Ultra High Temperature Treatment, but Not Pasteurization, Affects the Postprandial Kinetics of Milk Proteins in Humans

Magali Lacroix, Cyriaque Bon, Cécile Bos, Joëlle Léonil, Robert Benamouzig, Catherine Luengo, Jacques Fauquant, Daniel Tomé, and Claire Gaudichon

INRA, AgroParisTech. UMR 914 Nutrition Physiology and Ingestive Behavior, CRNH-IdeF, F-75005 Paris, France; Department of Gastroenterology, Avicenne Hospital, CRNH Ile de France, 93009 Bobigny, France; and INRA, Agrocampus Rennes, UMR1253, Science and Technology of Milk and Egg, F-35000 Rennes, France

Abstract

Although the chemical and physical modifications to milk proteins induced by technological treatments have been characterized extensively, their nutritional consequences have rarely been assessed in humans. We measured the effect of 2 technological treatments on the postprandial utilization of milk nitrogen (N), pasteurization (PAST) and ultra high temperature (UHT), compared with microfiltration (MF), using a sensitive method based on the use of milk proteins intrinsically labeled with $^{15}$N. Twenty-five subjects were studied after a 1-wk standardization of their diet. On the day of the investigation, they ingested a single test meal corresponding to 500 mL of either MF, PAST, or UHT defatted milk. Serum amino acid (AA) levels as well as the transfer of $^{15}$N into serum protein and AA, body urea, and urinary urea were determined throughout the 8-h postprandial period. The kinetics of dietary N transfer to serum AA, proteins, and urea did not differ between the MF and PAST groups. The transfer of dietary N to serum AA and protein and to body urea was significantly higher in UHT than in either the PAST or MF group. Postprandial deamination losses from dietary AA represented 25.9 ± 3.3% of ingested N in the UHT group, 18.5 ± 3.0% in the MF group, and 18.6 ± 3.7% in the PAST group ($P < 0.0001$). The higher anabolic use of dietary N in plasma proteins after UHT ingestion strongly suggests that these differences are due to modifications to digestive kinetics and the further metabolism of dietary proteins subsequent to this particular treatment of milk.


Introduction

The nutritional value of milk proteins is generally recognized as excellent (1–5). Differences do, however, exist between milk proteins, especially due to their digestion kinetics (6). However, the effect on milk protein utilization of the usual technological treatments employed to ensure milk conservation has rarely been assessed on the basis of in vivo criteria, and to date only limited studies have been conducted in humans. Heat treatments are generally assumed to impair protein quality (5). Pasteurized milk represents the majority of milk consumed worldwide (72% of sales in 2004), particularly in the UK, USA, Canada, and Netherlands, but ultra high temperature (UHT) milk is commonly used in many European countries, particularly in Belgium, France, Sweden, Spain, and Portugal, where UHT milk represents >90% of total milk consumption. In France, UHT milk ranks 2nd in terms of its contribution to protein intake in women and 5th in men (7). The main advantage of this process is that it ensures a long shelf life of 3 mo. Microfiltration has recently emerged as a new process that enables the maintenance of traditional milk flavor while ensuring microbiological stability for ~2 wk (8). The impact of this treatment on nutritional protein quality, when compared with standard treatments, is not known.

As a result of heat treatment, Maillard reaction products such as furosine may form (9) and some amino acid (AA) residues are rendered nutritionally unavailable because of the formation of lysinoalanine (10); furthermore, soluble proteins are altered (9). For these reasons, heat treatments compromise AA availability (11). For instance, heating has been reported to reduce protein digestibility (12,13). However, the heat treatments that were applied during the aforementioned studies were drastic. In addition, the effect of these treatments on the protein efficiency ratio (PER), one of the standard criteria used to determine protein quality, was not consistent in all studies. Some authors reported an altered PER (13,14), whereas others did not observe...
any modifications (15). Lastly, no study has measured in vivo in humans the nutritional impact of the treatments generally employed to stabilize microflora in milk.

Our objective was to assess in vivo in humans the impact of heat treatments on protein quality by studying dietary nitrogen (N) metabolism following a single meal. For this purpose, we compared microfiltered (MF), pasteurized (PAST), and UHT milks in which proteins were intrinsically labeled with \(^{15}\)N. We could thus follow the transfer kinetics of dietary N toward several N pools and assess protein quality as the net postprandial protein utilization (NPPU) for each type of milk treatment.

**Subjects and Methods**

**Experimental meals.** Milk was \(^{15}\)N-labeled in the Milk Production Unit (Unité de Production du Lait, INRA, Saint-Gilles, France) and then processed as detailed in Lacroix et al. (16). Briefly, milk was defatted and then microfiltered on a 1.4-μm membrane (GP7 Sterilox equipment) to produce MF milk. This reference milk was then either pasteurized (72°C for 20 s) or underwent UHT treatment (140°C for 5 s). The pattern of AA in the 3 meals did not differ significantly except for proline, which was found at lower levels in the UHT product (16). The test meals contained total milk protein and lactose from PAST (\(n = 8\)), MF (\(n = 8\)), or UHT milk (\(n = 8\)). Meals were isonitrogenous and isonitrogenous, containing 974 kJ, 23.3 g of protein, and 35 g of lactose in a total volume of 500 mL.

**Subjects.** Twenty-five men (\(n = 11\)) and women (\(n = 14\)) participated in this study. Written informed consent was obtained from all participants and the protocol was approved by the Institutional Review Board of Saint-Germain-en-Laye Hospital. All participants were certified as being in good health after a thorough examination performed by the medical staff of the Human Nutrition Research Center and routine biochemical tests. The purpose and the potential risks of the study, as well as the constraints due to the obligatory dietary standardization period, were fully explained to the subjects, who were randomly assigned to 1 of the 3 groups that would each receive different test meals. Subject characteristics and body composition did not differ significantly between the groups (Table 1).

**Standardization of the diet.** During the week preceding the day of the experiment, the subjects had to follow a dietary adaptation at home, adjusted to their body weight (BW) as verified during the prestudy medical examination. Dietary notebooks containing daily menus and the specific quantities of food to be consumed at each meal were delivered to all volunteers, as were kitchen scales (accurate to the nearest 2 g) and daily record sheets. The subjects were asked to ensure strict compliance with their respective diets but were allowed some equivalent food exchanges. They were also required to adhere to 3 main meals per day, with 1 snack per day. Daily menus were designed to provide 138 kJ/(kg BW-d), comprising 1.3 g protein/(kg BW-d), 4.2 g carbohydrates/(kg BW-d), and 1.2 g fat/(kg BW-d). The energy supply was determined in accordance with international recommendations for a normal BMI and moderately active person (17). Overall, the subjects exhibited good compliance with the standardization diet (data not shown) as their protein ingestion ranged from 1.21 g/(kg BW-d) in the PAST group to 1.26 g/(kg BW-d) in the UHT group, as computed from the dietary records.

**TABLE 1** Subject characteristics in each experimental group1

<table>
<thead>
<tr>
<th></th>
<th>MF</th>
<th>PAST</th>
<th>UHT</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>8 (3M, 5F)</td>
<td>8 (4M, 4F)</td>
<td>9 (4M, 5F)</td>
</tr>
<tr>
<td>Age, y</td>
<td>27.1 ± 7.8</td>
<td>23.5 ± 6.9</td>
<td>25.7 ± 6.5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.0 ± 2.0</td>
<td>22.8 ± 2.9</td>
<td>22.2 ± 2.6</td>
</tr>
<tr>
<td>BW, kg</td>
<td>63.5 ± 11.3</td>
<td>67.2 ± 11.9</td>
<td>64.8 ± 9.8</td>
</tr>
<tr>
<td>Total body water, L</td>
<td>37.5 ± 9.4</td>
<td>39.5 ± 7.2</td>
<td>38.9 ± 7.9</td>
</tr>
<tr>
<td>Fat mass, %</td>
<td>20.8 ± 8.0</td>
<td>20.5 ± 7.5</td>
<td>19.1 ± 7.9</td>
</tr>
</tbody>
</table>

1 Values are means ± SD.

**Experimental design.** After fasting overnight, the volunteers were admitted to the hospital on the morning of the experiment and a catheter was placed in a superficial forearm vein for blood sampling. Baseline blood and urine were then collected. Basal glycemia was measured immediately using a portable refractometer (Encore glucometer, Bayer Diagnostics). Volunteers were required to consume their respective liquid meal (MF, PAST, UHT) within 15 min and were then monitored for 8 h. Blood samples were taken every 30 min for the first 3 h and hourly for the remaining 5 h following consumption of the meal. Urine was collected every 2 h. Three hours after the experimental meal, each subject drank an accurately weighed dose (80 ± 6 mg/kg BW) of \(^2\)H\(_2\)O [99.94 atom percent (AP), Eurisio-Top] to determine total body water, which was then used to further calculate the body urea N pool. After immediate determination of the glucose concentration in blood samples, serum was obtained by centrifugation (3000 × g; 15 min at 4°C), aliquoted, and frozen at −20°C until later analysis. \(\gamma\)-Aminobutyric and \(\alpha\)-aminoacidic acids were added to 1 aliquot as internal standards for the subsequent determination of serum AA concentrations. Urine samples were stored at −4°C with thymol crystals and paraffin as preservatives to be processed within the next 48 h or frozen immediately at −20°C, depending on the analysis.

**Analytical procedures.** Serum urea, urinary creatinine, and urinary urea were determined using an enzymatic method (Dimension AutoMate, Dupont de Nemours). Serum AA concentrations were determined only in the UHT and MF groups, using an HPLC system (Bio-Tek Instruments). We delayed the analysis of serum AA in the PAST group, depending on the presence of any differences detected between the PAST and MF groups. Protein, urea, and AA fractions were isolated from serum, as well as ammonia and urea from urine, using a cation exchange resin as previously described (6,18). Sample N contents and \(^{15}\)N enrichments were measured using isotopic ratio mass spectrometer (Optima, Fisons Instruments) coupled with an elemental analyzer (NA 1500 series 2, Fisons Instruments) with atropine (Carlo Erba Instruments, Fisons) as the standard, as previously described (19).

Deuterium enrichments were analyzed using an isotopic ratio mass spectrometer coupled with a GC device (Multiflow-IRMS, Isoprim, Micromass), as detailed in Lacroix et al. (6).

**Calculations.** The time course of dietary N incorporation into the different pools monitored (serum free AA pool and proteins, body urea, urinary urea) was evaluated by the following general equation:

\[
N_{\text{net pool}}(t) = N_{\text{net pool}}(t) \times (E_0 - E(t))/E_{\text{meal}} - E(t)]/N_{\text{ingested}} \times 100,
\]

where \(N_{\text{net pool}}(t)\) is the N content of the pool (mmol N) at each time point \(t\), \(E_0\) is the \(^{15}\)N-enrichment (expressed as Atom %) in the pool sampled at time \(t\), \(E(t)\) is the baseline \(^{15}\)N-enrichment of the meal, and \(N_{\text{ingested}}\) is the N content (mmol) of the meal, as previously detailed (6).

\(N\) recovered in the different pools was always expressed as percent of the \(N\) amount ingested during our study, except for AA, where it was expressed as an enrichment excess ratio between sample and meal.

NPPU represented the postprandial retention of dietary N and was estimated using the following formula:

\[
\text{NPPU} = 100 - (N_{\text{net dem}} - N_{\text{total}}) \times 100/\text{ingested} N,
\]

where \(N_{\text{net dem}}\) is the loss of dietary N through urinary and body urea, and \(N_{\text{total}}\) the ideal losses that were assumed to be 5% of dietary N (1,4,16).

**Statistics.** The results are expressed as means ± SD. Differences between subjects in terms of body composition, compliance with the standardization diet, and variable values obtained at 8 h were tested by 1-way ANOVA (version 9.1, SAS). Differences among groups during the 8 h following meal ingestion were tested by repeated-measures ANOVA using the MIXED procedure. For plasma AA concentrations, the basal value was inserted as a covariate in the model. Moreover, differences from baseline values at each time point within groups were determined using the contrast statement in a mixed model with time as factor. For
each variable, we tested 5 different covariance structures for random statements (compound symmetry, unstructured, auto regressive 1, auto regressive moving average 1, and Toeplitz) and the most appropriate matrix was selected. Post-hoc tests were performed using the Tukey test. A P-value < 0.05 was considered significant.

Results

Blood glucose, serum urea, and AA concentrations. The postmeal responses of glycemia and urea were not influenced by the treatment (data not shown). Plasma AA concentrations were determined in the MF and UHT groups (Fig. 1), assuming that plasma AA in the PAST group would be similar to MF values. Compared with the baseline values, ingestion of the meal was followed by a marked increase in aminoacidemia in both groups, with more sustained hyperaminoacidemia in the MF group than in the UHT group for total (Fig. 1A), dispensable (Fig. 1B), and indispensable AA (Fig. 1C). However, there were no significant effects of group and group × time interactions for any variables. For lysine (Fig. 1D), as for each AA (not shown), postprandial concentrations did not differ significantly between groups.

Exogenous N transfer to serum AA, protein, and urea. The time courses of dietary N transfer into serum AA, urea N, and protein pools differed between groups (Fig. 2). A significant meal effect was observed whatever the N pool (P < 0.05), with a higher level of dietary N in circulating AA (Fig. 2A), serum urea (Fig. 2B), and protein (Fig. 2C) pools after the UHT meal than after either the PAST or MF meals. A significant meal × time interaction was also reported for dietary N appearance in serum AA and proteins. Eight h after meal ingestion, ~15% of dietary N was present in the body urea pool after the ingestion of UHT milk, whereas this value reached ~10% after the ingestion of MF or PAST milk. In serum proteins, dietary N recovery was 7.7 ± 1.2% in the UHT group compared with 6.1 ± 1% in the MF group and 6.4 ± 1.5% in the PAST group.

Dietary N losses in urinary urea and total deamination of dietary AA. At the end of the investigation, the amount of dietary N recovered in urinary urea represented 8.0 ± 2.1% and 8.1 ± 2.4% of ingested N in the MF and PAST groups, respectively, whereas the level was higher in the UHT group (11.7 ± 3.1% of ingested N; P < 0.05) (Fig. 3).

Finally, the sum of exogenous N still present 8 h after the meal in body urea, and that excreted in urinary urea (representing total deamination), differed (P < 0.0001) between the UHT group (25.9 ± 3.3% of ingested N) and the MF (18.5 ± 3.0% of ingested N) and PAST groups (18.6 ± 3.7% of ingested N). The postprandial retention of dietary proteins assessed as the NPPU (Table 2) was similar in the MF and PAST groups but was significantly reduced by 8% in the UHT group.

Discussion

This study aimed to evaluate in vivo in humans the protein quality of various types of processed milk that represent all the milk consumed in western countries. This question is important given the fact that it is generally assumed that milk protein quality is impaired after heat treatment. Proteins from PAST milk, which is the type most commonly consumed in the world, were associated with the same metabolic pattern as that originating from microfiltration, a treatment that involves no heating. In contrast, dietary N kinetics differed with UHT milk and were characterized by lower retention (68% vs. 76%) than MF milk. Interestingly, our results strongly suggest that a
modification of digestive kinetics was responsible, at least partly, for the higher postprandial deamination rate associated with UHT milk consumption, as indicated by the enhanced incorporation of dietary N in plasma proteins.

Study of the transfer of dietary N to deamination pools revealed that microfiltration and pasteurization exhibited similar dietary protein utilization during the postprandial phase, in contrast to UHT. Irreversible losses of dietary N accounted for 18.5% in the MF and PAST groups and 25% in the UHT group. These values are consistent with those already reported by us in humans using a similar methodology (1,18). To calculate postprandial retention, we applied a uniform digestibility coefficient of 95% that corresponded to the values obtained at the ileal level in humans using ileal tubes (1,4). Although heat treatments are suspected of impairing digestibility (20,21), proteins from UHT milk have been described as being as digestible as unheated proteins (16,22). Thus, the postprandial retention of MF and PAST milk was 76%, which fully agrees with the values that we had already obtained for total milk protein. In this context, UHT N retention (68%) is equivalent to that obtained for purified soy proteins (23).

Few assessments have been made of the nutritional value of products after household heat treatments. Povoa and Moraes-Santos (24) compared in rats the protein quality of raw, PAST, and UHT milk on the basis of PER values and found no differences between treatments. Using the 15N-labeling methodology, we previously found PAST, UHT, and raw milk did not differ in rats after the ingestion of a calibrated meal (16). We already noted that the rat model might not be sufficiently sensitive to detect small variations in postprandial N retention, especially due to the low amounts of urine produced over 6 h. Another reason may also have been differences in test meal composition. Indeed, a higher energy:protein ratio was used during the rat study. Energy macronutrients are known to markedly lower gastric emptying (4,25) and may buffer the specific effects of kinetics on protein metabolism (26). Consistent

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Postprandial retention of dietary nitrogen in humans after the ingestion of MF, PAST, and UHT milk¹</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>MF</td>
<td>8</td>
</tr>
<tr>
<td>PAST</td>
<td>8</td>
</tr>
<tr>
<td>UHT</td>
<td>9</td>
</tr>
</tbody>
</table>

¹ Values are means ± SD Means without a common letter differ, P < 0.05.
² PPUN, Postprandial retention of dietary N.
with this, our previous study in rats using the same products did not reveal any higher transfer of dietary N to splanchnic and plasma proteins after UHT milk ingestion, in contrast to our findings in humans. The present results thus strongly support the hypothesis that digestive kinetics are more rapid after UHT milk ingestion than with PAST or MF milk, leading to an increased transfer of dietary N into plasma protein and urea. We cannot exclude the possibility that lysine damage may also be responsible (at least in part) for the higher deamination level of dietary AA. This hypothesis is supported by the moderate but still substantial increase in furosine and lysinoalanine levels previously reported for UHT milk (27,28).

Nonetheless, several explanations may support our hypothesis of accelerated digestion kinetics as a result of UHT treatment. First, β-lactoglobulin is resistant to digestion and thus the AA released from this protein are mostly absorbed in the distal portion of the small intestine (29). It has been shown in vitro that heating β-lactoglobulin led to more rapid hydrolysis of this protein by the pancreatic juice (15). We thus suggest that AA from soluble proteins are more rapidly absorbed after heating. Moreover, the UHT treatment of milk causes structural changes in its protein system (30). The main change is the denaturation of whey proteins and their interaction with casein micelles. Consequently, it was expected that the behavior of the UHT milk would be similar to that of casein micelles, as observed with native casein (6). However, it has been shown that the interaction between casein micelles and whey proteins is also accompanied by a large increase in number of very small, soluble particles resulting from the desegregation of casein micelles (31,32). Consequently, by weakening the interaction forces between caseins, heat treatment provokes both a loss of the micellar framework and the formation of small aggregates. Correlatively, an increase in the amount of nonsedimentable casein has been observed in UHT milk following high-speed centrifugation (33). Two effects of these changes on milk properties may be softer coagulation and a higher susceptibility to enzymatic hydrolysis due to loosening of the micellar structure and the presence of small aggregates that may be more accessible to enzymes. The biphasic transfer of dietary N to plasma AA appears to constitute a good reflection of the structural heterogeneity resulting from UHT treatment.

Heat treatment markers that were previously quantified in the 3 milk products (16) were consistent with the postprandial retention measured. However, they are only qualitative markers and cannot quantitatively predict the nutritional value of a protein. Indeed, soluble proteins were found at levels that were 78% lower in UHT than in MF milk and lactulose levels were twice as high in UHT than in MF milk, suggesting a greater difference in quality between products than that actually measured in vivo; MF retention was only 12% higher than that of UHT. This clearly shows that in vivo studies are necessary to determine the effect of technological treatments on protein quality and to help establish the significance of variations in biochemical marker values.

In conclusion, our in vivo study in humans revealed that PAST milk, the most widely consumed processed milk in the world, exhibited similar postprandial utilization values of dietary N to MF milk, in contrast with UHT milk, which is consumed to a greater extent in Europe. However, the postprandial retention of proteins from UHT milk remained greater than that of nonheated whey proteins. We report an enhancement of the anabolic use of dietary N in plasma proteins following the ingestion of UHT milk, which strongly suggests that differences in postprandial retention are driven by a modification of digestive kinetics. This in turn results from structural modifications to proteins and especially the desegregation of casein micelles under UHT treatment. Further trials are necessary to confirm whether the higher deamination rate of UHT proteins is due only to more rapid gastric emptying or if it is also caused by the blockade of certain AA residues.

Acknowledgment
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Literature Cited


