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Effects of Pasteurization on Adiponectin and Insulin Concentrations in Donor Human Milk

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ABSTRACT: Although pasteurization is recommended before distributing donor human milk in North America, limited data are available on its impact on metabolic hormones in milk. We aimed to investigate the effects of pasteurization on adiponectin and insulin concentrations in donor human milk. The study investigates concentrations of components in donor human milk before and after Holder pasteurization. After the guidelines of the Human Milk Bank Association of North America, human milk samples were pooled to produce 17 distinct batches (4 individuals per batch) and pasteurized at 62.5°C for 30 min. Adiponectin, insulin, energy, fat, total protein, and glucose concentrations were measured pre- and postpasteurization. Pasteurization reduced milk adiponectin and insulin by 32.8 and 46.1%, respectively (both $p < 0.0001$). Adiponectin and insulin were significantly correlated with energy and fat milk composition ($r = 0.36–0.47$; all $p < 0.05$). Pasteurization effects on milk hormone concentrations remained significant after adjusting for fat and energy (beta ± SEE: −4.11 ± 1.27, $p = 0.003$ for adiponectin; −70.0 ± 15.0, $p < 0.0001$ for insulin). Holder pasteurization reduced adiponectin and insulin concentrations in donor human milk. In view of emerging knowledge on the importance of milk components, continued work to find the optimal pasteurization process that mitigates risks but promotes retention of bioactive components is needed. **(*Pediatr Res* 70: 278–281, 2011)**

The epidemic in childhood obesity is a major public health challenge, which is reflected in the rapidly increasing rates of youth onset type 2 diabetes (1,2). Epidemiological evidence has shown a protective effect of breastfeeding on obesity (3,4) and type 2 diabetes later in life (5). Human milk contains not only macro- and micronutrients but also an array of bioactive substances including insulin (6,7). More recently, adiponectin, an insulin-sensitizing hormone in serum (8), has been detected in human milk (9–11). Therefore, these metabolic hormones may explain the observed association of breastfeeding with reduced risk for metabolic disease in offspring later in life (12).

With improved knowledge about the benefits of breastfeeding, there has been increasing demand for donor milk when mother's own milk is not available (13). To protect recipients against disease transmission, donor milk undergoes safety screening and handling processes (14–17). These processes, however, vary among countries: donor milk is pasteurized at 62.5°C for 30 min (Holder method) before distribution in North America and the United Kingdom (14,15), whereas donor milk is pasteurized at a lower temperature or unpasteurized in other countries (16,17). Although various nutrient components remain intact after pasteurization, the pasteurization process is known to effect biological activity of a number of milk components (18). Although the concentrations of adiponectin and insulin have been reported in human milk previously (9,10), the effect of pasteurization on these metabolic hormones has not been investigated. With recent evidence from a randomized controlled study demonstrating that dietary intervention in infancy has long-term effects on beta cell autoimmunity (19), it is important to understand the impact of pasteurization on milk metabolic hormones, which may have a critical impact on infant metabolic trajectories (20,21). We therefore aimed to investigate the effects of Holder pasteurization on adiponectin and insulin concentrations in donor human milk.

METHODS

The study was approved by the Hospital for Sick Children Ethics Review Committee, and informed consent was obtained from all milk donors. The inclusion criteria of donor milk samples were that milk needed to be expressed at >1 mo postpartum and <1 yr. This was to avoid inclusion of colostrum and transitional milk samples and milk from an involuting mammary gland. Donor milk from 34 women, frozen immediately and stored at −20°C for <6 mo, were used for the current analysis. After thawing individual milk samples in a water bath at 37.5°C, milk was pooled and processed following the guidelines of the Human Milk Bank Association of North America (14). Distinct batches of 17 pooled samples were produced with each batch comprising milk from four women. Samples were divided into 2 sets of 17 batches to assess pre- and postpasteurization effects. Samples for postpasteurization analysis were processed in a Breast Milk Pasteurizer (T30/USA; Sterifeed, Medicare Colgate Ltd, United Kingdom), which involved submerging bottles into a preheated water bath (63.2°C) followed by a cool water bath (<9°C). A temperature probe was positioned in a centrally placed nonsample bottle to ensure milk samples were maintained at 62.5°C for 30 min. Pre- and postpasteurization samples were aliquoted and stored at −80°C until biochemical analysis.

Biochemical analysis and validation of adiponectin assay. Adiponectin concentration was measured using a RIA (Millipore, Linco Research, MO). This assay has an interassay coefficient of variation of 9.3% at 7.5 μg/L. To validate assay methods for adiponectin, whole milk samples were spiked with 5, 10, 20, or 40 ng/mL human adiponectin standards (Millipore, Linco Research) to determine the recovery of the added volume. To account for sample dilution effects due to the added spiking volume, an equivalent volume

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Abbreviation: HMW, high-molecular weight

of physiological saline containing 0 ng/mL adiponectin was added to control nonspiked samples. Therefore, each adiponectin spiked sample had its own control designated as baseline. To assess whether lipids interfered with the adiponectin assay, 12 nonspiked study samples (6 pre- and 6 postpasteurization pooled batches) were assayed using both whole and skim milk samples. Skim milk samples were centrifuged (3000 revolutions per minute for 15 min), and the fat layer was removed.

Insulin concentration in skim milk was measured using the electrochemiluminescence immunoassay (Modular Analytics E170; Roche, NJ). This assay shows 0.05% cross-reactivity to intact human proinsulin and the primary circulating split form (des 31, 32). Total energy was determined by bomb calorimetry using the 1241 Automatic Adiabatic Calorimeter (Parr Instrument Company, IL) according to the method described by Garza *et al.* (22). Total fat was determined using the Creamatocrit methodology described by Lucas *et al.* (23). Total protein was measured using the Bicinchoninic Acid protein assay kit (Sigma Chemical Co., MO) (24). Glucose concentration in skim milk was determined using the hexokinase enzymatic method (Modular Analytics E170; Roche). All study sample assays including nutrient compositions were performed on two sets (pre- and postpasteurization) of 17 pooled batches.

Statistical analysis. Data analyses were performed using SAS software, version 9.2 (SAS Institute, NC) and with the consideration of two-sided $p < 0.05$ as statistically significant for all analyses. Distributions of continuous variables were assessed for normality and were determined to follow a normal distribution.

Descriptive statistics for continuous variables were summarized as mean ± SD. Concentrations of milk components pre- and postpasteurization were compared using paired *t* tests. To assess correlations of adiponectin and insulin with potential covariate milk components, Spearman rank correlation analysis was performed.

To evaluate effects of pasteurization as a main exposure on the outcomes of adiponectin and insulin with adjustment for potential covariates, we used mixed model analysis that is similar to analysis of covariance except the analysis accounts for correlation within the batches related to the repeated measure design. Three models were tested for each outcome variable: 1) an unadjusted model; 2) a model adjusted for total fat; and 3) a model adjusted for total fat and energy.

RESULTS

Average recovery rates of spiked adiponectin from whole and skim milk samples were 105.5 ± 18.2% (mean ± SD) and 118.4 ± 36.0%, respectively. Nonspiked adiponectin concentrations of whole and skim milk samples were highly correlated ($r_{spearman} = 0.90$ and $r_{pearson} = 0.97$, both $p < 0.0001$; Fig. 1), and mean adiponectin concentrations of whole (12.3 ± 6.1 ng/mL) and skim (12.1 ± 5.4) milk were not significantly different. Based on these similar findings, adiponectin concentrations in study milk samples were measured using whole milk.

Pasteurization reduced the concentration of milk adiponectin by 32.8% (pre- and postmean ± SD: 13.9 ± 4.8 *vs* 9.3 ± 3.0 ng/mL; $p < 0.0001$) and insulin by 46.1% (162.8 ± 64.2 *vs* 87.8 ± 26.3 pmol/L; $p < 0.0001$), whereas changes in energy, total fat, total protein, and glucose concentrations were modest, ranging 0–8.9% (Table 1).

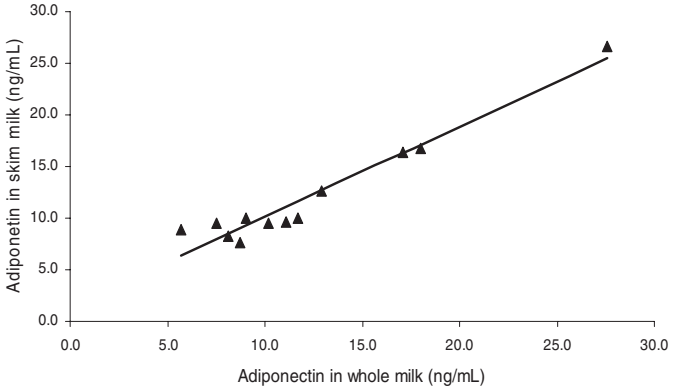

Figure 1. Comparison of adiponectin concentrations in skim and whole milk. The *solid line* represents the linear regression line ($r_{spearman} = 0.90$ and $r_{pearson} = 0.97$).

As assessed by Spearman correlation, adiponectin was significantly correlated with energy and fat composition in milk ($r = 0.47$ and 0.41, respectively; both $p < 0.05$) but not with protein and glucose ($r = 0.04$ and -0.08, respectively). Similarly, insulin was significantly correlated with energy and fat composition in milk ($r = 0.41$ and 0.36, respectively; both $p < 0.05$) but not with protein and glucose ($r = -0.21$ and 0.09, respectively).

The effects of pasteurization as a main exposure on the outcome of adiponectin or insulin were assessed with adjustment for significantly correlated potential covariates. Pasteurization effects on milk hormone concentrations remained significant after adjusting for fat and energy and accounting for correlations within the same batches (beta ± SEE: -4.11 ± 1.27, $p = 0.003$ for adiponectin; -70.0 ± 15.0, $p < 0.0001$ for insulin; Table 2).

DISCUSSION

We report that Holder pasteurization recommended in North America reduced adiponectin and insulin concentrations in donor human milk. Pasteurization effects on milk hormone concentrations remained significant with adjustment for potential covariates including milk fat.

To prevent transmission of bacteria and pathogens including HIV and human T-lymphotropic virus, donor milk banks have implemented safety screening and handling processes (14–17). Although donor milk is Holder pasteurized before distribution in North America and the United Kingdom (14,15), some countries have implemented more detailed screening processes followed by a lower temperature pasteurization or no pasteurization (16,17). Although a number of nutrients are unaffected by pasteurization (18), the pasteurization process deactivates or reduces the activity of several bioactive components in human milk including immunological proteins (18,25). Our results, which demonstrate that pasteurization reduced adiponectin and insulin concentrations in donor milk, raise concerns considering these hormones may have an important role in the metabolic development of infants (20,21). Although the molecular mechanism by which these metabolic hormones in milk may provide protection against developing metabolic disease later in life is not completely understood, potential mechanisms and physiological roles of these hormones influencing infant metabolic trajectories have been reviewed (12,20,21). Evidence indicates that oral administration of insulin stimulates gut maturation (21) and that adiponectin receptors are present in the fetal small intestine (20). These milk metabolic hormones, therefore, may have a direct role in the optimal metabolic development of infants and subsequently in reducing susceptibility to future metabolic disease. This may be especially important for the primary recipients of donor milk, preterm very LBW infants who are at increased risk for insulin resistance and type 2 diabetes later in life (26–28).

Table 1. *Concentrations of human milk components pre- and postpasteurization*

Component	Mean ± SD		Mean of differences ± SEM	*p**	Observed change (%)
	Pre	Post			
Adiponectin (ng/mL)	13.91 ± 4.84	9.34 ± 2.96	−4.57 ± 0.61	<0.0001	−32.8
Insulin (pmol/L)	162.8 ± 64.2	87.8 ± 26.3	−74.9 ± 11.1	<0.0001	−46.1
Energy (kcal/dL)	71.5 ± 9.9	69.4 ± 8.8	−2.2 ± 1.5	0.17	−2.9
Fat (g/L)	4.29 ± 0.95	3.91 ± 0.81	−0.38 ± 0.15	0.02	−8.9
Glucose (mmol/L)	0.97 ± 0.25	1.11 ± 0.22	0.13 ± 0.03	0.0007	1.4
Protein (g/L)	14.8 ± 1.5	14.8 ± 1.1	−0.02 ± 0.37	0.97	<0.1

* Paired *t* test.

Table 2. *Milk adiponectin and insulin concentrations pre- and postpasteurization*

Models	Adiponectin (95% CI), ng/mL				Insulin (95% CI), pmol/L			
	Beta ± SEM	Pre	Post	*p*	Beta ± SEE	Pre	Post	*p*
1	−4.57 ± 1.36	14.8 (11.9–17.6)	10.2 (8.0–12.4)	0.002	−74.9 ± 15.5	186.2 (151.4–220.9)	111.2 (89.3–133.2)	<0.0001
2	−4.03 ± 1.30	15.0 (12.2–17.9)	11.0 (8.6–13.4)	0.004	−69.7 ± 15.0	187.0 (153.8–220.2)	117.4 (96.2–138.5)	<0.0001
3	−4.11 ± 1.27	14.5 (11.8–17.1)	10.4 (8.1–12.6)	0.003	−70.0 ± 15.0	184.9 (151.2–218.6)	114.9 (92.5–137.4)	<0.0001

Mixed models were used accounting for correlations within the same batches. Models are 1) unadjusted, 2) adjusted for total fat, and 3) adjusted for total fat and energy.

In human serum, high-molecular weight (HMW) adiponectin is known to be the most biologically active form that has stronger associations with diabetes risk than total adiponectin (8). It must be noted that we used the total adiponectin assay that is not specific to the HMW form of adiponectin. Adiponectin in human milk, however, has been reported to be almost entirely composed of the HMW form (20). We also note that the heat treatment at 70°C for 10 min denatures adiponectin from trimeric to monomeric forms (29). Therefore, it is possible that Holder pasteurization might have reconfigured the adiponectin molecule such that it was unrecognizable to the assay. This unrecognizable molecule, however, is likely to be also nonfunctional. If this nonfunctional molecule in pasteurized milk was detected by the assay, pasteurized milk would have even greater reduction in its bioactivity. However, the functional analysis was not performed in the current study, which is another limitation of the study. In addition, we report the pasteurization effects on donor milk components but not other donor milk handling processes including freeze-thaw cycles. Previously, Bronsky *et al.* (10) reported a strong correlation between adiponectin concentrations before and after two freeze-thaw cycles ($r = 0.894$, $p < 0.0001$). Although Holder pasteurization is likely a major attributor that alters adiponectin and insulin concentrations, we cannot conclude that the impact of the overall donor milk processing protocol on milk composition is not greater than the pasteurization effects reported here. We also cannot separate effects of each pasteurization step, including heat treatment and container changes, on concentrations of milk components based on our results. Although the magnitude of changes in pre- and postpasteurization fat and glucose concentrations were small, they were significant. We speculate that the fat content might have been reduced during the donor milk handling process as a result of multiple container transfers. As for glucose concentrations, the pasteurization process might have caused molecular reconfigurations exposing more glucose molecules to be recognized by the assay. These hypotheses, however, were not tested in the current study. The strength of our report is that we investigated the practical impact of Holder pasteurization on donor milk following the standardized processing protocol (14).

In conclusion, Holder pasteurization, currently recommended by the Human Milk Banking Association of North America, reduced adiponectin and insulin concentrations in donor human milk. Because insulin and adiponectin are known to be involved in the pathophysiology of diabetes (8), the impact of the reduction in these milk hormones on susceptibility for developing type 2 diabetes later in life warrants further investigation. Variation in heat treatment options including a reduction in the length of pasteurization has been shown to preserve insulin-like growth factors (30). In addition, reducing pasteurization temperature to 57°C improved immunological protein retention while effectively removing 99.9% of inoculated bacterial species (25). With recent evidence from a randomized controlled study demonstrating long-term benefit of dietary intervention in infancy (19), it is important to understand the effect of pasteurization on milk components that may have a critical impact on infant metabolic trajectories (20,21). In view of emerging knowledge on the importance of milk components on human health outcomes, comprehensive risk and benefit assessments to find the optimal pasteurization process that mitigates risks but promotes retention of bioactive components is needed.

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