

Coelomic Fluid Leptin Concentration in Normal First-Trimester Pregnancies and Missed Miscarriages

G. Makrydimas^a H. Vandecruys^b A. Sotiriadis^a L. Lakasing^b K. Spencer^c
K.H. Nicolaides^b

^aDepartment of Obstetrics and Gynaecology, Ioannina University Hospital, Ioannina, Greece;

^bHarris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, and

^cDepartment of Clinical Biochemistry, Harold Wood Hospital, Romford, Essex, UK

Key Words

Leptin · Miscarriage · Coelomic fluid · Coelocentesis

Abstract

Objective: Investigation of the possible role of leptin in early pregnancy failure. **Methods:** Leptin concentration was measured in maternal serum, coelomic fluid and amniotic fluid from 15 singleton pregnancies with live fetuses and 7 missed miscarriages at 7–10 weeks of gestation. **Results:** In the pregnancies with live fetuses, the median leptin concentration was significantly higher in coelomic fluid (median 33.1 ng/ml) than in maternal serum (median 8.1 ng/ml) or amniotic fluid (median 0.5 ng/ml). In the pregnancies with missed miscarriage, compared to those with live fetuses, the median leptin concentration in coelomic fluid was higher (median 45.3 ng/ml), but in maternal serum it was not significantly different (median 5.5 ng/ml). **Conclusions:** The high coelomic fluid leptin concentration suggests that embryonic death may be preceded by impaired oxygenation of the placenta that stimulates production of leptin.

Copyright © 2005 S. Karger AG, Basel

Introduction

Leptin, whose name derives from the Greek word for thin, is a protein encoded by the obesity gene and in the non-pregnant state it is exclusively secreted by adipocytes [1, 2]. It is implicated in suppression of food intake and increase in energy consumption and these actions are mediated through leptin-specific receptors [3, 4].

Leptin is also produced by the placenta and in the first trimester maternal serum levels are twice as high as in non-pregnant controls [5, 6]. Although the exact role of leptin in pregnancy is not known, it is thought to be important in the control of the functional integrity of the fetoplacental unit and is implicated in the pathogenesis of recurrent miscarriage and pre-eclampsia [7–9]. In women with a history of recurrent miscarriage, maternal plasma leptin concentration in the first trimester is decreased in those who finally miscarry. In contrast, pre-eclampsia is associated with increased maternal plasma levels, especially if the hypertensive disease is associated with fetal growth restriction [10–12].

The aim of this study is to investigate the possible role of leptin in sporadic early pregnancy loss by examining the concentration of leptin in coelomic fluid from pregnancies with a missed miscarriage.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2005 S. Karger AG, Basel
1015–3837/05/0205–0406\$22.00/0

Accessible online at:
www.karger.com/ftd

Dr. G. Makrydimas
Department of Obstetrics and Gynaecology
Ioannina University Hospital
Ioannina (Greece)
Tel. +30 651 99783, Fax +30 651 99788, E-Mail grmak@otenet.gr

Methods

Coelomic fluid (2–3 ml) was obtained by coelocentesis, which was performed immediately before elective suction termination of pregnancy for psychosocial reasons from 15 singleton pregnancies with live fetuses at 7–10 weeks of gestation. Coelocentesis was also performed before evacuation of retained products of conception in 7 pregnancies at 7–10 weeks in which routine ultrasound examination had demonstrated missed miscarriage; in all cases the fetus was visible but there was no heart activity. None of the women examined had a previous miscarriage. All patients were examined at the Department of Obstetrics and Gynecology in Ioannina University Hospital, Greece. The women gave written consent to participate in the study that was approved by hospital ethics committee. In all cases maternal blood (5 ml) was obtained from the antecubital vein and the serum was separated and frozen at -80°C . Subsequently, coelocentesis was performed under general anaesthesia. The external genitalia and the vagina were carefully cleansed with an antiseptic solution. Transvaginal sonography, with a 5-MHZ ultrasound transducer (Toshiba SSA-220A, Tokyo, Japan) covered with a sterile rubber, was then performed. The fetal crown-rump length was measured and the amniotic membrane, coelomic space and yolk sac were identified. A 20-G needle was introduced transvaginally into the coelomic cavity, through a guide attached to the transducer and fluid was aspirated. Subsequently, a new 20-G needle was used to aspirate amniotic fluid but this was successfully obtained in only 7 of the pregnancies with live fetuses and 2 of the missed miscarriages, because in early pregnancy the amniotic cavity is considerably smaller than the coelomic cavity.

The concentration of leptin in maternal serum, amniotic fluid and coelomic fluid was measured using the DRG ELISA kit EIA-1863 (DRG Instruments GmbH, Germany). Competitive binding to sites of a polyclonal antiserum of an unknown concentration of leptin from a study sample with a known concentration of leptin conjugate immobilized onto microtitre wells was conducted. This was followed by enzyme complex binding to the antiserum. Unbound enzyme complex was washed off and substrate solutions added. The concentration of Leptin in the sample is inversely proportional to the optical density at 450 nm. All samples were measured at a dilution of 1 in 5 in assay buffer and measured in duplicate across two assays. The kit assay sensitivity was 0.2 ng/ml and the coefficient of variation of the duplicate results was less than 10% in all cases.

Statistical Analysis

Mann-Whitney U test was used to determine the significance of differences in median concentration of leptin in coelomic fluid, amniotic fluid and maternal serum in the pregnancies with live fetuses and those with missed miscarriages. Wilcoxon signed rank test was used to determine the possible significance of the association in leptin concentration between the various compartments.

Results

In the pregnancies with live fetuses, the median gestation was 7.9 (range 6–10.6) weeks and the median leptin concentration was significantly higher in coelomic fluid

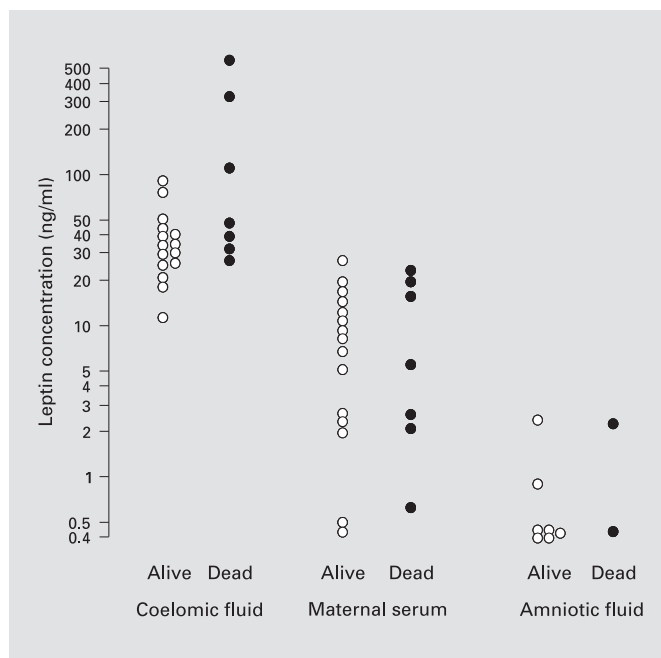


Fig. 1. Individual values and median leptin concentration in coelomic fluid, maternal serum and amniotic fluid in pregnancies with live fetuses and those with missed miscarriages.

(median 33.1 ng/ml, range 11.3–77.6 ng/ml) than in maternal serum (median 8.1 ng/ml, range 0.5–27.0 ng/ml; $p = 0.018$) or amniotic fluid (median 0.5 ng/ml, range 0.5–3.0 ng/ml; $p = 0.001$) (fig. 1). There was a significant association between coelomic fluid and maternal serum leptin concentration ($r = 0.525$, $n = 15$, $p = 0.044$). In contrast, there was no significant association in leptin concentration between coelomic fluid and amniotic fluid ($r = 0.078$, $n = 7$, $p = 0.867$) and between maternal serum and amniotic fluid ($r = 0.291$, $n = 7$, $p = 0.527$).

In the pregnancies with missed miscarriage, the median gestation was 9 (range 8–10) weeks, but according to the crown-rump length measurement the estimated interval between embryonic death and sampling was 1–24 (median 18) days. The median leptin concentration was significantly higher in coelomic fluid (median 45.3 ng/ml, range 26.9–638.0 ng/ml) than in maternal serum (median 5.5 ng/ml, range 0.6–23.1 ng/ml; $p = 0.018$) or amniotic fluid (2 cases only 0.5 and 2.9 ng/ml) (fig. 1). There was no significant association in leptin concentration in coelomic fluid and maternal serum ($r = 0.004$, $n = 7$, $p = 0.993$).

In the pregnancies with missed miscarriage, compared to those with live fetuses, the median leptin concentration

in coelomic fluid was higher ($u = 19.000$, $p = 0.017$), but in maternal serum it was not significantly different ($u = 49.000$, $p = 0.837$).

Discussion

The finding of this study that in pregnancy with a live fetus the median concentration of leptin in coelomic fluid is four times higher than in maternal serum provides supportive evidence for high production of this protein by trophoblast. Similar findings have been reported in relation to other trophoblast metabolites, where higher concentration in coelomic fluid than maternal serum has been attributed to the close anatomical proximity of trophoblast to the coelomic cavity that are separated by only loose mesenchymal tissue [13, 14]. The observed maternal hyperleptinaemia in early pregnancy could be due to upregulation of adipose leptin mRNA, as shown in rodent studies [15, 16], although in humans the most likely explanation is increased trophoblastic production, because maternal serum concentration falls dramatically to pre-pregnancy levels within a few hours following delivery of the placenta [5].

The association between the concentration of leptin in coelomic fluid and that in maternal serum was weak. There are several potential explanations for this. Firstly, maternal serum concentrations reflect not only the trophoblast-derived leptin, but the additional contribution from adipocytes. Secondly, leptin metabolism and clearance may be slower in the coelomic cavity compared with the maternal circulation.

The leptin concentration in amniotic fluid was about 60 times lower than in coelomic fluid, which is of a similar magnitude reported previously for the total protein

concentration in these compartments [17]. This finding presumably reflects the poor permeability of the amniotic membrane to such proteins. Consequently, studies aimed at investigating the mechanisms involved in early pregnancy failure should be conducted on coelomic rather than amniotic fluid as this more accurately reflects early trophoblast function.

In the pregnancies with missed miscarriage, compared to those with live fetuses, the median leptin concentration in maternal serum was not significantly different. The median interval between embryonic death and coelocentesis was 18 days, so these findings suggest that placental protein metabolic function does not decline immediately after embryonic death but persists for at least 2–3 weeks. These findings are consistent with the results of a previous study of coelocentesis in missed miscarriages, which reported that the coelomic fluid total protein concentration was similar to that in live pregnancies, unless there was histological evidence of advanced placental necrosis, in which case the concentrations were substantially reduced [18].

In the pregnancies with missed miscarriage the median coelomic fluid leptin concentration was increased. In addition, previous studies have reported that in pregnancies with severe pre-eclampsia both maternal serum levels of leptin and placental expression of leptin mRNA are increased [11, 12]. Since both pre-eclampsia and missed miscarriage are associated with impaired placentation and placental oxidative stress, a common mechanism may be responsible for the increased production of leptin [19]. Interestingly, in women with recurrent pregnancy loss, a reduction in maternal leptin concentration has been reported [10] and this suggests that the pathophysiology of recurrent miscarriage may be different from that of sporadic miscarriage.

References

- 1 Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM: Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994;37:425–432.
- 2 Auwerx J, Staels B: Leptin. *Lancet* 1998;351:737–742.
- 3 Magni P, Motta M, Martini L: Leptin: A possible link between food intake, energy expenditure and reproductive function. *Regul Pept* 2000;92:51–56.
- 4 Zhang Y, Olbort M, Schwarzer K, Nuesslein-Hildesheim B, Nicolson M, Murphy E, Kowalski TJ, Schmidt I, Leibel RL: The leptin receptor mediates apparent autocrine regulation of leptin gene expression. *Biochem Biophys Res Commun* 1997;240:492–495.
- 5 Masuzaki H, Ogawa Y, Sagawa N, Hosoda K, Matsumoto T, Mise H, Nishimura H, Yoshimasa Y, Tanaka I, Mori T, Nakao K: Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. *Nat Med* 1997;3:1029–1033.
- 6 Butte NF, Hopkinson JM, Nicolson MA: Leptin in human reproduction: Serum leptin levels in pregnant and lactating women. *J Clin Endocrinol Metab* 1997;82:585–589.
- 7 Sagawa N, Yura S, Itoh H, Kakui K, Takemura M, Nuamah MA, Ogawa Y, Masuzaki H, Nakao K, Fujii S: Possible role of placental leptin in pregnancy. *Endocrine* 2002;19:65–71.
- 8 Bajoria R, Sooranna SR, Ward BS, Chatterjee R: Prospective function of placental leptin at maternal-fetal interface. *Placenta* 2002;23:103–115.

- 9 Lage M, Garcia-Mayor RV, Tomé MA, Cordido F, Valle-Inclan F, Considine RV, Caro JF, Dieguez C, Casanueva FF: Serum leptin levels in women throughout the pregnancy and the postpartum period and in women suffering spontaneous abortion. *Clin Endocrinol* 1999; 50:211–216.
- 10 Laird SM, Quinton ND, Anstie B, Li TC, Blakemore AIF: Leptin and leptin-binding activity in women with recurrent miscarriage: Correlation with pregnancy outcome. *Hum Reprod* 2001; 16:2008–2013.
- 11 Teppa RJ, Ness RB, Crombleholme WR, Roberts JM: Free Leptin is increased in normal pregnancy and further increased in preeclampsia. *Metabolism* 2000; 49:1043–1048.
- 12 Mise H, Sagawa N, Matsumoto T, Yura S, Nanno H, Itoh H, Mori T, Masuzaki H, Hosoda K, Ogawa Y, Nakao K: Augmented placental production of leptin in preeclampsia: Possible involvement of placental hypoxia. *J Clin Endocrinol Metab*. 1998; 83:3225–3229.
- 13 Jones CPJ, Jauniaux E: Ultrastructure of the materno-embryonic interface in the first trimester of pregnancy. *Micron* 1995; 2:145–173.
- 14 Jauniaux E, Gulbis B: Fluid compartments of the embryonic environment. *Hum Reprod Update* 2000; 6:268–278.
- 15 Tomimatsu T, Yamaguchi M, Murakami T, Ogura K, Sakata M, Mitsuda N, Kanzaki T, Kurachi H, Irahara M, Miyake A, Shima K, Aono T, Murata Y: Increase of mouse leptin production by adipose tissue after midpregnancy: Gestational profile of serum leptin concentration. *Biochem Biophys Res Commun* 1997; 240:213–215.
- 16 Kawai M, Yamaguchi M, Murakami T, Shima K, Murata Y, Kishi K: The placenta is not the main source of leptin production in pregnant rat: Gestational profile of leptin in plasma and adipose tissues. *Biochem Biophys Res Commun* 1997; 240:798–802.
- 17 Jauniaux E, Gulbis B, Jurkovic D, Schaaps JP, Campbell S, Meuris S: Protein and steroid levels in embryonic cavities in early human pregnancy. *Hum Reprod* 1993; 8:782–787.
- 18 Jauniaux E, Gulbis B, Nagy AM, Jurkovic D, Campbell S, Meuris S: Coelomic fluid chorionic gonadotrophin and protein concentrations in normal and complicated first trimester human pregnancies. *Hum Reprod* 1995; 10: 214–220.
- 19 Hempstock J, Jauniaux E, Greenwold N, Burton GJ: The contribution of placental oxidative stress to early pregnancy failure. *Hum Pathol* 2003 34:1265–1275.