



Appearance of Ellagic Acid Metabolites from Pomegranate Juice in Breast Milk: A Case Report

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Abstract

Pomegranate Juice (PomJ) contains Ellagitannins (ETs), the largest known group of polyphenol antioxidants with greater antioxidant activity than red wine or green tea. Maternal polyphenol consumption benefitting infant health could lead to greater incentive to breastfeed. In order to demonstrate the presence of EA metabolites in breast milk after consumption of PomJ, two healthy women with full-term, exclusively breast-fed infants, consumed 8 ounces of PomJ daily for two weeks. Breast milk and urine samples obtained on day 1, 7 and 14. Samples analyzed with Liquid Chromatography-Mass Spectrometry (LC-MS/MS) to identify EA metabolites. As a result, mothers had detectable and comparable EA metabolites (DMEAG and UAG) after 14 days of PomJ consumption. This pilot study demonstrates the presence of EA metabolites in breast milk after consumption of PomJ. Delivering phenolic compounds via breast milk could be a way to promote infant health and development.

Keywords: Ellagic acid metabolites; Pomegranate juice; Breastfeeding; Breast milk; Antioxidant; Polyphenols

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Introduction

Pomegranate (*Punica granatum*) Juice (PomJ) is remarkable in containing Ellagitannins (ETs), the largest known polyphenol antioxidant in a beverage with greater antioxidant activity than either red wine or green tea [1]. In addition, pomegranate extract inhibits the proliferation of human cancer cells and modulates inflammatory subcellular signal pathways and apoptosis [2]. In recent years, most health advantages of pomegranate have been attributed to the presence of ETs, mainly punicalagins and Ellagic Acid (EA) [1-4]. During processing of the whole fruits, ETs are extracted in significant amounts, subsequently enriching pomegranate juice with at least 2 g/L of punicalagins [2]. Research from our group and others have showed what appeared in the plasma after ET ingestion was EA. ETs are hydrolyzed in the intestine and absorbed as EA over six hours after administration of 6 ounces of Pomegranate Juice (PJ) [5-8]. After PJ consumption, the remaining ETs and EA are retained unabsorbed in the gut lumen where they likely interact with the complex intestinal bacteria of the microbiome. Previous research from our laboratory and others has shown that bacteria in the intestine are able to form a family of urolithins from the EA remaining in the gut [6,9,10]. There is individual variation of individuals to produce either urolithin A, urolithin B, isourolithin or to be non-producers [11-13]. These differences called metabotypes have also been related to biomarkers for cardiovascular disease and other chronic diseases of aging [11,12].

Breast feeding plays a critical role in the psychological and physical well-being of the newborn infant. It has been established that prebiotic carbohydrates, found in breast milk as the third most common component after lipids and lactose, support the development of specific bacterial species which may aid in the establishment of the immune system [14]. ETs affect bacteria in the adult gut with acute and chronic exposure [13] and could also affect the microbiome of newborn infants. Demonstrating health benefits of pomegranate consumption on infant health could lead to greater incentive for women to breast feed. This case report is to demonstrate for the first time whether pomegranate metabolites are secreted into breast milk.

Case Presentation

Subjects

A total of 2 healthy women with full term infants, exclusively breastfed, were in this case report.

Table 1: Characteristics of the study participants.

	Case 1	Case 2
Mother age (year)	41	37
Gestational Age at delivery (weeks)	38.7	40.0
Infant age at start of PJ (month)	1.5	6
Ethnicity of mother	Caucasian	Chinese
Ethnicity of infant	Caucasian	Chinese/Caucasian

Informed consent was obtained, the participants were investigators and it was a pilot study. The study protocol is now in place and approved by IRB. The demographic data is shown in Table 1. Both mothers were in excellent health without any chronic medical conditions. Both infants were delivered vaginally at an academic medical center without any complications.

The mothers consumed 8 oz of pomegranate juice (POM Wonderful LLC, Los Angeles, CA) daily for 2 weeks. Mothers were asked to maintain their usual diet pattern and abstain from consuming other polyphenol-rich foods during this 2-week period. Breast milk and urine samples were obtained from the mothers on day 1 and day 14. For breast milk collection, the nipple was cleaned with cloth; 10 ml of expressed milk was collected and frozen immediately.

Reagents and instruments

All solvents were HPLC grade and purchased from Fisher Scientific (Tustin, CA, USA). The HPLC-UV analyses were carried out on a Waters Alliance 2690 system equipped with a photo diode array detector (Waters Corp., Milford, MA, USA) and data was processed using Waters Millennium software, version 3.02.

LC-MS/MS Analyses of pomegranate juice metabolites in breast milk and urine

One mL urine samples were processed as previous reported [6]. 5 mL breast milk was mixed with 20 mL acetonitrile by vortex. The mixture was centrifuged at 4000 rpm for 10 min. The acetonitrile phase was dried using speedvac and reconstituted in 1 mL water, and further purified using by Solid-Phase Extraction (SPE) on C18 cartridges (Waters WAT 036945) and eluted with 2 mL methanol. The elutant was SpeedVac to dry, reconstituted in 200 μ l methanol:water 1:1 (v:v), and analyzed for ETs and ET metabolites, Dimethyl Ellagic Acid Glucuronide (DMEAG) and Urolithin A-Glucuronide (UAG) by LC-MS/MS system to determine the presence and levels of as previously reported [6]. Each breast milk and urine samples were separately extracted three times. The concentrations were estimated based on urolithin A standard. The conversion of UAG to UA was estimated by using β -glucuronidase to catalyze hydrolysis of β -D-glucuronic acid residues from UAG in human urine samples.

Table 2: Levels of PomJ metabolites in urine and breast milk.

I.D.	Day	Urine (nmol/L)		Breast Milk (nmol/L)	
		UAG	DMEAG	UAG	DMEAG
Case 1	1	3931 \pm 294	128 \pm 14	5.93 \pm 1.48	0.40 \pm 0.20
	7	17785 \pm 985	567 \pm 59	24.82 \pm 3.59	1.12 \pm 0.30
	14	16564 \pm 726	596 \pm 39	36.28 \pm 4.58	0.53 \pm 0.23
Case 2	1	ND	ND	ND	ND
	7	150 \pm 18	69 \pm 9	ND	ND
	14	7647 \pm 664	671 \pm 23	21.52 \pm 3.35	0.92 \pm 0.23
Detection Limit		12.37	1.0	4.95	0.40

Data are mean \pm STD (n=3). *below detectable limit

Results

In these 2 subjects tested for the presence of conjugated EA and UA (DMEAG and UAG) in breast milk, both of them had detectable and comparable DMEAG and UAG after 14 day's PomJ consumption (Table 2). In the urine, the concentrations of DMEAG and UAG were at much higher concentration compared to breast milk. For both subjects, a trend of increase of DMEAG and UAG concentration was observed from day 1-14. The absence of DMEAG in breast milk was associated with absence or very low DMEAG in the urine (Table 2).

Discussion

To the best of our knowledge, this is the first human case report describing the presence of ellagic acid metabolites in the breast milk. Pomegranate has a long history of use for medicinal purposes [15]. The potent antioxidant property as well as other biological activities of pomegranate has been mainly attributed hydrolysable tannins (ETs) including punicalagins and EA as well as anthocyanins and other polyphenols found in pomegranate extract and juice [16]. Pomegranate ETs are hydrolyzed to EA in the stomach. EA can then be absorbed into the bloodstream or further transformed to bacterial-derived metabolites including urolithins A-D in the intestine. Urolithins can be absorbed from the colon, conjugated, and excreted in the urine [6,9,10]. Depending on the individual's gut microbiota, variability of the urolithin formation has been observed. Among healthy overweight/obese individuals about 57% formed urolithin A (UA), 31% urolithin B (UB) and 12% did not form urolithins [11,13]. EA and urolithins are both bioavailable. Our previous bioavailability study demonstrated that after consumption of 180 mL of PomJ the metabolite DMEAG occurred the same day and plasma EA reached the maximum concentration at 0.06 μ mol/L + 0.01 μ mol/L 1 h after PomJ consumption. While an increase from 0.04 μ mol/L to 0.11 μ mol/L of conjugated urolithin A in plasma occurred between 0.5 and 6 h and in all participants conjugated UA and UB occurred in urine 24 h after consumption of PomJ [6]. It is interesting to see that breast milk containing UAG at the level between 5 nmol/L to 36 nmol/L (0.005 μ mol/L to 0.036 μ mol/L), within the range of previous plasma UAG level [6]. More and more evidence has indicated that EA as well as its microbial metabolite urolithins are involved in many biological activities. The antioxidant, anti-inflammation, and antimutagenic effects of EA have been studied [17]. Furthermore, neonatal Hypoxic-Ischemic Encephalopathy (HIE) is a major cause of morbidity and mortality in human newborns with motor and cognitive sequelae frequently seen in survivors [18,19]. HIE has been associated with increases in Reactive Oxygen Species (ROS) and recent studies suggest that new avenues of treatments of neonatal HIE should include selective responses to the generation of oxidative damage [20,21]. Phenolic phytochemicals are known for their antioxidant capacity, and studies have indicated the neuroprotective effects of dietary supplementation with food rich in phenolic compounds, such as green tea polyphenol EGCG, blueberry, and pomegranate [22-27].

Conclusion

Breast milk is the perfect nutrition for infants, a result of millions of years of evolution. In addition to providing a source of nutrition, breast milk contains a diverse array of microbiota and a myriad of biologically active components that are thought to guide the infant's developing mucosal immune system as well as neonatal gut microbiota. In this case report, we showed for the first time that ellagic acid metabolites (DMEAG and UAG) are present in the breast

milk, and the concentrations are within the range of blood levels. This report provides the basis for future study to investigate the impact of phenolic compounds in breast milk on neonatal brain development and their role in the development of the neonatal gut microbiome.

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