BIOACTIVE FACTORS IN MILK: COMPARISONS ACROSS NONHUMAN PRIMATES AND HUMANS

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Introduction

Milk is not just a food, but also a means by which mammalian mothers signal biochemically to their offspring. Milk contains a host of signaling molecules that influence growth and development of offspring. Some of these signaling molecules have been implicated as risk factors or as being protective against the early development of metabolic diseases in humans, such as obesity and diabetes. Growth factors in milk are thought to have important effects on gut development. These signaling molecules have begun to be examined in human breast milk. We sought to extend that examination to the anthropoid primates. We investigated whether commercially available assays designed to measure two growth factors (EGF and TGF-β_2), their receptors (EGF-R and TGF-RIII), and the metabolic hormone adiponectin in human biological samples were effective for non-human primate milks.

Methods

Milk samples for gorilla (N=1), orangutan (N=1), baboon (N=9), squirrel monkey (N=5) and common marmoset (N=5) came from the Smithsonian National Zoological Park's Milk Repository; milk samples for rhesus macaque (N=59) came from individuals housed at the California National Primate Research Center. The milk samples from baboon and common marmoset represented a single time point in mid lactation (4 months and 1 month, respectively) from multiple females. The common marmoset samples were pooled to obtain sufficient sample volume. The rhesus macaque samples represented two time points: early lactation and mid lactation. The orangutan milks were from a single female from infant age 6 months through one year. The gorilla samples were from a single female and were collected every week from the first week of life until the infant was 42 months old.

Parallelism and recovery protocols

We performed parallelism and recovery tests to validate that the component kits were able to accurately measure our analytes of interest. Briefly, we pooled several aliquots of milk from different time points, or from different individuals (where available), for each species. These pooled samples were serially diluted and run in an assay along with the standard curve supplied by the manufacturer. We then tested the parallelism of the slopes of the standard and serially diluted pooled curves. Since the component kits used were not developed for use with milk, we

utilized recovery tests in order to test whether anything in the sample matrix interfered with the assay. To do this, we spiked samples from our species of interest with controls provided in the kit, and measured the % value recovered.

Results and Discussion

All molecules were detectable in gorilla milk; all but TGF-β2 were detectable in orangutan milk and all but TGF-RIII were detected in rhesus macaque milk (Table 1). Only two molecules (EGF and adiponectin) were successfully detected in baboon milk. None were unambiguously detected in milks from either of the New World monkeys (Table 1).

Milk adiponectin concentration in the two great apes was low compared to human breast milk (gorilla=4.8±.5 ng/ml, orangutan = 1.4±.1 ng/ml, human range = 5 – 80 ng/ml). Macaque milk adiponectin (6.9±.4 ng/ml) was higher than ape milk, and appeared to increase with infant age. Milk EGF concentrations were highest in gorilla milk (29.7±.9 ng/ml), followed by orangutan (7.8±.4 ng/ml) and macaque (2.9±.2 ng/ml); these values are all below those found in mature human milk (75±12 ng/ml). The values for adiponectin and EGF in milk declined with infant age in orangutan; in gorilla milk concentrations were high in the first months, and then declined to fairly stable levels until 2-3 years after birth, when they increased. However, when expressed on a per energy basis the values did not differ with age for orangutan and the variation was reduced at all ages in gorilla. These data indicate that potent signaling molecules such as EGF and adiponectin are in ape milk at physiological concentrations for an extended time during lactation. However, human breast milk on average contains higher concentrations.

Table 1. Results of assay tests for different molecules in milks from six primate species.

	EGF	**EGF-R**	**TGF-β2**	**TGF-RIII**	**Adiponectin**
Gorilla	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Orangutan	**Yes**	**Yes**	Not detected	**Yes**	**Yes**
Rhesus macaque	**Yes**	**Yes**	**Yes**	Not detected	**Yes**
Baboon	**Yes**	Failed	Not tested	Failed	**Yes**
Common marmoset	Not detected	Not tested	Not tested	Not tested	Not detected
Squirrel monkey	Barely detected	Not detected	Not tested	Not tested	Not detected