Nutrient-Induced Maternal Hyperinsulinemia and Metabolic Programming in the Progeny

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Fetal Programming due to an Altered Nutritional Experience in utero

Although the early development of living creatures is primarily influenced by the genetic instructions acquired at the time of conception, the environment under which the organism develops limits the expression of these genetic instructions. Fetal and neonatal growth in mammals is a complex process involving cross talk between the fetal genome, the maternal intrauterine environment and the early postnatal nutritional experience. Hence the well-being of the mother (with optimal nutrition) during pregnancy is of pivotal importance for optimal growth of the fetus and, in this context, the quality and quantity of nutrition in the mother have been identified as important factors contributing to the metabolic programming of the fetus. Metabolic programming is the phenomenon by which a nutritional stress/stimulus overlapping with the critical window of early development of specific organs permanently alters the physiology and metabolism of the organism thereby predisposing it for adult-onset disease conditions.

Several epidemiological studies have provided compelling evidence for the role of metabolic programming in the etiology of adult-onset diseases thereby emphasizing the importance of adequate nutrition during fetal development. The fetal origins hypothesis, first proposed by Barker, suggests that disproportionate size at birth of the newborn due to an adverse intrauterine environment during pregnancy complicated with malnutrition (protein or caloric) is highly correlated with the increased incidence of cardiovascular diseases, type-2 diabetes and hypertension during later periods in life [1, 2]. Studies
with animal models have provided additional support for the role of metabolic programming in the etiology of adult-onset diseases. Many animal models have been developed mainly to explore the role of fetal nutritional experience and in utero programming of adult-onset diseases. The consequences of maternal protein restriction, global caloric restriction and diabetes during pregnancy for the progeny have been extensively investigated [3–6]. Pregnancy complicated with protein malnutrition results in rat pups with significant changes in pancreatic islets including reduced islet vascularization, β-cell proliferative capacity, and rightward shift in the insulin secretory response to a glucose stimulus in their postnatal life [3]. Additional changes include malformation of hypothalamic nuclei and compromised metabolic capacities of liver, muscle and adipose tissues in adult progeny [3, 7]. Global caloric restriction in rats during the last 2 weeks of pregnancy causes glucose intolerance in the adult progeny [8]. In humans, a moderate diabetic pregnancy frequently results in fetal macrosomia, whereas in the case of a severe diabetic pregnancy intrauterine growth retardation is observed [5]. In animal models of a diabetic pregnancy, it has been shown that a mild diabetic pregnancy causes impairment in glucose homeostasis immediately after birth resulting in glucose intolerance in adulthood and that this diabetogenic tendency is transmitted between generations [5]. Severe diabetes during pregnancy induces β-cell hyperactivity and hypertrophy resulting in fetal hyperinsulinemia in response to the increased glucose encountered by the fetus. But due to β-cell exhaustion, the islets eventually become depleted of insulin and appear degranulated [5].

**Metabolic Programming also Extends into the Immediate Postnatal Period**

McCance [9] was the first to demonstrate that, by adjusting litter size in rats, under- or overnutrition during the suckling period results in an altered growth trajectory for life in these rats. Other studies indicate altered plasma insulin levels, modified islet function at the level of insulin secretion and gene expression and alterations in hypothalamic neuronal activity in the post-weaning period of rats subjected to under- or overnutrition in their suckling period [10, 11].

**Maternal Early Life Nutritional Experience: An Altered Intrauterine Environment in the Mother and Metabolic Programming of the Progeny**

Studies from our laboratory have demonstrated that in addition to the effects of under- or overnutrition during pregnancy, the immediate postnatal period is also vulnerable to metabolic programming via a dietary modification in the form of caloric redistribution during this period. The artificial rearing technique provides the opportunity to rear suckling rat pups away from nursing dams on any desired milk formula [12]. In our ‘Pup-in-a-Cup’ model 4-day-old rat pups
are reared artificially away from their nursing dams on a high-carbohydrate (HC) milk formula until day 24 when they are weaned onto lab chow. The HC milk formula is isocaloric and isonitrogenous to rat milk but the caloric distribution of the macronutrients is altered. The caloric distribution of the macronutrients is 56% carbohydrates, 24% protein and 20% fat in the HC milk formula, and 8% carbohydrate, 24% protein and 68% fat in rat milk. This switch in the source of calories from fat-rich in rat milk to carbohydrate-rich in the HC milk formula given to newborn rat pups without any change in the total caloric availability results in alterations in metabolic processes for life [13, 14]. A milk formula similar to rat milk in its caloric distribution was also included in our studies as an internal control to demonstrate that the artificial rearing protocol per se does not induce metabolic programming [13, 14].

Metabolic processes programmed in response to this altered dietary pattern in the immediate postnatal period are expressed not only in adulthood of the same generation in the absence of the stimulus that triggered these responses but are also spontaneously transmitted to the progeny via the female [15]. The HC female is a unique model for metabolic programming of the progeny because the altered intrauterine environment in the HC mother is encountered not due to any dietary manipulation during pregnancy but due to the dietary modulation for a brief period of 3 weeks only in its immediate postnatal life. This experience is sufficient to set up a vicious cycle of transmission of the HC phenotype (chronic hyperinsulinemia and adult-onset obesity) to the progeny [15]. In this context, the HC rat model is different from other animal models for studying maternal–fetal interactions for programming of the progeny. The mechanisms supporting the onset of the HC phenotype in the adult life of rats due to the HC dietary intervention in their neonatal life and the transmission of this phenotype to the progeny are described below.

**Early Metabolic Adaptations**

The immediate metabolic responses to the HC milk formula are depicted in figure 1a. In response to the HC milk formula, artificially reared rat pups develop hyperinsulinemia within 24 h and maintain this hyperinsulinemic condition during the entire period of the dietary intervention up to postnatal day 24 [16]. During this period, the HC rats maintain normoglycemia and their body weights are comparable to age-matched mother-fed (MF) control rats [17]. Adaptations at the biochemical, cellular and molecular levels in pancreatic islets of these HC rats support the onset and maintenance of hyperinsulinemia in the HC rats during this period [18, 19]. Leftward shift in the response to a glucose stimulus, increased hexokinase activity, and increased response to incretins and neuropeptides even at sub-basal glucose concentrations are the important biochemical changes in the islets of these HC rats [17, 20]. Additionally, the islets isolated from 12-day-old HC rats secrete moderate amounts of insulin in the simultaneous absence of glucose and calcium [20]. It is interesting to note that 10 times more norepinephrine concentrations are
required to completely inhibit insulin secretion by islets from 12-day-old HC rats suggesting an altered neuroendocrine regulation of insulin secretion in these islets [20]. An increased number of small islets and an increase in the number of islets per unit area characterize the cellular adaptations [21]. At the molecular level significant changes include upregulation of gene transcription of the pancreatic duodenal homeobox transcription factor-1 (PDX-1) and pre-proinsulin genes [22]. Gene array analysis indicates increased gene expression of several clusters of genes involved in a wide array of cellular functions [23].

**Adult-Onset Metabolic Adaptations during the Pre-Pregnancy and Pregnancy States**

The metabolic processes programmed in the immediate postnatal period are expressed in adulthood even after withdrawal of the HC milk formula at the time of weaning [24, 25]. Hyperinsulinemia initiated in the immediate postnatal period persists into adulthood accompanied by alterations in the insulin secretory response to glucose and other secretagogues [25, 26]. Interestingly, up to postnatal day 55 there are similar weight gains for HC rats
compared to age-matched MF control rats [15]. Thereafter there is a spurt in
the weight gain in the case of HC rats, and by postnatal day 100 they are sig-
ificantly heavier compared to age-matched MF rats (fig. 1a) [15]. The HC
maternal intrauterine environment is therefore characterized by increased
body weight, as well as significantly increased plasma insulin levels and nor-
mal plasma glucose levels (fig. 2) during pre-pregnancy and pregnancy [15].

In order to establish that the suckling period does not contribute to the trans-
mission of the HC phenotype to the progeny, pups after birth were reared by
normal foster mothers. It was observed that regardless of whether the pups
were reared by their own natural mothers or by foster mothers, all progeny
acquired the HC phenotype. Also, it was shown that the macronutrient com-
position was similar in rat milk obtained from MF and HC mothers, which
provides additional support for the hypothesis that the suckling period is not
imperative for metabolic programming and it is the intrauterine environment
that is important for the observed generational effect in the HC rats [15].

Cross-breeding experiments which demonstrated that the acquisition of the
HC phenotype by the progeny occurred only via the HC female (unpublished
observations) further confirm the role of fetal development in a HC mother
for metabolic programming of the progeny.

**Metabolic Programming of the Progeny due to Maternal Hyperinsulinemia during Pregnancy in the HC Female**

Figure 1b provides an overview of the profile of the HC progeny. For stud-
ies on generational transfer of the HC phenotype, HC females were bred with
normal male rats. The progeny were reared by normal foster females and were
weaned onto lab chow on postnatal day 24. In order to decipher the mechanisms that contribute to hyperinsulinemia in the HC progeny, biochemical and molecular studies were carried out in islets isolated from 28-day-old HC rats. As seen in figure 3 the HC progeny are not hyperinsulinemic during the suckling period but their plasma insulin levels begin to increase almost as soon as they are weaned onto lab chow on postnatal day 24 [27]. By postnatal day 28 the plasma insulin levels in the HC progeny are significantly higher compared to age-matched MF control rats [27]. Figure 4 describes the insulin secretory response to a glucose stimulus by islets obtained from HC progeny rats at different ages. Islets isolated from HC progeny rats do not demonstrate any change in the insulin secretory capacity up to postnatal day 24 but then after an increased insulin secretory capacity is evident, and on postnatal day 28 they demonstrate a marked leftward shift in the response to a glucose stimulus and are able to secrete significant amounts of insulin at all the glucose concentrations tested [27]. This altered insulin secretory response is supported by a significant increase in hexokinase activity. Free fatty acids that are increased in the plasma of 28-day-old HC progeny rats significantly augment insulin secretion in a dose-dependent manner in the presence of basal glucose by these islets (table 1) [27]. At the molecular level there is a marked increase in the gene expression of preproinsulin, PDX-1, USF-1 and β2/Neuro D in 28-day-old HC progeny islets (fig. 5a). The putative pathway for regulation of expression of the preproinsulin gene in islets and the

**Fig. 3.** Plasma insulin levels in high-carbohydrate progeny (●) and control rats (○) from postnatal day 4 up to 35. All newborn rats were reared by foster mothers until postnatal day 24 [27]. The results are means ± SEM of 4 independent experiments. *p < 0.05 compared to age-matched controls.
contribution of the molecular events in 28-day-old HC progeny islets to the hyperinsulinemic condition in these rats can be observed in figure 5b.

Plasma insulin levels in both male and female HC progeny rats continue to be significantly increased compared to age-matched MF control rats on postnatal days 45, 65 and 100 [15]. The insulin secretory pattern observed in the immediate post-weaning period is sustained into the adulthood of the progeny rats [26]. In the progeny, there is no significant difference between the birth weight as well as the weight gain profile up to postnatal day 55 [15]. However, an increase in weight gain is observed thereafter and by postnatal day 100 the HC progeny are significantly heavier compared to controls.

**Table 1.** Effect of palmitate on insulin secretion by islets from 28-day-old HC progeny rats

<table>
<thead>
<tr>
<th>Glucose mM</th>
<th>Additions</th>
<th>Insulin secreted, fmol/20 islets/60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MF</td>
<td>HC</td>
</tr>
<tr>
<td>5.5</td>
<td>None</td>
<td>1.95 ± 0.02</td>
</tr>
<tr>
<td>5.5</td>
<td>Sodium palmitate 125 (\mu M)</td>
<td>1.95 ± 0.05</td>
</tr>
<tr>
<td>5.5</td>
<td>Sodium palmitate 250 (\mu M)</td>
<td>2.03 ± 0.03</td>
</tr>
</tbody>
</table>

The results are means ± SEM of 4 independent experiments [27]. MF = Mother-fed; HC = high carbohydrate. *p < 0.05 compared to the corresponding MF treatment.
The liver and epididymal adipose tissue are significantly heavier in the progeny males on postnatal day 100 [15]. The increase in the enzyme activities of fatty acid synthase and glucose-6-phosphate dehydrogenase in the liver and adipose tissue of 100-day-old HC progeny rats suggests that chronic hyperinsulinemia predisposes these rats to the onset of obesity via increased lipogenesis in target organs.

For the HC progeny the only difference in their lives compared to their age-matched controls is the fetal development in the HC intrauterine maternal environment.

\(~15\%\) increase for males and \(~10\%\) increase for females; fig. 1b).
environment. So the question arises does any metabolic programming occur during fetal life that predisposes them to express the HC phenotype in adulthood. In support of this proposition, we have observed that fetal plasma insulin levels are already elevated in the HC fetuses on gestation day 21 (unpublished observations). The high-fat content of rat milk may be responsible for suppressing this hyperinsulinemia in the suckling period of the HC progeny and once these rats are exposed to lab chow with a high carbohydrate content, hyperinsulinemia is reestablished. More detailed studies are needed to characterize the extent of fetal programming in the HC progeny.

**Concluding Remarks**

The results obtained from the HC model emphasize the importance of balanced nutrition in the immediate postnatal period, as the consequences of an altered nutritional status during this period not only affect the recipient rats in their own adulthood but a vicious cycle of transfer to the progeny occurs via the maternal intrauterine environment. Mere fetal development in the hyperinsulinemic HC female enables the expression of the HC phenotype in the adulthood of the progeny. In the case of diabetic pregnancy, increased glucose supply to the fetus programs the progeny for glucose intolerance in adulthood. In the low-protein diet model or total caloric restriction animal models, the lack of essential nutrients contributes to metabolic programming of the progeny. In HC pregnancy there is neither a lack of specific nutrients nor a change in the total caloric availability. Maternal hyperinsulinemia and insulin resistance govern the intrauterine environment in the HC female. Our studies are the first to demonstrate that not only are the changes in the total caloric content via under- or overnutrition responsible for metabolic programming, but also the quality of nutrition via a redistribution of the source of calories without altering caloric intake also contributes to this phenomenon. The significant observation from our results is that an increase in the availability of calories from carbohydrates for just 3 weeks in the immediate postnatal period programs an altered intrauterine environment in the HC female rats in their adulthood, which confers the potential for the later expression of the HC phenotype in the progeny. Collectively, the observations from different types of nutritional experiences in either the fetal and/or the immediate postnatal life indicate that metabolic programming of target tissues such as pancreatic islets and hypothalamus during the period of exposure finally culminates in the expression of the metabolic syndrome in adulthood causing diseases such as type-2 diabetes, cardiovascular disorders, etc., with varying degrees of severity.

The question arises, do these observations have any relevance to the high incidence of obesity in humans observed in recent decades? It is tempting to speculate based on the dietary practices of infant feeding that metabolic...
programming of target tissues in these infants may have some relevance to
the surge in the incidence of obesity and related disorders in adults observed
in recent decades. For example, in light of the results from the HC rat model
one wonders if formula feeding in combination with the early introduction
of infant foods, such as cereal and fruit juices (both high in carbohydrate
calories), over the past several decades is one of the factors contributing to
the obesity epidemic of the 20th century. Such dietary practices not only
introduce carbohydrate-derived calories early in life but also, due to the mode
of feeding (for example, bottle, spoon, etc.), increase their total availability
and predispose these infants for metabolic programming due to increased
availability of carbohydrate-derived calories. Such feeding procedures not
only predispose the infants to adult-onset metabolic diseases in their adult-
hood but also via the female (due to an obese, hyperinsulinemic and insulin-
resistant pregnancy) could set up a cycle of transmission to the next
generation. This generational effect could be amplified by dietary practices in
their infancy setting the stage for a more unfavorable intrauterine environ-
ment from one generation to the next. Although only a hypothesis at this
stage, it merits an in-depth investigation as yet another factor in the etiology
of adult-onset obesity and other disorders associated with obesity.

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Discussion

Dr. Zhu: Thank you for a very interesting study and a very nice presentation. I have noticed that your high carbohydrate (HC) animals had a very high insulin level in plasma but they had a normal glucose level. How did it happen? Is it related to insulin resistance or to some problems with the insulin receptor?

Dr. Patel: There are two aspects in the maintenance of hyperinsulinemia without changing the blood glucose levels. In the first generation of animals, as we give the HC diet, plasma insulin increases and, to our surprise, plasma glucagon also increases. Even then the insulin:glucagon ratio is still higher and favors the insulin action. These animals maintain normoglycemia because there is also an effect at the receptor level. As I showed there is a reduction in the insulin receptor in the hypothalamus which also has insulin-responsive cells.

Dr. Kramer: It was fascinating and also very scary. I have two questions. First, have you extended the manipulations back to affect the carbohydrate versus fat content of the maternal diet while the fetus is still in utero, rather than in the postnatal period? And second, if you followed the pups into subsequent generations, how permanent is this or is it dampened out after a certain number of generations?

Dr. Patel: The second question first. I really don’t want to scare you but I think it looks like the effects are quite strong, at least in this animal model. The second
question is for how many generations might this go on. We have studied only 3 genera-
tions and to that point it looks as though the pattern repeats itself without any nutri-
tional interventions in the second and the third generations. We haven't considered it
in terms of its overall effect, in terms of the level of plasma insulin which is very high,
and the eventual outcome is the development of obesity. These animals do become
obese in every generation. Regarding the effect of maternal diet, we have not done
experiments in which we gave different diets to HC animals during pregnancy because
they are already hyperinsulinemic and obese, and giving a high sucrose or high fat diet
is just going to add more fat and more weight gain. As a different approach we are cur-
tently doing a series of experiments in which the females are reared on different diets
in the post-weaning period. If you give a high sucrose or high fat diet it is well known
that rats eventually become obese in post-weaning life. So these animals have been
placed on these diets and they developed obesity. These are currently ongoing exper-
iments that ask the same question: would they transfer the same phenomenon to the
next generation. These rats don't have an early life nutritional experience, but they
have the post-weaning experience in terms of dietary interventions which result in
hyperinsulinemia, insulin resistance and obesity. Whether they would transfer the
same phenotype to the next generation would depend on the outcome of the ongoing
experiments. I don't have the data to fully answer on your question.

**Dr. Lönnerdal:** Very fascinating model, and I am sure you have been tempted to
speculate about human infants and I would like you to do that too. Formula-fed infants
receive a diet which is very similar with regard to carbohydrate as compared to breast
milk. On the other hand they are fed a high protein diet, they get more calories from
protein than breast-fed infants, they have higher resting levels of the insulinogenic
amino acids, they have higher resting values of insulin. How would you compare your
model with the carbohydrate differences to the protein differences in the human
situation?

**Dr. Patel:** This can only be done through extrapolation with some cautious inter-
pretation because of species differences. One difference that comes to mind is that
human milk is relatively rich in carbohydrate, about 42% of calories come from car-
bohydrate, fat is about 51%, and the protein calorie content (7%) is very low. In rat
milk it is just the opposite as it is high in fat (68%), high in protein (24%) and low in
carbohydrate (8%). What we know from animal studies is that a change in the carbo-
hydrate content in rat milk from 8 to 55% causes a very marked change in the insulin
level. Even if we just double it from 8 to 16%, we still see the same impact on the early
nutritional programming into the 1st generation of animals.

Going back to the human situation I think nature has devised human milk for
human babies. I think the modification that can come, and this is just speculation, is
due to an early introduction of baby foods which are quite prevalent in developed
countries and even in developing countries. If you want to use baby foods as supple-
ments, you have to be very cautious in terms of what you want to put in that supple-
ment. I think the baby foods currently available in the United States are high in
carbohydrate (about 90%) of the calories coming from carbohydrate, the remaining
from protein and none from fat). The disturbing part is that the majority of carbohy-
drate calories come from simple sugars such as sucrose. So baby foods such as juices,
cereals and fruits are loaded with carbohydrates and largely with simple sugars. If, on
a theoretical basis, you provide 20 or 30% of daily calories from baby foods compared
to milk formula or breast milk, then the distribution of the total calories in a given day
is highly modified compared to milk feeding alone. So there are now more calories
coming from carbohydrates compared to fat and, for an extended period of months,
it might have a significant programming effect impacting on the development of
obesity later in life. That is my speculation from the available data from animals to
humans.
**Dr. Butte:** I was thinking about how your model might apply to the hyperinsulinemia that we see in childhood obesity. Clamp data have shown that it is not a problem at the pancreatic islet level of insulin secretion but rather insulin resistance at the periphery [1]. So can you speculate on how that model might apply to childhood obesity?

**Dr. Patel:** I am sorry I didn't get the first part.

**Dr. Butte:** Your model seems to have its effect primarily at the pancreatic level, on the islets and in insulin secretion, but clamp studies have shown that in obese children the problem is not with insulin secretion but rather with insulin resistance.

**Dr. Patel:** There are two parts. I think the pancreatic effect is immediate because of the dietary change. I think what really makes it permanent is hyperinsulinemia which develops within 24 h after initiation of this HC milk formula and that has an effect on the development of the hypothalamus. We did an interesting experiment showing that feeding the HC formula for a shorter time (8 days only) does all the observed biochemical changes but it does not program them for the long-term. We fed HC formula to animals from day 4 to day 12 and then switched back to rat milk. As I showed a number of parameters were modified by day 12: islet size; islet numbers, and even the hypothalamic levels of neuropeptides. Only 8 days experience with this HC formula wasn't sufficient because when we looked on days 55 and 100, these animals were perfectly normal without hyperinsulinemia.

For the second part, insulin resistance comes much later in life because we also measured the leptin concentration in suckling pups and adult animals. In the suckling pups there is no change in the plasma leptin level but during adulthood there is an increase in plasma leptin which results in leptin resistance, and they start gaining body weight during the time of leptin resistance. We have measured insulin-signaling pathways in muscle, liver and adipose tissue, and found definite changes in terms of tissue specificity. We saw insulin resistance in the skeletal muscle and liver but not in adipose tissue, which allowed them to synthesize more lipids for storage.

**Dr. Uauy:** Have you explored different simple carbohydrates like fructose versus glucose in terms of their insulinogenic potential in your model? The other question is, have you seen some of the recent data about leptin playing a role in neurogenesis in the hypothalamus? Could these responses actually be hidden early on, mediated by hormone effects on the hypothalamus?

**Dr. Patel:** Good questions. We struggled initially to make rat milk the way we wanted, to change from high fat to HC. The physical property of rat milk is such that it provides the function that nature has intended. We first added more lactose but that didn't work because the animals developed cataracts when they opened the eyes. We also tried glucose, fructose and galactose, but nothing worked because of the amount water that moved in with it. The animals were bloating up and died very early. So when we added Polycose, it worked well. We added extra carbohydrate as Polycose in addition to lactose, and the milk was refrigerated to avoid fermentation of sugars.

The second question was about the leptin programming. I think leptin is not really a major factor in this early programming because it does not change. Some of the ongoing studies are now looking at the changes in neuropeptides and their connection to the other secondary neurons, and so the structural basis needs to be examined in these animals.

**Dr. Hornstra:** In your palmitate study, did you have the opportunity to compare the results with other fatty acids as well? In other words, is this a specific palmitate effect or a nonspecific fatty acid effect? Can you tell us something about that?

**Dr. Patel:** No, we didn't go through a great deal of investigation. We wanted to show whether fatty acids have any effect on insulin secretion. So we just measured the effect of palmitate. Future studies will have to be done with different fatty acids, such as saturated, unsaturated and polyunsaturated fatty acids.
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Dr. Bleker: Did you look back to see whether there was some relationship with birth weight in the first generation?

Dr. Patel: There is no change in the birth weight of the progeny of the HC mothers which are obese, insulin-resistant and hyperinsulinemic. We didn’t see any significant effect on the average body weight, and even their growth rate during the suckling period remained very similar. The only change was in body weight after 55 days or so in second-generation rats.

Dr. Bleker: My question was because you try to imagine some influence already in fetal life, which might preprogram the results you obtain. Do you understand my question?

Dr. Patel: Not quite.

Dr. Bleker: Did you see any difference in birth weight in the second generation between the 2 groups?

Dr. Patel: There was no significant change.

Dr. Yajnik: Is there any change in the body composition of the fetus? You said there was no difference in birth weight, is there any difference in body fat percent?

Dr. Patel: That is a good question. We haven’t measured body composition so I cannot answer whether there was any difference and, if any at all, whether there was more fat, but that needs to be done.

Dr. Yajnik: Are you saying that they are hyperinsulinemic but not insulin-resistant to begin with? Are they in that case insulin-sensitive before they become insulin-resistant?

Dr. Patel: We are currently doing the insulin-sensitivity test in the 1st and 2nd generation of animals, so I don’t have the data to answer that question directly. One of the experiments we are planning to do is to give the drugs which will enhance their insulin sensitivity to see whether that would decrease hyperinsulinemia in the 1st generation of animals.

Dr. Yajnik: In that case you are saying hyperinsulinemia is secondary to insulin resistance?

Dr. Patel: Hyperinsulinemia comes from the very first day of treatment, so it has to be a metabolic response to the diet, and anything else that follows like insulin resistance has to be because of hyperinsulinemia and it cannot be vice versa.

Dr. Yajnik: The reason I am asking this is because to put on weight theoretically you need to be insulin-sensitive, and you said that adipose tissue is insulin-sensitive and that is why they are becoming fat. This is a perpetual sort of problem in following up the little story that if you measure at different stages. I suspect in early childhood people are more insulin-sensitive, certainly in fat tissue. Then they become fatter and become insulin-resistant because fat cells secrete a lot of substances which make you insulin-resistant. You have a unique opportunity to look at this in the animal model.

Dr. Patel: It would be a good way to look at insulin sensitivity in these suckling rats because they are already hyperinsulinemic at an early stage. We have not done any specific experiments yet, but it would be nice to see how soon they develop insulin resistance, and if they develop it whether there are tissue-specific differences as we have seen in adult animals.

Dr. Yang: A lot of human studies show that the fetal insulin level may just be related to the maternal glucose level, not to insulin because insulin cannot cross the placenta. So the fetal insulin levels may only be related to maternal blood and this would explain the difference between your results and the human studies.

Dr. Patel: That is correct; maternal insulin does not cross the placenta. Fetal hyperinsulinemia is due to insulin synthesized on the fetal side. Under maternal diabetic conditions the high levels of glucose and other nutrients which are passed on to the fetus cause hyperinsulinemia in the fetus. In the case of our HC rats, the mother is hyperinsulinemic but normoglycemic and, in spite of that, the fetus is developing hyperinsulinemia. So there are other causative factors which increase the biosynthesis
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of insulin and the development of hyperinsulinemia in the HC fetus. I don’t think it is a nutrient, but some other parameters on the maternal side predispose the fetus to develop hyperinsulinemia.

Dr. Moore: Is there any evidence that by manipulating the early diet to such an extent you can alter production of any of the appetite hormones? I mean when your rats return to lab chow are they consuming equal calorific intakes or is there any difference in appetite because of the early manipulations?

Dr. Patel: That is a good question. The HC pups are controlled in terms of how much they eat and their calorific intake is equalized to maintain the body weight to that of the mother-fed pups, so they grow normally. Once they are weaned, their food consumption is very similar to that of the control animals. The food consumption increases as they start gaining weight beyond day 55, but during the first month of postnatal life these animals eat normally and grow normally.

Dr. Endres: I think your studies have been very helpful in the context of the ongoing debate on whether there is an inverse relationship between breast feeding and later obesity, because most of the studies in humans of course have been retrospective ones. We had a discussion 2 years ago and you sent me some fascinating reprints showing that the metabolic programming is going through into the second generation. Do you intend to repeat the studies or are you already doing studies with changing protein levels? My question is related to another study presented about 2 years ago in an ESPGHAN meeting by Heywood et al. [2]. They used different protein concentrations in mice showing results similar to yours.

Dr. Patel: We have maintained most of the other nutrients similar to rat milk in composition for both the high fat and HC formulas. Our intent was to change only the calorific distribution between carbohydrate and fat. I think this experimental design or model is very appropriate to do a lot of other changes, it is not limited to changes in carbohydrate only but any other nutrient that one wants to change in early postnatal life. The experimental approach is very powerful in terms of examining the effects of essential fatty acids, and anything else that one wants to study.

Dr. Endres: The lactose content is about the same in human breast milk and in infant formulae. Thus, I would not expect that any obesity deriving from breast milk substitutes in contrast to breast-feeding is due to the carbohydrate. I would expect it is something else, for example the protein level which is probably too high in many infant formulae.

Dr. Patel: Human milk and milk formula for babies may be very similar in terms of macronutrient compositions. Our experimental design suggests that carbohydrate intake, whether given in the form of milk or as a food supplement, can make a difference. It is not the change in the composition of milk formula that is given to human babies, but it is the food supplement which changes the overall calorific content and the calorific composition for the human baby with a preponderance of carbohydrate calories because of the mixing of milk formula and a food supplement. It would be desirable not to change the carbohydrate and fat distribution. Supplement as such would not be a bad idea; the question is what you put in a supplement and how that might influence the outcome later in life.

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