

Rate of [^{15}N]leucine incorporation and determination of nitrogenous fractions from gastro-jejunal secretion in fasting humans

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Summary — The aim of this study was to quantify the nitrogen fraction flow rates in gastro-jejunal secretions in fasting humans and to determine the [^{15}N] leucine incorporation into the secreted proteins. A double lumen intestinal perfusion method was used in 5 healthy volunteers. Plasma and gastro-jejunal juices were collected during a 15-h intravenous [^{15}N] leucine infusion. Total, soluble and insoluble nitrogen, amino acids and [^{15}N] leucine enrichment were measured. The total nitrogen flow rate was $7.2 \pm 1.9 \text{ mmol}\cdot\text{h}^{-1}$ and 58% was ethanol soluble. The amino-acid composition remained constant and glycine was the most abundant. The plasma [^{15}N] leucine enrichment at the isotopic plateau was $4.8 \pm 0.9 \text{ mol}\%$ excess. The [^{15}N] leucine enrichment in the intestinal chyme increased asymptotically to reach a plateau after 5 h. The [^{15}N] leucine enrichment at the plateau and the fractional synthesis rate of secreted proteins were $1.6 \pm 0.5 \text{ mol}\%$ excess and $21.5 \pm 3.3\%\cdot\text{h}^{-1}$, respectively. These results show that the composition of the basal gastro-jejunal secretion is very stable. A part of this secretion is composed of proteins with rapid synthesis rates.

human / jejunum / endogenous nitrogen / [^{15}N] leucine / protein turnover

Résumé — Taux d'incorporation de leucine dans les protéines sécrétées et quantification des flux azotés des sécrétions jéjunales chez l'homme à jeun. Cette étude a pour but de quantifier les flux azotés des sécrétions jéjunales chez l'homme à jeun et de mesurer le taux d'incorporation de [^{15}N] leucine dans les protéines sécrétées. Cinq volontaires sains ont été intubés avec une sonde jéjunale biluminale permettant la perfusion d'un marqueur non absorbable. Le plasma et les sécrétions gastro-jéjunales ont été collectés pendant les 15 h de perfusion intraveineuse de [^{15}N] leucine. L'azote total, soluble et insoluble, les acides aminés et l'enrichissement en [^{15}N] leucine ont été mesurés. Le débit d'azote ($7,2 \pm 1,9 \text{ mmol}\cdot\text{h}^{-1}$) était constant et 58% était sous forme soluble dans l'éthanol. Les proportions des acides aminés étaient constantes et la glycine était largement majoritaire. L'enrichissement

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plasmatique en [^{15}N] leucine au plateau isotopique était de 4,8 mol % excess. La cinétique d'enrichissement dans les sécrétions digestives présentait un profil asymptotique atteignant un plateau au bout de 5 h. L'enrichissement en [^{15}N] leucine au plateau était de $1,6 \pm 0,5$ mol % excess et le taux de synthèse protéique était de $21,5 \pm 3,3$ % $\cdot\text{h}^{-1}$. Ces résultats montrent d'une part la stabilité de la composition des sécrétions digestives et des flux azotés en conditions basales. D'autre part, ils indiquent que les sécrétions jéjunales sont composées de protéines à vitesse de renouvellement très rapide.

homme / jéjunum / azote endogène / [^{15}N] leucine / taux de renouvellement protéique

INTRODUCTION

The gut is known to play an important role in amino-acid and protein metabolism. Protein digestion and absorption are the first steps in the assimilation of protein components into the amino-acid body pool. The mechanisms of intestinal nitrogen digestion, absorption and recycling have been widely described, but many aspects remain unknown particularly in man (Young, 1991). One of the main problems encountered in protein digestion studies is the contribution of endogenous protein secretion and recycling. Indeed, endogenous proteins that originate from saliva, gastric and pancreatic juices and intestinal mucosa are secreted into the intestinal lumen and mixed with the ingested exogenous nitrogen. This secretion of endogenous proteins in the human gut was estimated to be 10–60 g $\cdot\text{d}^{-1}$ with high individual variation (Florent and Bernier, 1984; Alpers, 1987; Mahé *et al*, 1992). Moreover, it has been shown in animals that the amount and the nature of protein in the diet influences the level of intestinal secretion including gastric, pancreatic and biliary secretions (Coring *et al*, 1989; Fushiki *et al*, 1989).

Isotope tracer techniques are of interest in the study of nitrogen fluxes and protein metabolism. Radioactive tracers have been used in pigs (Simon *et al*, 1983) and rats (Costa de Oliveira and Sgarbieri, 1986) in order to measure the intestinal secretion of endogenous nitrogen, and in humans after acute pancreatitis (Ogden *et al*, 1993) in order to evaluate changes in the synthesis

of pancreatic enzymes. For ethical reasons, stable isotopes are more appropriate to the study of human metabolism (Wolfe, 1992). However, the labelling of the intestinal endogenous protein after intravenous [^{15}N]amino-acid infusion has only been performed in pigs (Souffrant *et al*, 1986; De Lange *et al*, 1990; Krawielitzki *et al*, 1990; Huisman *et al*, 1992; Souffrant *et al*, 1993). Neither the rate of incorporation of a stable isotope-labelled amino acid into digestive secretions in humans nor the time required to achieve the isotopic plateau are known. The aims of the present study were, first, to characterize biochemically nitrogen fluxes in the gastro-jejunal lumen of fasting humans and, second, to measure the rate of [^{15}N]leucine incorporation into these secreted proteins with constant tracer infusion of labelled leucine.

MATERIALS AND METHODS

Subjects

Five healthy volunteers (1 female and 4 males) of normal weight (body mass index = 21.3 kg $\cdot\text{m}^{-2}$), aged from 22 to 26 years (average = 23 years) participated in the study. They were selected according to the following criteria: (i) no history of gastro-intestinal surgery; (ii) absence of gastro-intestinal system disorders; (iii) nonpregnant; and (iv) a stable, satisfactory nutritional status and a stable body weight. The protocol was previously approved by the Ethics Committee of the University Hospital (Nantes, France). All subjects gave their consent to participation in this study.

Experimental design

The volunteers were admitted to the Clinical Research Center the day before the experiment and a double lumen jejunal tube was introduced into the gastro-intestinal tract. The intestinal 'slow marker' perfusion technique has been described previously (Modigliani *et al*, 1973; Mahé *et al*, 1992). The jejunal tube was used: (a) to perfuse polyethyleneglycol-4000 (PEG-4000) (Prolabo, Paris, France) into the duodenum; and (b) to aspirate the jejunal contents. The perfusion site of PEG-4000 was located at the angle of Treitz and the aspiration site 20 cm distally. After an overnight fasting, the position of the tube was verified by radioscopy. A small catheter (Vigon, France) was placed in a forearm vein for blood sampling. Another catheter was placed in a contralateral vein in the other forearm in order to infuse the [¹⁵N]leucine solution (99% enrichment, Tracer Technologies Inc, Somerville, USA). At T₀, a priming dose of [¹⁵N]leucine (10 µmol.kg⁻¹) was given, followed by a constant infusion of [¹⁵N]leucine (10 µmol.kg⁻¹.h⁻¹) and a saline solution (150 mM Na Cl) containing 10 g.l⁻¹ of PEG-4000 was perfused into the intestine at a flow rate of 2 ml.min⁻¹. The subjects were asked to drink 50 ml water (Vittel, France) every hour for 15 h. Blood samples and jejunal effluents were collected hourly. Intestinal samples were obtained by continuous suction through the distal opening of the intestinal tube. Aspirates were collected over ice and pooled at 60-min intervals. Blood samples were collected on heparin and the plasma was immediately separated from whole blood by centrifugation and frozen at -20°C until analysis.

Analytical methods

The volume and pH of the effluents were measured after homogenization. Effluents were treated with 0.1 mM diisopropylfluorophosphate (Sigma, Saint-Quentin-Fallavier, France) to prevent enzymatic degradation of the proteins, and then frozen at -20°C and freeze-dried. The PEG-4000 concentration in the samples was measured by a turbidimetric method (Hyden, 1955). The jejunal nitrogen content was determined by the pyrochemiluminescence technique (Antek 720-771 system, Antek Instruments GmbH, Düsseldorf, Germany).

Protein precipitation and polyacrylamide gel electrophoresis

Precipitable and soluble nitrogen were measured after ethanol precipitation of the dry samples as previously described (Mahé *et al*, 1994). Samples (25 mg) were mixed with 1 ml of 70% ethanol and 1 ml hexane, and allowed to flocculate at 4°C for 1 h. They were centrifuged at 2 500 *g* at 4°C for 25 min. The upper phase (hexane) was discarded and the ethanol phase was collected. The pellet was washed once more with 1 ml of ethanol and both ethanol supernatants were combined. The pellet and the supernatant were dried under reduced pressure with a vacuum concentrator and dissolved in 1 ml of water. The pellet was considered to be composed of proteins and the dried supernatants contained peptides and free amino acids. The samples from jejunal effluents were analysed using sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE) with 15% polyacrylamide gels in the presence of 0.1% SDS (Weber and Osborn, 1969). Proteins of known molecular weights were used as internal standards: bovine serum albumin (67 kDa), human immunoglobulin G (50 kDa), porcine pepsin (34.5 kDa), porcine trypsin (24kDa) and β-lactoglobulin (18.36 kDa).

Amino-acid isolation, derivatization and gas chromatographic mass spectrometry

The amino-acid composition of the jejunal effluents was determined after acid hydrolysis (110°C, 24 h, 6 N HCl, under vacuum) with an automatic analyser LKB Alpha Plus (Pharmacia LKB Biochrom, Cambridge, UK). Cysteine, methionine and tryptophan were not determined. The free amino acids, after acid hydrolysis, were extracted with cation exchange columns (Dowex AG-50X8, Mesh 100; 200, BioRad, France) following the method described by Bier and Christopherson (1979). Plasma-free amino acids were also extracted with cation exchange columns after acidifying the plasma with 1 M acetic acid. The free amino acids were mixed with 600 µl of an esterification reagent containing acetyl chloride and *n*-propanol (v/v; 1:5) and then heated at 110°C for 30 min. The reagent excess was evaporated under N₂ and 50 µl of heptafluorobutyric anhydride (Interchim, Monluçon, France) was

added. The mixture was allowed to incubate at 60°C for 30 min and then dried under N₂. The mass spectrometer was a Hewlett-Packard 5971A interfaced to a 5890A gas chromatograph. The mass spectrometer was operated in the positive chemical ionization mode and the enrichment of [¹⁵N]leucine in plasma and jejunal effluents was determined by single ion monitoring of the ions m/z 282 and 283.

Calculation and statistical analysis

The leucine flux at the steady state (Q) in plasma was calculated according to the model described by Waterlow *et al* (1978). The fractional synthetic rate (FSR) of proteins was calculated according to the relation

$$FSR = (E_{t_2} - E_{t_1}) \times 100 / [E_p (t_2 - t_1)]$$

where E_p and E_t were the enrichment of the precursor pool and the digestive proteins, respectively (Wolfe, 1992). To determine E_p , the experimental values of the [¹⁵N]leucine enrichment $E(t)$ were fitted to a sigmoidal curve for a 0–8 h period according to the relation

$$E(t) = b / [1 + \exp(a - t)]$$

in which a and b were the inflection point and the asymptotic value of the curve, respectively. Results were expressed as mean \pm SD. Statistical analyses were performed by using Tukey's studentized range test and the equation of the enrichment curve was adjusted by a non-linear regression (ANOVA; SAS 6.03; SAS Institute Inc Cary, NC, USA).

RESULTS

Nitrogen characterization of gastro-jejunal effluents

Total nitrogen was measured in the jejunal effluents, collected 20 cm below the angle of Treitz and pooled at 60 min intervals over the 15 h. Nitrogen flow was found to be significant ($P < 0.05$) with a mean rate of 7.2 ± 1.9 mmol N•h⁻¹. The ethanol soluble and

insoluble fractions of the total nitrogen are shown in figure 1. Ethanol precipitation gave an estimation of the balance between protein (insoluble) and peptide or amino-acid (soluble) fractions. The ethanol-soluble fraction represented 58.8% of the total nitrogen. The flow rates of soluble and precipitable nitrogen were 4.2 ± 1 and 2.9 ± 0.7 mmol•h⁻¹, respectively. For both fractions, peaks were observed at 3 and 9 h although no statistical variation was found. The SDS-PAGE analysis of the protein fraction showed the presence of 3 groups of bands present in large amounts (fig 2). The first appeared in the 35–60 kDa range, with a band of high intensity near 50 kDa. The second was detected in the 24–30 kDa range and contained 3–5 bands. The third was made up of small molecules with a molecular weight under 20 kDa. No great variation was observed in the electrophoretic pattern either during the sampling time or among the 5 subjects.

Amino-acid profile of gastro-jejunal effluents

The amino-acid composition of the jejunal effluents was measured every 60 min over 15 h (fig 3 and table I). The jejunal flow rate of total amino acids over the 15 h period was 54.1 ± 36.32 mmol (3.74 ± 1.8 mmol•h⁻¹). The highest flow rate was observed for glycine (9.56 ± 5.59 mmol) which was the most abundant amino acid ($14.7 \pm 4.3\%$) and presented a kinetic profile similar to that of taurine. The secretion of the other amino acids ranged between 1.11 ± 0.72 and 5.19 ± 3.80 mmol for histidine and glutamic acid, respectively. The flow rate of leucine was 3.82 ± 2.62 mmol. Intestinal urea flow rate was also measured and represented 5.26 ± 4.34 mmol, and NH₃ originating from deaminations was 8.07 ± 5.3 mmol (not shown). Although variations in the amino-acid flow rates were observed, their relative proportions remained constant. Aspar-

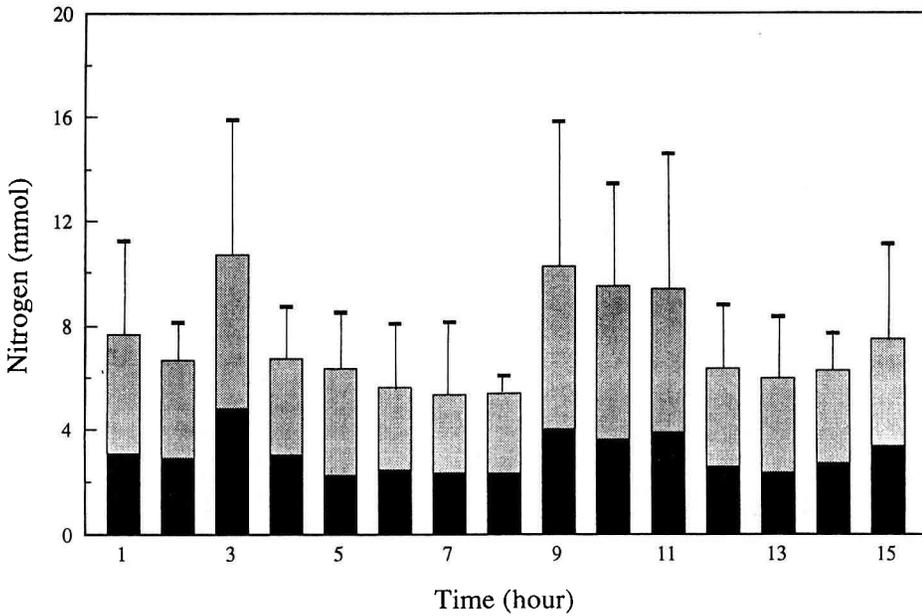


Fig 1. Precipitable (■) and soluble (▨) nitrogen flow rates in the jejunum of fasting humans. Each value represents the mean of 5 subjects. Standard deviations were calculated for total nitrogen and are represented by T.

tic acid, threonine, serine, glutamic acid, proline, valine and leucine were present in the same proportions (5–8%).

[¹⁵N]Leucine enrichment in plasma and in gastro-jejunal secretions

The [¹⁵N]leucine enrichment in the jejunal effluents is shown in figure 4. It showed an

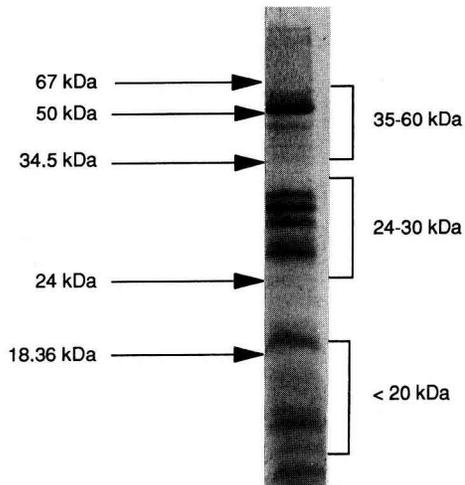


Fig 2. Typical coomassie blue stain of SDS-PAGE of jejunal effluents in fasting humans. Bovine serum albumin (67 kDa), human immunoglobulin G (50 kDa), porcine pepsine (34.5 kDa), porcine trypsin (24 kDa) and β-lactoglobulin (18.36 kDa) were used as reference proteins. Their molecular weights are indicated in the left-hand column.

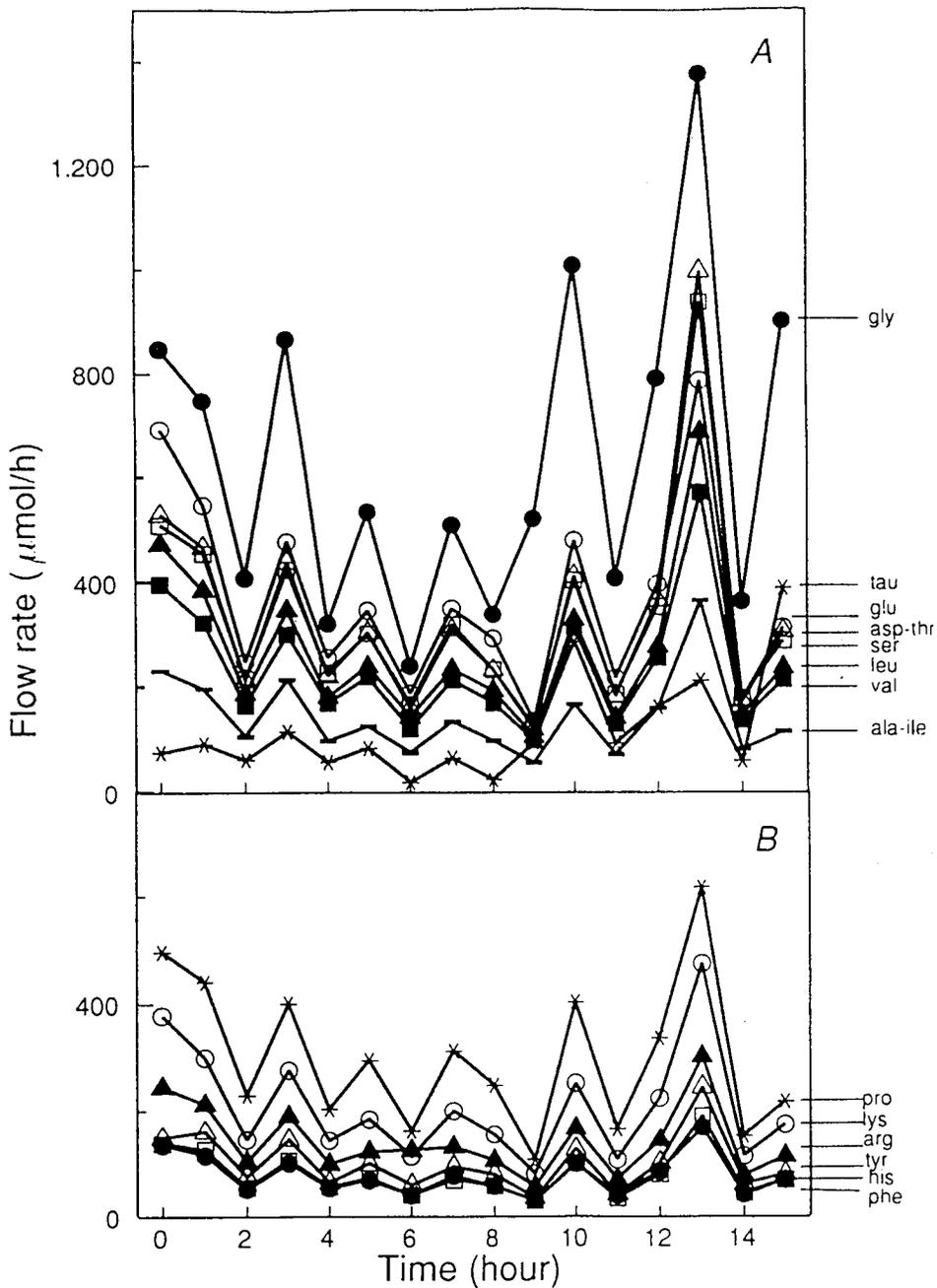


Fig 3. Amino-acid flow rate in the jejunum of fasting humans. **A** Flow rate of tau (*), asp and thr (Δ), ser (\square), glu (O), ala and ile (-), gly (\bullet), val (\blacksquare), and leu (\blacktriangle); and **B** flow rate of pro (*), lys (O), tyr (Δ), phe (\square), his (\bullet), and arg (\blacktriangle). Each value represents the mean of 5 subjects.

Table 1. Amino-acid flow rates over a 15 h period and relative composition in the intestinal lumen.

Amino acid	Amount of amino acids (mmol)	Relative proportion%
Taurine	2.05 ± 1.89	2.8 ± 1.1
Aspartic acid	4.76 ± 3.89	6.9 ± 2.1
Threonine	3.52 ± 2.19	5.8 ± 1.8
Serine	4.81 ± 3.47	7.3 ± 2.3
Glutamic acid	5.19 ± 3.80	7.8 ± 2.5
Proline	4.30 ± 2.98	6.8 ± 2.3
Glycine	9.56 ± 5.59	14.7 ± 4.3
Alanine	2.04 ± 1.40	3.3 ± 1.1
Valine	3.39 ± 2.21	4.6 ± 1.5
Isoleucine	2.10 ± 1.38	3.7 ± 1.1
Leucine	3.82 ± 2.62	5.6 ± 1.7
Tyrosine	1.56 ± 1.04	2.9 ± 0.9
Phenylalanine	1.13 ± 0.79	1.8 ± 0.6
Lysine	2.92 ± 1.99	4.1 ± 0.3
Histidine	1.11 ± 0.71	3 ± 1.1
Arginine	2.00 ± 1.28	3.4 ± 0.1
Total	54.16 ± 36.32	

Values represent mean ± SD ($n = 5$). The relative proportion of each amino acid is the ratio $[aa] \times 100 / \sum [aa]$.

initial lag time of 2 h, a linear increase between 2 and 5 h period before levelling off to a relative plateau value (E_2) between 5 and 8 h, followed by a regular drift that appeared after 8 h. The [^{15}N]leucine enrich-

ment was fitted according to a sigmoidal relationship for the 0–8 period, in order to calculate both the isotopic [^{15}N]leucine enrichment at the plateau of the precursor pool (E_2), which represents the asymptote

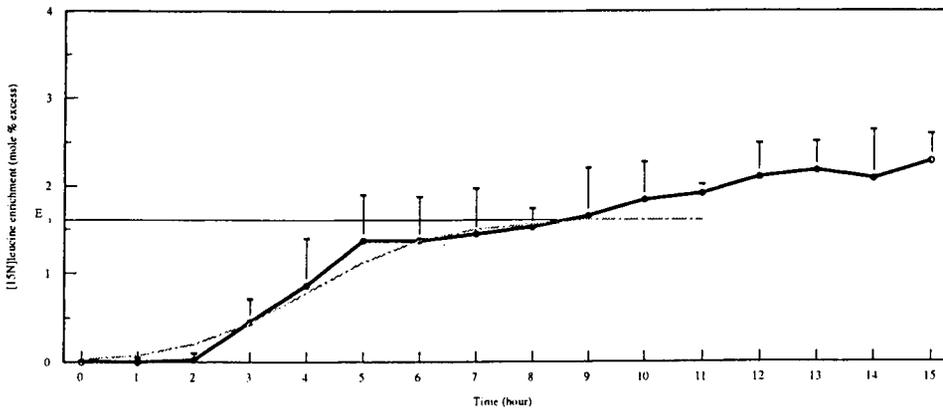


Fig 4. Kinetics of [^{15}N]leucine incorporation in jejunal effluents. Each value represents the mean ± SD of 5 subjects. The parameters of the sigmoidal model (---) are $a = 4.13 \pm 0.72$ and $b = 1.6 \pm 0.563$. E_2 represents the asymptote value of the sigmoidal curve and corresponds to [^{15}N]leucine enrichment of the precursor pool at the plateau.

Table II. Plasma leucine turnover (*Q*) and fractional synthetic rate (*FSR*) of intestinal proteins.

Subject number	Plasma		Intestine	
	E_1 : Plateau enrichment (mpe)	Q : Leucine flux ($\mu\text{mol.kg}^{-1}.\text{h}^{-1}$)	E_2 : Asymptotic enrichment (mpe)	FSR $\%.\text{h}^{-1}$
1	4.9 ± 0.6	191.9	1.59 ± 0.1	20.12
2	4.14 ± 0.6	228.8	1.49 ± 0.1	21.48
3	6.31 ± 0.9	146.9	2.21 ± 0.1	17.19
4	3.95 ± 0.7	240.5	1.75 ± 0.1	27.42
5	4.57 ± 0.5	206.4	0.93 ± 0.2	21.51
Mean(SD)	4.77 ± 0.9	202.9 ± 36.6	1.6 ± 0.5	21.54 ± 3.33

Q was calculated from plasma [^{15}N]leucine enrichment (E_1) at the plateau. *FSR* was calculated from the intestinal [^{15}N]leucine enrichment kinetics and the asymptotic intestinal [^{15}N]leucine enrichment (E_2) as precursor pool enrichment (fig 4).

value, and the *FSR* of intestinally secreted proteins. The equation fitted to the experimental values was $E(t) = 1.6/[1 + \exp(4.13 - t)]$. Individual and mean values are reported in table II. The mean value of plasma [^{15}N]leucine enrichment at the isotopic plateau (E_1), which was reached 2 h after the beginning of the infusion, was 4.8 ± 0.9 mol% excess (mpe). The mean value of the turnover of leucine in plasma was $Q = 202.9 \pm 36.6 \mu\text{mol.kg}^{-1}.\text{h}^{-1}$. In the intestinally secreted proteins and subsequently in their precursor pool, the [^{15}N]leucine enrichment was 1.6 ± 0.5 mpe and the mean value of the *FSR* was $21.5 \pm 3.3\%.\text{h}^{-1}$.

DISCUSSION

In the present study, the double lumen tube technique was used to collect effluents at the proximal jejunum level in order to quantify the gastro-jejunal secretion of nitrogen in fasting humans. In addition, a constant intravenous infusion of [^{15}N]leucine was performed in order to measure the labelling of endogenous proteins secreted into the gastro-jejunal lumen.

The composition of the nitrogenous basal secretion at the collection site appeared relatively constant though maxima are seen at the subjects usual meal times. Amino-acid flow rates were similar to the total nitrogen flux and the relative proportions of the different amino acids remained constant. Glycine proved to be the most abundant amino acid; it is abundant in bile where it conjugates with biliary acids. The maxima of glycine also corresponded to the usual meal times and may have originated from a stimulation of biliary secretion through an hypophyseal induction (Singer, 1986); this could be a major factor responsible for the variations in nitrogen flux (Alpers, 1987). The relative proportion of the protein fractions also remained constant and its electrophoretic profile showed no variation in any of the subjects throughout the experimental period. The endogenous nitrogen flux was $7.2 \pm 1.9 \text{ mmol.h}^{-1}$, which corresponds to approximately 15 g protein/d. This value is consistent with our previous data (Mahé *et al*, 1992) where the estimation of endogenous nitrogen secretion was $2.5 \text{ mmol.20 min}^{-1}$ (15.7 g.d^{-1}). Secretion of endogenous intestinal proteins has previ-

ously been evaluated in the human gut to be 30–60 g.d⁻¹ and is dependent on the level of stimulation including that provided by a meal (Florent and Bernier, 1984). In fact, as the effluents were collected 20 cm below the angle of Treitz, they are made up of salivary, gastric, pancreatic, biliary and intestinal secretions, cell protein loss, and plasma proteins. In addition, part of the secreted nitrogen could have been reabsorbed in the duodenum, just next to the collection site and consequently the basal nitrogen secretion value was underestimated. Moreover, the high level of ethanol-soluble nitrogen strongly suggests that an important part of the secreted proteins had already been hydrolyzed in the duodenum.

In this study, [¹⁵N]leucine was used to label the endogenous nitrogen fraction in the jejunum of fasting human volunteers. This tracer, which has previously been employed in pigs in order to differentiate exogenous nitrogen, proved to be accurate in a digestion study (De Lange *et al*, 1992). The plasma [¹⁵N]leucine enrichment stabilized after 2 h. The enrichment of 4.8 mpe obtained after infusing [¹⁵N]leucine into subjects at a rate of 10 $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$ is consistent with Tessari's results where the enrichment was 5.4 mpe with an infusion rate of 9.6 $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$ (Tessari *et al*, 1985). The leucine turnover ($203 \pm 37 \mu\text{mol.kg}^{-1}.\text{h}^{-1}$) is in agreement with values reported in the literature where post-absorptive [¹⁵N]leucine flux ranged between 150 and 250 $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$ (Matthews *et al*, 1981; Tessari *et al*, 1985; Cheng *et al*, 1987). The gastro-jejunal flow rate of leucine calculated from the amino-acid analysis was $4.0 \pm 2.1 \mu\text{mol.kg}^{-1}.\text{h}^{-1}$ which represents only 2% of the total plasma leucine flux. Under these conditions, a significant [¹⁵N]leucine enrichment was detected in the intestinal lumen leucine pool after a 2-h delay. This delay represents the time needed for plasma leucine to be incorporated from the precursor pools (cellular

tRNA) into synthesized proteins and subsequently to be secreted into the lumen. When pancreatic enzymes were hormonally stimulated, it took the infused [¹⁴C]leucine 80 min to appear in the duodenal juice proteins (O'Keefe *et al*, 1991). The jejunal [¹⁵N]leucine enrichment is biphasic, with a first plateau achieved after 5 h and a subsequent drift observed after 8 h. This is consistent with the observation of Ogden *et al* (1993) who reported a plateau of [¹⁴C]leucine enrichment in pancreatic secretions after 4 h of infusion. We applied several types of equations to our experimental enrichment kinetics. An exponential equation was suitable to model the isotopic incorporation into proteins such as fibronectin (Carraro *et al*, 1991) and apolipoproteins (Cohn *et al*, 1990). In the case of gastro-jejunal proteins, this kind of equation did not fit the enrichment profile well and a sigmoidal model proved to be much more suitable. The precursor pool enrichment of leucine incorporated into the gastro-jejunal secretions was 1.6 ± 0.5 mpe. This confirms that plasma leucine is not the direct precursor pool of the intestinally secreted proteins, an assumption that has been previously formulated (Alpers, 1972). An underestimation of the isotopic enrichment of the precursor pool for lumenally secreted proteins could originate from the reabsorption and possible subsequent transamination of labelled leucine in the intestine. However, the intestinal leucine flux only represents 2% of the whole body leucine turnover. Furthermore, if about 50% of luminal proteins are absorbed in the duodenum, the perturbation accounts for only 0.5% and is probably not significant in our experimental conditions. This underestimation must be negligible because leucine is largely transaminated in the muscles (Harper *et al*, 1984). The drift of leucine enrichment observed after 8 h could not be fully explained by a recycling of leucine since the increase due to isotope recycling is about $0.2\text{--}1\%.\text{h}^{-1}$ (Carraro *et al*, 1991;

Wolfe, 1992). The second phase of the kinetic enrichment may be due to the presence of a low synthesis rate protein pool, which probably contains plasma proteins.

The mean *FSR* of luminally secreted proteins was $21.5 \pm 3.4\% \cdot h^{-1}$. In fact, this value represented a composite of individual fast and slow turnover proteins. The plateau corresponds to the enrichment of such rapidly synthesized proteins as pancreatic enzymes. Ogden *et al* (1993) estimated that the turnover rates of duodenal juice proteins and of trypsin in healthy humans were about 30 and $20\% \cdot h^{-1}$, respectively. In comparison, the *FSR* of fibronectin is about $1.5\% \cdot h^{-1}$ (Carraro *et al*, 1991) and the *FSR* of skeletal muscle protein is $0.05\text{--}0.08\% \cdot h^{-1}$ (Cheng *et al*, 1987; Garlick *et al*, 1989; McNurlan *et al*, 1991).

Our results were obtained with a one-pool model and with the approximation of a single protein secretion whereas proteins in the luminal effluents originate from gastric, pancreatic and bile secretions, as well as plasma (Florent and Bernier, 1984). They probably include serum albumin (69 kDa), the heavy chains of secretory immunoglobulins (55 kDa for IgA and 50 kDa for IgG, as a band of high intensity migrates in the same position as the IgG standard), pepsin (34 kDa), carboxypeptidases A and B (34 and 35 kDa), chymotrypsin A and B, trypsin, elastase (24–25 kDa), and biliary proteins. The relatively high levels of both fractions below 20 kDa and of ethanol-soluble nitrogen strongly suggests that an important part of the secreted proteins are rapidly hydrolyzed and cannot be detected in their native form.

In conclusion, the present study shows that both the nitrogen and the amino-acid composition of the basal gastro-jejunal secretion are very stable. This secretion is composed of proteins with rapid synthesis rates probably corresponding to digestive enzymes as well as proteins with low synthesis rates. We showed that it is possible to label endogenous protein in the human

intestine with [^{15}N]leucine. This method can be used to determine the endogenous nitrogen flow rates in the gut lumen in the post-absorptive state. Regarding our results, the meal must be given 6–8 h after the beginning of the [^{15}N]leucine infusion when the isotopic plateau is reached. Moreover, further studies are needed to define the nature of the different luminal proteins and their individual synthesis rates as well as their responses to different stimuli.

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REFERENCES

- Alpers DH (1972) Protein synthesis in intestinal mucosa: the effect of route of administration of precursor amino acids. *J Clin Invest* 51, 167-173
- Alpers DH (1987) Digestion and absorption of carbohydrates and proteins. In: *Physiology of the Gastrointestinal Tract* (LR Johnson ed) (Raven Press, ed) New York, 1469-1487
- Bier DM, Christopherson HL (1979) Rapid micromethod for determination of ^{15}N enrichment in plasma lysine: application to measurement of whole body protein turnover. *Anal Biochem* 94, 242-248
- Carraro F, Rosenblatt J, Wolfe RR (1991) Isotopic determination of fibronectin synthesis in humans. *Metabolism* 40, 553-561
- Cheng KN, Pacy PJ, Dworzak F, Ford GC, Hallyday D (1987) Influence of fasting on leucine and muscle protein metabolism across the human forearm determined using L-[1- ^{13}C , ^{15}N] leucine as the tracer. *Clin Sci* 73, 241-246
- Cohn JS, Wagner DA, Cohn SD, Millar JS, Schaeffer EJ (1990) Measurement of very low density and low density lipoprotein apolipoprotein (apo)B-100 and high density lipoprotein apoA-1 production in human subjects using deuterated leucine. *J Clin Invest* 85, 804-811
- Corring T, Juste C, Lhoste EF (1989) Nutritional regulation of pancreatic and biliary secretions. *Nutr Res Rev* 2, 161-180

- Costa de Oliveira A, Sgarbieri VC (1986) Effect of diets containing dry beans (*Phaseolus vulgaris*, L) on the excretion of endogenous nitrogen. *J Nutr* 116, 2387-2392
- De Lange CFM, Souffrant WB, Sauer WC (1990) Real ileal protein or amino acid digestibilities in feedstuffs for growing pigs as determined with ¹⁵N-isotope dilution technique. *J Anim Sci* 68, 109-118
- De Lange CFM, Sauer WC, Souffrant WB, Lien KA (1992) ¹⁵N-leucine and ¹⁵N-isoleucine isotope dilution techniques versus the ¹⁵N-isotope dilution technique for determining the recovery of endogenous protein and amino acids in digesta collected from the distal ileum in pigs. *J Anim Sci* 70, 1848-1856
- Florent CH, Bernier JJ (1984) *Intestinal Absorption and Secretion* (Skadhauge E, Heintze K eds) Lancaster, MTP Press
- Fushiki T, Kajiura H, Fukuoka SI, Kido K, Semba T, Iwai K (1989) Evidence for an intraluminal mediator in rat pancreatic enzyme secretion: reconstitution of the pancreatic response with dietary protein, trypsin and the monitor peptide. *J Nutr* 119, 622-627
- Garlick PJ, Wernerman J, McNurlan MA *et al* (1989) Measurement of the rate of protein synthesis in muscle of postabsorptive young men by injection of a 'flooding dose' of [1-¹³C]leucine. *Clin Sci* 77, 329-336
- Harper AE, Miller RH, Block KP (1984) Branched-chain amino-acid metabolism. *Ann Rev Nutr* 4, 409-454
- Huisman J, Heinz TH, Van der Poel AFB, Leeuwen PV, Souffrant WB, Verstegen MWA (1992) True protein digestibility and amounts of endogenous protein measured with the ¹⁵N-dilution technique in piglets fed on peas (*Pisum sativum*) and common beans (*Phaseolus vulgaris*). *Br J Nutr* 68, 101-110
- Hyden S (1955) A turbidimetric method for the determination of higher polyethylene glycols in biological materials. *Kungl Lantbrukshögskolans Annaler* 22, 139-145
- Krawiellitzi K, Zebrowska T, Schadereit R *et al* (1990) Determination of nitrogen absorption and nitrogen secretion in different sections of the pig's intestine by digesta exchange between ¹⁵N labelled and unlabelled animals. *Arch Anim Nutr* 40, 25-37
- Mahé S, Huneau JF, Marteau P, Thuillier F, Tome D (1992) Gastro-ileal nitrogen and electrolyte movements after bovine milk ingestion in humans. *Am J Clin Nutr* 56, 410-416
- Mahé S, Roos N, Benamouzig R *et al* (1994) True exogenous and endogenous nitrogen fractions in the human jejunum after ingestion of small amounts of ¹⁵N-labeled casein. *J Nutr* 124, 548-555
- Matthews DE, Bier DM, Rennie MJ *et al* (1981) Regulation of leucine metabolism in man: a stable isotope study. *Science* 214, 1129-1131
- McNurlan MA, Essen P, Heys SD, Buchan V, Garlick PJ, Wernerman J (1991) Measurement of protein synthesis in human skeletal muscle: further investigation of the flooding dose technique. *Clin Sci* 81, 557-564
- Modigliani R, Rambaud JC, Bernier JJ (1973) The method of intraluminal perfusion of the human small intestine. I. Principle and Technique. *Digestion* 9, 176-192
- Ogden JM, O'Keefe SJ, Louw JA, Adams G, Marks IN (1993) Duodenal juice total protein and pancreatic enzyme synthesis, turnover, and secretion in patients after acute pancreatitis. *Gut* 34, 1261-1266
- O'Keefe SJD, Bennet WM, Haymond MW (1991) Measurement of pancreatic enzyme turnover in man. *FASEB J* 5, A563, 1120.
- Simon O, Zebrowska T, Bergner H, Münchmeyer R (1983) Investigations on the pancreatic and stomach secretion in pigs by means of continuous infusion of ¹⁴C-amino acids. *Arch Tierärz* 33, 9-22
- Singer MV (1986) Réponse sécrétoire pancréatique aux stimulants intestinaux. *Gastroenterol Clin Biol* 10, 504-512
- Souffrant WB, Darcy-Vrillon B, Corring T *et al* (1986) Recycling of endogenous nitrogen in the pig (preliminary results of a collaborative study). *Arch Anim Nutr* 36, 269-274
- Souffrant WB, Rérat A, Laplace JP *et al* (1993) Exogenous and endogenous contributions to nitrogen fluxes in the digestive tract of pigs fed a casein diet. III. Recycling of endogenous nitrogen. *Reprod Nutr Dev* 33, 373-382
- Tessari P, Tsalikian E, Schwenk WF, Nissen ST, Haymond MW (1985) Effects of [¹⁵N]leucine infused at low rates on leucine metabolism in humans. *Am J Physiol* 249, E121-E130
- Waterlow JC, Golden MH, Garlick PJ (1978) Protein turnover in man measured with ¹⁵N: comparison of end products and dose regimes. *Am J Physiol* 235, E165-E174
- Weber K, Osborn M (1969) The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J Biol Chem* 224, 4406-4412
- Wolfe RR (1992) *Radioactive and Stable Isotope Tracers in biomedicine. Principles and Practice of Kinetic Analysis*. Wiley and Sons, Wiley-Liss Inc, New York
- Young VR (1991) Nutrient interactions with reference to amino-acid and protein metabolism in non-ruminants; particular emphasis on protein-energy relations in man. *Z Ernährungswiss* 30, 239-267