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Short Communication

Thermoultrasonication, ultraviolet-C irradiation, and high-pressure processing: Novel techniques to preserve insulin in donor human milk

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SUMMARY

Background & aims: Donor human milk (DHM) is recommended as the first alternative for preterm infants if their mother's own milk is not available or if the quantity is not sufficient. The most commonly used technique to eliminate microbial contaminants in DHM is holder pasteurization (HoP). However, the heating process during HoP partially destroys milk bioactive factors such as insulin. Therefore, innovative techniques have been developed as alternatives to HoP. The objective of this study was to determine the effect of HoP, high-temperature–short-time (HTST), thermoultrasonication (TUS), ultraviolet-C irradiation (UV-C), and high-pressure processing (HPP) on the insulin concentration in DHM.

Methods: Milk samples from 28 non-diabetic mothers were collected. The milk samples were aliquoted and either left untreated or treated with HoP (62.5 °C; 30 min), HTST (72 °C; 15 s), TUS (60 W; 6 min), UV-C (4863 J/L), or HPP (500 MPa; 5 min).

Results: The mean insulin concentration in untreated milk was 79 ± 41 pmol/L. The mean insulin retention rate was 67% for HoP, 78% for HTST, 97% for TUS, 94% for UV-C, and 106% for HPP. The mean insulin concentration in milk treated with HoP was significantly lower compared to untreated milk ($p = 0.01$).

Conclusion: TUS, UV-C, and HPP preserve insulin in DHM. The insulin concentration in DHM is affected to a larger extent by HoP than by HTST. These results indicate that TUS, UV-C, and HPP may serve as alternatives to HoP.

1. Introduction

Pasteurized donor human milk (DHM) is recommended as the first alternative for preterm infants if their mother's own milk is not available or if the quantity is not sufficient [1]. Pasteurization of DHM ensures microbiological safety. The currently recommended method is holder pasteurization (HoP), which includes heating the milk for 30 min at 62.5 °C [2]. However, this heating process partially destroys bioactive factors, thereby reducing the quality of DHM [2].

To improve the quality of DHM, innovative techniques, such as high-temperature–short-time (HTST), thermoultrasonication (TUS), ultraviolet-C irradiation (UV-C), and high-pressure processing (HPP) are currently under investigation as alternatives for HoP [2,3]. In several studies, higher retention rates of various nutritional and bioactive factors (e.g., immunological components and enzymes) were achieved using these techniques compared to HoP, while pathogens were still adequately inactivated [2,3]. The effect of these innovative techniques on milk hormones, such as insulin, has rarely been investigated [2,3].

Milk insulin appears to be a key factor for optimal gastrointestinal development given that this hormone stimulated intestinal

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maturation *in vitro* and in small-scale *in vivo* experiments [4]. The insulin concentration is reported to be significantly reduced in DHM after HoP [2]. Therefore, the gastrointestinal development and clinical outcomes of preterm infants who are fed DHM may improve by innovative techniques that better preserve insulin in DHM.

As such, the objective of this study was to determine the effect of HoP, HTST, TUS, UV-C, and HPP on the insulin concentration in DHM.

2. Materials and methods

Non-diabetic lactating mothers were recruited at Amsterdam University Medical Centers (Amsterdam, The Netherlands). Written informed consent was obtained from all participants. The study protocol was approved by the local Medical Ethical Committee.

2.1. Sample collection and preparation

Milk samples (complete expression of all milk in one breast) were collected in disposable polypropylene bottles. If the amount was less than 200 mL, milk from multiple days was pooled. The date of milk collection was written on the bottles by the mother, and the milk was stored at −20 °C. If the milk was collected at home, samples were stored at −20 °C in home freezers and then transported on dry ice to the hospital. Twenty-four hours before pasteurization, the milk was thawed at 4 °C.

After thawing, the milk sample from each mother was divided into six tubes. One tube remained untreated as a reference. The other five tubes underwent one of the following: HoP, HTST, TUS, UV-C, or HPP. After treatment, the samples were stored at −20 °C until analysis. In a preliminary study by our group, we showed that the human milk insulin concentration is not affected by freeze-thaw cycles at −20 °C [6].

The processing details are described in Section S1 in the Supplementary Appendix.

2.2. Sample analysis

Macronutrient analysis was performed using a commercially available human milk analyzer (MIRIS, Uppsala, Sweden). The milk insulin concentration was determined using a luminescence immunometric assay (Atellica, Siemens Medical Solutions Diagnostics, Malvern, USA) as published previously by our group [6].

2.3. Statistical analysis

The milk insulin retention rate was calculated as a percentage of the insulin concentration in untreated milk, which was set at 100%. The range of agreement was set at ± 10% of the insulin concentration in untreated milk. Variables were expressed as the mean ± standard deviation (SD), median (interquartile range [IQR]), or as the frequency, depending on their distribution. The mean insulin concentrations were compared using an analysis of variance (ANOVA). A p-value <0.05 was considered to be statistically significant.

3. Results

Milk from 28 mothers was collected at a median of 4 (IQR, 2–7) months postpartum. The median gestational age at delivery was 39.6 (IQR, 37.5–40.9) weeks, and 10 (36%) infants were male. The median body mass index of the mothers was 24 (20–25) kg/m^2 at the time of milk collection.

The mean insulin retention rate was 67% for HoP, 78% for HTST, 97% for TUS, 94% for UV-C, and 106% for HPP (Table 1). The mean insulin concentration was significantly lower only in the HoP-treated samples compared to untreated milk ($p = 0.01$).

Figure 1 shows the retention rate of all individual milk samples after either HoP, HTST, TUS, UV-C, and HPP. Samples with a retention rate that was within the range of agreement were as follows: 4 (14%) for HoP, 13 (46%) for HTST, 22 (79%) for TUS, 23 (82%) for UV-C, and 21 (75%) for HPP. A decreased milk insulin concentration (i.e., retention rate <90%) was observed in 24 (86%) of the samples treated with HoP, 15 (54%) of the samples treated with HTST, 2 (7%) of the samples treated with TUS, and 5 (18%) of the samples treated with UV-C. None of the HPP-treated samples showed a decreased insulin concentration. An increased milk insulin concentration (i.e., retention rate >110%) was observed in 4 (14%) TUS-treated samples and 7 (25%) HPP-treated samples.

4. Discussion

To the best of our knowledge, this is the first study that investigated the effect of HoP, HTST, TUS, UV-C, and HPP on the insulin concentration in DHM simultaneously. TUS, UV-C, and HPP did not affect the DHM insulin concentration, while the insulin concentration was decreased by HoP and HTST. The insulin concentration in DHM was affected to a larger extent by HoP than by HTST.

All techniques must meet the highest safety standards because vulnerable preterm infants are the usual recipients of DHM. In our preliminary study, the application of TUS (60 W; 6 min), UV-C (4683 J/L), and HPP (500 MPa; 5 min), resulted in a 5-$\log_{10}$ reduction of *Enterobacteriaceae* and *Staphylococci* species as required by human milk banking guidelines (Table S1 in the Supplementary Appendix). The HTST parameters that are used showed similar results as HoP for eliminating microbial contaminants in human milk in a previous study [3].

The milk insulin concentration was significantly decreased after HoP, with an average retention rate of 67%. This is consistent with two previous studies, which showed an insulin retention rate of 68% and 54% respectively after HoP [2]. The mean milk insulin concentration after HTST treatment was not significantly different from untreated milk, but the mean retention rate was outside the range of agreement, which suggests milk insulin degradation. The insulin degradation during HoP and HTST is probably caused by the thermal instability of insulin at a temperature above 60 °C [7]. The temperature during TUS remained below this insulin thermal instability threshold, and no heating of the samples occurred during either HPP or UV-C, which may explain the milk insulin preservation when applying these innovative methods [7].

A small milk insulin concentration increase was observed in 4 (14%) and 7 (25%) samples treated with TUS and HPP respectively. This is comparable to the milk leptin concentration increase after HPP treatment as observed by Wesolowska et al. [8]. They hypothesized that the increase might be caused by a small release of leptin incorporated in fat globules due to the high pressure. A similar process might occur with other hormones, such as insulin. However, additional studies are needed to investigate this hypothesis.

The biological effect of milk insulin has been investigated *in vitro* and in small-scale *in vivo* experiments [4]. The small intestinal mass and intestinal disaccharidase activity were significantly higher in piglets and rats that were treated with either enteral recombinant human insulin (rh-insulin) or recombinant porcine insulin compared to the respective control [4]. The effect on the intestine seems to be mediated by insulin receptors, which has been observed on the apical and basolateral membrane of enterocytes [4]. Consistent with animal studies, the intestinal lactase activity

Table 1
Effect of HoP, HTST, TUS, UV-C, and HPP on insulin in DHM.

	Untreated DHM N = 28	HoP N = 28	HTST N = 28	TUS N = 28	UV-C N = 28	HPP N = 28
Insulin concentration (pmol/L), mean ± SD	79 ± 40	53 ± 26	62 ± 33	77 ± 36	74 ± 38	84 ± 42
Retention rate (%), mean	–	67	78	97	94	106
P-value	–	0.01	0.10	0.90	0.63	0.58

HoP, holder pasteurization; HTST, high-temperature–short-time; TUS, thermoultrasonication; UV-C, ultraviolet-C irradiation; HPP, high-pressure processing; SD, standard deviation; DHM: donor human milk.

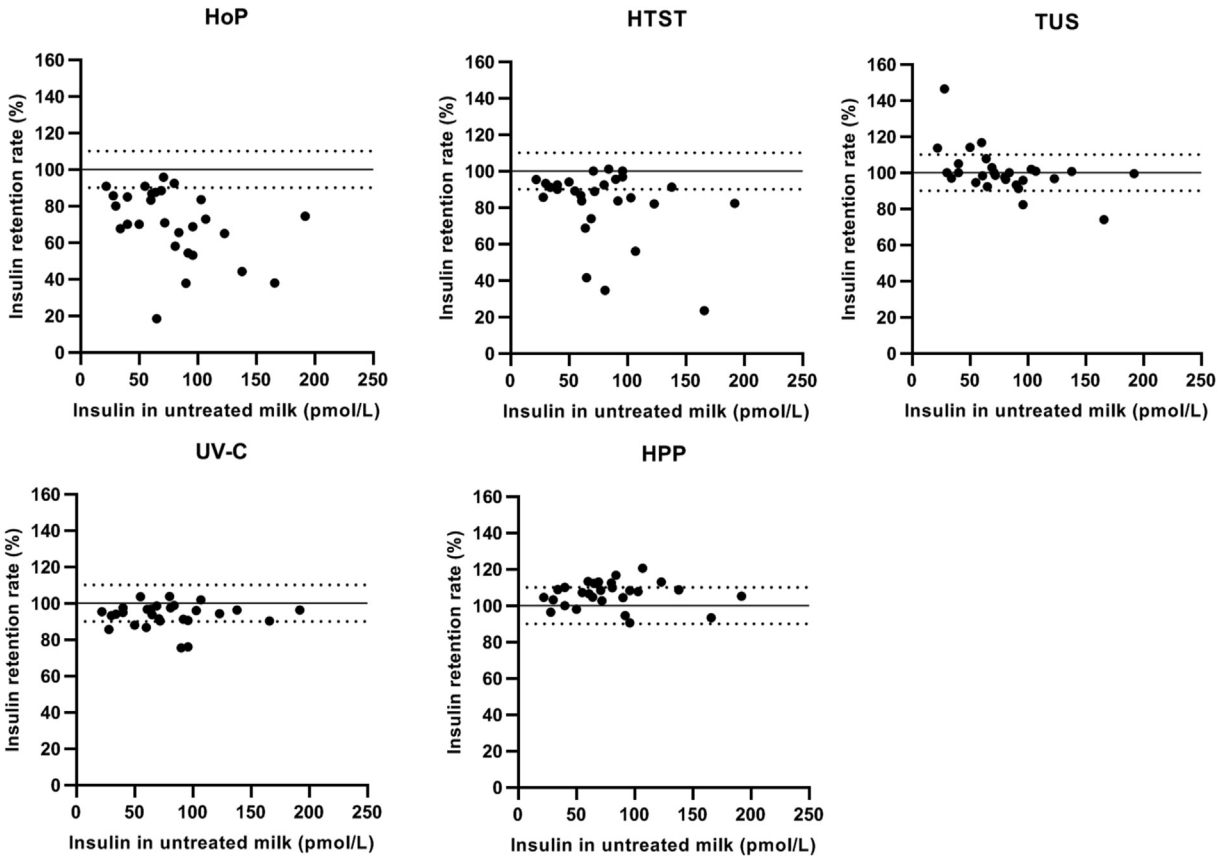

Fig. 1. The human milk insulin retention rate after HoP (A), HTST (B), TUS (C), UV-C (D), and HPP (E) (n = 28). The insulin retention rate was calculated as a percentage of the insulin concentration in untreated milk which was set at 100% (identity line). The range of agreement was set at ± 10% of the insulin concentration in untreated milk (dashed lines). HoP, holder pasteurization; HTST, high-temperature–short-time; TUS, thermoultrasonication; UV-C, ultraviolet-C irradiation; HPP, high-pressure processing.

was significantly higher in six preterm infants who received enteral rh-insulin compared to a historical control group [4]. In addition, time to achieve full enteral feeding was significantly reduced in preterm infants who received rh-insulin-supplemented formula compared to placebo-supplemented formula in a small clinical trial [5]. Thus, milk insulin has been suggested to be a key factor for optimal intestinal development and function, especially in preterm infants. Therefore, the higher occurrence of feeding intolerance and growth restriction in preterm infants fed DHM relative to preterm infants fed their mother's own milk might be because DHM is generally treated by HoP, which significantly decreases the insulin concentration [9].

Besides insulin, several other bioactive factors in DHM were shown to be better preserved by innovative techniques compared to HoP. For example, UV-C (4863 J/L) did not affect bile salt-stimulated lipase or alkaline phosphatase activity [12]. Additionally, the concentrations of secretory immunoglobulin A (IgA), lactoferrin, and lysozyme levels in DHM were higher after UV-C compared to HoP [10]. Furthermore, the IgA, IgG, and IgM retention rates were significantly higher in DHM after HPP treatment (500 MPa; 5 min) compared to HoP [11]. Additional studies are needed to investigate whether the improved DHM quality results in improved clinical outcomes of preterm infants who are fed DHM.

In conclusion, TUS, UV-C, and HPP all completely preserve milk insulin in DHM. The insulin concentration in DHM is affected to a larger extent by HoP than by HTST. These results indicate that TUS, UV-C, and HPP may be alternatives to HoP. Additional studies are needed to further investigate the effect of these methods on DHM safety and on the preservation of other bioactive components in DHM before implementing them in human milk banks.

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Author contributions

Elise Mank: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing – Original Draft, Project Administration. Eva Kontopodi: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – Original Draft, Project Administration. Annemieke C. Heijboer: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing – Review and Editing, Supervision. Ruurd M. van Elburg: Conceptualization, Methodology, Writing – Review and Editing, Supervision. Kasper Hettinga: Conceptualization, Methodology, Writing – Review and Editing, Supervision. Johannes B. van Goudoever: Conceptualization, Methodology, Writing – Review and Editing, Supervision. Letty van Toledo: Conceptualization, Methodology, Writing – Review and Editing, Supervision.

Conflict of interest

J.B.v.G. is member of the National Health Council, Chair of the Committee on Nutrition and Pregnancy, and Director of the Dutch National Human Milk Bank. He does not receive any honorarium for his services.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2021.09.028.

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