Fat Tolerance Test in Pregnancy: Intralipid© Loading Test

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Hyperlipemia occurs during pregnancy. The present study deals with lipid metabolism in the female by comparing the response of the pregnant with that of non-pregnant females to a lipid loading test with 10% Intralipid© given intravenously. The results were: (i) Exogenous and endogenous triglycerides (TG) were significantly higher in the pregnant group. (ii) The K2 value was significantly lower in the pregnant group. (iii) The level of FFA in the non-pregnant group rose rapidly 5 min after Intralipid load. In contrast, the pregnant group showed a gradual rise which reached a maximum one hr after administration of Intralipid. The delay of the lipid metabolism during pregnancy might be one of the reason of hyperlipemia. Hyperlipemia in pregnancy; Intralipid© loading; K2 value; serum TG and FFA concentration

It is well known that the pregnant female needs increased amount of nutritional factors to provide for the growing fetus and for the delivery process, and moreover, the metabolic environment is markedly different from that of the non-pregnant female. Lipid metabolism during pregnancy has been studied since the first report by Becquerel (1845) on hyperlipemia in pregnancy.

As a result, it is now known that there is little difference between early pregnancy and non-pregnancy but from the second trimester, serum lipids rise throughout the course of the pregnancy to reach their highest level at term. In order to analyze the origin of this hyperlipemia in the pregnant female, we administered a transient load of Intralipid© intravenously to pregnant and non-pregnant females, and measured the changes in triglycerides (TG) and FFA.

MATERIALS AND METHODS

The pregnant group consisted of 12 females in the 40 weeks of normal pregnancy, and the non-pregnant group consisted of 12 healthy volunteers in the 20-30 age group. The test was performed after a 12-hr period of fasting.

Fat emulsion of which bean oil is the main component (10% Intralipid©, Green Cross Co., Japan. Same as AB Vitrum, Stockholm, Sweden) was given by a single injection at the time of early morning fasting. Intralipid comprises TG 10%, phospholipids 1.2%.

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glycerin 2.5%, and water 86.3%. Its TG fatty acid composition is \( C_{18:2} 51\% \), \( C_{18:0} 18\% \) and \( C_{18:1} 19\% \). Intralipid was given rapidly as a 1 ml/kg intravenous bolus and the changes with time were observed. Blood specimens were taken at fixed times after Intralipid injection, centrifuged at 2,000 rpm for 10 min, and the serum was stored in ice. Exogenous and endogenous TG were separated by the P.V.P. (polyvinylpyrrolidone) gradient method of Gordis (1962) and measured by the method of Fletcher (1968). Exogenous TG as measured at the same time by the method of Lewis et al. (1972) using a micro-nephelometer (MN 102 type, Toshiba Co., Japan). Using the blood levels of exogenous TG, the \( K_2 \) value of Hallberg (1964) (fractional removal rate of Intralipid) was calculated. Total TG was measured with a TG kit (Sanko Junyaku, Ltd., Japan) based on the method of Fletcher (1968), and free fatty acids were also measured with a Wako Kit (Wako Junyaku, Ltd., Japan) based on the method of Duncombe (1964).

The structural fatty acids of TG and FFA were analyzed by extracting the serum lipids by the method of Folch et al. (1957), and separating each lipid fraction on the thin-layer chromatography. A silica-gel plate and a development solution containing ethylene glycol, ether, and acetic acid in the ratio of 30:20:1 was used. The separated spots representing lipids were exposed to iodine gas and removed. Anhydrous benzene 0.5 ml and 5% methanol chloride 2.0 ml were added, heated at 100°C for 4 hr in water bath to cause methylation, extracted with ethylene glycol and used as the sample for gas chromatography (Yanaco Model G-80. Yanagimoto Co., Japan).

**RESULTS**

Changes in serum TG

Immediately after Intralipid load, marked changes were seen in both TG and FFA. First of all, the TG in pregnant group rose on an average from 261 mg/100 ml before Intralipid load to 342 mg/100 ml after, an increase of approximately 31%. Thereafter, the level decreased with time as shown in Fig. 1 so that the level was 272 mg/100 ml, the pre-load level, at 120 min after the load. In the non-pregnant group, the TG rose from 119 mg/100 ml before Intralipid load to 226 mg/100 ml after, an increase of approximately 90%, and thereafter the level decreased to the pre-load level at 120 min after the load.

Next, the endogenous and exogenous levels of TG were studied. The early morning fasting level of endogenous TG before Intralipid load was 223 mg/100 ml in the pregnant group and 89 mg/100 ml in the non-pregnant group, that is, the level in the pregnant group was approximately 2.5 times that in the non-pregnant group. After 60 min, the level was almost similar in the pregnant group, while it increased to 119 mg/100 ml in the non-pregnant group, but this difference was not statistically significant at \( p<0.05 \).

The early morning fasting level of exogenous TG before Intralipid load was 5 mg/100 ml in the non-pregnant group, and 30 mg/100 ml in the pregnant group. This difference was statistically significant (\( p<0.01 \)). After the load, both group showed their highest levels at 5 min after administration. The increases were comparable (non-pregnant 124 mg/100 ml, pregnant 123 mg/100 ml). At 60 min after administration, the level decreased rapidly to 14 mg/100 ml in the non-pregnant group and to 25 mg/100 ml in the pregnant group (Fig. 2, Table 1).

The clearance of the exogenous TG from the blood was measured by estimating the \( K_2 \) value. In the non-pregnant group, the value was \( 2.798\pm0.545\% \) min
using the P.V.P. method, and 2.810±0.435% with the nephrometer method. In the pregnant group, the values was 1.886±0.669% min with the P.V.P. method, and 1.621±0.632% min with the nephrometer method. This indicates that the administered exogenous TG (Intralipid) remained longer in the blood in the pregnant group than in the non-pregnant group (Fig. 3).

Changes in serum FFA

The changes in FFA were studied. Both groups showed increased levels 5 min after administration. The early morning fasting level of FFA was 402 µEq/liter in the non-pregnant group and 484 µEq/liter in the pregnant group (Fig. 4).
In the non-pregnant group, the level reached a high value of 639 µEq/liter 5 min after Intralipid load and maintained a high level thereafter, and it was 631 µEq/liter at 120 min. In contrast, the pregnant group showed a slow rise to 563 µEq/liter 5 min after administration and the highest level of 706 µEq/liter 30 min after Intralipid load.
Changes in the fatty acid composition of TG

Changes in the fatty acids after Intralipid load are illustrated in Fig. 5. The changes were most marked 5 min after the load with an abrupt decrease in C_{16:0} and an increase in C_{18:2}. The changes were less, however, in the non-pregnant group. The changes in the fatty acid composition of exogenous TG in the pregnant group showed exactly the same pattern as that of the changes in total TG. The range of changes was wider in exogenous TG than in total TG.

It is interesting that, regardless of being pregnant or non-pregnant, C_{18:1} and C_{18:2} abruptly increased and C_{16:0} rapidly decreased.

Changes in the fatty acid composition of FFA

Fig. 6 shows the changes in the fatty acid composition of FFA. As for TG, C_{18:2} increased and C_{16:0} decreased by 30 min after the load, although the changes were not marked as those for TG. This result indicates that the administered Intralipid was rapidly broken down to FFA and caused the changes in fatty acid composition.

![Graph showing changes in fatty acid composition](image)

**Fig. 6. Changes in the fatty acid composition of FFA.**

**DISCUSSION**

The genesis of hyperlipemia during pregnancy is complicated because of the presence of the developing fetus in addition to the general etiological mechanisms for hyperlipemia. Therefore, we investigated changes in lipids in pregnant and non-pregnant females by measuring fasting levels of serum lipids and the response to a lipid load.

Exogenous lipid, Intralipid, as administered intravenously and the effects on serum TG metabolism were analyzed. The serum TG concentration after Intralipid load was highest after 5 min. The levels at that time averaged 1.9 times the preload level in the non-pregnant group, and 1.3 times in the pregnant group. This was due to the high pre-load level in the pregnant group. The difference
between before and 5 min after Intralipid load was 107 mg/100 ml in the non-pregnant and 81 mg/100 ml in the pregnant group.

Intralipid used for the loading is taken up by the liver, adipose tissue and other tissues, and disappears from the blood. It is used as one source of energy in adipose tissue, and the rest is stored as fat deposit. In the liver, it is used partially as an immediate energy source after hydrolysis. The majority, however, is esterified to TG, phospholipids and cholesterol, then reappears in the blood as lipoprotein.

The K2 value was first studied by Hallberg (1964). In this tolerance test, the disappearance of injected fat emulsion from blood was characterized by 2 rates: K1, the maximal removal rate, and K2, the fractional removal rate of the injected triglycerides. The K2 phase was thought to be mainly due to triglyceride lipase, pinocytosis, phagocytosis and to have little correlation with disease process. As for the relationship with K2, it is said to be low in hyperlipemia, coronary artery disease, atherosclerosis, and old people. Therefore, low values, in general, suggest decreased ability to control lipids (Boberg et al. 1969).

The K2 value in the present test was significantly low in the pregnant as compared with the non-pregnant, indicating that the exogenous TG remained longer in the blood. Furthermore, the early morning fasting exogenous TG levels were also significantly higher in the pregnant group. This is interpreted as indicating that the TG ingested at the previous evening meal remained longer in the blood in the pregnant group.

On the other hand, high fasting levels of endogenous TG were characteristic of the pregnant group. This was thought to be due to increases in phospholipids and lipoprotein during pregnancy accompanied by the break-down of exogenous lipids and the promotion of pathways for the lipid syntheses.

Another characteristic was the marked decrease in C16:0 and the large increase in C18:2 after Intralipid load. C16:0 and C18:1 occur in approximately equal amounts in Intralipid, but C18:1 increased in the same way as C18:2, whereas C16:0 decreased. This suggests that differences in the number of carbon atoms result in differences in the speed of utilization. Fatty acids of fraction also showed an increase in C18:2 in a short time. This was probably due to extremely rapid hydrolysis of the administered exogenous TG by triglyceride lipase.

The present experiments show that the ability to control exogenously administered TG is decreased in the pregnant female. Assuming that the blood volume is increased, the pregnant female may control exogenous TG with almost the same speed as the non-pregnant female, and it implies the need for calculation based on blood volume.

However, one of the reasons for the hypertriglyceridemia during pregnancy may be a decreased control ability suggested by low K2 values.

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References


