JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Consumption of a Functional Fermented Milk Containing Collagen Hydrolysate Improves the Concentration of Collagen-Specific Amino Acids in Plasma

STEPHANE WALRAND,[†] ELENI CHIOTELLI,*[,‡] FLORENCE NOIRT,[‡] SANDRINE MWEWA,[§] AND TAOUS LASSEL[‡]

INRA, UMR1019, UNH, Clermont-Ferrand, F-63000, France, Danone Research, Centre Daniel Carasso, R.D. 128, 91767 Palaiseau Cedex, France, and Umanis France, 7-9 Av Paul Vaillant Couturier, 92301 Levallois-Perret, France

Clinical studies have shown that collagen hydrolysate (CH) may be able to protect joints from damage, strengthen joints, and reduce pain from conditions like osteoarthritis. CH is a collection of amino acids and bioactive peptides, which allows for easy absorption into the blood stream and distribution in tissues. However, although various matrices have been studied, the absorption of specific amino acids from CH added to a fresh fermented milk product (FMP) was not studied. The primary objective of the present study was to compare the plasma concentrations of four representative amino acids from the CH (glycine, proline, hydroxyproline, and hydroxylysine) contained in a single administration of a FMP with that of a single administration of an equal amount of neat hydrolyzed collagen. These four amino acids were chosen because they have already been used as markers of CH absorption rate and bioavailability. This was a single-center, randomized open, and crossover study with two periods, which was performed in 15 healthy male subjects. The subjects received randomly and in fasted state a single dose of product 1 (10 g of CH in 100 mL of FMP) and product 2 (10 g of CH dissolved in 100 mL of water) separated by at least 5 days. After administration, the subjects were assessed for plasma concentrations of amino acids and for urine concentrations of hydroxyproline. After FMP administration, mean values of the maximal concentration (C_{max}) of the four amino acids were greater than after ingredient administration ($p < 0.05$). This effect was related to an increased C_{max} of proline ($p < 0.05$). In conclusion, because of their physicochemical characteristics, the fermentation process, and the great homogeneity of the preparation, this milk product improves the plasma concentration of amino acids from CH, that is, proline. The present study suggests an interesting role for FMP containing CH to improve the plasmatic availability of collagen-specific amino acids. Hence, this FMP product could be of potential interest in the management of joint diseases.

KEYWORDS: Fermented milk product; collagen hydrolysate; hydroxyproline

INTRODUCTION

Collagen hydrolysate (CH) is obtained by enzymatic hydrolysis of collagenous tissue (bone, hide, and hide split) from mammals. The special characteristic of CH is its amino acid (AA) composition, which is identical to type II collagen, thus providing high levels of glycine and proline, two AAs essential for the stability and regeneration of cartilage. Type II collagen makes up approximatively 70% of the joint cartilage and provides the joint with its tensile strength and stiffness. To synthetize a single picogram of type II collagen, more than 1 billion glycine molecules and 620 million proline molecules are required (*1*). In the absence of these AAs, the anabolic phase of cartilage metabolism can be impaired. To date, clinical studies have shown that CH may be able to protect joints from damage and reduce pain from conditions like osteoarthritis.

CH has long been used in pharmaceuticals and food supplements. This product is generally recognized as a safe food ingredient by regulatory agencies. After processing, CH does not gel any further, giving it the advantage of being soluble in water. Proteins such as CH taken in oral form are enzymatically digested to their peptides and AA components in the gastrointestinal tract; therefore, CH presents a good digestibility. However, even if the clinical use of CH is associated with minimal adverse effects, gastrointestinal side effects, character-

* To whom correspondence should be addressed. Tel: +33 1 69 35 72 12. Fax: +33 1 69 35 70 42. E-mail: eleni.chiotelli@danone.com.
[†] INRA.
[‡] Danone Research.
[§] Umanis France.

10.1021/jf800691f CCC: $40.75
Published on Web 08/16/2008

ized by fullness and unpleasant taste, have been described (*2*). In this context, although CHs from different origins have been studied (*3*), no study exists in which CH was provided within a fresh fermented milk product (FMP), and in most of the studies, CH has been administrated alone in a water solution. FMP constitutes a very interesting vector for this type of ingredient. Because of its physicochemical characteristics, it allows a great homogeneity of preparations, while the quantities provided can be perfectly controlled (standardized portions). In addition, unpleasant taste could be overcome by the addition of CH in a fruit-flavored FMP. Then, CH could be easily integrated as a part of people's daily lives, within a tasty and nutritionally balanced fresh dairy product, to improve joint function in a pleasant way. Of note, the consumption of CH as part of a FMP may improve the acceptability by people of a long-term treatment with CH.

However, whether the dairy composition or the fermentation process might impact CH digestion and absorption is an unanswered question. Peptides and AAs from milk proteins and CH can be liberated during milk fermentation with proteolytic enzymes, as it has already been observed (*4, 5*). Additional hydrolysis of CH by bacteria used for milk fermentation may change the profile of AA in plasma after CH consumption. Therefore, it is of interest to compare the digestion and absorption of CH, whether ingested on its own or in the form of a FMP. The primary objective of this study is to compare the plasma concentrations of four representative AAs from the CH (glycine, proline, hydroxyproline, and hydroxylysine) contained in a single administration of a FMP with that of a single administration of an equal amount of neat CH. These four AAs have been chosen because they are major components of collagen and because they play essential roles in collagen structure and function (*3, 6, 7*). A distinctive feature of collagen is the regular arrangement of AAs in a very specific tripeptide sequence repeat, with glycine being mandatory at every third position. The sequence often follows the pattern glycine−X−proline or glycine−X−hydroxyproline, where X may be any of the various other AA residues. Glycine−proline−hydroxyproline occurs frequently. Hence, a high glycine content is found in collagen; that is, approximatively 50% of collagen AAs are glycine. In addition, hydroxyproline and proline permit the sharp twisting of the collagen helix and provide stability to the triple helical structure of collagen by forming hydrogen bonds (*8*). Hydroxylysine is also most widely known as a component of collagen. For these reasons, glycine, proline, hydroxyproline, and hydroxylysine plasma concentrations have often been used as markers for CH digestibility, absorption rate, bioavailability, and action (*3, 6, 7*). In addition, because it is frequently asserted that plasma levels of AAs are difficult to interpret (*9*), the calculation of the sum of collagen-specific AAs after FMP administration has been done to take the physiological situation, that is, CH supplementation, fully into account.

MATERIALS AND METHODS

Subjects. Fifteen adult men (ages, 39.7 ± 3.2 years) were recruited. The detailed subject characteristics are given in **Table 1**. All were considered as healthy according to their detailed medical histories, physical examinations, and laboratory tests and had body mass indexes (BMI) within 18 and 25 kg/m^2. None were taking medication that would affect the studied parameters (corticosteroids, estrogen replacement, β-adrenergic blockers, and anticoagulants). All subjects were sedentary, and individuals who participated in a regular exercise program for more than 30 min twice a week were excluded. All smoking subjects were also excluded.

Table 1. Anthropometric Characteristics of the Population

$n = 15$	means	min	max
age (years)	39.7 ± 3.2	35	45
weight (kg)	72.2 ± 5.7	64	83
height (cm)	177.2 ± 5.1	172	187
BMI (kg/m^2)	22.99 ± 1.38	20.7	24.8
waist circumference (cm)	80.8 ± 3.6	74.0	86.0

Table 2. Composition of the Control Product and FMP[a]

quantity per 100 g container	control product	FMP
total proteins (g)	10.0	12.1
from CH (g)	10.0	10.0
total AAs (g)	9.2	12.0
from CH (g)	9.2	9.2
proline (g)	1.21	1.22
from CH (g)	1.21	1.21
hydroxyproline	1.09	1.09
from CH (g)	1.09	1.09
glycine (g)	2.09	2.15
from CH (g)	2.09	2.09
carbohydrates (g)		7.1
lipids (g)		1.4
calcium (mg)		151

[a] Values are means ± SEM; $n = 15$ subjects. Control product: CH was consumed in the form of a powder dissolved in 100 mL of water.

The purposes of the study were fully explained, and written informed consent was obtained from each participant. The subjects were recruited and studied in a phase I centre (Therapharm Research, Caen, France). After it was approved by the Independent Ethics Committee (IEC) of Caen, France (agreement 2006-26), the study was carried out in accordance with the Declaration of Helsinki, with local legislation and regulations governing clinical trials and with the requirements of good clinical practice.

Study Protocol. A randomized open cross-over design with two periods was used to compare the effects on plasma AA concentrations of a single administration of a CH-containing FMP with that of a single administration of an equal amount of neat CH (**Figure 1**). Practically, each subject attended an inclusion visit 14 days before administration and two assessment visits of 1 day each during which the subjects received the following products according to a randomization list:

Product 1: One hundred milliliters of FMP containing 10 g of CH. The FMP was drinkable yogurt. The source of collagen was pig bones. CH was generated via enzymatic hydrolysis. The compositions of CH and FMP are given in **Table 2**.

Product 2: Ten grams of CH as a powder dissolved in 100 mL of water (**Table 2**).

One week before CH administration, subjects had to follow dietary recommendations that were designed to reduce the consumption of foods rich in the components under study, for example, gelatin, to limit interference with the compounds being tested. In the last 24 h before product administration, protein intake was also controlled and limited to 0.8 g/kg/day. The wash-out period lasted 5−7 days. The allocation of the sequence of products to the subjects was determined randomly and performed before the beginning of the study. Although this was an open study, the plasma determinations were completed blindly for each administered product.

On the study days, a catheter was inserted into a forearm vein, and blood was sampled at regular intervals, that is, −0.25, 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, and 8 h after product administration. Urine samples (cumulatively, at times 0−3, 3−6, 6−9, 9−15, and 15−24 h postingestion after bladder emptying at $T = 0$ h) were also collected. Plasma and urine samples were kept at −80 °C until analyses.

Analyses. *Plasma and Urine AA Concentrations.* Plasma was deproteinized with 30% (v/w) sulfosalicylic acid solution and centrifuged at 4000*g* for 10 min at 4 °C. AAs were separated and quantified by ion exchange chromatography with ninhydrin derivatization using an AA analyzer (Aminotac, JLC-500/V Jeol, Tokyo, Japan). This

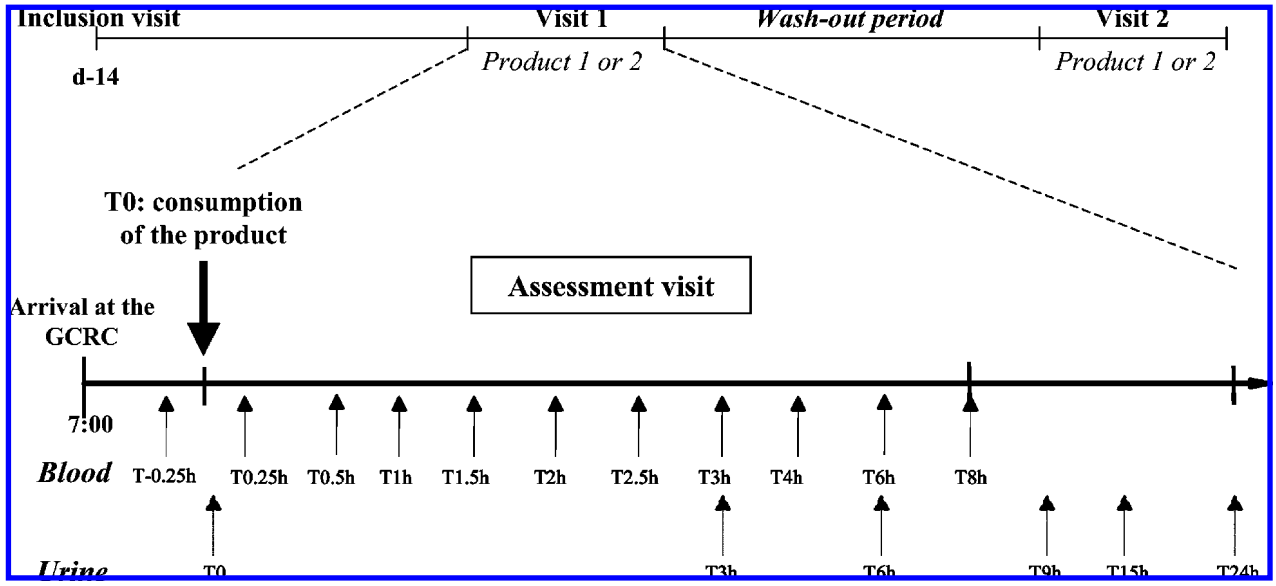

Figure 1. Experimental design of the cross-over study. A randomized open cross-over design with two assessment visits was used. GCRC, General Clinical Research Center.

allowed us to determine a CH index expressed as the sum of the plasmatic concentrations (μmol/L) of four AA (proline, hydroxyproline, glycine, and hydroxylysine) used for which area under the curve (AUC) from $T = 0$ to $T = 8$ h, that is, concentration $\times$ hours, and C_{max} values were calculated. The determination of the sum of essential (leucine, isoleucine, valine, threonine, histidine, methionine, lysine, and phenylalanine), nonessential (glutamate, glycine, alanine, arginine, asparagine, aspartate, citrulline, cystine, glutamine, ornithine, proline, serine, taurine, and tyrosine), and total (essential + nonessential + hydroxyproline + hydroxylysine) AA plasmatic concentrations was performed, and AUC and C_{max} values were calculated. T_{max} was also measured as the time after administration of the products when the maximum plasma concentration was reached. The urine hydoxyproline concentration was measured by using the same procedure and was expressed as μmol/L.

Statistical Analysis. The comparison between the two product groups was performed using a mixed analysis of variance on repeated measures model with product (product 1 vs product 2), period (assessment visit), time ($T = 0$ to $T = 8$ h for each period), and the interaction product $\times$ time as fixed effects and subject as the random effect on the raw data and a mixed analysis of variance model with product, period as fixed effects, and subject as random effects on AUC, C_{max}, and T_{max}. In both analyses, the carry-over (or residual) effect, that is, the influence of the first period on the second period, was checked. In the case of carry-over effect, a second analysis on the first period was performed to assess the product effect. In the case of interaction between product and time, an analysis for each time point was performed. The normality hypothesis was assessed using the analysis of the residual distribution. A $p < 0.05$ was considered significant. The analysis was performed using SAS software version 8.2 for Windows XP, and the model described above was implemented with the mixed procedure from SAS/STAT package.

RESULTS

Plasma and Urine AA Concentrations. *Plasma AAs.* After administration of CH-containing FMP, mean values of C_{max} for the four AAs representative of collagen AA composition (glycine, proline, hydroxyproline, and hydroxylysine) in plasma were significantly greater ($p < 0.04$) than after administration of CH in water (**Figures 2A**,**B** and **3** and **Table 3**). Mean values of AUC of the sum of the four AAs after administration of CH in FMP were not changed in comparison to those reported after the ingestion of the control product. The comparison between the two product groups was also performed on the sum of four AA raw data using a mixed analysis of variance (ANOVA) on a repeated measures model with product, period, time, and carry-over effect as fixed effects and subject as the random effect. No statistically significant product or period effect was observed. However, a statistically significant variation of the sum of the four AA concentrations in plasma with time ($p < 0.0001$) and an interaction between time and product were shown ($p = 0.001$). Hence, an a posteriori analysis of each time point was performed and revealed that the sum of the plasmatic concentrations of these four AA was significantly greater after the administration of CH in FMP than after the control product at $T = 1$ h ($p = 0.009$) and $T = 1.5$ h ($p = 0.005$) postadministration (**Figure 3**). Additional statistical analysis was performed for AUC and C_{max} parameters for each of the four AAs separately using the same previous ANOVA technique. Except for an increase in C_{max} of proline after administration of CH-containing FMP ($p = 0.015$), no significant difference between treatments was demonstrated for AUC of proline or for C_{max} and AUC parameters of glycine, hydroxylysine, and hydroxyproline.

After the fermented milk administration, mean values of C_{max} of the sum of essential AAs were greater than after the control product intake ($p = 0.011$, **Figure 2**). No modification was noticed concerning the AUC of the plasmatic concentration of essential AAs between $T = 0$ and $T = 8$ h. No statistically significant product or period effect was observed. However, a statistically significant variation of the sum of essential AA concentrations in plasma with time ($p < 0.0001$) and interactions between time and product ($p = 0.001$) were reported. An analysis of each time point was performed and revealed that the sum of the essential AA concentration was statistically higher in subjects under CH-containing FMP than in subjects under the control product at $T = 0.5$ h ($p = 0.014$), $T = 1$ h ($p = 0.005$), and $T = 1.5$ h ($p = 0.001$) postdose.

A greater C_{max} value of nonessential AA concentration in plasma was observed after the FMP intake in comparison with the control product ($p = 0.002$, **Figure 2**). The AUC was not changed between the two periods. No statistically significant product or period effect was shown. However, a statistically significant variation of the sum of plasma nonessential AA concentrations with time ($p < 0.0001$) and interaction between time and product ($p = 0.001$) were demonstrated. An analysis of each time point was performed and revealed that the sum of nonessential AAs was statistically greater in subjects under FMP

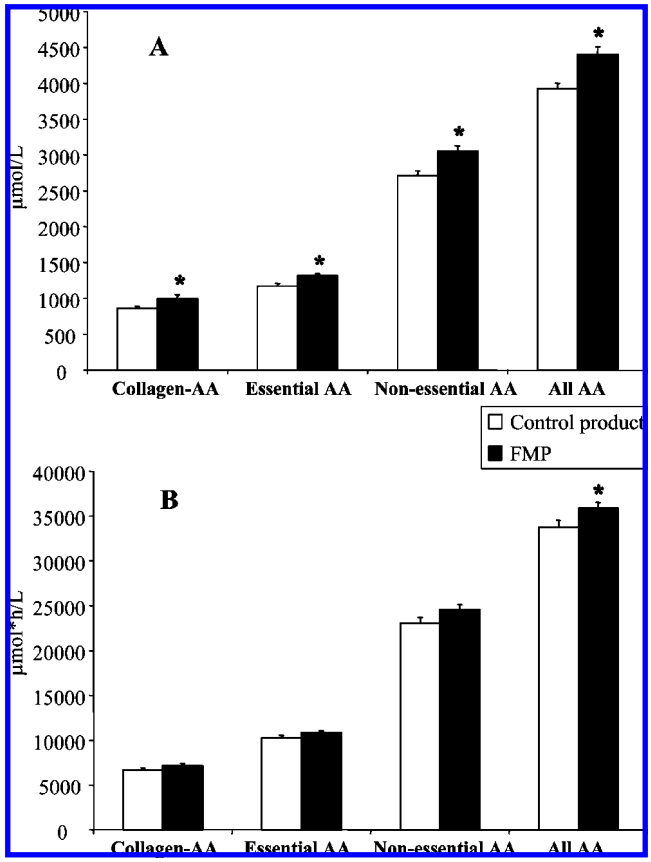

Figure 2. Maximal concentration (**A**) and AUC (**B**) of plasma collagen representative, essential, nonessential, and total AA concentrations after administration of the control product or FMP. Values are means ± SEM; $n = 15$ subjects. Plasma AA concentrations are expressed as AUC (μmol h/L) and maximal concentration (C_{max}, μmol/L). Collagen-AA is the sum of the plasma concentrations of the four AAs representative of the AA compositions of collagen (glycine, proline, hydroxylysine, and hydroxyproline). Essential, nonessential, and all AAs are the sum of plasma concentrations of essential, nonessential, and total AAs, respectively. A mixed analysis of variance on repeated measures model was performed to discriminate among effects of product, period, and time and their interaction. A statistically significant variation with time ($p < 0.0001$) and an interaction between time and product ($p < 0.005$) were observed for the sum of the collagen-representative, essential, and nonessential AA concentrations in plasma. A statistically significant variation of the sum of all AA concentrations with treatment ($p = 0.043$), time ($p < 0.0001$), and interactions between time and product ($p = 0.0005$) and period and product ($p = 0.040$) were reported. *$p < 0.05$ vs the control product.

than in subjects under control product at $T = 0.5$ h ($p = 0.032$), $T = 1$ h ($p = 0.003$), and $T = 1.5$ h ($p = 0.002$) postdose.

When the CH was administered as a FMP, mean values of AUC ($p = 0.053$) and C_{max} ($p = 0.001$) of all AAs were significantly greater than when it was given dissolved in water. A statistically significant variation of the sum of all AA concentrations with treatment ($p = 0.043$), time ($p < 0.0001$), and interactions between time and product ($p < 0.001$) and period and product ($p = 0.040$) were reported. An analysis for each time point was performed and showed that the sum of all AAs was statistically greater in subjects under FMP than in subjects under the control product at $T = 0.5$ h ($p = 0.014$), $T = 1$ h ($p < 0.001$), and $T = 1.5$ h ($p < 0.001$). postadministration.

Urinary AAs. Urinary hydroxyproline concentrations were similar between the two groups of treatment with 104.9 and 100.1 μmol/L after CH-containing FMP and control product administration, respectively.

Table 3. AUC and Maximal Plasma Concentration of the Sum of the Four Collagen-Specific AAs and of Glycine, Proline, Hydroxyproline, and Hydroxylysine Concentrations after Administration of the Control Product or FMP[a]

	control product	FMP	*p* values
	sum		
AUC (mmol h/L)	6689.8 ± 216.9	7150.4 ± 285.8	0.224
C_{max} (mmol/L)	865.6 ± 25.1	1000.2 ± 52.8	0.038
	glycine		
AUC (μU h/mL)	3861.7 ± 175.6	3998.0 ± 175.6	0.612
C_{max} (μU/mL)	511.6 ± 19.8	568.7 ± 30.5	0.145
	proline		
AUC (g h/L)	2406.3 ± 107.9	2686.2 ± 138.6	0.131
C_{max} (g/L)	300.4 ± 11.9	359.8 ± 19.9	0.015
	hydroxyproline		
AUC (g h/L)	395.3 ± 21.2	437.3 ± 32.3	0.138
C_{max} (g/L)	63.7 ± 4.1	76.6 ± 8.0	0.261
	hydroxylysine		
AUC (g h/L)	26.5 ± 1.0	28.9 ± 1.4	0.09
C_{max} (g/L)	5.7 ± 0.3	6.8 ± 0.5	0.207

[a] Values are means ± SEM;, $n = 15$ subjects. Sum, sum of the four collagen-specific AA, that is, glycine + proline + hydroxyproline + hydrolysine. AUC between $T = 0$ and $T = 8$ h; C_{max}, maximal concentration.

DISCUSSION

In recent years, potential improvement of degenerative joint disease after oral administration of CH has received increasing attention (*10*). However, the question as to collagen absorption from the gut, the dietary vector to be used, and its therapeutic mechanism remain essentially unsolved. Hence, before speculating about the mechanism of the therapeutic effectiveness of CH, the question must be clarified as to whether CH can be absorbed from the intestine and furthermore in what form and quantity. Few investigations have looked at the time course of CH absorption as well as its subsequent distribution in various tissues and organs. Oesser et al. (*11*) have determined the bioavailability of CH or its corresponding AA mixture after oral administration in mice. They have shown that more than 90% of the administrated CH was removed from the gut within the first 6 h subsequent to oral administration. This rapid transmucosal transit of collagen correlates well with our results concerning the high digestibility of CH, that is, the time peak of blood AA concentration only at 1−1.5 h after the ingestion. Of note, our study revealed that the sum of the plasmatic concentrations of the four representative AAs (proline, hydroxyproline, glycine, and hydroxylysine) was significantly higher when the CH was administrated as a FMP than as an ingredient dissolved in water at $T = 1$ h ($p = 0.0090$) and $T = 1.5$ h ($p = 0.0051$) postadministration. This result indicates that the profile and the amount of AAs in human blood are different according to the vector used to provide collagen. Nevertheless, it has to be noticed that the consumption of CH as part of the FMP exclusively increased the C_{max} of proline, so that the possibility that the increased C_{max} of proline is due to the ingestion of proline-rich caseins can not be eliminated. However, we measured the composition, that is, AA content, of each product. The proline content of FMP is negligible in comparison with CH (see **Table 2**), showing that increased proline concentration in blood after FMP consumption is only due to the presence of CH in FMP. Taking this into consideration, our data highlight the high digestibility of CH. H. Greiling (University of

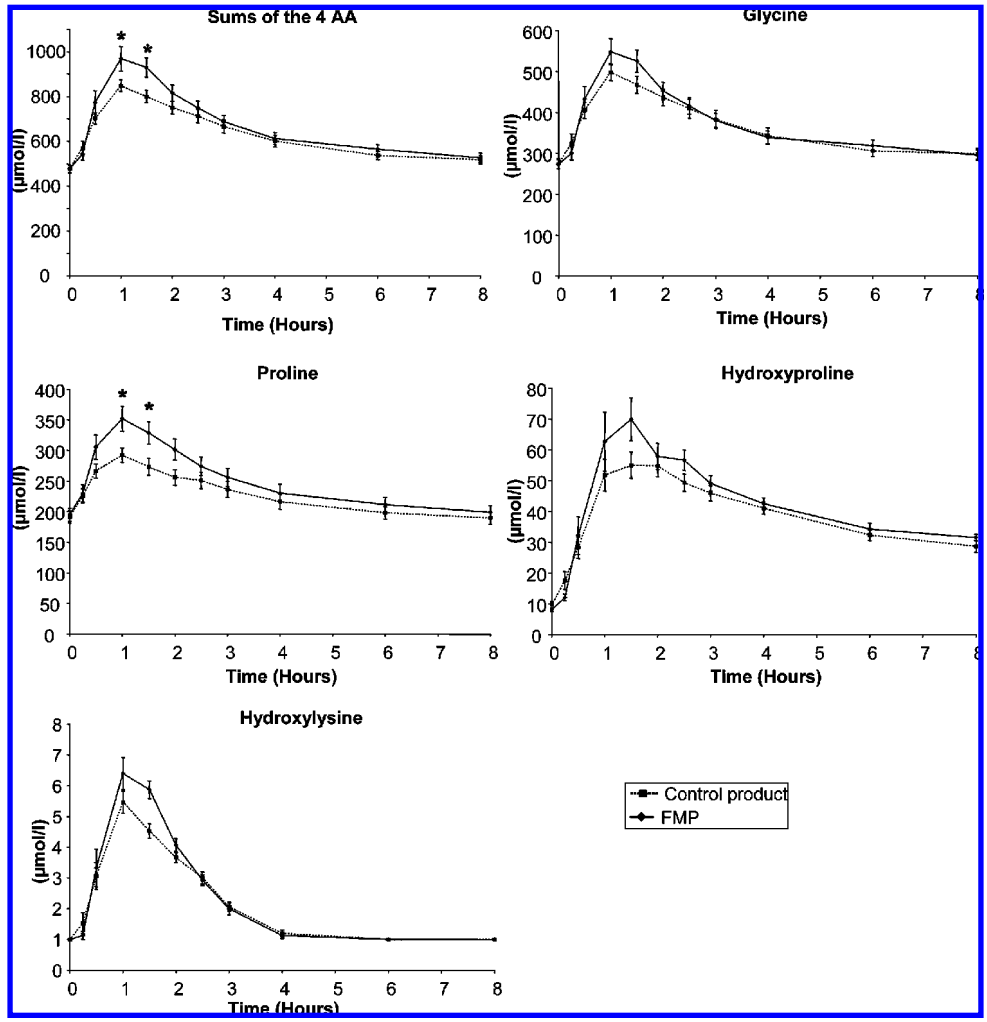

Figure 3. Kinetics of the sum of the four collagen-specific AA and of glycine, proline, hydroxyproline, hydroxylysine after oral administration of the control product, or FMP. Values are means ± SEM; $n = 15$ subjects. Collagen-AA is the sum of the plasma concentrations of the four AAs representative of the AA compositions of collagen (glycine, proline, hydroxylysine, and hydroxyproline). The sum of the plasmatic concentrations of the four AA and of proline was significantly greater after the administration of CH in FMP than after the control product at $T = 1$ h and $T = 1.5$ h postadministration. *$p < 0.05$ vs the control product.

Technology RWTH, Aachen, Germany, personal communication) has determined AA concentrations in serum subsequent to oral administration of CH (10 g/day) in humans. A substantial increase in blood collagen-specific AAs such as hydroxyproline, hydroxylysine, glycine, and proline was observed in this study. The effect of an oral CH substitution (10 g/day) given over a period of 4.5 months was also investigated in a randomized and placebo-controlled study on 60 male students (*12*). In relation to the control group, the data for blood concentrations of glycine, proline, and hydroxyproline in the collagen group showed increased levels in a highly significant way (*12*). Overall, these findings confirm the high digestibility of CH even if it is embedded in a FMP matrix.

In the present study, the concentration and the rate appearance of AAs in plasma were similar or even greater in the FMP group in comparison with ingredients dissolved in water. The intraluminal digestion of proteins and peptides is processed by pancreatic proteolytic enzymes and brush border oligopeptidases, which have a functionally significant role in the terminal stages of protein digestion (*13*). The products of luminal proteolysis are free AAs and small peptides having a chain length of 2−6 AA residues. Results of in vitro experiments have shown that AA absorption is an active, carrier-mediated, and tightly regulated mechanism that is dependent on a gradient of sodium ions across the brush border membrane of the intestinal cells (*14, 15*). Of note, the rate of AA absorption is influenced by the proportion and the composition of the entire meal (*16*). This observation suggests that protease and peptidase as well as AA carrier activities are regulated by the composition of ingested food, for example, the presence of peptides, in particular bioactive peptides (*16*). In the present study, bioactive peptides derived from milk proteins may have been released and activated by enzymatic proteolysis, that is, by the action of proteinases and peptidases from lactic acid bacteria used for fermentation (*17, 18*). In addition, in the human gastrointestinal tract, digestive enzymes will contribute to the further breakdown of long casein-derived oligopeptides, which may also lead to release of bioactive peptides (*19, 20*). Once they are liberated in the body, bioactive peptides can act as regulatory compounds with hormonelike activity on different intestinal and peripheral target sites (*19, 20*). Interestingly, some of these bioactive peptides, for example, opioid peptides, derived from FMP, appear to participate in the control of gastrointestinal functions (*21*). For example, orally given milk-derived opioid peptides are able to modulate absorption processes in the gut (*19*). Thus, we can postulate that the production of bioactive peptides in the FMP may have participated in the greater AAs absorption, that is, appearance of proline in plasma, in the FMP group as

compared to the control group, by enhancing the process of protein digestion and absorption in the small bowel. However, this hypothesis needs further clarification.

Hydroxyproline is an index of collagen catabolism. In our study, urinary hydroxyproline was not modified by the FMP dietary supplementation, indicating that collagen degradation was unaltered, which is not surprising given the very short duration of the supplementation. However, one would have expected a higher urinary hydroxyproline elimination after CH ingestion. Interestingly, previous studies (*22, 23*) have clearly shown that increasing calcium supply can decrease urinary hydroxyproline by 15−35%. Additionally, this effect on urinary hydroxyproline can occur within a few hours (*23*), indicating that urinary hydroxyproline is a sensitive marker of nutritional calcium intake (*22*). Therefore, the presence of calcium in the FMP may explain the lack of increase in urinary hydroxyproline grouping our study.

In conclusion, FMP can be considered as a possible and well-tolerated vector for CH. Our results suggest that CH is resistant to decomposition and modification induced by the fermentation process. The digestion rate of AAs contained in collagen was not modified by the FMP likely because of the physicochemical characteristics and the great homogeneity of the preparation. The value of this functional food product may therefore relate not only on the AA composition of the CH but also on the combined effect with other proteins or peptides, for example, milk proteins and bioactive peptides, of the FMP. As a consequence, it can be regarded as a valuable nutritional component because of its excellent digestibility.

ACKNOWLEDGMENT

We thank Luc Cynober and Nathalie Neveu (Pharmacy School, Paris 5, France) for the analysis of AA pharmacokinetics and Patrick Borel (INRA, Marseille, France) for the analysis of cholesterol levels in plasma.

LITERATURE CITED

(1) Clark, K. L. Nutritional considerations in joint health. *Clin. Sports Med.* **2007**, *26*, 101–118.
(2) Moskowitz, R. W. Role of collagen hydrolysate in bone and joint disease. *Semin. Arthritis Rheum.* **2000**, *30*, 87–99.
(3) Ohara, H.; Matsumoto, H.; Ito, K.; Iwai, K.; Sato, K. Comparison of quantity and structures of hydroxyproline-containing peptides in human blood after oral ingestion of gelatin hydrolysates from different sources. *J. Agric. Food Chem.* **2007**, *55*, 1532–1535.
(4) Gobbetti, M.; Stepaniak, L.; De Angelis, M.; Corsetti, A.; Di Cagno, R. Latent bioactive peptides in milk proteins: Proteolytic activation and significance in dairy processing. *Crit. Rev. Food Sci. Nutr.* **2002**, *42*, 223–239.
(5) Korhonen, H.; Pihlanto, A. Technological options for the production of health-promoting proteins and peptides derived from milk and colostrum. *Curr. Pharm. Des.* **2007**, *13*, 829–843.
(6) Iwai, K.; Hasegawa, T.; Taguchi, Y.; Morimatsu, F.; Sato, K.; Nakamura, Y.; Higashi, A.; Kido, Y.; Nakabo, Y.; Ohtsuki, K. Identification of food-derived collagen peptides in human blood after oral ingestion of gelatin hydrolysates. *J. Agric. Food Chem.* **2005**, *53*, 6531–6536.
(7) Rein, M. S.; Barbieri, R. L.; Welch, W.; Gleason, R. E.; Caulfield, J. P.; Friedman, A. J. The concentrations of collagen-associated amino acids are higher in GnRH agonist-treated uterine myomas. *Obstet. Gynecol.* **1993**, *82*, 901–905.
(8) Bhattacharjee, A.; Bansal, M. Collagen structure: the Madras triple helix and the current scenario. *IUBMB Life* **2005**, *57*, 161–172.
(9) Cynober, L. A. Plasma amino acid levels with a note on membrane transport: Characteristics, regulation, and metabolic significance. *Nutrition* **2002**, *18*, 761–766.
(10) Bello, A. E.; Oesser, S. Collagen hydrolysate for the treatment of osteoarthritis and other joint disorders: A review of the literature. *Curr. Med. Res. Opin.* **2006**, *22*, 2221–2232.
(11) Oesser, S.; Adam, M.; Babel, W.; Seifert, J. Oral administration of (14)C labeled gelatin hydrolysate leads to an accumulation of radioactivity in cartilage of mice (C57/BL). *J. Nutr.* **1999**, *129*, 1891–1895.
(12) Beuker, F.; Stehle, P.; Ritsh, M. Hydroxyproline concentration during collagen hydrolysate supplementation to humans. *Int. J. Sport Med.* **1993**, *1*, 88.
(13) Silk, D. B.; Grimble, G. K.; Rees, R. G. Protein digestion and amino acid and peptide absorption. *Proc. Nutr. Soc.* **1985**, *44*, 63–72.
(14) Matthews, D. M. Intestinal absorption of amino acids and peptides. *Proc. Nutr. Soc.* **1972**, *31*, 171–177.
(15) Walrand, S.; Cynober, L. Acides aminés et trophicité intestinale. *Hepato-Gastroenterology* **2001**, *8*, 53–66.
(16) Buraczewska, L., III. Absorption of constituents of protein hydrolysates. *Acta Physiol. Pol.* **1981**, *32*, 569–584.
(17) Laffineur, E.; Genetet, N.; Leonil, J. Immunomodulatory activity of beta-casein permeate medium fermented by lactic acid bacteria. *J. Dairy Sci.* **1996**, *79*, 2112–2120.
(18) LeBlanc, J. G.; Matar, C.; Valdez, J. C.; LeBlanc, J.; Perdigon, G. Immunomodulating effects of peptidic fractions issued from milk fermented with *Lactobacillus helveticus*. *J. Dairy Sci.* **2002**, *85*, 2733–2742.
(19) Meisel, H.; Bockelmann, W. Bioactive peptides encrypted in milk proteins: Proteolytic activation and thropho-functional properties. *Antonie Van Leeuwenhoek* **1999**, *76*, 207–215.
(20) Shimizu, M. Food-derived peptides and intestinal functions. *Biofactors* **2004**, *21*, 43–47.
(21) Teschemacher, H.; Koch, G.; Brantl, V. Milk protein-derived opioid receptor ligands. *Biopolymers* **1997**, *43*, 99–117.
(22) Fardellone, P.; Brazier, M.; Kamel, S.; Gueris, J.; Graulet, A. M.; Lienard, J.; Sebert, J. L. Biochemical effects of calcium supplementation in postmenopausal women: influence of dietary calcium intake. *Am. J. Clin. Nutr.* **1998**, *67*, 1273–1278.
(23) Horowitz, M.; Need, A. G.; Morris, H. A.; Wishart, J.; Nordin, B. E. Biochemical effects of calcium supplementation in postmenopausal osteoporosis. *Eur. J. Clin. Nutr.* **1988**, *42*, 775–778.

Received for review March 6, 2008. Revised manuscript received July 11, 2008. Accepted July 18, 2008. This study was sponsored by DANONE Research Center.

JF800691F