JOURNAL OF PROTEOMICS 88 (2013) 47–57

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Review

# Proteomics as a tool to explore human milk in health and disease☆

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ARTICLE INFO

Available online 13 April 2013

*Keywords:*
Milk
Human
Proteome
Mastitis

ABSTRACT

Proteins in milk have wide range of functions, they are carriers of minerals or chemically vulnerable and insoluble vitamins and other compounds, stabilisers of large aggregates or micelles of lipids, and components of both innate and acquired immune defence systems. Together with other components of milk, proteins may also contribute to the selection and establishment of appropriate microbiome in the gut of the infant. The proteome of mammalian milk is now known to be dynamic and changes radically with time after birth from colostrum to mature lactation. Significantly, immune and innate defence proteins appear in milk during infection of the mammary gland and possibly also during systemic infections. The understanding of the human milk proteome and how it changes with time during lactation and in disease is developing rapidly, and is to a large extent informed by proteomics of the milks of non-human mammals, domestic animals in particular. We review general methods now being applied for proteomic analysis of human milk. Moreover we place emphasis on how the milk proteome may change in different ways in response to disease, mastitis in particular, how such changes may be specific to pathogen types, and we give some insights about evolution.
This article is part of a Special Issue entitled: New Horizons and Applications for Proteomics [EuPA 2012].



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http://dx.doi.org/10.1016/j.jprot.2013.04.008

## Contents



## 1. Introduction

The aim of this review is to discuss study design in the exploration of the milk proteome, and how currently used and developing proteomic technologies can be applied and adapted for analysis of milks. One problem with milks is that, in common with other secretions, some protein types are in such superabundance that they obscure minor components that may be equally important, albeit with different biological activities. This broad dynamic range necessitates appropriate fractionation in order to achieve satisfactory proteomic coverage. The review will follow and highlight considerations required to undertake a proteomic study, dealing with the selection of samples, study design, and the type of techniques to use. There is also some brief discussion of broader issues, particularly focussing on changes in the milk proteome during the course of lactation, comparison between different species of mammals, and the effect of maternal infection on the milk proteome and how genetic polymorphisms may have evolved in milk proteins.

### 1.1. *Mammalian milks*

The components of mammalian milks have evolved to provide an optimised source of nutrients, growth factors and immune protection to an infant, and also to establish a gut microbiome appropriate for an initial diet of milk and progression through weaning to the solid food suitable for each species [1–3]. The major constituents of milk are, broadly, lipids, for energy metabolism, membrane construction, brain development and as precursors of signalling lipids; carbohydrates for energy metabolism, protein and lipid glycosylations, and oligosaccharides as inhibitors of bacterial adhesion; and proteins.

The protein composition of milks is sensitive to the type of placenta that a species has. Humans have haemochorial placentae that engage in the translocation of, for instance, large quantities of immunoglobulins (but only IgG) during the third trimester of gestation. Species such as cows, sheep, goats and horses have epitheliochorial placentae that do not permit this, so they acquire their immunoglobulins immediately after birth in colostrum. In the mature phase of lactation, once the colostral phase is over, many milk proteins will have functions that are common to all mammals (e.g. the caseins), but others may have functions that are species-specific. Examples of the latter would be proteins whose operation requires the existence of specific cell surface receptors that may only operate to bind and translocate a milk protein in the same species. For instance, there are good examples of certain proteins in human milk that promote neonatal development in specific tissues, especially in brain development [4] and maturation of the gut microbiota [5,6]. It must also not be forgotten that some proteins that are abundant in the milks used as bases for artificial formulae (predominately bovine) are absent in human milk, some of these, such as β-lactoglobulin, are also targets of allergic responses in humans [7,8].

There is an incomplete understanding of the functions of the proteins of human milk, partly due to an incomplete inventory of the human milk proteome [9]. Evidence that breastfeeding is correlated with a lower prevalence of obesity, diabetes, and cardiovascular disease in childhood and later life compared to infants fed on an artificial formulae diet [10–13], demands a fuller understanding of the proteins of human milk in addition to other essential nutrients such as lipids, carbohydrates, minerals and vitamins [14]. It is now well-known that the feeding of neonates with artificial formulae results in initial and persistent colonisation with a microbiome that is radically different from that of breast fed infants [15–17]. It is notable that breastfeeding results in enhanced colonisation by *Bifidobacterium* species whose genome sequences indicate specialisation for use of human milk oligosaccharides and similar host-derived metabolites [18,19]. In addition, the fact that bovine milk-based formula contains proteins that are inactive or not appropriately operative in humans means that refining and improving artificial formulae will ultimately be more challenging than initially anticipated. It could also be that improved understanding