Influence of the Protein Digestion Rate on Protein Turnover in Young and Elderly Subjects[1,2]

Martial Dangin,*† Yves Boirie,*[3] Christelle Guillet* and Bernard Beaufrère*

*Unité du Métabolisme Protéino-Energétique, Université d'Auvergne/Institut National de la Recherche Agronomique, Centre de Recherche en Nutrition Humaine, Clermont-Ferrand, France and †Nestec Ltd., Nestlé Research Center, Lausanne, Switzerland

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ABSTRACT It has long been recognized that numerous dietary parameters, such as the amount and type of protein and nonprotein energy sources, affect protein metabolism. More recently, we demonstrated that the protein digestion rate is an independent factor regulating postprandial protein gain. Indeed, in young men, using a non-steady-state approach and intrinsically labeled milk protein fractions [whey protein (WP) and casein (CAS)] we showed that a slow digested dietary protein (CAS) induced a greater protein gain than a fast one (WP). The mechanisms of this gain also differed according to the protein rate of digestion. WP stimulated amino acid oxidation and protein synthesis without modifying proteolysis, whereas CAS increased amino acid oxidation and protein synthesis to a lesser extent and strongly inhibited proteolysis. These results led to the concept of "slow" and "fast" protein and were confirmed by further experiments during which the meals tested presented different digestion rates but were otherwise identical in terms of amino acid profile. We also analyzed the effects of fat and carbohydrates added to CAS and WP. Our preliminary results suggest that added nonprotein energy sources to CAS and WP attenuated the differences in both the protein digestion rate and protein gain. Finally, and in contrast to young subjects, a "fast" protein may be more beneficial than a "slow" one in elderly subjects, to limit body protein loss. However, long-term studies are needed to confirm this age-related effect. J. Nutr. 132: 3228S–3233S, 2002.

KEY WORDS: • *protein turnover* • *dietary protein* • *digestion* • *non-steady state* • *milk proteins*

Preservation of protein homeostasis by an appropriate dietary strategy is crucial to sustain growth, maintenance and recovery and/or to limit losses of body protein mass after stress. To achieve these goals, it is necessary to characterize nutritional factors modulating protein metabolism.

Regulation of protein homeostasis during feeding results from a complex interplay among numerous factors, including protein intake, nonprotein energy sources [i.e., fat and carbohydrate content (1)] and the physiological or pathological state of the subjects (2,3). When considering protein intake, it has long been recognized that the quantity and quality of protein, i.e., its digestibility and its composition in indispensable and conditionally indispensable amino acids (AA), can modulate protein metabolism (4).

More recently, we have identified that protein digestion rate is a regulating factor of postprandial protein gain. As will be discussed further, this factor might offer an adjunctive dietary strategy to prevent protein losses in various pathophysiological situations. In this review, after a brief summary of our current knowledge on postprandial modifications of whole-body kinetics, we report the studies that led to the concept of "slow" and "fast" protein. We also describe the evolution of the concept in more physiological situations and its potential applications.

Protein metabolism in response to continuous feeding

Feeding induces a net protein accretion that compensates for protein losses occurring during the postabsorptive phase (5). Conceptually, net protein gain may result from inhibition of proteolysis, stimulation of protein synthesis or any coordinated changes of these two parameters which lead to a rate of protein synthesis higher than the rate of proteolysis. There is a general agreement that feeding stimulates AA oxidation according to the plasma AA level reached. Feeding also induces a 30–50% decrease in endogenous leucine rate of ap-

[1] Presented as part of a symposium, "Protein Metabolism in Response to Ingestion Pattern and Composition of Proteins," given at Nutrition Week 2002, San Diego, CA, February 27, 2002. This program was sponsored by the American College of Nutrition and was supported by grants from the National Dairy Council. Guest editors for this symposium were Robert R. Wolfe, University of Texas Medical Branch and Shriners Burn Hospital, Galveston, TX, and Sharon L. Miller, Director of Nutrition Research, National Dairy Council, Rosemont, IL.

[2] Supported by Nestlé Research Center, Institut National de la Recherche Agronomique, Région Auvergne and the French Ministry of Research.

[3] To whom correspondence should be addressed.
E-mail: boirie@clermont.inra.fr.

[4] Abbreviations used: AA, amino acid; CAA, free AA mimicking the composition of casein; CAS, casein; E-CAS, casein + energy; Leu R_a, leucine rate of appearance; Leu R_d, leucine rate of disappearance; NOLD, nonoxidative leucine disposal; WP, whey protein; E-WP, whey protein + energy.

0022-3166/02 $3.00 © 2002 American Society for Nutritional Sciences.

pearance (Leu R_a), i.e., proteolysis, which is mostly attributed to glucose-induced hyperinsulinemia (6). Conversely, inconsistent results have been obtained on whole-body protein synthesis. Indeed, protein synthesis was stimulated in some studies (7–9) or did not change in others (7,10,11). These apparent discrepancies may partly be explained by differences in AA availability, as assessed by plasma AA concentration. For instance, gradual increases in AA concentration by variable intravenous infusion of AA induce a dose-dependent stimulation of protein synthesis (12). A similar interpretation could be made from results obtained with continuous feeding using various amounts of dietary protein or AA. Protein intake $>1.5\ g \cdot kg^{-1} \cdot d^{-1}$, which induces strong hyperaminoacidemia, generally stimulates protein synthesis (7–9), whereas such a stimulation was not observed with lower protein intake (7,10,11). The AA levels may act not only as substrates for protein synthesis but also as signals to modulate gene expression (13). Together, these data suggest that both AA availability and insulin action are important determinants of the regulation of protein metabolism.

Measurement of postprandial protein metabolism: steady-state vs. non-steady-state approach

Most of our knowledge on whole-body protein gain and its mechanisms during the postprandial period is based on the isotopic dilution of a labeled AA (usually leucine) in the precursor pool of protein synthesis, i.e., free AA pool (14). In addition, for the sake of simplicity, most of these studies have been performed at steady state, i.e., when pool size (concentration and volume of distribution) of the tracer and the tracee are constant. To reach this steady state during feeding, nutrients are administered continuously through nasogastric tubes (8,15,16) or intravenously (12,17), or repeated small meals are given at regular intervals (2,7,18).

However, the relevance of steady state measurements for the physiological feeding is questionable. Indeed, feeding in normal man is intermittent and generally consists of two or three meals per day, which is a non-steady-state situation. Moreover, the modifications of the two major factors affecting protein metabolism, i.e., AA and insulin, are quite different after a single meal than during a continuous feeding. Typically, after a single meal there is an acute but transient elevation in plasma AA concentration whereas, with the same amount of protein, continuous feeding results in a smaller but more prolonged increase of aminoacidemia (19). Similar observations are done for plasma insulin. During a single meal hyperinsulinemia is higher and more transient than during prolonged feeding. As aforementioned, it seems logical that the differences in the magnitude of variations of aminoacidemia and insulinemia may affect protein metabolism.

Consequently, the reasons that led us to study the effect of a single meal on protein metabolism were that *1*) compared with continuous feeding, it is more representative of human nutritional behavior, 2) modifications of plasma AA and insulin following a single meal ingestion are different from those observed at steady state and 3) these differential changes may affect protein metabolism parameters.

Non-steady-state approach of protein metabolism after ingestion of a single meal

To study protein metabolism after ingestion of one single meal, a new methodological approach was used. Non-steady-state equations initially developed by Steele (20) and widely applied to study glucose, ketone and glycerol metabolisms were adapted to assess leucine kinetics (21,22). Briefly, total leucine rate of disappearance (Leu R_d) and Leu R_a from the free AA pool are calculated from the changes in tracer and tracee concentrations in this compartment during a continuous constant intravenous infusion of labeled leucine (23).

Total Leu R_a is the sum of the entry rate of leucine derived from whole-body proteolysis (endogenous Leu R_a) and of the exogenous Leu R_a. To characterize endogenous Leu R_a, exogenous Leu R_a is calculated by using the specific equations developed by Proietto et al. (24), and it is subtracted to total Leu R_a.

Total Leu R_d corresponds to the sum of leucine either oxidized or used for protein synthesis [nonoxidative leucine disposal (NOLD)]. When L-[1-^{13}C]-leucine is chosen as the intravenous tracer, leucine oxidation in non-steady state can be assessed by determining simultaneously and serially the [^{13}C] O_2 rate of production and ketoisocaproate enrichments (immediate precursor of leucine decarboxylation) as previously described (23). To evaluate NOLD, leucine oxidation is subtracted to total Leu R_d.

The choice of an appropriate oral tracer is a crucial issue. Basically, the tracer should represent the metabolic fate of the tracee. In steady-state conditions, it has been shown that free L-[1-^{13}C]-leucine added to CAS induced similar enrichments and fluxes to those observed with intrinsically L-[1-^{13}C]-leucine-labeled CAS (18). By contrast, in non-steady state, i.e., after a single meal ingestion, the time course of appearance in plasma of a free tracer is different from that of the same tracer bound to protein, as discussed below (23). Indeed, protein and free AA behave differently at several digestive steps such as gastric emptying rate, dietary protein hydrolysis and absorption process. Therefore, in our experimental conditions only intrinsically labeled proteins were adequate as oral tracers.

Production of intrinsically labeled milk proteins and their use for the study of human protein metabolism

Labeled bovine milk proteins were produced by infusing lactating cows with a large amount of L-[1-^{13}C]-leucine for 24–32 h (25). Milk was collected during and after the tracer infusion period. The two major bovine milk protein fractions—casein (CAS) and whey proteins (WP)—were isolated and purified by microfiltration and ultrafiltration membrane techniques, respectively (25). More than 3 kg of native labeled proteins were obtained in one experiment with sufficient enrichment (two thirds of these proteins had enrichments ranging from 10 to 19 mol percent excess) to be used in human protein metabolism studies. The yield of recovery of the tracer infused in these proteins was between 22 and 27%, making this labeling method expensive. However, these proteins are highly digestible and provide a well balanced AA composition, and they are widely consumed in human nutrition (4).

To verify whether free tracers added to unlabeled proteins reflect the metabolic fate of the protein-bound AA, young men ingested intrinsically labeled WP, CAS or free labeled leucine added to an identical amount of unlabeled WP or CAS. The data obtained demonstrated that the plasma leucine enrichment profile was dependent upon the molecular form of the tracer (**Fig. 1**A). With WP and the free tracer, the peak of enrichment of the oral tracer was 50% higher and occurred 40 min earlier than with labeled leucine incorporated into dietary protein (23). With CAS, those differences were much more marked (Fig. 1A). Indeed, with free [5,5,5-$^{2}H_3$]-leucine added to CAS, the increase in plasma enrichment was fast, high and transient, whereas the enrichment of the tracer

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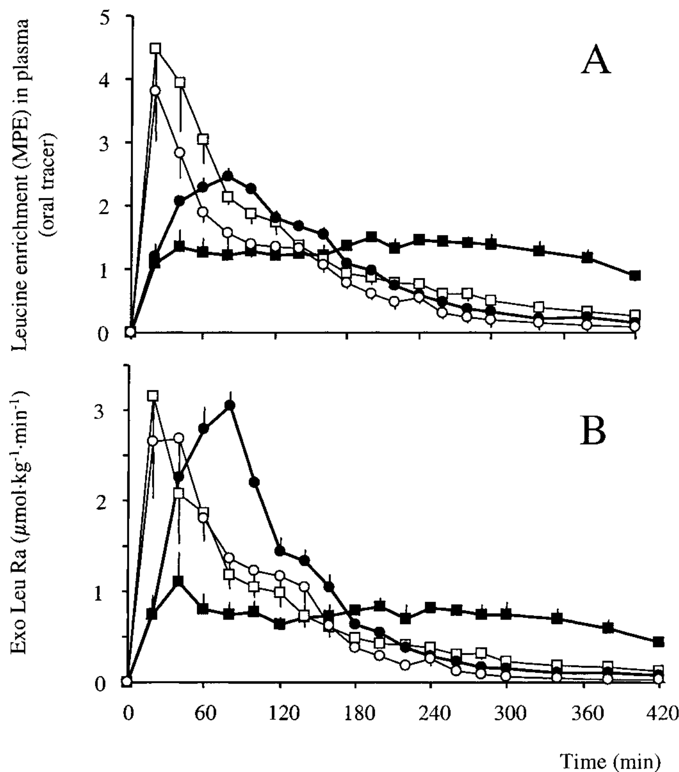

FIGURE 1 Time-dependent evolution of plasma enrichments of the orally administered tracer (*A*) and exogenous dietary leucine rate of appearance (Leu R_a) (Exo Leu R_a; *B*) after ingestion of intrinsically [1-^{13}C]-leucine-labeled casein (CAS) (■) and, whey protein (WP) (●) or unlabeled CAS (□) and WP (○) added with free [5,5,5-$^{2}H_3$]-leucine. The enrichments in the meals are similar (~3.4 mol percent excess). Results are expressed as mean ± SEM.

bound to CAS was slower, lower and more persistent. Furthermore, regardless of the protein considered, with the free tracer, enrichment of oral labeled leucine in plasma was even higher than in the meal. Thus, the free tracer does not reflect the metabolic fate of AA bound to the dietary protein. Because plasma enrichment of the oral tracer was used to calculate the exogenous (Fig. 1*B*) and endogenous Leu R_a, free oral tracer led to aberrant results on those parameters (23). This error was particularly striking with whole-body proteolysis, for which values were close to zero or even negative in some subjects, when using the free oral tracer for calculations.

Those data demonstrated not only that intrinsically labeled protein is essential to study the effect of a single meal on whole-body protein metabolism but also that the Leu R_a issued from labeled CAS or WP were quite different (Fig. 1*B*). After WP ingestion, dietary leucine appeared rapidly and transiently in the peripheral blood, whereas after CAS its Leu R_a was lower and more prolonged. These results were in accordance with previous studies that showed WP and CAS to be rapidly and slowly digested, respectively (26,27). These differences are probably related to the physicochemical behavior of WP and CAS in the digestive tract (26,27). Indeed, WP, which remains soluble in the stomach, is emptied rapidly. By contrast, the gastric acidic environment converts the initial CAS liquid meal into a solid clot, which is released slowly from the stomach. The delay of CAS emptying might also be explained by a release of opioid peptides that were reported to slow down gastrointestinal motility (28).

The slow and fast protein concept

Those differences in protein digestion rate between CAS and WP induce variable postprandial plasma AA levels, which could influence postprandial protein gain. To test this hypothesis, we first compared the effects of a liquid protein meal containing either CAS or WP on plasma AA on insulin concentration, protein metabolism parameters and postprandial leucine balance (29). For the sake of simplicity, the meals were composed exclusively of CAS or WP dispersed in water. Because of the differences in AA composition of CAS and WP, it was not possible to obtain a CAS meal that contained both the same leucine and nitrogen contents as the WP meal. Therefore, we chose to compare CAS and WP when identical amounts of leucine were provided, nitrogen content being higher with CAS than with WP (**Table 1**). No other energy source was added to the meals.

As previously shown (Fig. 1*B*), the R_a of leucine derived from WP was faster, higher and more transient than that of CAS. The different rates of digestion affected plasma AA levels but not insulin concentration (29). Indeed, the elevation in aminoacidemia was high and of short duration with WP, whereas the increment was lower but more prolonged with CAS, despite the higher nitrogen intake with CAS. With WP, NOLD, an index of protein synthesis, was stimulated by 68% and proteolysis was unaffected. The effects of CAS were

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TABLE 1

Characteristics of the meals ingested and postprandial leucine balance[1]

Meal	Protein digestion rate	Protein intake, *g/kg BW*	Leucine intake, *µmol/kg BW*	Leucine balance, *µmol/kg BW*
CAS[2]	Slow	0.61 ± 0.01	379 ± 1	141 ± 39[3]
WP[2]	Fast	0.45 ± 0.02	359 ± 18	11 ± 15
CAS[4]	Slow	0.44 ± 0.01	289 ± 8	38 ± 13[3]
CAA[4]	Fast	0.45 ± 0.02	289 ± 15	−12 ± 11
RP-WP[4]	Slow	0.46 ± 0.02	393 ± 15	87 ± 25[3]
WP[4]	Fast	0.45 ± 0.02	360 ± 18	6 ± 19

[1] Meals were composed of CAS, CAA or WP given as a single meal (WP) or as repeated small meals (RP-WP). BW, Body weight. Results are expressed as mean ± SEM.
[2] Derived from Ref. 29.
[3] Slow digested meal significantly higher ($P < 0.05$; *t* test) than its faster counterpart.
[4] Derived from Ref. 30.

quite different: NOLD was slightly increased and proteolysis was inhibited by ~30% for a long period of time (29). Most importantly, the total amount of leucine oxidized over the 7 h that followed meal ingestion was higher with WP than with CAS, despite identical leucine intake. Because postprandial leucine balance was calculated as the difference between leucine intake and leucine oxidized, WP resulted in a significantly lower balance than CAS (Table 1).

These results strongly suggested that the digestion rate of dietary protein affects protein metabolism parameters and postprandial protein gain. In young subjects, slowly digested protein without nonprotein energy source was more efficient than a rapidly digested protein to improve postprandial protein gain.

Validation of slow and fast protein concept

Although clear-cut differences in postprandial protein gain between CAS and WP were observed, validation of these initial data were required to address two criticisms. In our initial work (29), leucine content was controlled but nitrogen intake, i.e., the amount of essential and nonessential AA, was higher with CAS than with WP. Additionally, the specific AA profile of the two proteins might have affected protein metabolism directly.

Therefore, another study was performed to compare postprandial leucine kinetics and mainly leucine balance after ingestion of different protein meals of identical AA profile and nitrogen content (Table 1) but of different digestion rate (30). For that purpose, two pairs of studies were performed in healthy young men. In the first pair of studies, modifications induced by ingestion of CAS, a slowly digested meal, were compared with those induced by a mixture of free AA mimicking the composition of CAS (CAA), a nitrogen source previously described as rapidly digested (31). The second pair of studies compared the effects of a single WP meal, a rapidly digested protein, with a sequence of small meals made of WP given every 20 min during 240 min (RP-WP), taken as a paradigm of a slow digested protein.

As expected, CAA and WP were rapidly digested and induced strong, fast and transient hyperaminoacidemia (30). By contrast, CAS and RP-WP were slowly digested and resulted in slower but more prolonged increases in plasma AA levels. The results concerning endogenous Leu R_a and NOLD were in agreement with those observed in our former work (29). After ingestion of a "fast meal" endogenous Leu R_a was inhibited moderately and transiently, whereas with a "slow meal" the inhibition was persistent and more marked (30). With the "fast meal" NOLD was strongly stimulated, whereas it was nearly absent with the "slow meal." In addition and as previously observed, postprandial leucine balance was lower with the "fast" than with the "slow meal" (Table 1).

Collectively our studies support the general idea that the magnitude and the duration of changes in AA availability determine the anabolic effects of the protein digestion rate. Indeed, results concerning AA oxidation and protein synthesis are in agreement with a dose-dependent stimulation during gradual increases in AA availability (12). The persistent inhibition of proteolysis induced by the "slow proteins" is unlikely due to insulin, because insulinemia was either not different (29) or higher with the "fast proteins" (30). It is more tempting to attribute this effect to duration of the postprandial hyperaminoacidemia, because AA have been shown to inhibit proteolysis (12) and the hyperaminoacidemia is much more prolonged with "slow " than with "fast" proteins.

In summary, the protein digestion rate is unequivocally an independent regulating factor of postprandial protein retention (30). Slowly digested protein, by inhibiting proteolysis, induces a higher postprandial protein gain than rapidly digested protein, which stimulates protein synthesis but also oxidation (29,30). However, extrapolations of these findings to human nutrition require verification of the sustainment of such effects in more practical feeding situations.

Digestion rate and postprandial utilization of CAS and WP within mixed meals

Dietary carbohydrates and lipids have been reported to affect gastric emptying (32) and to modulate protein metabolism by postprandial changes in substrates and/or in hormonal concentrations (33). Therefore, the absorption rate of dietary proteins and their impact on postprandial protein metabolism will likely be affected by these nonprotein energy sources.

To verify this hypothesis, two test meals, differing only in their protein composition (E-CAS containing CAS + energy vs E-WP containing WP + energy) and providing an identical amount of leucine (296 μmol/kg body weight), were ingested in two separate occasions by healthy young men (34). As previously discussed (29), the amount of protein ingested was necessarily lower with E-WP than with E-CAS. Each test meal was otherwise composed of identical amounts of carbohydrates and fat (0.75 and 0.13 g/kg body weight, respectively).

Our preliminary results suggest that, in complete meals, *i*) E-WP was still more rapidly absorbed than E-CAS and *ii*) postprandial leucine balance was still lower with the meal containing WP than with meal composed of CAS, although the differences in digestion rate and in leucine balance were less marked than when the proteins were given alone (30). However, a more conclusive overview of the effects of CAS and WP in mixed meals will require adjustment of differences in nitrogen content between CAS and WP meals and confirmation of these preliminary results in young men ingesting an identical amount of protein.

Perspectives: elderly subjects as a target population

Duration and magnitude of elevated AA are key factors responsible for the effects of slow and fast proteins. Therefore, insight into potential applications in human nutrition of slow and fast proteins can be theoretically extrapolated from studies that have manipulated AA availability. In this respect, body protein loss occurring in the elderly population may be prevented by the modulation of AA availability.

Recently, it has been demonstrated in elderly women that protein feeding pattern modulated the enhanced protein retention because of an increase of daily protein intake from 0.75 to 1.05 g protein · kg^{-1} · day^{-1} (35). Indeed, when 80% of the daily protein intake was consumed at lunch (a "pulse" pattern of feeding), the increase of nitrogen balance was higher than when the same intake was evenly distributed over four meals (a "spread" pattern). The better efficacy of the pulse pattern was attributed to the improvement of the responsiveness of protein synthesis to feeding via an increased disposal of AA to muscle. It was also possibly due to a better protein-sparing effect in the postabsorptive state. In other words, the fact that the evening meal of the pulse pattern contained little protein was probably responsible for the lower postabsorptive losses during the night after this meal. Therefore, both the high-protein lunch and the low-protein dinner contributed to the global protein-sparing effect (35). On the surface, these

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results may appear to be inconsistent with the data obtained with the slow/fast proteins, because one might equate a "fast" protein with a pulse condition. However, the same group then reported that the positive effect of the pulse pattern did not exist in young women (36). In fact, in this age group, there was a strong trend ($P = 0.16$) for a better (>50%) nitrogen balance with the "spread" pattern than with the "pulse" one. These results strongly suggest that the effect of the pattern of feeding on protein metabolism depends on age, with opposite effects in young and elderly subjects. In keeping with this hypothesis, we very recently completed short-term studies with slow/fast proteins in elderly subjects. Our preliminary results confirm that, contrary to what we had observed in young people, a fast protein (i.e., WP) induced a better post-prandial leucine balance than a slow one (37). Thus, the effects of the modifications of kinetics of delivery of dietary AA, whether they are achieved by modifying the pattern of intake or by using "slow or fast" proteins, are clearly age dependent. These series of studies also strongly suggest that, in healthy elderly people, short-term optimization of protein retention could be achieved by changing the pattern of protein feeding and/or the rate of absorption of the proteins. Further studies are needed to confirm this possibility over longer periods and in frail subjects.

For other populations, the relevance of slow or fast protein depends on alterations of protein metabolism that have to be prevented or corrected. Applications in different pathophysiological conditions are only speculative and require further investigation. However, as highlighted elsewhere (38), the slow and fast protein concept may represent an adjunctive dietary strategy to optimize protein deposition in preterm infants, patients with wasting disorders (e.g., protein-energy malnutrition, critically ill patients) or subjects recovering from metabolic stress. It may also be applied to pathologies, such as renal diseases or hepatic encephalopathy, in which excessive AA concentrations have to be avoided while preserving the anabolic action of dietary proteins.

Conclusion

Dietary factors, such as quantity, digestibility and AA profile of proteins, regulate postprandial protein metabolism. In addition, there is growing body of evidence that the protein digestion rate is an independent regulating factor of postprandial protein gain. Controlling protein digestion kinetics could represent an adjunctive strategy to optimize postprandial protein gain in situations in which protein wasting has to be prevented. Further studies should emphasize the clinical applications of this concept under more prolonged periods of time and with various methodological approaches.

ACKNOWLEDGMENTS

We thank P. Gachon, P. Rousset, C. Giraudet, M. Brandolini and L. Morin for their technical and nursing assistance, S. Corny for tracer preparations, J. Fauquant and J. L. Maubois for milk proteins separation, C. Garcia-Rodenas, K. Reiffers-Magnani and O. Ballèvre for their invaluable assistance, and C. Bouteloup-Demange for medical examination of the volunteers.

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