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Whole-Body Protein Metabolism Assessed by Leucine and Glutamine Kinetics in Adult Patients With Active Celiac Disease

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To assess the effect of increased renewal of intestinal epithelial cells on leucine and glutamine (Gln) turnover, 4-hour intravenous infusions of L-[1-^{13}C]leucine and L-[2-^{15}N]Gln were administered to five adult patients with active celiac disease in the postabsorptive state. There was a 35% increase in leucine flux (micromoles per kilogram per hour) in patients (117 ± 17) compared with healthy controls (96 ± 11, $P < .03$). Gln flux was increased by 13% in patients (377 ± 35) versus controls (335 ± 16, $P < .04$). These results suggest that active celiac disease, characterized by villous atrophy and crypt cell hyperplasia, is associated with a dramatic increase in whole-body protein breakdown as assessed by ^{13}C-leucine, which may contribute per se to the protein malnutrition status of the patients. The increase in Gln utilization as assessed by L-[2-^{15}N]Gln was moderate, but may have been offset due to the villose atrophy and ensuing reduced intestinal epithelial cell mass. The results are consistent with the concept that increased renewal of intestinal epithelial cells represents a sizable fraction of whole-body protein turnover and that Gln is an important fuel for epithelial intestinal cells in vivo.

CELIAC DISEASE is characterized by alterations in small intestine mucosa that predominantly affect the proximal segments, ie, the duodenum and jejunum. The typical histological lesion is subtotal or total villous atrophy associated with a marked increase in the renewal of intestinal crypt cells.[1] In celiac patients who are nonresponsive to a gluten-free diet, villous atrophy may extend to the entire small bowel, resulting in malabsorption of most nutrients with ensuing protein energy malnutrition.[2,3] The renewal of enterocytes is presumed to account for 10% to 20% of the whole-body protein turnover rate.[4,5] Besides, glutamine (Gln) is considered an important energy substrate for rapidly proliferating cells, including epithelial cells of the intestinal mucosa.[6,7] Intestinal Gln metabolism also supplies nitrogen for the synthesis of purines and pyrimidines, the building blocks of nucleic acids that are in high demand during mitosis.[8,9] We have previously demonstrated a 20% reduction of Gln utilization associated with a reduction in small bowel mass following subtotal enterectomy in humans[10,11]; we hypothesized an opposite need for Gln in patients with active celiac disease, in which an increase in crypt cell mitosis aimed at compensating for the villous atrophy is usually observed. In addition, the model of diffuse celiac disease may serve to assess the impact of a marked increase in crypt cell renewal of the small intestine on the overall protein economy of the body. The purpose of this study was therefore to assess the kinetic parameters of protein metabolism in celiac patients using stable isotope–labeled leucine and Gln tracer infusion in vivo.

MATERIALS AND METHODS

Materials

L-[1-^{13}C]leucine (99.3% ^{13}C), L-[2-^{15}N]Gln (99% ^{15}N), and sodium [^{13}C]bicarbonate (99% ^{13}C) were purchased from Tracer Technologies (Somerville, MA). Before the infusion study, sterile solutions of the three labeled tracers were prepared using aseptic technique. Accurately weighed amounts of each labeled compound were dissolved in known volumes of sterile, pyrogen-free 0.9% NaCl solution and filtered through a 0.22-μm membrane filter (Millipore, Bedford, MA) before use. A sample of the solution was initially verified to be pyrogen-free and sterile before administration to human subjects. Solutions were prepared no more than 24 hours before use and were kept at 4°C.

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Table 1. Nutritional Characteristics of Patients With Celiac Disease

Group	Subject No.	Sex	Age (yr)	Nonprotein Intake (kcal/kg/d)*	Protein Intake (g/kg/d)*	Ideal Body Weight (%)	Albumin (g/L)
Patients	1	F	47	54	1.8	78	27
	2	F	44	32	2.1	90	19
	3	F	72	18	1.5	107	38
	4	F	74	52	1.9	94	25
	5	F	55	24	1.5	88	25
Patients *v*	(n = 5)		58 ± 14	36 ± 15	1.8 ± 0.3	91 ± 10	27 ± 7
Controls	(n = 6)		33 ± 14	32 ± 2	1.3 ± 0.2	102 ± 8	39 ± 3

NOTE. Results for patients *v* controls are the mean ± SD.
Abbreviation: F, female.
*All but patient no. 2 were nutritionally supported parenterally.

Subjects

Five celiac patients and six control subjects were studied at the Saint-Lazare Hospital Nutrition Unit (Table 1). Control subjects were healthy sedentary individuals with stable body weight before the study. Their usual dietary intake was 32 ± 2.4 kcal/kg/d (mean ± SD) of nonprotein energy and 1.3 ± 0.19 g protein/kg/d. Before the study, four of the five patients received parenteral nutrition, either short-term for 2 months in two patients or long-term at home for 50 and 120 months, respectively, in two other patients for celiac disease either unresponsive to a gluten-free diet (patients no. 1 and 3) or recurrent after deviation from the diet regimen (patients no. 4 and 5). Patient no. 2 was on oral nutrition but made few errors in his gluten-free diet before the study. None of the patients received steroids in the last 6 weeks prior to the study. Their intake was 36 ± 14.7 kcal nonprotein energy/kg/d and 1.8 ± 0.3 g protein/kg/d. Celiac disease activity was assessed by proximal jejunal (all five patients) and distal ileal (all but patient no. 2) biopsies, and typical histological lesions were demonstrated with villous atrophy graded as either grade II, III, or IV, partial, subtotal, or total villous atrophy, respectively. Characteristics of the intestinal status of the patients are indicated in Table 2, with jejunoileal diffuse villous atrophy demonstrated in four of the five. Patients received detailed information on the purpose and potential risks of the protocol and provided written consent before the study, in accordance with procedures recommended by the Ethics Committee of our Hospital.

Infusion Protocol

Each subject fasted overnight (>12 hours) and remained fasting during the following 4-hour tracer infusion protocol. On the study day at 7:30 AM, two intravenous catheters were placed, the first in a forearm vein for isotope infusion and the second in a superficial vein of the contralateral hand for blood sampling. At 8:00 AM, baseline blood samples were obtained for determination of background isotopic enrichment in plasma amino acids. Immediately thereafter, a priming dose of [^{13}C]leucine (~4 μmol · kg^{-1}), [^{15}N]Gln (~7 μmol · kg^{-1}), and sodium [^{13}C]bicarbonate (~10 mg) was injected, and infusion of the amino acid tracers was then started and maintained for 4 hours at a constant rate of 4 and 7 μmol · kg^{-1} · h^{-1} for labeled leucine and Gln, respectively, using a calibrated syringe pump. Blood was drawn at baseline and at 20-minute intervals during the last 120 minutes, ie, between 10:00 AM and 12:00 noon. During the sampling period, the hand remained in a heated box (60° to 65°C) to produce arterialized venous blood samples.

Analytical Procedures

Heparinized blood samples were centrifuged at 4°C immediately after sampling. The plasma was separated and frozen at −20°C until further processing. Plasma leucine was analyzed as the *N*-trifluoroacetyl, *N*-butyl derivative,[12] α-ketoisocaproate (KIC) as the quinoxalinol-trimethylsilyl derivative,[13] and Gln as the *N*-acetyl, *n*-propyl derivative.[14] Stable isotopic enrichment in plasma amino acids was determined by electron-impact gas chromatography–mass spectrometry using a Nermag R1010T instrument (Nermag, Argenteuil, France). Selected ion monitoring of ions at *m/z* 227 and 228 was used to quantify the [^{13}C]leucine to natural leucine molar ratio, *m/z* 232 and 233 for the [^{13}C]KIC to natural KIC molar ratio, and *m/z* 187 and 186 for the [^{15}N]Gln to natural Gln molar ratio.

Calculations

The flux (Q) for Gln and leucine into the plasma compartment was calculated via the standard isotope dilution expression,[15] $Q = i[Ei/Ep - 1]$, where i and Q are the tracer infusion rate and amino acid appearance rate, respectively, in micromoles per kilogram per hour and Ei and Ep are the tracer enrichment in the infusate and plasma at steady state (mole percent excess), respectively. Isotopic steady state was defined by a coefficient of variation less than 10% in tracer enrichment over the last 2 hours of infusion. Because leucine is an essential amino acid, leucine flux (Q leu) is entirely derived from protein breakdown in the postabsorptive state. In contrast, the nonessential amino acid Gln has two inflow components to its flow[16]: release from protein breakdown (B Gln) and Gln de novo synthesis (D Gln). The former is

Table 2. Histological and Biological Characteristics of Patients With Celiac Disease

Patient No.	Small Bowel Histology*	Gluten-Free Diet	Steatorrhea (g/d)	α_1AT Clearance (mL/d)
1	Villose atrophy grade III and IV, crypt hyperplasia	Unresponsive	30	173
2	Villose atrophy grade III and IV, crypt hyperplasia	Not strict	18	86
3	Villose atrophy grade III and IV, crypt hyperplasia	Unresponsive	ND	ND
4	Villose atrophy grade III and IV, crypt hyperplasia	Not strict	22	48
5	Villose atrophy grade II and III, no crypt hyperplasia	Not strict	19	44
Mean ± SD			22 ± 5	88 ± 60
Usual reference			<6	<15

Abbreviations: α_1AT, α_1-antitrypsin; ND, no data.
*Histological data obtained at both proximal (duodenal-jejunal) and distal (terminal ileum) levels of the small intestine in all but patient no. 2.

estimated as B Gln = k · Q Leu, where k is the assumed ratio of Gln to leucine in body protein, 6.95[17,18] and 8.0 g amino acid/100 g protein, respectively; therefore, D Gln = Q Gln − B Gln. For leucine kinetics, leucine flux (Q Leu) was calculated using either plasma ^{13}C-leucine enrichment (Ep Leu) or plasma ^{13}C-KIC enrichment (Ep KIC) at steady state.

Statistical Analysis

The data are presented as the mean ± SD. During tracer infusion, the steady state for plasma amino acid enrichment was defined by a coefficient of variation less than 10% of the measured parameter over the period considered. Data for controls and patients were compared using a nonparametric Wilcoxon test with P less than .05 as the level of significance.

RESULTS

Nutritional characteristics (percent ideal body weight and serum albumin) for the patients and controls are reported in Table 1. All patients except one (patient no. 3) presented with protein-energy malnutrition. All patients had active celiac disease as demonstrated by villous atrophy with crypt cell hyperplasia in four of the five patients; the fifth patient presented with partial and subtotal villous atrophy without crypt cell hyperplasia. Villous atrophy extended further than the proximal jejunum as demonstrated on ileal biopsy in all but patient no. 5, in whom ileal biopsy was not attempted. Malabsorption syndrome with fat output far greater than normal was documented (Table 2). Celiac disease was not associated with lymphoma, as documented with intestinal biopsy and abdominal scan.

The parameters of leucine and Gln metabolism, ie, flux rates, were significantly increased in the patients in comparison to controls (Figs 1 and 2). The percent increase of the flux was 36%, 35%, and 13% for leucine (117 ± 17 *v* 96 ± 11 μmol/kg/h, P = .03), KIC (156 ± 29 *v* 111 ± 18 μmol/kg/h, P = .07), and Gln (379 ± 35 *v* 335 ± 16 μmol/kg/h, P = .04), respectively, in patients versus controls, taking into account for the comparison the median of fluxes in patients and controls. The de novo Gln synthesis rate was not significantly different in patients and controls (280 ± 28 *v* 251 ± 17 μmol/kg/h).

DISCUSSION

The results of the present study show that whole-body leucine and Gln metabolism, measured in the postabsorptive state with infusion of stable isotope–labeled leucine and Gln, is significantly accelerated in patients with celiac disease in comparison to controls.

The mean 13% increase in Gln turnover demonstrated in celiac patients can be considered physiologically significant, as noted by Matthews and Campbell.[19] They proposed that a threshold of 10% was physiologically significant for changes in postabsorptive Gln flux; indeed, when normal subjects were submitted to changes in dietary protein over a range from 0.1 to 2.2 g/kg/d, a change in Gln turnover of plus 9% and minus 9% was observed with a low- and high-protein diet, respectively, compared with the recommended 0.8 g protein/kg/d.[19] Since our patients received a generous amount of protein in the diet, ie, 1.8 g/kg/d, the 13% increase we observed cannot be attributed to insufficient protein intake. Such an increase can be interpreted in the context of intestinal mucosal damage as documented in our patients with active celiac disease by histological examination of both proximal jejunal and distal ileal mucosa, where total villose atrophy and crypt cell hyperplasia were observed.[1] In this situation, epithelial morphological cell counting between controls and patients with active celiac disease indicated a threefold to sixfold increase in the crypt cell renewal rate associated with a near-total disappearance of the villi enterocyte population,[1,20,21] resulting in an estimated net total decrease of greater than 50% in the total enterocyte cell mass.[21] The significant but modest increase in whole-body Gln turnover, which was not as high as expected, can therefore be viewed as the result of an increase in crypt cell renewal coexisting with diffuse villose atrophy. Enterocytes play an important role in Gln metabolism because this amino acid is both a major fuel and a nitrogen donor for rapidly dividing intestinal cells.[22] It is therefore worth noting that in patient no. 5

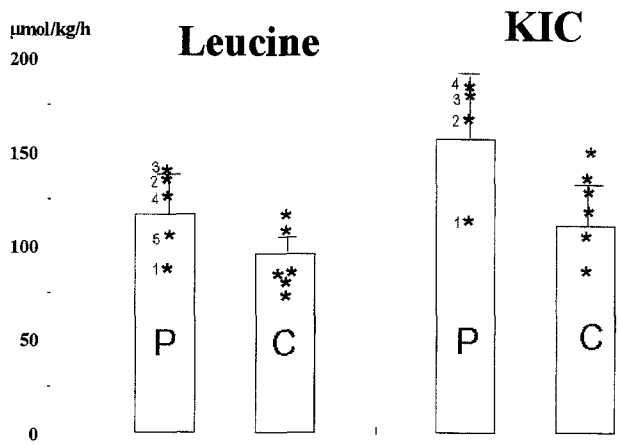

Fig 1. Whole-body protein turnover in celiac patients as assessed by ^{13}C-leucine and ^{13}C-KIC fluxes. Results are the mean ± SD. C, controls (n = 6); P, patients (leucine, n = 5; KIC, n = 4). Wilcoxon test, patients *v* controls: leucine flux, P = .03; KIC flux, P = .07. *Individual data and subject no. of patients.

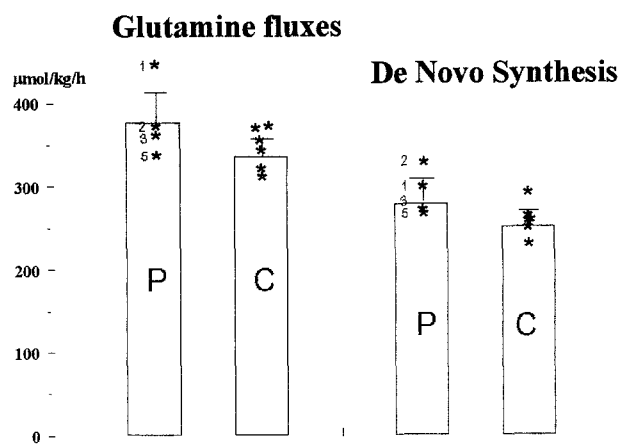

Fig 2. Whole-body ^{15}N-glutamine turnover in celiac patients. Results are the mean ± SD. C, controls (n = 6); P, patients (n = 4). Wilcoxon test, patients *v* controls: glutamine flux, P = .04. *Individual data and subject no. of patients.

with partial and subtotal villose atrophy but without crypt cell hyperplasia, the increase in Gln flux was only 4%, ie, not physiologically significant, whereas in the other three patients with subtotal and total villose atrophy with crypt cell hyperplasia, the median increase was 16% (8% to 27%). Our data are therefore consistent with the hypothesis that Gln is predominantly used by stem cells located in the crypt cell compartment from which epithelial villi cells derive. The role of the intestinal tract as a major site of Gln utilization was demonstrated by an approximately 75% arterioportal difference in the Gln concentration in healthy dogs.[9] This was confirmed in several animal studies[23-26] and in only a few human studies.[24] We have previously demonstrated in both adult and pediatric short-bowel patients, as a model of an absolute decrease in enterocyte mass,[27] a 20% decrease in the rate of whole-body Gln turnover.[10,11] Thus, we speculate that the 13% increase in whole-body Gln turnover we observed in the current study may have been the result of an increase in crypt cell renewal concomitant with a decrease in Gln metabolism in relation to a net decrease in the total enterocyte mass. Nevertheless, our data contribute to the concept of Gln as an important fuel for intestinal cells in the crypt cell compartment. Small bowel status per se is therefore capable of significantly modifying the ^{15}N-Gln whole-body turnover rate.

In our celiac patients, whole-body leucine flux was also significantly increased in comparison to controls. The increase was 36% and 35% when calculated from ^{13}C-leucine and ^{13}C-KIC enrichment, respectively, far greater than the 13% increase in ^{15}N-Gln turnover. Because protein breakdown is the only source of this essential amino acid in the postabsorptive state, leucine flux reflects leucine release from protein breakdown. As nitrogen transfer from leucine to Gln has been estimated to contribute about 9% of Gln nitrogen flux,[28] the 35% increase in leucine flux could be, in theory, a contributor to the increased Gln production. However, our data do not favor such a mechanism, since the de novo Gln synthesis rate was not significantly different between celiac patients and controls. One can question the role of both the level and route of protein intake in the leucine flux difference noted in the postabsorptive state between patients and controls. Concerning protein intake, both controls and patients received a generous intake[29] of 1.3 and 1.8 g/kg/d, respectively. A significant increase in leucine release from protein breakdown of 13% (8% to 18%)[29-31] was indeed documented when healthy subjects were switched from 0.6 to 0.8 g/kg body weight/d, a medium recommended protein intake, to 1.5 to 1.7 g/kg/d, a generous protein intake. We therefore believe that the slight difference in protein intake cannot account for the 35% increase in leucine flux between patients and controls in the current study. Regarding the route of nutrient intake, controls were fed orally, whereas four of the five patients received parenteral nutrition because of severe malabsorption. In humans, using intravenous tracer techniques similar to the current study, it has been estimated that whole-body protein turnover is indeed reduced 20% when parenteral nutrition is used even for a short period.[4] This route of nutrition induces, at least in animals, a hypoplastic state with a decreased crypt cell proliferative rate and epithelial cell renewal in the small intestine.[32] This is exactly the opposite of what is observed in active celiac disease patients, in whom the increased intestinal epithelial cell renewal rate is largely higher than in controls.[20] Thus, the observed increase in leucine flux does not seem to be the consequence of the level or route of protein intake. The 35% increase in leucine flux in celiac patients was also far greater than the 15% increase induced in healthy subjects by hypercortisolemia.[16] Although levels of the stress hormones were not measured in our patients, the increased leucine flux observed in the current study is unlikely to result from alterations in hormone secretion, since our patients were free of intercurrent infection, did not receive corticosteroids, and were not postsurgical. In addition, our in vivo data are in agreement with the 50% to 200% increase in the mucosal intestinal fractional protein synthesis rate recently demonstrated in celiac patients with ^{13}C-leucine.[5] The latter results, as well as our own, were observed despite the subtotal or total villose atrophy and the resulting net decrease in enterocyte cell mass. By contrast, in short-bowel adult patients with a normal remnant small bowel, we did not find a change in ^{13}C-leucine flux in comparison to controls.[10] This study therefore demonstrated that the intestinal mucosal damage observed in celiac patients can dramatically alter whole-body protein turnover.

In healthy human subjects, although the mass of the small intestine is only one eighth of the mass of skeletal muscle, its protein turnover may represent up to 20% of whole-body protein turnover.[4,33] Protein malnutrition was documented in our celiac patients despite the fact that they received intravenous appropriate calories and a generous protein intake. We therefore speculate that protein malnutrition may be a consequence of the increased protein breakdown aimed at meeting the increased amino acid requirement for increased protein synthesis in the small intestine. Thus, the observed increased whole-body turnover rates of leucine and Gln driven by the increased cellular turnover at the intestinal level may have contributed to the malnutrition status of our celiac patients. Along the same lines, growth retardation, well documented in children with untreated celiac disease,[34] could not be entirely accounted for by malabsorption of nutrients or anorexia. Indeed, muscle wasting in severe intestinal disease has been suggested to be a consequence of increased intestinal protein and energy metabolism.[33]

In summary, the current study demonstrates that patients with active celiac disease in the postabsorptive state present with a significant 35% increase in whole-body leucine flux, reflecting a dramatic increase in protein breakdown. It is tempting to speculate that this change is caused by the already documented marked increase in mucosal intestinal protein synthesis in the crypt compartment.[5] Such a dramatic increase in protein breakdown, apart from malabsorption or anorexia, certainly may contribute to the malnutrition status of celiac patients. In addition, we documented a modest but physiologically significant 13% increase in Gln turnover. This change, not as large as expected, may have been blunted due to the reduction in total intestinal cell mass.[20,21] Our study thus clearly demonstrates that small-bowel disease can dramatically alter whole-body protein turnover as assessed by both essential and nonessential amino acid kinetics.

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