**Supplemental File 1: Milk hormone assay methods**

Within 20 minutes of the full breast expression, milk was gently mixed, placed on ice, and divided into ten aliquots. All aliquots were stored at −80°C until analyses. Prior to analyses, frozen aliquots (never previously thawed) were then thawed on ice. Milk fat was separated from the aqueous phase by centrifugation at 3,000 × g for 10 minutes. The resulting skimmed milk was assayed as follows. Insulin was assayed using ELISA kits from EMD Millipore (St. Charles, MO; catalog number EZHIASF-14K) following the manufacturer’s protocol. Total adiponectin was measured using an ELISA kit from Mercodia (Uppsala, Sweden; catalog number 10-1193-01), without dilution. Leptin was measured using an ELISA kit from R&D Systems (Minneapolis, MN; catalog number DLP00) after 4-fold dilution. Samples with leptin concentrations above the standard curve were repeated after further dilution.

Assay kits were evaluated for suitability for use with skimmed breast milk using spike recovery and linearity experiments. To assess spike recovery for each assay, known quantities of the kit standard were added to a composite of skimmed milk samples to yield at least three concentrations within the dynamic range of the assay. Recovery ranges for each assay were as follows: insulin, 88-115%; adiponectin, 98-120%; and leptin, 90-94%. Similarly, linearity of each assay was determined by diluting the composite milk sample to yield three levels of analyte within the standard curve. Ranges of percent of expected values for each assay were as follows: insulin, 93-111%; adiponectin, 94-111%; leptin 104-108%.

Inter-assay variability was 6.2% for insulin, 5.9% for adiponectin, and 5.1% for leptin. Intra-assay variability was 5.1% for insulin, 2.0% for adiponectin, and 5.6% for leptin. In our hands, limits of quantitation were 0.24 µU/ml for insulin, 1 ng/ml for adiponectin, and 7.86 pg/ml for leptin.