Leptin identification and quantification in human and bovine milk and infant formulas

This is the author's manuscript

Original Citation:

Availability:
This version is available http://hdl.handle.net/2318/1663749 since 2018-03-26T11:39:28Z

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**OVERVIEW**

- The LC-MS MS and HR MS/MS behavior of leptin was evaluated by the use of a Q-Exactive Fusion Tribrid analyzer. Bottom-up and top-down approaches were compared.
- We aimed to quantify leptin in human milk and infant formulas, so an immunoaffinity extraction was performed in comparison with direct detection.
- The protein analysis was detected in human milk at 6-7 pg/µL.

**INTRODUCTION**

Leptin is a small protein (16 kDa) present in plasma and milk. It plays a key role in the regulation of body weight and its concentration is affected by many physiological parameters especially body weight variation and lipodystrophic conditions. Leptin is encoded by the ob gene on human chromosome 7 and it is mainly secreted by adipocytes. It is possible that serum leptin concentration in breastfed infants is associated to early adipose tissue production and to the leptins levels in milk. In order to understand the role of different factors (feeding, formula, breast milk) on leptin protein production and risk of obesity, we aimed to develop a sensitive LC-MS/MS method to evaluate leptin content in different milk matrices.

**METHOD 1**

**Quantitation of leptin after hydrolysis**

- After digestion of purified samples leptin was quantified in samples (besides identification of interfering unknown proteins: lactotransferrin and β-lactoglobulin). The method was applied to investigate the potential presence of leptin in two different commercial infant formulas. In this kind of food supplements leptin resulted < LLOQ (3 ags).
- Finally leptin concentration was quantified in human breast milk samples, obtaining values of 6-7 ags.
- The developed method displays a better sensitivity compared to ELISA and can be used for milk and plasma leptin dosage.

**METHOD 2**

**Experimental**

The human milk was prepared and purified following an immunoaffinity protocol in order to detect leptin as intact protein.

**Sample preparation:** Purification of human milk with glass micro-inertialization kit and monoclonal antibody (Leptin 3H11). Make up 2,5 µl of heating antibodies in 87,5 µL of glycerol containing buffered and add 11 µL of NaOH 0,2 M for degrading step. Bind the antibody antibody to the column resin and equilibrate the latter with 10, 75, 450 buffer (aqueous solution of βC 2000), commercial milk and with blocked antibody antibody. Elute analyze (leptin protein) with glycine HCl solution pH 2,5-2,0 M and re-equilibrate the eluting buffer 10 µL per 5 min.

**RESULTS**

Evaluation of leptin in breast milk and commercial infant formulas

**APPLICATION TO MILK SAMPLES**

**Leptin identification and quantification in human milk and infant formulas**

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**Leptin_1ppm_001**

Leptin_1ppm_001

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**References:**