Maternal nutritional status changed dramatically with the rapid social and economic development in China. It remains unclear whether maternal nutritional status is related to concentration of various proteins in human milk. The purposes of the current paper were to examine the relationship between maternal pre-pregnancy body mass index (BMI) and concentration of alpha-lactalbumin, beta-casein, lactoferrin, alpha-s1 casein, kappa-casein, serum albumin in human milk. This was a cross-sectional study. Human milk proteins were measured using ultra-high performance liquid chromatography-tandem/mass spectrometry with isotopic dilution. The concentrations of various human milk proteins excluding lactoferrin did not differ among the 3 maternal BMI groups (all P>0.05). Human milk alpha-lactoalbumin, beta-casein, alpha-s1 casein, kappa-casein and albumin level may be consistent across different pre-pregnant nutritional status regardless of lactation stage.