Leptin receptor expression in human umbilical cord leucocytes

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Received 18 January 1999; accepted 1 February 1999

Abstract: We have assessed the expression of leptin receptor on human umbilical cord blood leucocytes using flow cytometry, by a specific antibody, in 12 normal newborns (seven females and five males). The expression was significantly ($P < 0.0001$) higher in polymorphonuclear cells (70 ± 6%) as compared to monocytes (23 ± 4%) and lymphocytes (16 ± 4%). There was no correlation between the weight or the sex of the newborn and the effect of leptin or the expression of its receptor. Future studies should ascertain the type of leptin receptor, its function and the role of this cytokine in cord blood cells.

Med Sci Res 27:231–232 © 1999 Lippincott Williams & Wilkins

Keywords: leptin, leptin receptor, umbilical cord leucocytes

Introduction: Leptin is a hormone, encoded by the *ob* gene, whose major source is the adipose tissue [1]. Its levels increase at night and its circulating concentrations reflect body fat stores [2]. The hormone has been discovered in the serum of newborns [3] and its levels correlated with birth weight [4]. Nevertheless, Casibiell *et al.* [5] found that levels of the hormone were significantly lower in umbilical veins as compared to umbilical arteries suggesting that the placenta is one of the major sources of leptin in fetal circulation.

At birth, the levels of leptin decline rapidly [5]. The hormone is probably still supplied by the mother since it is secreted in the colostrum and/or breast milk [7]. Thus leptin may be an important modulator of substrate supply in the newborns.

The aim of our study was to assess the expression of leptin receptors on neonatal leucocytes.

Materials and methods: In order to ascertain the expression of leptin receptors on cord blood leucocytes, whole blood was obtained from 12 umbilical cords of normal at term (38–40 weeks of gestation) neonates (seven females and five males, mean weight = 3.1 ± 0.1 kg) delivered by caesarean in the morning (8–10 am) Written consent from the mothers was obtained and the Ethical Committee of the Institute approved the protocol.

The blood was collected in sterile Falcon® tubes containing EDTA as anticoagulant. The erythrocytes were lysed using buffered ammonium chloride. Then the leucocytes were washed and resuspended in PBS-gel (2 mM EDTA labelling with goat anti-human leptin receptor (Santa Cruz Biotechnology, Sta. Cruz, CA).

Briefly, 1×10^6 cells (total leucocytes) were incubated with PBS-gel–0.2% sodium-azide at 4°C for 10 min, then labelled with 0.5 µg of anti-leptin antibody for 30 min at 4°C, washed twice with PBS gel-azide –0.1% bovine serum albumin, incubated with 0.5 µg of rabbit-anti goat immunoglobulin G-FITC and finally washed with PBS-gel. Samples incubated with goat serum plus anti-goat IgG-FITC were used as controls.

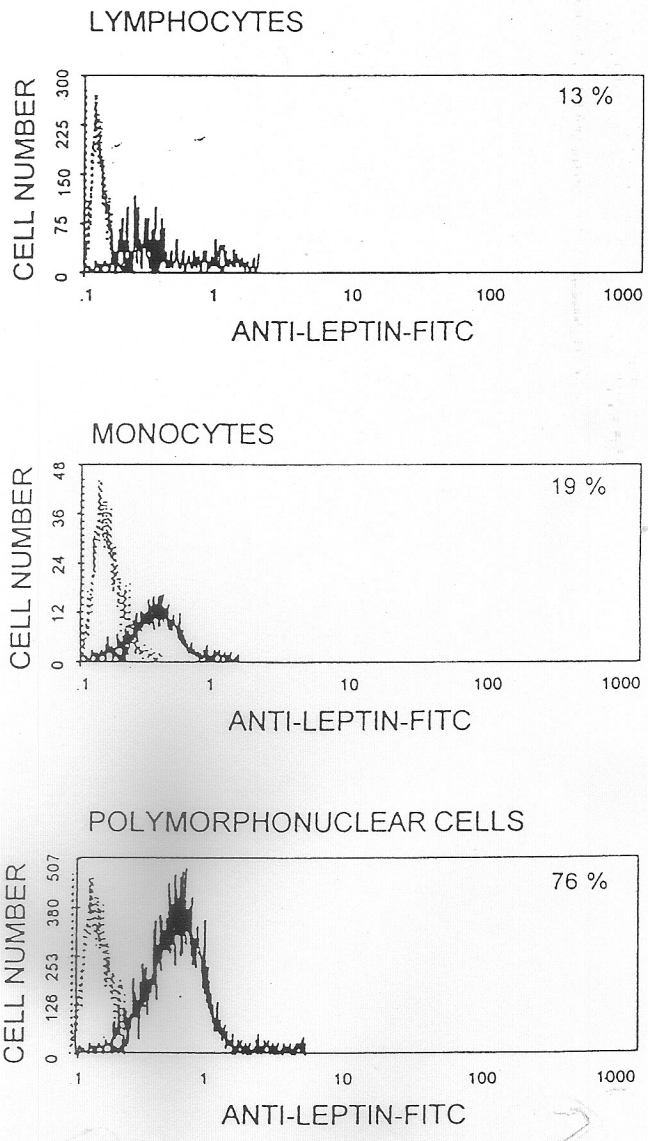

Figure 1. Expression of leptin receptor in different cord blood leucocyte populations. A typical flow cytometry analysis of the cells obtained from the umbilical cord is represented. The dotted lines correspond to the non-specific binding and the dark continuous

The expression of leptin receptor (assessed in an EPICS ELITE II Coulter Corporation, Miami, FL, USA) on lymphocytes, monocytes and polymorphonuclear cells was performed using three bitmaps on a forward light scatter (FALS) versus side scatter (FALS vs SS) cytogram. Each cell population was defined by granularity and also by antigen expression. The lymphocyte map contained 66.1 ± 8.3% CD3+, 15.6 ± 3.4% CD 19+, 5.5 ± 3.2% CD56% cells. The monocyte map contained 95 ± 2.8% CD14+ cells and the polymorphonuclear cell map 94.5 ± 3.6% CD16b+ cells.

Results: Figure 1 illustrates the expression of leptin receptors in the three types of leucocytes studied. The expression of leptin receptors was significantly ($P < 0.0001$) higher in polymorphonuclear cells (70 ± 6%) as compared to monocytes (23 ± 4%) and lymphocytes (16 ± 4%). The expression was independent of the weight and sex of the neonate.

Discussion: Leptin receptors belong to the superfamily of cytokine receptors [7]. Several types of leptin receptors (recognized by the same antibody) have been reported: a long form (OBR1), 302 cytoplasmic residues, and several, widely expressed, short forms (OBRs). Bjorbaek *et al.* [8] have shown that in response to leptin, OBR1, and to a less extent OBRs, underwent tyrosine phosphorylation by JAK2 activation. This suggests that the effect of leptin is dependent on the expression of OBR1.

A differential expression of OBR receptors is observed in cord blood leucocytes although we are unable to identify which type of receptor is present. Future studies should ascertain the type of receptor and its function in cord blood leucocytes.

Acknowledgements: This study was supported by grant from Consejo de Desarrollo Científico y Humanístico de la Universidad Central de Venezuela (CDCH) # 09-36-3590-98 and 09.033.98. The authors are grateful to Dr Jenny Garmendia for useful comments.

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