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Lipid Metabolism During the Perinatal Phase, and its Implications on Postnatal Development

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Abstract: During pregnancy, lipid metabolism plays a major role to warrant the availability of substrates to the foetus. By using different experimental designs in the rat we have been able to answer several questions that were open about the short- and long-term effects of alterations of lipid metabolism during the perinatal stage. The first one was to demonstrate the importance of maternal body fat depot accumulation during the first half of pregnancy. We found that conditions like undernutrition circumscribed to this specific period when foetal growth is still small, that impede such fat accumulation not only restrain intrauterine development but also have long-term consequences, as shown by an impaired glucose tolerance when adults. Secondly, undernutrition during suckling has major long-term effect decreasing body weight, even though food intake was kept normal from the weaning period. Present findings also show that a diet rich in ω-3 fatty acids during pregnancy and lactation has negative effects on offspring development, but cross fostered experiments showed that the effect was a consequence of the intake of these fatty acids during the lactation period rather than during pregnancy. Pups from dams that were fed a fish oil-rich diet during pregnancy and lactation were found to have altered glucose/insulin relationship at the age of 10 weeks. Since a ω-3 fatty acid-rich diet decreases milk yield during lactation, additional experiments were carried out to determine whether decreased food intake, altered dietary fatty acid composition, or both were responsible for the long-term effects on the glucose/insulin axis. Results show that the decreased food intake caused by a ω-3 fatty acid-rich diet rather than the change in milk composition during suckling was responsible for the reduced pancreatic glucose responsiveness to insulin release at 16 weeks of age. In conclusion, present findings indicate that impaired maternal fat accumulation during early pregnancy and food intake during lactation, rather than a difference in dietary fatty acid composition have a greater influence on postnatal development and affect glucose/insulin relationships in adult rats.

Key words: Pregnancy, undernutrition, rats, fish oil diet, olive oil diet, suckling, postnatal development, insulin, glucose, long-term consequences

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There is now evidence that impaired foetal and early postnatal growth followed by an accelerated weight gain thereafter confers an increased susceptibility for the development of adult chronic disease such as type 2 diabetes, obesity and cardiovascular disease [1, 2]. Early nutrition influences development and can cause adaptive and permanent changes in structure, physiology and metabolism [3].

During pregnancy, the availability of nutrients to the foetus depends on those crossing the placenta from maternal circulation, which depends on maternal nutrition [4, 5]. However, we do not know yet the impact of maternal nutrition on foetal development. Although dietary interventions in pregnancy on birth weight in humans have lead to the erroneous view that foetal nutrition is poorly affected by maternal nutrition [6], there are studies in sheep and rats showing that maternal undernutrition during pregnancy leads to intrauterine growth retardation and produces long-term effects in the offspring, altering glucose tolerance [7, 8].

It is proposed that maternal nutrition must be extended beyond a mother's diet to include her body composition and metabolism [5]. Lipid metabolism plays a major role in maternal metabolic adaptations to warrant the availability of substrates to the foetus [5, 9, 10]. We therefore hypothesized that the accumulation of fat depots that takes place in maternal tissues during pregnancy plays a key role in the availability of nutrients to the foetus and in its subsequent growth and health.

We were interested in determining how changes in the capability of the mother to accumulate fat depots during different stages of gestation, and in maternal nutrition during pregnancy and lactation, have short- and long-term consequences on offspring development and on the susceptibility for causing adult disease. Due to ethical and methodological limitations, appropriate experimental models are needed to answer these questions. By using the rat, we applied different experimental designs to study the effects of alterations in maternal fat depot accumulation and nutrition during the perinatal stage on lipid metabolism, and its consequences on postnatal development and susceptibility to alter the glucose/insulin relationships. Thus, by presenting data from our own laboratory of studies in the rat, we will analyse here the changes of fat depot accumulation that take place along pregnancy and the role of maternal undernutrition circumscribed to specific periods of pregnancy and lactation, and changes in dietary fatty acids, on offspring growth and long-term effects in glucose/insulin relationships.

Fat depot accumulation in normal pregnancy

Fat accumulation is a characteristic feature of pregnancy, occurring in both women [11–13] and experimental animals [14–17]. The accumulation of maternal fat in maternal depots takes place during the first two-thirds of gestation but stops, or even declines, during the last third [11, 14, 18, 19], as a consequence of enhanced adipose tissue lipolytic activity.

During early pregnancy, body fat accumulation seems to be the result of both hyperphagia and increased lipid synthesis. Hyperphagia during pregnancy occurs in both women [20, 21] and in rats [16, 22]. Both, fatty acid synthesis and the conversion of glucose to form glyceride glycerol, have been found to increase progressively in rat adipose tissue until day 20 of gestation and then, sharply decline on day 21, just before parturition [23, 24].

An enhanced responsiveness to insulin has been recently proposed as the hormonal change being responsible for the anabolic events taking place in adipose tissue during early pregnancy that result in fat depot accumulation [25].

During late pregnancy, lipoprotein lipase (LPL) activity in rat adipose tissue has consistently been found decreased [18, 26, 27, 28]. Postheparin LPL activity has also been found to decrease in pregnant women during the third trimester of gestation [29]. Thus, it is proposed that fat uptake by adipose tissue decreases during late pregnancy, and this change, together with the enhanced lipolytic activity, results in the net accelerated breakdown of fat depots during the last trimester of pregnancy, which coincides with the phase of maximal foetal growth [18, 30]. Increased lipolysis of adipose tissue fat stores occurs both in women and rats during the last third of gestation [31–35].

The products of adipose tissue lipolysis, free fatty acids (FFA) and glycerol, are released into the circulation. Since the placental transfer of these products is quantitatively low [36], their main fate is maternal liver [37] where, after conversion into active forms, acyl-CoA and glycerol-3-phosphate respectively, they are re-esterified for the synthesis of triacylglycerols that are released into the circulation as part of very low density lipoproteins (VLDLs). Since insulin inhibits both adipose tissue lipolytic activity [38, 39] and hepatic VLDL secretion [40] but increases LPL activity [41], the insulin-resistant condition of late pregnancy contributes to both the increased lipolysis of fat stores [42] and the increased VLDL production, although for the latter, the enhanced estrogen concentration at late pregnancy seems to be its major activator [43].

In addition to the use of the lipolytic products in the

resynthesis of triacylglycerols described above, glycerol may be used for glucose synthesis and FFA for β-oxidation to acetyl-CoA, leading to energy production and synthesis of ketone bodies; these pathways also increase markedly under fasting conditions in late pregnancy [44–47].

The preferential use of glycerol for gluconeogenesis and the efficient placental transfer of the newly-formed glucose may be of major importance to the foetus under such fasting conditions, where the availability of other essential substrates like amino acids is reduced [44, 48]. The enhanced maternal ketogenesis during fasting also benefits the foetus in two ways: i) ketone bodies are used by maternal tissues thus sparing glucose for essential functions and delivery to the foetus. ii) Placental transfer of ketone bodies is very efficient [49], and ketone bodies may be used by the foetus as oxidative fuels [50] as well as substrates for brain lipid synthesis [51].

Short- and long-term effects of maternal undernutrition during the first half of pregnancy

We have previously described that a decrease in the capacity of the mother to accumulate fat depots during the first half of gestation, as result of hypothyroidism, greatly compromises normal catabolic adaptations of late pregnancy and impairs foetal growth [52, 53]. It seems then that maternal accumulation of fat depots during early pregnancy may play a key role in the availability of nutrients to the foetus. To investigate this possibility we determined the consequences of undernutrition circumscribed to the first half of pregnancy in the rat, in order to avoid maternal fat depot accumulation, on short- and long-term in their offspring.

Age matched female rats were mated, and from the day of appearance of spermatozoids in vaginal smears (day 0 of pregnancy) they were divided into two groups. One group was fed *ad libitum* (controls) whereas the other group was allowed to eat 60% of the amount of food consumed by controls (underfed). Animals were kept on this feeding conditions until day 12 of gestation, when the increase in maternal body weight from the onset of pregnancy was 68.9 ± 1.5 g in controls whereas it was just 16.3 ± 4.1 g in the underfed rats ($p < 0.001$). This finding is interpreted in the sense of an incapacity of the underfed pregnant rat to increase her fat depots, since during this period of pregnancy the increase of foetal-placental structures ("conceptus") is very small [30], and most of the increase in maternal body weight during early pregnancy corresponds to her fat accumulation [18]. From day 12 of pregnancy, all animals were allowed to eat *ad libitum*, and maternal body weight increased in parallel in both groups, in such a way that at day 20 of pregnancy the increase in body weight during pregnancy of controls was 162.6 ± 4.8 g, whereas it was 113.1 ± 4.5 g in the rats that were underfed ($p < 0.001$). Thus, impaired accumulation of fat depots, as a consequence of underfeeding during early pregnancy, is not overcome when a normal feeding condition is instaurated during the second half of gestation.

At the time of delivery, both the number of alive newborns per litter and their body weight were significantly lower in the rats that were underfed during early pregnancy as compared to the controls. This finding shows that despite of the small foetal growth that takes place during the first half of pregnancy, an impairment of the mother to accumulate fat depots during this specific phase clearly damages the normal intrauterine development, with consequences that are seen at the time of birth.

During lactation, newborns from dams that were underfed during early pregnancy and those from control dams were allowed to suckle from their respective mother fed *ad libitum*. It was found that at the time of weaning (21 days after delivery) pups from both groups had a similar body weight. Although this finding indicates that the negative effect caused by maternal undernutrition during early pregnancy on intrauterine development disappeared by allowing a free access of food during suckling, it does not discard the possibility of long-term consequences.

In order of determining whether maternal undernutrition during early pregnancy caused any long-term effect on the insulin-glucose axis, pups were studied when they were 16 weeks old. They were subjected to an oral glucose tolerance test by giving 2 g glucose/Kg body weight, and collecting blood from the tail at 0, 5, 10, 15, 20, 30, 45 and 60 min thereafter. The area under the curve of plasma insulin and glucose along the 60 min was calculated, and the corresponding ratios are shown in Figure 1. It is clearly seen that both adult male and female pups from rats that were underfed during early pregnancy have an impaired response to the glucose load, as shown by a significant increase in the ratio of the area under the curve for insulin and glucose. Although a more direct experimental design, like the hyperinsulinemic euglycemic clamp or the minimal model, would be needed to test insulin sensitivity, the augmented ratio of the area under the curve of plasma insulin and glucose seen in the adult pups from mothers that were underfed during the first half of pregnancy after an oral glucose load indicat an impaired insulin responsiveness.

Thus, present finding shows that maternal undernutrition circumscribed to just the first half of pregnancy has negative effects on intrauterine development and a long-term effect impairing glucose tolerance in adults.

Long-term effects of undernutrition during early postnatal life

Since negative effects on development and on glucose/insulin relationship could be also the result of an undernutrition condition just during suckling, we also used an animal model to test this possibility. In order of decreasing the amount of milk intake, litter size from untreated rats were adjusted to 16 pups per litter (underfed pups) whereas controls were adjusted to 9 pups per litter. At weaning (day 21 of age), underfed pups (i.e. those of litters having 16 pups) weighed less than controls ($p < 0.001$), demonstrating a decreased milk intake in these pups. From this time on, all pups were allowed to eat *ad libitum*, but at 16 weeks of age, body weights remained lower in those pups that were underfed during suckling than in their controls, showing that they were unable to catch up the body weight of the controls.

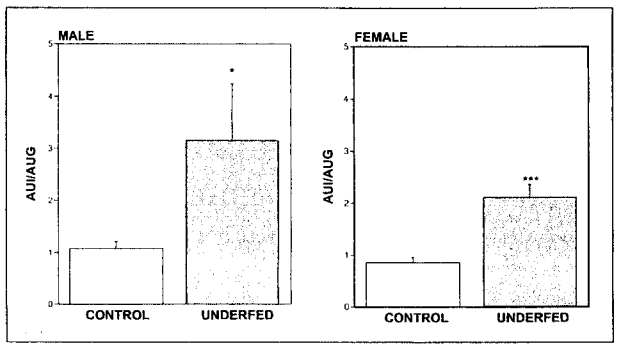

Figure 1: Ratio of the area under the curve of plasma insulin (AUI) and glucose (AUG) during an oral glucose tolerance test (2 g of oral glucose/Kg body weight) in 16 weeks old pups from dams that were either fed *ad libitum* throughout pregnancy (controls) or were underfed during the first half of pregnancy (available only 60% of the food eaten by the controls during just the first 12 days of pregnancy). Methodological details for the oral glucose tolerance tests as previously reported [58]. Statistical comparison between underfed and control rats: * = $p < 0.05$, *** = $p < 0.001$.

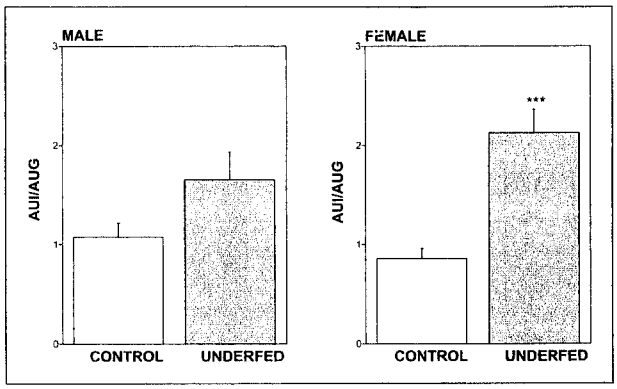

Figure 2: Ratio of the area under the curve ratio of plasma insulin (AUI) and glucose (AUG) during an oral glucose tolerance test (2 g of oral glucose/Kg body weight) in 16 weeks old pups born from rats fed normal chow diet that were either kept under control- (9 pups per litter) or underfed-conditions (16 pups per litter) during suckling. All the rats were allowed to eat *ad libitum* from the time of weaning until the study. Methodological details for the glucose tolerance tests as previously reported [58]. Statistical comparison between underfed and control rats: *** = $p < 0.001$.

At 16 weeks of age, oral glucose tolerance tests were performed following the same protocol described above. As shown in Figure 2, the ratio of the area under the curve for insulin versus the area under the curve of glucose was higher in the pups that were underfed during suckling, either males or females. These findings therefore indicate that decreased food intake circumscribed to the suckling period not only causes a permanent growth retardation but impairs glucose tolerance when adults, with the subsequent risk for developing overt diabetes.

Short- and long-term effect of modifying dietary fatty acid composition during pregnancy and lactation

Under the base of the above results, we wanted to determine whether a change in dietary fatty acids during pregnancy and lactation in the rat affected the offspring outcome. With this aim, female rats were allowed to eat *ad libitum* from mating until the end of lactation a semisynthetic diet having 10% of either fish oil (FOD) or olive oil (OOD) as the only non-vitamin fat component. The composition of the diets were as previously described, [54, 55] and their proportion of fatty acids is shown in Figure 3, where it appears that the FOD had higher proportion of ω-3 fatty acids (eicosapentaenoic and docosahexaenoic acids, 20:5 ω-3 and 22:6 ω-3, respectively) but lower proportion of oleic acid (18:1) than the OOD.

At birth, newborns from dams fed FOD weighed less than those from dams fed OOD ($p < 0.01$), and this difference was further enhanced along the suckling period despite that the litter size was always adjusted to 8 pups per litter. As already reported [55], the increase in body size and the acquisition of psychomotor reflexes during suckling were delayed in pups suckled by dams fed FOD as compared to those of OOD. As shown in Figure 4, the estimated milk yield of the dams fed FOD was lower than those fed OOD. Therefore, the delayed postnatal development of pups from dams fed FOD could be the result of

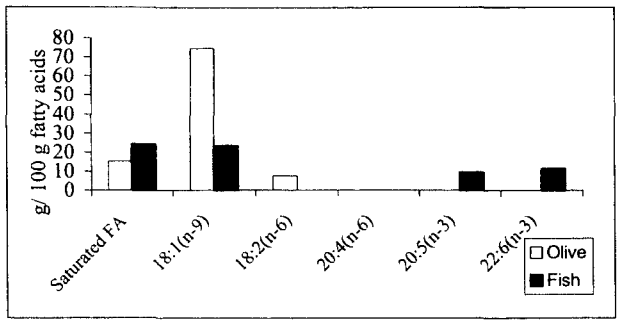

Figure 3: Fatty acid composition of semisynthetic diets containing 10% of either olive oil (OOD) or fish oil (FOD) as the only non-vitamin lipidic component. Methodological details as in [55].

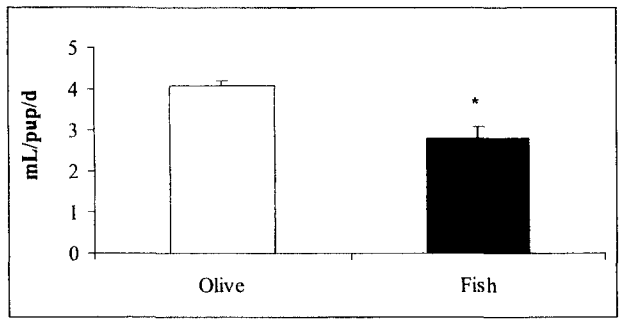

Figure 4: Estimated milk yield at day 10 of lactation, measured as described [59], in lactating rats fed with either olive oil diet (OOD) or fish oil diet (FOD). Statistical comparison: * = $p < 0.05$.

both the different fatty acid composition as well as the decline in milk intake.

We also wanted to determine whether these differences in the postnatal development of the pups from the two groups were due to the effects of the type of food eaten by the mother either during pregnancy or lactation. Thus, an experiment of cross-fostered was designed, where newborns from dams fed FOD during pregnancy were lactated by dams fed OOD (FOD-OOD) and vice versa (OOD-FOD). For comparison, we also studied pups that were suckled from dams that during lactation were fed the same diet that during pregnancy (OOD-OOD, and FOD-FOD). The indexes of acquisition of one representative psychomotor reflex, the air righting reflex, during suckling are shown in Figure 5. It is seen that pups suckled by dams fed OOD during lactation but coming from dams fed FOD during pregnancy have the same value for the acquisition of the psychomotor reflexes than those whose mother was fed OOD for the whole time (OOD-OOD). However, when pups born from dams that were fed OOD during pregnancy were suckled by dams fed FOD, a decreased capacity to acquire the psychomotor reflexes was observed, attaining the same level of pups from the FOD-FOD group.

These findings therefore show that a change in the composition of fatty acids in the diet during lactation affects more the postnatal development than when the change is circumscribed to pregnancy. Although during pregnancy the amount of food intake between dams fed FOD do not differ to that of OOD, there is a possibility that an additive effect of altered dietary fatty acid composition plus the undernutrition caused by decreased milk yield during lactation would be affecting the development of pups from dams fed FOD during lactation.

Long-term effects of changes in dietary fatty acids composition during suckling in the rat

Under the findings summarized in the previous section, it was decided to determine whether the two factors together, an enhanced intake of ω-3 fatty acids and undernutrition during suckling, had any long-term effect on the glucose/insulin axis. With this purpose, pups suckled by dams fed either FOD or OOD and kept in litters of 8 pups each, were allowed to eat *ad libitum* from the time of weaning. It was found that the decreased body weight of pups from FOD dams was maintained at 7 and 10 weeks of age in males and in females. At this later age, the oral glucose

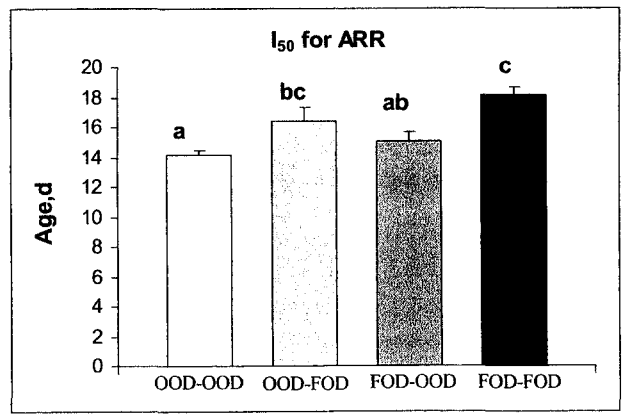

Figure 5: Acquisition of air righting reflex in suckling newborns, expressed as the day that 50% of the litter acquired the mature response (I_{50}), from rats fed fish oil diet (FOD) during pregnancy and lactation (FOD-FOD), olive oil diet (OOD) during pregnancy and FOD during lactation (OOD-FOD), FOD during pregnancy and OOD during lactation (FOD-OOD), or OOD during both pregnancy and lactation (OOD-OOD). Different letters indicate significant differences between the groups ($p < 0.05$). Methodological details as in [55, 60].

tolerance test showed a similar increase in plasma glucose levels but smaller increases in plasma insulin in those pups that were suckled by dams fed FOD than in those that suckled from dams fed OOD. Thus, whereas the area under the curve for plasma glucose was similar in the two groups, the area under the curve for the change of insulin was significantly lower in both females and males that were suckled by dams fed FOD as compared to those from OOD ($p < 0.05$), indicating either an impaired pancreatic insulin release, an enhanced insulin sensitivity, or both.

Since the altered oral glucose tolerance test in pups that were suckled by dams fed FOD could be the result of either the increased ω-3 fatty acids in maternal milk or the decreased food intake as result of the decreased milk yield (see above), two additional experiments were performed to determine between these two posibilities. In the first one, pups that were suckled by dams fed OOD that had 8 pups per litter during lactation (controls) were compared to those from dams that were fed OOD but had 16 pups per litter during lactation (underfed). As shown in Figure 6, at the time of weaning, the underfed pups weighed much less than their controls. At this time, pups from both groups were fed *ad libitum* regular chow diet, but still at 16 weeks of age pups that were underfed during suckling remained having a lower body weight than controls, the difference being statistically significant for males as well as for females. At this age (16 weeks old), oral glucose tolerance test showed a similar increase of plasma glucose in those pups that were underfed during suckling as compared to their controls but the increase in plasma insulin was lower in the former group (Figure 7). This differential response between the two groups was similar in females as in male rats, and shows that decreased food intake during suckling contributes to the lower insulin release after the glucose load when adults. This finding agrees with those previously reported [56] showing a persistently reduced pancreatic glucose-responsiveness in rats subjected to large litters until weaning.

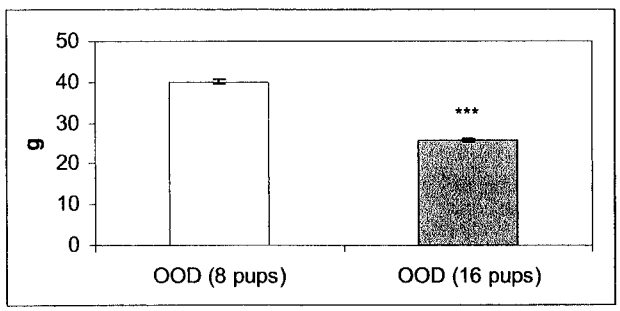

Figure 6: Body weight at the time of weaning (21 days) of pups from rats fed an olive oil diet (OOD) during pregnancy and lactation, that during lactation had litter size adjusted to either 8 (controls) or 16 pups (underfed)/dam. Statistical comparison: *** = $p < 0.001$.

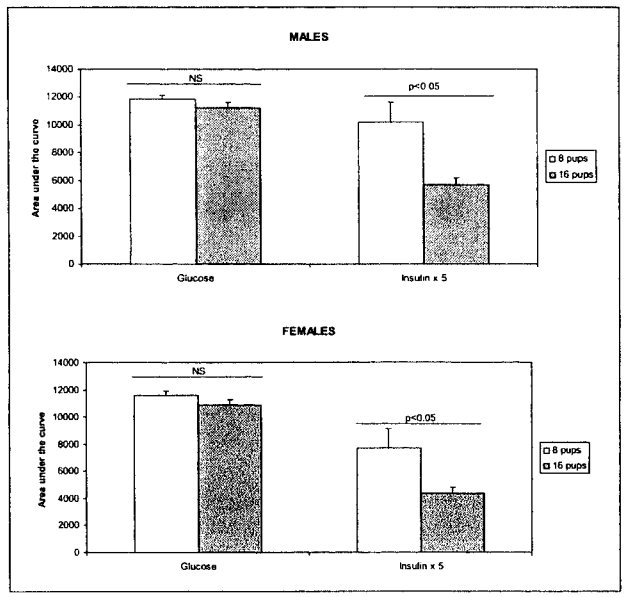

Figure 7: Area under the curve of plasma glucose and insulin during an oral glucose tolerance test (2g /Kg) of 16 weeks old male and female pups that during suckling were underfed (16 pups/litter) as compared to controls (8 pups/litter). Methoological details for the glucose tolerance tests as previously reported [58].

The second experiment was addressed to determine whether an enhanced intake of ω-3 fatty acids during suckling but unchanged food intake could have long-term effects on the glucose/insulin axis. For this purpose, newborns from normally fed rats were suckled either by dams fed the OOD and with litter adjusted to 8 pups (OOD-8) or by dams fed FOD and with litter adjusted to 4 pups (FOD-4). The estimated milk yield per pup at the peak of the lactation period (15 days) was similar in both groups, and the body weight at the time of weaning did not differ between them, showing a similar nutritional condition in both groups. As shown in Figure 8, milk composition of dams fed FOD had higher proportion of ω-3 fatty acids but lower proportion of oleic acid (18:1) than in those fed OOD. Thus, we had two groups of rats with a similar food intake during suckling but with a different proportion of fatty acids in their diet. From weaning, both groups were allowed to eat *ad libitum* a regular rat chow and were studied at 16 weeks of age. At this time their body weight did not differ between the groups. After an oral glucose load (2g/Kg) the increase in plasma glucose and plasma insulin was similar in the two groups (OOD-8 and FOD-4), and this was so both in female and male rats. In fact the ratios of the area under the curve for insulin and glucose were similar in the OOD-8 and FOD-4 pups (data not shown).

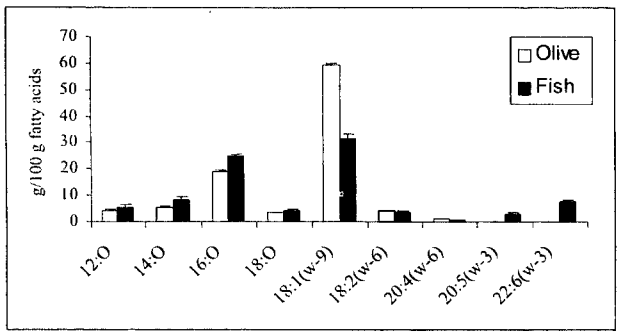

Figure 8: Fatty acid composition of milk at day 10 of lactation in rats fed either olive oil (OOD) or fish oil (FOD). Methodological details as previously described [55].

Although as recently reviewed, changes in the glucose tolerance tests in pups as a result of maternal undernutrition during the perinatal stages could be transitory, ranging from enhanced glucose tolerance in early adult life (6–12 weeks), to unchanged at 44 weeks of age and even decreased at 15 months of age [57], present findings indicate that a change in the dietary fatty acid composition without affecting the amount of food intake during suckling in the rat does not have either short- or long-term effect in body weight nor affecting long-term glucose/insulin relationships.

Conclusions and final considerations

Several conclusions may arise from these findings:

1. During pregnancy, maternal fat depot accumulation takes place during the first half of pregnancy, when foetal growth is still small. The main endocrine factor responsible for this change seems to be maternal hyperinsulinemia and the enhanced adipose tissue insulin sensitivity that takes place at this stage. During late pregnancy, the net catabolic condition of maternal adipose tissue driven by insulin resistance allow the availability of substrates to sustain foetal growth, despite that lipids cross with difficulty the placenta.
2. Maternal undernutrition during the first half of gestation greatly impairs her capacity to accumulate fat depots, and compromises the catabolic adaptations normally taking place during late pregnancy. This causes a subsequent undernutrition condition in the foetus during the stage of its maximal growth rate. Decreased offspring body weight at birth found in those animals from dams that were underfed during the first half of gestation disappears during suckling, but an impaired glucose tolerance remains at 16 weeks of age.
3. Undernutrition during suckling caused by increasing litter size or by feeding lactating dams fed a fish oil-rich diet that decreases milk yield, permanently impairs normal development and alter glucose tolerance when adults.
4. Substantial changes in dietary fatty acids composition during lactation but normal food intake in the suckling pups do not affect their growth nor alter glucose/insulin relationships when adults.

Extrapolation of these conclusions to humans must be made with caution, but present findings give an experimental support of the current hypothesis that undernutrition during specific perinatal stages alter foetal programming events leading to altered birth weight and permanent structural and functional changes that predispose to disease in adult life. Current results extend these conclusions by emphasizing the importance of maternal nutrition during early pregnancy. In fact, any nutritional or endocrine insult that would limit the mother's capacity to accumulate fat depots during early pregnancy compromises the catabolic events that normally take place during late gestation, since the availability of susbtrates crossing the placenta, which at this stage are highly needed to sustain the rapid foetal growth would be limited.

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