a high prevalence of low 25-hydroxyvitamin D (25OHD) levels in our population, so we are using 25OHD as a marker of whether we need to supplement. There are not any studies that tell us how much to increase 25OHD throughout pregnancy or what the effect would be on the newborn.

Dr Poston: A recent publication describes a large study looking at different supplement doses of vitamin D in pregnant women. The researchers gave the women up to 4000 units and found that 2000 were sufficient. A dose of 4000 units did not confer any extra benefit. Importantly, the researchers did not report any adverse effect throughout the whole of the study at any dose.

Dr Godfrey: I think there are concerns other than the immediate outcomes of high doses.

Dr Poston: Later outcomes.

Dr Godfrey: Exactly. For our institutional review board, we were tabled to enroll women with high vitamin D levels at the outset.

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Dr Shamir: People now would be reluctant to give more than 2000 units.

Dr Yajnik: In the Pune Maternal Nutrition Study, we found the opposite association. Higher maternal vitamin D levels were associated with a smaller baby. Part of the reason is that the vitamin D status in these women who work in the farms is quite good, and vitamin D is partly a surrogate for maternal physical activity, which is considerably high. Thus, there was quite a bit of compounding by maternal activity.

Dr Atkinson: Other studies done by Prentice and colleagues in Gambia [Prentice A et al. *Acta Paediatr*. 2009;98:1360-1362] found no association between maternal vitamin D status in pregnancy (all mothers had serum 25OHD >50 nmol/L) and infant outcomes of birth weight, weight, length, or head circumference during the 1st year, or bone outcomes, including bone mineral content, bone width, or area or size-adjusted bone mineral content of the radius or whole body.

So, I think the jury is still out on the long-term effects of exposure to maternal vitamin D status in pregnancy.