ELSEVIER

Biochimica et Biophysica Acta 1215 (1994) 275–279

BBA Biochimica et Biophysica Acta

Effect of starvation on lipoprotein lipase activity in different tissues during gestation in the rat

Pilar López-Luna [a], Juana Olea [b], Emilio Herrera [b,*]

[a] *Departamento de Fisiología y Farmacología, Universidad de Alcalá de Henares, 28034-Madrid, Spain*
[b] *Departamento de Investigación, Hospital Universitario Ramón y Cajal, Ctra. Colmenar Km 9, 28034-Madrid, Spain*

Received 28 February 1994; revised 13 July 1994

Abstract

This study was addressed to determine whether the tissue-specific LPL activity response to fasting differs between nonpregnant and pregnant rats over the course of pregnancy. Fed and 24-h fasted rats were studied at days 12, 15 or 20 of gestation and were compared to virgin controls. In fed rats at days 15 and 20 of gestation LPL activity decreased in lumbar adipose tissue and the heart and liver, and increased in mammary gland tissue. Fasting decreased LPL activity in lumbar adipose tissue in 12 day pregnant and virgin rats and in mammary gland tissue in pregnant rats at 15 and 20 days of gestation and in virgin rats, whereas it increased LPL activity in heart tissue in rats at day 15 and 20 and in liver at day 20 of gestation. Plasma triacylglycerols were higher in 20 day pregnant rats than in the other groups when fed and this difference was even more noticeable in the fasting condition where the plasma β-hydroxybutyrate level also reached the highest value in the 20 day pregnant rats. Since tissue LPL activity controls the hydrolysis and uptake of circulating triacylgylcerols, the present results indicate that in fed rats after the 15th day of gestation circulating triacylglycerols are preferentially taken up by the mammary gland instead of being taken up by adipose tissue and heart. However, after fasting, circulating triacylglycerols are driven to the heart and liver in the late pregnant rat, and become a major source for fatty acid oxidation, an effect that seems to be specially evident in the liver of the 20 day pregnant rat where there is an intense increase in LPL activity and the triacylglycerols become preferential substrates for ketone body production.

Keywords: Lipoprtein lipase; Triacylglycerol; Gestation; (Rat)

1. Introduction

Lipoprotein lipase (LPL) is an enzyme which plays a major role in the regulation of triacylglycerol metabolism. In its active form, LPL is found bound to the luminal surface of capillary endothelial cells where it catalyses the hydrolysis of triacylglycerols from circulating chylomicrons and very-low-density proteins (VLDL), into free fatty acids and 2-monoacylglycerol or even glycerol that are taken up and utilized by the subjacent tissue [1–3]. LPL activity is found in practically every extrahepatic tissue and adipose tissue, heart and lactating mammary glands have the highest transcriptional and catalytic activity for this enzyme [2]. However, although LPL activity has been found in neonatal liver [4–8], the capacity for LPL hepatic synthesis is markedly reduced in the adult liver [9], where what little LPL activity is detected is mainly the result of the hepatic uptake of the enzyme from circulation [2].

LPL activity is modulated in an organ and tissue-specific manner; thus circulating triacylglycerols for fatty acid utilization are channelled towards one organ or another. The relationship between the LPL activity in different tissues and the development of hyperlipemia during late gestation has been studied in humans and in rats [10–15]. The changes occurring in mammary gland LPL activity during late gestation are probably triggered by a decline in the plasma progesterone concentration and the subsequent release of prolactin that occurs during the last days of pregnancy [6,16]. However, the decline in LPL found in white adipose tissue during late gestation seems to be the result of the insulin resistance that is detected at the end of gestation [17,18]. Although these two changes are modulated by different hormonal mechanisms, they direct circulating triacylglycerols toward the mammary gland where

* Corresponding author.

SDI 0005-2760(94)00144-8

they can be taken up and used for milk synthesis instead of being stored as fat depots [19,20]. In addition, LPL activity is also highly sensitive in a tissue specific manner to food intake. Thus, while fasting results in a reduction in LPL activity in adipose tissue it increases this activity in the heart [21–24], thus allowing fatty acids to be diverted away from storage in adipose tissues to meet the metabolic demands of the heart under conditions of caloric deprivation. The response of LPL activity to fasting also seems to differ in certain organs in the pregnant versus the nonpregnant condition. For example, whereas LPL activity in liver has been found to increase in the 21 day pregnant rat after a 24-h fast, it practically does not change in the virgin animal [14,25–27]. However, whether this different response to fasting in the pregnant versus the nonpregnant condition also occurs with LPL activity in other tissues, exactly when during pregnancy the differential response occurs, what the factor(s) are that are responsible for it or even what its physiological implications might be, are all unknown. To approach an answer to these questions, the present study examines the effect of 24-h starvation on LPL in different tissues in virgin rats and in pregnant rats at the 12th, 15th and 20th days of gestation.

2. Materials and methods

Female Wistar rats, weighing initially 160 to 180 g, were mated and the day spermatozoids appeared in vaginal smears was considered day 0 of gestation. All animals were kept under light and temperature controlled conditions (12 h on/12 h off; 22–23°C). Pregnant animals were randomly placed in groups and studied on days 12, 15 and 20 of gestation. Age- and sex-matched virgin animals were also studied in parallel with the experimental animals.

All rats had continuous access to Purina chow diet ad libitum (Panlab, Barcelona, Spain); but the fasted rats were deprived of food, but not water, for 24 h before study. All animals were sacrificed by decapitation and blood was collected from the neck wound for plasma separation. It was kept at −80°C until analyzed for triacylglycerols using a commercial enzymatic kit (Menarini) and β-hydroxybutyrate [28]. The liver, heart, lumbar fat pads and mammary glands were quickly removed and immediately placed in liquid nitrogen before freezing at −80°C until LPL analysis.

LPL activity was determined by assaying the enzyme in acetone/diethyl ether-dried powders as previously described [4]. The LPL assays were carried out in the presence and absence of 1 M NaCl, and LPL activity values were calculated as a function of the inhibition caused by the presence of the NaCl. It has previously been demonstrated in our laboratory that this experimental procedure measures the lipase activity that corresponds to LPL, but not to hepatic lipase [26]. Values were expressed as pkatals (pmol of substrate transformed per second) per amount of protein measured in the extract [29], with the exception of values in adipose and mammary tissues that were expressed as pkat/g of fresh tissue since these tissue homogenates had to be supplemented with preheated (60°C) plasma to ensure quantitative extraction. Results are ex-

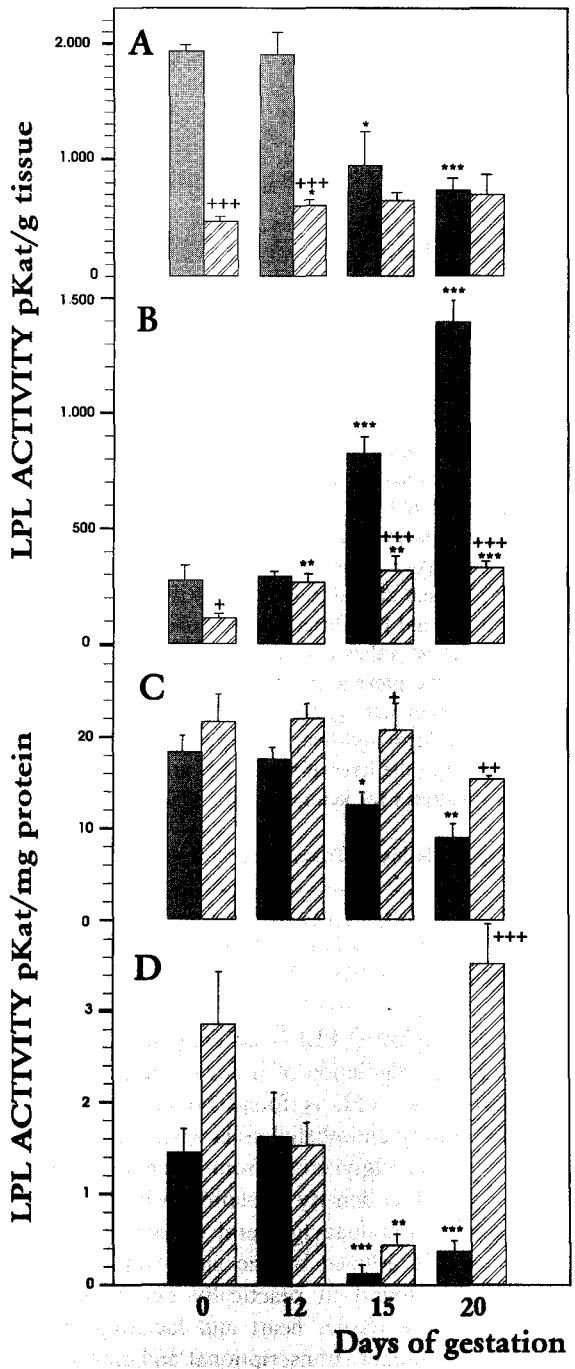

Fig. 1. Lipoprotein lipase activity in lumbar fat adipose tissue (A), mammary gland (B), heart (C) and liver (D) in fed (solid bars) and 24-h starved (cross-hatched bars) virgin (day 0) and gestating rats at different days of gestation. Values are means ± S.E. for at least 6 rats per group. * Statistical comparisons versus virgin rats, and $^{+}$ statistical comparisons between fed and fasted rats of the same gestational day: * or $^{+}P < 0.05$, ** or $^{++}P < 0.01$, *** or $^{+++}P < 0.001$.

Table 1
Plasma β-hydroxybutyrate and triacylglycerol levels in fed and 24 h-fasted rats at different days of gestation.

Gestation time:	β-Hydroxybutyrate (nmol/ml)			Triacylglycerols (mg/dl)		
	Fed	Fasted	P	Fed	Fasted	P
0 days	103.0 ± 22.6	1791.0 ± 104.6	< 0.001	63.5 ± 14.5	49.7 ± 11.0	
12 days	92.1 ± 14.2	1446.0 ± 102.2*	< 0.001	73.8 ± 10.0	45.3 ± 6.8	< 0.05
15 days	92.1 ± 18.1	1269.3 ± 153.1*	< 0.001	85.0 ± 10.5	39.5 ± 8.0**	< 0.01
20 days	110.5 ± 19.1	3531.0 ± 520.6**	< 0.001	131.4 ± 9.6**	293.0 ± 11.5***	< 0.001

P = Statistical comparison between fed and fasted rats.
* = Statistical comparison vs. virgin rats (0 days): * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

pressed as mean ± S.E.; statistical comparison between groups was done using Student's t test.

3. Results

As shown in Fig. 1A, LPL activity in lumbar adipose tissue was similar in fed virgin rats (day 0) and in 12 day pregnant rats whereas it decreased very intensely in 15 day pregnant rats, the values decreasing even further in 20 day pregnant rats. After 24 h fasting LPL activity in the lumbar adipose tissue decreased very intensely in virgin and 12 day pregnant rats while it did not change in the 15 and 20 day pregnant animals (Fig. 1A). LPL activity in mammary gland tissue did not differ between virgin and 12 day pregnant rats when fed, but it increased progresively and very intensely at days 15 and 20 of gestation (Fig. 1B). Fasting for 24 h decreased the LPL activity in the mammary gland tissue from virgin rats but had no effect in that from 12 day pregnant rats (Fig. 1B). However, 24 h fasting had a very intense inhibitory effect on mammary gland LPL activity in 15 and 20 day pregnant rats, even though the absolute values for LPL activity in the fasted pregnant rats always remained above those in the virgin rats (Fig. 1B). As shown in Fig. 1C, LPL in heart did not change in fed rats at day 12 of gestation as compared to virgin animals whereas it decreased significantly and progressively at days 15 and 20 of gestation. After 24 h fasting LPL, activity in heart increased slightly but not significantly in virgin and 12 day pregnant rats whereas it was significantly increased in both 15 and 20 day pregnant rats (Fig. 1C). LPL activity in liver showed much lower values than in any of the other tissues in either fed or fasted rats (Fig. 1D). In fed rats, LPL activity in liver did not change in 12 day pregnant rats versus virgin animals whereas it decreased to practically undetectable values at day 15 of gestation, and remained as low on day 20 of gestation. Whereas 24 h fasting had no effect on liver LPL activity in virgin rats or 12- and 15-day pregnant rats, it produced a significant increase in 20 day pregnant rats (Fig. 1D).

Table 1 summarizes the plasma concentration of β-hydroxybutyrate and triacylglycerols in fed and 24 h fasted rats. Plasma levels of β-hydroxybutyrate were practically undetectable in fed rats but they significantly increased with fasting in all the groups. Although the values of β-hydroxybutyrate reached with fasting in both the 12 and 15 day pregnant rats were lower than in virgin rats, in the 20 day pregnant rats the increase was greater than in any of the other groups attaining values that were significantly higher than in virgin rats. As also shown in Table 1, plasma triacylglycerol levels progressively increase with gestation in fed rats, although values in 20 day pregnant rats were the only ones that differed significantly from those in virgin rats. Although fasting does not modify plasma triacylglycerol levels in virgin rats, it significantly decreases them in 12 and 15 day pregnant rats and intensely increases them in 20 day pregnant rats to a level that is significantly different when compared both to the same group when fed and to fasted virgin controls (Table 1).

4. Discussion

The present study shows changes in LPL activity in white adipose tissue, heart, liver and mammary gland in fed and 24-h fasted condition at different days of gestation in the rat. Although the results in mammary gland do not permit identification of the cellular origin of the measured LPL, previous studies in mice have shown that in this tissue LPL is produced in adipocytes in the interstitial spaces [30,31]. The present finding, that the effects of fasting on mammary gland LPL from day 15 of gestation on are similar to the effects of fasting on adipose tissue LPL at earlier stages of gestation in the rat, supports the same origin for the mammary gland LPL in the rat as in the mouse. Although LPL activity in all the studied tissues did not differ between 12 day pregnant rats and virgin controls, major changes appeared at day 15 of gestation and they were further enhanced by day 20 of gestation. It seems then that hormonal changes already occurring at the 15th day of gestation must be responsible for the LPL changes found during late gestation. Among these changes we can note hyperinsulinemia. It is known that, whereas insulin inhibits LPL activity in heart [2,32] it enhances it in mammary gland [33,34]; therefore the decrease in LPL activity in heart and the increase in mammary gland seen in fed rats from the 15th day of gestation could be

triggered by the hyperinsulinemia that is already developed at this stage of gestation [35]. Although maternal insulin resistance present during late gestation [36–41] would decrease the potential role for hyperinsulinemia in these changes, neither heart, nor even more markedly, mammary gland, manifest reduced insulin sensitivity at this gestational time [42]. On the contrary, although insulin enhances lumbar adipose tissue LPL activity under nonpregnant conditions [22–24,32,43,44], the activity in this tissue is very sensitive to the insulin resistance present during late gestation [17,18]. This sensitivity justifies the low LPL found despite the hyperinsulinemia in the lumbar adipose tissue during late pregnancy.

The opposite change in LPL activity seen from the 15th day of gestation on in adipose tissue versus mammary gland seems to have an important physiological role in driving circulating triacylglycerols to the mammary gland for milk synthesis instead of towards adipose tissue for storage. This reasoning agrees with our previous findings of a reduced uptake of hydrolytic products from VLDL triacylglycerols by isolated adipocytes in the lumbar fat pads of 20 day pregnant rats in vitro [45,46] and the intense uptake of labelled lipids by mammary gland after oral [^{14}C]triolein administration to rats at this same stage of gestation [19]. The inverse change in LPL activity between these tissues justifies the lack of change in plasma triacylglycerols seen in fed 15 day pregnant rats. The situation at the 20th day of gestation in fed animals, however, differs in the sense that plasma triacylglycerols increase despite the diverging changes in LPL activity in the lumbar adipose tissue and mammary gland which are even more exaggerated than at day 15. Nevertheless, we know that at late gestation, intracellular adipose tissue lipolytic activity is highly enhanced [14,47] and this would be responsible for an increase in the arrival of lipolytic products to the liver and the subsequent increase in triacylglycerol synthesis and release into the circulation, as has been demonstrated in the perfused liver of the late pregnant rat [48].

The intense hypertriglyceridemia developed with fasting in 20 day pregnant rats requires special comment. In the fasting condition intracellular lipolytic activity in adipose tissue of the late pregnant rat becomes maximally stimulated [14,47,49], facilitating an active use of lipolytic products for triacylglycerol synthesis by the liver. Besides, as seen in this study, the decline in LPL activity in mammary gland with fasting is also maximal at this late stage of gestation, and we have previously found that inhibiting the LPL induction normally occurring in the mammary gland of fed pregnant rats increases plasma triacylglycerol levels [6,50]. It seems then that conditions that restrained the increase in LPL activity normally seen in the mammary gland at late gestation, as is the case here in fasted 20 day pregnant rats, would decrease the clearance of circulating triacylglycerols which, together with an enhanced production of triacylglycerols by the liver, would be responsible for the increase in plasma triacylglycerols seen in these animals.

The increase in circulating triacylglycerols in the fasted 20 day pregnant rat may affect, or even be responsible for, the specific increase in LPL activity seen in the liver of the fasted 20 day pregnant rat, an increase which was, however, not seen at earlier stages of gestation. The increase of LPL activity in the liver of the 24-h starved late pregnant rat seen in the present study confirms previous findings [14,25–27]. A similar increase in liver LPL activity has been found previously after the intravenous administration of Intralipid to fasted nonpregnant rats [51], probably as the result of being carried by the triacylglycerol-rich lipoprotein remnants [52,53]. Since no LPL mRNA has been found in the liver of the adult rat [2] and the LPL synthesis by the liver is negligible [9], it is proposed that a mechanism like the one mentioned for Intralipid contributes to the increase in LPL activity in the liver of the 24-h starved late pregnant rat. As previously proposed [14], although only temporarily, this would permit the liver of the fasted late pregnant rat to switch from a triacylglycerol exporting organ into a triacylglycerol accepting one and allow the use of circulating triacylglycerols as substrates for ketone body synthesis. That this may be the case is also suggested by the exaggerated increase in the plasma β-hydroxybutyrate level seen here in the fasted 20 day pregnant rat but not at earlier stages of gestation when neither plasma triacylglycerols nor liver LPL activity are augmented. Since ketone bodies easily cross the placenta [54] and are even used by the fetus for brain development [55,56], this efficient mechanism of using circulating triacylglycerols as a substrate for ketogenesis by the mother's liver, would allow the rapidly growing fetus to benefit from maternal hypertriglyceridemia under conditions of food restriction at this late stage of gestation.

Acknowledgements

This study was conducted with a grant from the Fondo de Investigaciones Sanitarias de la Seguridad Social, Ministerio de Sanidad, Spain (grant 92/0407). We thank Carol F. Warren from the ICE of Alcalá de Henares University for editorial help.

References

[1] Wang, C.-S., Hartsuck, J. and McConathy, W.J. (1992) Biochim. Biophys. Acta 1123, 1–17.
[2] Braun, J.E.A. and Severson, D.L. (1992) Biochem. J. 287, 337–347.
[3] Lasunción, M.A. and Herrera, E. (1981) Horm. Metabol. Res. 13, 335–339.
[4] Llobera, M., Montes, A. and Herrera, E. (1979) Biochem. Biophys. Res. Commun. 91, 272–277.
[5] Grinberg, D.R., Ramirez, I., Vilaró, S., Reina, M., Llobera, M. and Herrera, E. (1985) Biochim. Biophys. Acta 833, 217–222.

[6] Ramirez, I., Llobera, M. and Herrera, E. (1983) Metabolism 32, 333–341.
[7] Chajek, T., Stein, O. and Stein, Y. (1977) Atheriosclerosis 26, 549–561.
[8] Burgaya, F., Peinado-Onsurbe, J., Vilaró, S., Llobera, M. and Ramirez, I. (1989) Biochem. J. 259, 159–166.
[9] Vilaró, S., Llobera, M., Bengtsson-Olivecrona, G. and Olivecrona, T. (1988) Biochem. J. 249, 549–556.
[10] Kinnunen, P.K., Unnérus, H.A., Ranta, T., Ehnholm, C., Nikkilä, E.A. and Seppälä, M. (1980) Eur. J. Clin. Invest. 10, 469–474.
[11] Herrera, E., Martín, A., Montelongo, A., Domínguez, M. and Lasunción, M.A. (1992) Avanc. Diabet. 5 (Supl. 1), 73–84.
[12] Hamosh, M., Clary, T.R., Chernick, S.S. and Scow, R.O. (1970) Biochim. Biophys. Acta 210, 473–482.
[13] Hamosh, M., Chernick, S.S. and Scow, R.O. (1972) Isr. J. Med. Sci. 8, 834–835.
[14] Herrera, E., Lasunción, M.A., Gomez Coronado, D., Aranda, P., Lopez Luna, P. and Maier, I. (1988) Am. J. Obstet. Gynecol. 158, 1575–1583.
[15] Otway, S. and Robinson, D.S. (1968) Biochem. J. 106, 677–682.
[16] Spooner, P.M., Garrison, M.M. and Scow, R.O. (1977) J. Clin. Invest. 60, 702–708.
[17] Herrera, E., Ramos, P. and Martín, A. (1990) in Frontiers in diabetes research. Lessons from animal diabetes III (Shafrir, E., ed.), pp. 551–554, Smith-Gordon, London.
[18] Martín, A., Ramos. P. and Herrera, E. (1993) in Physiologic basis of perinatal care (Medina, J.M. and Quero, J., eds.), pp. 117–122, Ediciones Ergon, Madrid.
[19] Argiles, J. and Herrera, E. (1989) Metabolism 38, 104–108.
[20] Herrera, E., Ramos, P., López-Luna, P. and Lasunción, M.A. (1994) in Dairy products in human health and nutrition (Serrano Ríos, M., Sastre, A., Perez Juez, M.A., Entrala, A. and De Sabesti, C., eds.), pp. 189–197, A.A. Balkema, Rotterdam.
[21] Borensztajn, J. and Robinson, D.S. (1970) J. Lipid Res. 11, 111–117.
[22] Doolittle, M.H., Ben-Zeev, O., Elovson, J., Martin, D. and Kirchgessner, T.G. (1990) J. Biol. Chem. 265, 4570–4577.
[23] Ladu, M.J., Kapsas, H. and Palmer, W.K. (1991) Am. J. Physiol. 260, R953–R959.
[24] Oliver, J.D. and Rogers, M.P. (1993) Biochem. J. 292, 525–530.
[25] Testar, X., Llobera, M. and Herrera, E. (1988) Metabolism 37, 1008–1014.
[26] Testar, X., Llobera, M. and Herrera, E. (1985) Biochem. Soc. Trans. 13, 134.
[27] Vilaró, S., Testar, X., Ramirez, I. and Llobera, M. (1990) Biol. Neonate 57, 37–45.
[28] Kientsch-Engel, R.I. and Siess, E.A. (1985) in Methods of enzymatic analysis. Vol. VIII (Bergmeyer, H.U., Bergmeyer, J. and Grabl, M., eds.), pp. 60–69, VCH, Weinheim.
[29] Zak, B. and Cohen, J. (1961) Clin. Chim. Acta 6, 665–676.
[30] Jensen, D.R., Bessesen, D.H., Etienne, J., Eckel, R.H. and Neville, M.C. (1991) J. Lipid Res. 32, 733–742.
[31] Jensen, D.R., Gavigan, S., Sawicki, V., Witsell, D.L., Eckel, R.H. and Neville, M.C. (1994) Biochem. J. 298, 321–327.
[32] Robinson, D. and Speake, B. (1989) Biochem. Soc. Trans. 17, 40–42.
[33] Neville, M.C., Waxman, L.J., Jensen, D. and Eckel, R.H. (1991) J. Lipid Res. 32, 251–257.
[34] Da Costa, T.H.M. and Williamson, D.H. (1993) Biochem. J. 290, 557–561.
[35] Zorzano, A., Palacín, M. and Testar, X. (1992) in Perinatal biochemistry (Herrera, E. and Knopp, R.H., eds.), pp. 69–92, CRC Press, Boca Raton.
[36] Leturque, A., Burnol, A.-F., Ferré, P. and Girard, J. (1984) Am. J. Physiol. 246, E25–E31.
[37] Cousins, L. (1991) Diabetes 40, 39–43.
[38] Leturque, A., Ferré, P., Satabin, P., Kervran, A. and Girard, J. (1980) Diabetologia 19, 521–528.
[39] Knopp, R.H., Ruder, H.J., Herrera, E. and Freinkel, N. (1970) Acta Endocrinol. (Copenh) 65, 325–360.
[40] Ryan, E.A., O'Sullivan, M.J. and Skyler, J.S. (1985) Diabetes 34, 380–389.
[41] Martín, A., Zorzano, A., Caruncho, I. and Herrera, E. (1986) Diabet. Metab. 12, 302–307.
[42] Ramos, R. (1993) Doctoral Thesis: Papel de la sensibilidad insulínica en la actividad de la lipoproteína lipasa tisular en gestación, Universidad Autónoma de Madrid, Madrid.
[43] Appel, B. and Fried, S.K. (1992) Am. J. Physiol. 262, E695–E699.
[44] Lamarche, B., Després, J.-P., Moorjani, S., Nadeau, A., Lupien, P.-J., Tremblay, A., Thériault, G. and Bouchard, C. (1993) Int. J. Obes. 17, 255–261.
[45] Herrera, E., Gomez Coronado, D. and Lasunción, M.A. (1987) Biol. Neonate 51, 70–77.
[46] Lasunción, M.A. and Herrera, E. (1981) Biochem. Biophys. Res. Commun. 98, 227–233.
[47] Knopp, R.H., Herrera, E. and Freinkel, N. (1970) J. Clin. Invest. 49, 1438–1446.
[48] Wasfi, I., Weinstein, I. and Heimberg, M. (1980) Biochim. Biophys. Acta 619, 471–481.
[49] Chaves, J.M. and Herrera, E. (1978) Biochem. Biophys. Res. Commun. 85, 1299–1306.
[50] Herrera, E., Lasunción, M.A., Martín, A. and Zorzano, A. (1992) in Perinatal biochemistry (Herrera, E. and Knopp, R.H., eds.), pp. 1–18, CRC Press, Boca Raton.
[51] Vilaró, S., Reina, M., Ramírez, I. and Llobera, M. (1986) Biochem. J. 236, 273–278.
[52] Vilaró, S., Llobera, M., Bengtsson-Olivecrona, G. and Olivecrona, T. (1988) Am. J. Physiol. 254, G711–G722.
[53] Vilaró, S., Ramirez, I., Bengtsson-Olivecrona, G., Olivecrona, T. and Llobera, M. (1988) Biochim. Biophys. Acta 959, 106–117.
[54] Herrera, E., Lasunción, M.A. and Asunción, M. (1992) in Fetal and neonatal physiology (Polin, R. and Fox, W.W., eds.), pp. 291–298, W.B. Saunders, Philadelphia.
[55] Shambaugh, G.E. (1985) Fed. Proc. 44, 2347–2351.
[56] Shambaugh, G.E., III, Metzger, B.E. and Radosevich, J.A. (1992) in Perinatal biochemistry (Herrera, E. and Knopp, R.H., eds.), pp. 213–231, CRC Press, Boca Raton.