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## **EXPERIMENTAL MODELS FOR STUDYING PERINATAL LIPID METABOLISM**

*Long-term effects of perinatal undernutrition*

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**Abstract:** By using different experimental designs in the rat we have been able to answer several unanswered questions on the short- and long-term effects of alterations of lipid metabolism during the perinatal stage. The first was to demonstrate the importance of maternal body fat accumulation during the first half of pregnancy, since undernutrition in this critical period when fetal growth is slow, impedes fat depot accumulation and not only restrains intrauterine development but has long-term consequences, as shown by an impaired glucose tolerance when adults. Secondly, undernutrition during suckling has major long-term effect of decreasing body weight, even though food intake is kept normal from the weaning period. Our findings also show that a diet rich in n-3 fatty acids during pregnancy and lactation has adverse effects on offspring development, but cross fostered experiments showed that this 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 n-3 fatty acid-rich diet decreases milk yield during lactation, additional experiments were carried out to determine whether decreased food intake or 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 n-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 major effects 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.

## **1. INTRODUCTION**

Impaired fetal and early postnatal growth confers an increased susceptibility for the development of adult chronic disease such as type 2 diabetes, obesity and cardiovascular disease (3; 15). Early nutrition influences development and can cause adaptive and permanent changes in structure, physiology and metabolism (14). During pregnancy, the availability of nutrients to the foetus depends on those crossing the placenta from maternal circulation, which depends on maternal nutrition. In order to determine how changes in maternal nutrition during pregnancy and lactation have short- and long-term consequences on offspring development and susceptibility for causing adult disease, appropriate experimental models are needed, due to ethical and methodological limitations. By using the rat, we applied different experimental designs to study the effects of alterations in maternal nutrition during the perinatal stage on lipid metabolism, and its consequences on postnatal development and susceptibility to alter the glucose/insulin relationships.

## **2. SHORT- AND LONG-TERM EFFECTS OF MATERNAL UNDERNUTRITION DURING THE FIRST HALF OF PREGNANCY**

During pregnancy, the concept of maternal nutrition must be extended beyond a mother's diet to include her body composition and metabolism (6). Lipid metabolism plays a major role in maternal metabolic adaptations to warrant the availability of substrates to the foetus (7; 8). The accumulation of fat depots in maternal tissues is a constant characteristic feature in pregnancy (11; 19; 20), and takes place mainly during the first half of gestation, when opposite to the insulin resistant condition that occurs during late pregnancy, there is even an enhanced sensitivity of adipose tissue to insulin (17). A decrease in the capacity of the mother to accumulate fat depots during this early part of

gestation, as result of hypothyroidism, greatly compromises normal catabolic adaptations of late pregnancy and impairs fetal growth (4; 5). Thus, it is hypothesized that maternal accumulation of fat depots during early pregnancy may play a key role in the availability of nutrients to the foetus and in its subsequent growth and health. To investigate this possibility we determined the effect of undernutrition circumscribed to the first half of pregnancy in the rat, in order to avoid maternal fat depot accumulation in short and long-term effects 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 maintained 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  $68.9 \pm 1.5$  g in controls whereas it was just  $16.3 \pm 4.1$  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 fetal-placental structures ("conceptus") is very small (10), and most of the increase in maternal body weight during early pregnancy corresponds to her fat accumulation (9). From day 12 of pregnancy, all the 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  $162.6 \pm 4.8$  g, whereas it was  $113.1 \pm 4.5$  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 resumed 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 fetal 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. 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.

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 rat, 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 ratio for insulin and glucose.

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.

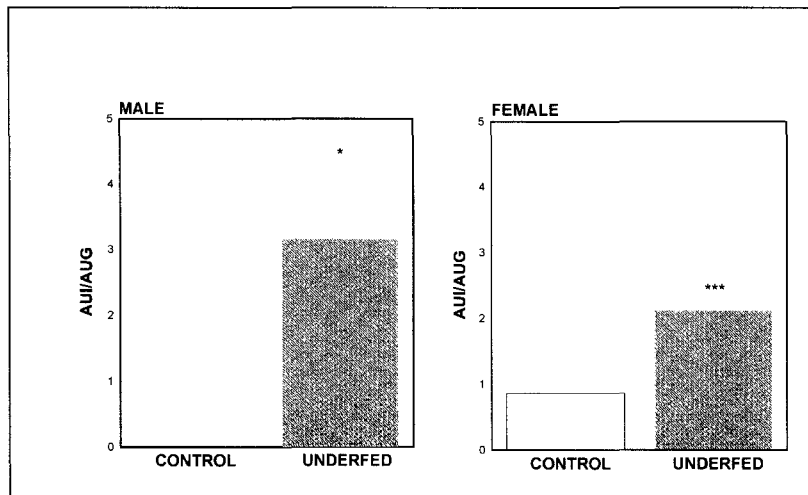


Figure 1. Ratio of the area under the curve of plasma insulin (AUI) and glucose (AUG) during an oral glucose tolerance test (2 g of oral glucose/Kg body weight) in 16 weeks old pups from dams that were either fed *ad libitum* throughout pregnancy (controls) or were underfed during the first half of pregnancy (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 (13). Statistical comparison between underfed and control rats: \*= $p < 0.05$ , \*\*\*= $p < 0.001$ .

### 3. 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.

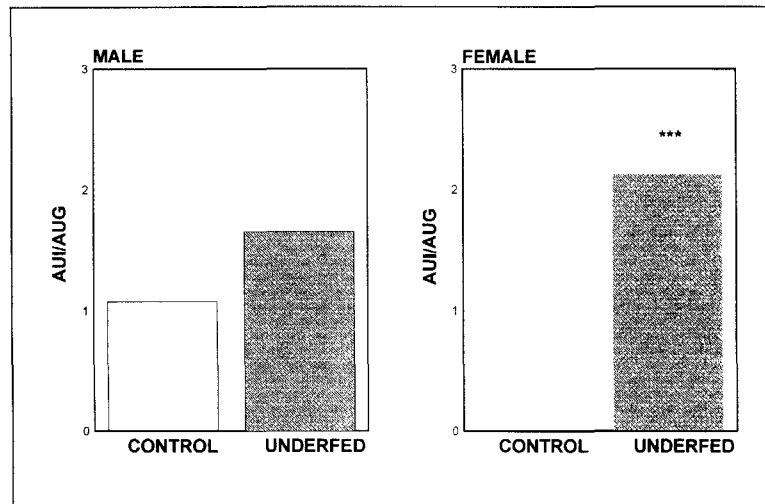


Figure 2. Ratio of the area under the curve ratio of plasma insulin (AUI) and glucose (AUG) during an oral glucose tolerance test (2 g of oral glucose/Kg body weight) in 16 weeks old pups born from rats fed normal chow diet that were either kept under control-conditions (controls, 9 pups per litter) or underfed-conditions (underfed, 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 (13). 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. It is interesting to notice that the effect of underfeeding during suckling impairing the oral glucose tolerance is smaller than that shown by those pups from mothers that were underfed during early pregnancy (figure 1).

#### 4. SHORT-AND LONG-TERM EFFECT OF MODIFYING DIETARY FATTY ACID COMPOSITION DURING PREGNANCY AND LACTATION

Under the base of 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 a 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, (1; 2) and their proportion of fatty acids is shown in figure 3, where it appears that the FOD had higher proportion of  $\omega$ -3 fatty acids (eicosapentaenoic and docosahexaenoic acids, 20:5  $\omega$ -3 and 22:6  $\omega$ -3, respectively) but lower proportion of oleic acid (18:1) than the OOD.

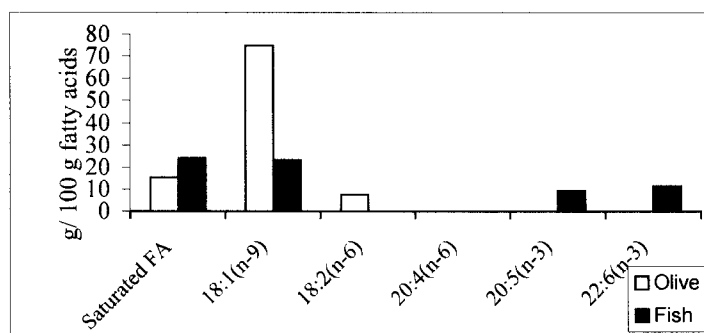


Figure 3. Fatty acid composition of semisynthetic diets containing 10% of either olive oil (OOD) or fish oil (FOD) as the only non-vitamin lipidic component. Methodological details as in (2).

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

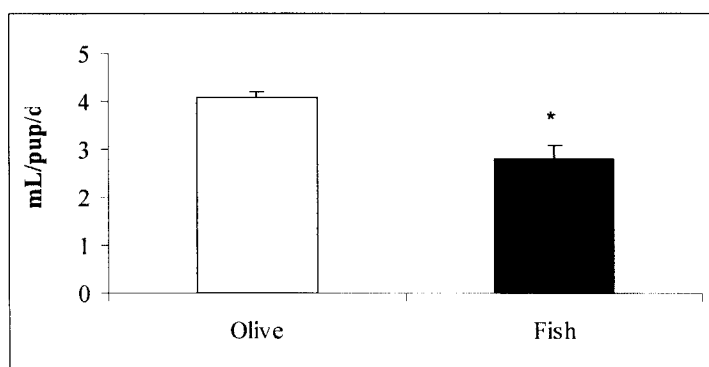


Figure 4. Estimated milk yield at day 10 of lactation, measured as described (18), in lactating rats fed with either olive oil diet (OOD) or fish oil diet (FOD). Statistical comparison:  $*=p<0.05$ .

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.

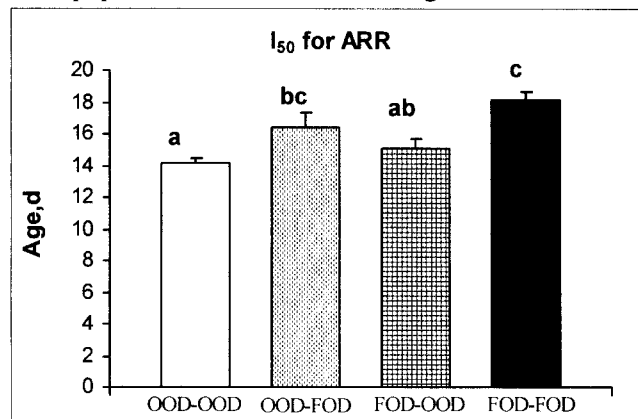


Figure 5. Acquisition of air righting reflex in suckling newborns (12), expressed as the day that 50% of the litter acquired the mature response ( $I_{50}$ ), from rats fed fish oil diet (FOD) during pregnancy and lactation (FOD-FOD), olive oil diet (OOD) during pregnancy and FOD during lactation (OOD-FOD), 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 ( $p < 0.05$ ).

## 5. 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  $\omega$ -3 fatty acids and undernutrition during suckling, have 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 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.

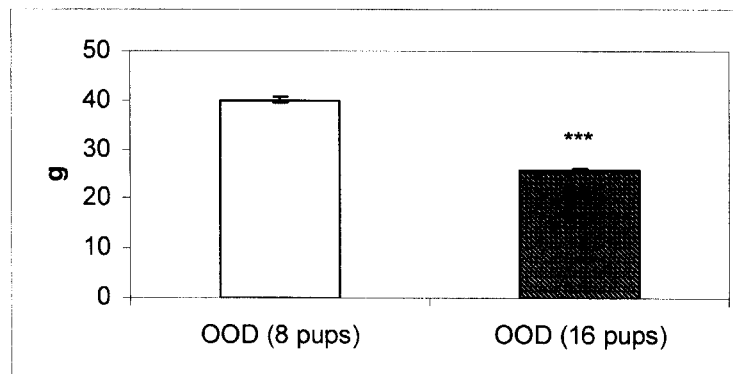


Figure 6. Body weight at the time of weaning (21 days) of pups from rats fed an olive oil diet (OOD) during pregnancy and lactation, that during lactation had litter size adjusted to either 8 (controls) or 16 pups (underfed)/dam. Statistical comparison: \*\*\*= $p < 0.001$ .

Since the altered oral glucose tolerance test in pups that were suckled by dams fed FOD could be the result of either the increased  $\omega$ -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 possibilities. 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 (21) showing a persistently reduced pancreatic glucose-responsiveness in rats subjected to large litters until weaning.

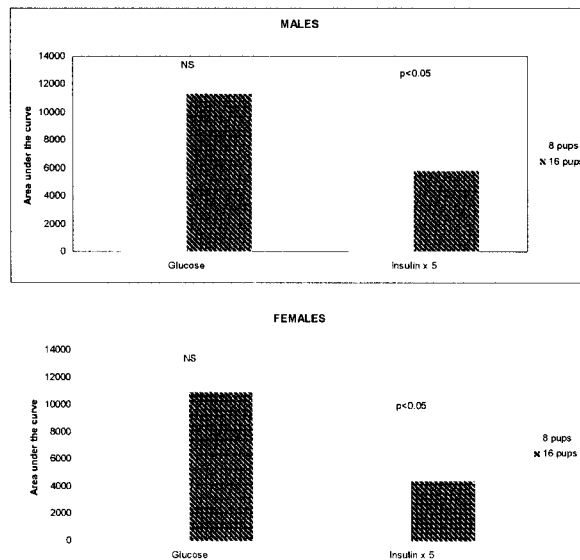


Figure 7. Ratio of the area under the curve of plasma insulin (AUI) and glucose (AUG) during an oral glucose tolerance test (2g /Kg) of 16 weeks old male and female pups that were underfed during suckling (16 pups/litter) as compared to controls (8 pups/litter,

during suckling). Methodological details for the glucose tolerance tests as previously reported (13).

The second experiment was addressed to determine whether an enhanced intake of  $\omega$ -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 by dams that were fed a semisynthetic diet having 10% of olive oil as the only non-vitamin fat and had 8 pups per litter (OOD-8) were compared to others that were suckled by dams fed the same diet but having 10% fish oil instead of the olive oil and had 4 pups per litter (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  $\omega$ -3 fatty acids but lower proportion of oleic acid (18:1) than in those fed OOD. Thus we had two rat groups 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 was similar in the OOD-8 and FOD-4 pups. Although as recently reviewed, changes in the glucose tolerance tests in pups as result of by 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 (16), 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.

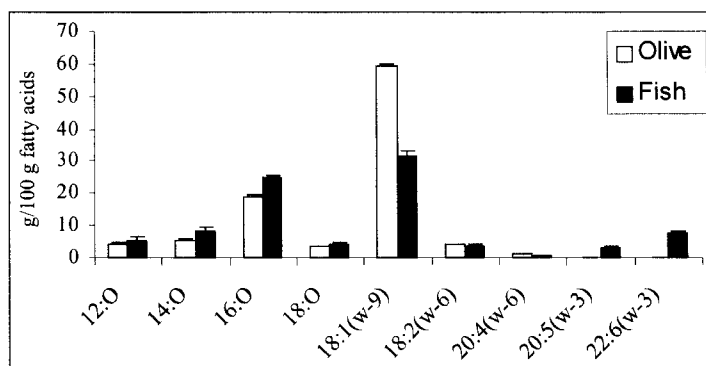


Figure 8. Fatty acid composition of milk at day 10 of lactation in rats fed either olive oil (OOD) or fish oil (FOD). Methodological details as previously described (2).

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