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Corticosteroids Increase Protein Breakdown and Loss in Newly Diagnosed Pediatric Crohn Disease

STEVEN J. STEINER, JOSHUA D. NOE, AND SCOTT C. DENNE

Department of Pediatrics, James Whitcomb Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, Indiana 46202

ABSTRACT: Children with Crohn disease have altered growth and body composition. Previous studies have demonstrated decreased protein breakdown after either corticosteroid or anti-TNF-α therapy. The aim of this study was to evaluate whole body protein metabolism during corticosteroid therapy in children with newly diagnosed Crohn disease. Children with suspected Crohn disease and children with abdominal symptoms not consistent with Crohn disease underwent outpatient metabolic assessment. Patients diagnosed with Crohn disease and prescribed corticosteroid therapy returned in 2 wk for repeat metabolic assessment. Using the stable isotopes [d5] phenylalanine, [1-^{13}C] leucine, and [$^{15}N_2$] urea, protein kinetics were determined in the fasting state. Thirty-one children (18 controls and 13 newly diagnosed with Crohn disease) completed the study. There were no significant differences in protein breakdown or loss between patients with Crohn disease at diagnosis and controls. After corticosteroid therapy in patients with Crohn disease, the rates of appearance of phenylalanine (32%) and leucine (26%) increased significantly, reflecting increased protein breakdown, and the rate of appearance of urea also increased significantly (273%), reflecting increased protein loss. Whole body protein breakdown and loss increased significantly after 2 wk of corticosteroid therapy in children with newly diagnosed Crohn disease, which may have profound effects on body composition. **(*Pediatr Res* 70: 484–488, 2011)**

Children with Crohn disease suffer from growth impairment before diagnosis, and despite therapy, continue to have growth difficulties which may persist to altered adult growth outcomes. At diagnosis, these children suffer from not only linear growth impairment but also deficits in lean body mass (1) and bone mineral density (2). These deficits may result from altered nutritional intake, malabsorption, and inflammation. A prospective observational study demonstrated increases in BMI and fat mass in the 2 y after diagnosis of pediatric Crohn disease; however, no significant change in lean body mass and persistent deficits in bone mineral content were observed (2). Emerging evidence suggests gender differences in body composition may exist in pediatric patients with Crohn disease. Females with Crohn disease may have more persistent lean body mass deficits than males (3). Current therapeutic strategies may not be resulting in significant and important improvements in lean body mass in these patients.

Both malnutrition and inflammation may be observed in pediatric patients with Crohn disease, and they may have opposing effects on substrate metabolism. In patients with chronic malnutrition, there are marked reductions in whole body protein turnover (4), including protein synthesis and breakdown, and in urea excretion, a marker of protein loss (5). In these patients, adaptations to reduction in protein intake result in minimization of protein loss. However, inflammation leads to increased whole body protein metabolism. Injection of TNF-α, an inflammatory cytokine, resulted in increased whole body protein turnover and synthesis and worsening nitrogen balance (6). These two opposing forces prevent normal adaptation to malnutrition in patients with ongoing inflammation and present a therapeutic challenge in children with newly diagnosed Crohn disease.

Rates of protein metabolism may play a key role in acquiring and maintaining lean body mass, which in turn may be critical for bone mineral density acquisition and linear growth (7). Altered rates of protein metabolism are present in children with active Crohn disease, and the severity of inflammation in Crohn disease correlates with the degree of protein breakdown (8). The effect of therapy for Crohn disease on protein metabolism has been studied in children. In children with active Crohn disease, protein breakdown decreased after treatment with either sulfasalazine or prednisolone (9). Similarly, we demonstrated reductions in protein breakdown 2 wk after a single dose of infliximab, an anti-TNF-α antibody, for the treatment of active Crohn disease (10).

Acute corticosteroid therapy results in increased whole body protein oxidation and decreased whole body protein synthesis in healthy subjects (11), and there may be a dose-response gradient with worsening whole body protein metabolism at increased steroid doses (12). Patients with Cushing's syndrome and high levels of corticosteroid production have significantly reduced lean body mass (13). Because corticosteroids are known to result in significant changes in body composition and protein metabolism, we aimed to examine the effects of high-dose corticosteroid therapy on whole body protein metabolism in children with newly diagnosed Crohn disease. Our hypothesis was that corti-

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Correspondence: Steven J. Steiner, M.D., Division of Pediatric Gastroenterology/Hepatology/Nutrition, James Whitcomb Riley Hospital for Children, Indiana University School of Medicine, 702 Barnhill Drive/ROC 4210, Indianapolis, IN 46202-5225; e-mail: ssteiner@iupui.edu
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J.D.N. is currently at Children's Hospital of Wisconsin, Milwaukee, WI 53201.

Abbreviations: ESR, erythrocyte sedimentation rate; I_{Phe}, infusion rate of tracer phenylalanine; **KIC,** a-ketoisocaproic acid; **PCDAI,** pediatric Crohn disease activity index; Q_{PT}, phenylalanine hydroxylation to tyrosine; R_a, rate of appearance

costeroids will result in adverse changes in protein metabolism despite their potent anti-inflammatory effects and patients' improvement in clinical disease.

MATERIALS AND METHODS

Children aged 6 to 17 y with suspected inflammatory bowel disease who were scheduled to undergo esophagogastroduodenoscopy and colonoscopy were recruited for this study. In addition, children with chronic abdominal pain who were scheduled to undergo esophagogastroduodenoscopy and colonoscopy were recruited to serve as controls, provided their result of endoscopic examination was normal. We elected to enroll these control patients to compare protein metabolism in these children to children with Crohn disease before therapy. Children with newly diagnosed Crohn disease who underwent endoscopic evaluation and completed the initial metabolic study as described below were asked to undergo a second outpatient metabolic study 2 wk after the initiation of corticosteroid therapy for their inflammatory bowel disease. The initial treatment of newly diagnosed inflammatory bowel disease consisted of oral prednisone at 2 mg/kg/d, up to a maximum of 60 mg/d. After 2 wk of oral prednisone therapy, these patients were admitted to the General Clinical Research Center (GCRC), after an overnight fast, for their metabolic study. During each visit, if the patient was diagnosed with Crohn disease, an investigator performed the Pediatric Crohn Disease Activity Index (PCDAI) (14).

Stable isotope infusions. Children were admitted to the outpatient surgery area after an overnight fast. Intravenous catheters were inserted in both arms, and a priming dose of stable isotopes, equivalent to 90 min of constant infusion, was administered. In addition, the priming dose contained [d_4] tyrosine at a dose of 0.44 μmol/kg. The priming dose of stable isotopes was given approximately 90 min before scheduled start of endoscopic examination. Thereafter, constant infusions of [1-^{13}C] leucine (5 μmol/kg/h), [d_5] phenylalanine (2.5 μmol/kg/h), [d_2] tyrosine (1.4 μmol/kg/h), and [$^{15}N_2$] urea (5 μmol/kg/h) dissolved in normal saline were delivered through an i.v. catheter *via* an infusion pump. Blood samples (3 mL) were obtained at 120, 140, 160, and 180 min. Blood was immediately analyzed for plasma glucose concentration, and the remainder of the sample was frozen at −70°C for later analysis. All stable isotopes were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA).

The enrichments of leucine, phenylalanine, tyrosine, α-ketoisocaproic acid (KIC), and urea were determined by electron impact ionization and selected ion monitoring on a gas-chromatograph-mass spectrometer [Hewlett-Packard (Agilent) Model 5988 A, Santa Clara, CA]. The enrichments were determined by monitoring ions 302 and 303 (leucine), 234 and 239 (phenylalanine), 466, 468, and 470 (tyrosine), and 231 and 233 (urea) after derivatization to the tertiary butyldimethylsilyl derivatives (15,16). The plasma enrichment of KIC was determined after derivatization to the *O*-trimethylsilylquinoxalinol by monitoring ions 232 and 233 (15,17).

Plasma enrichments were used to calculate the rates of appearance (R_a) of these amino acids. By studying phenylalanine metabolism, both primary and reciprocal pools of leucine metabolism, and urea metabolism, three different independent protein kinetics assays were used, resulting in a comprehensive analysis of whole body protein metabolism in these children. The total rates of appearance of leucine, phenylalanine, and tyrosine were each calculated by measuring tracer dilution at steady state as modified for stable isotopic tracers (18,19):

$$R_{3\,a} = [(100/EP) - 1] \times I$$

where *R*a is the rate of appearance of the amino acid; EP is the steady-state enrichment of the specific isotope; and *I* is the rate of tracer infusion.

Phenylalanine hydroxylation to tyrosine was calculated as follows (20):

$$Q_{PT} = \text{Tyr } R_a \times (^2H_4 \text{ Tyr}/^2H_5 \text{ Phe}) \times [\text{Phe } R_a/(I_{Phe} + \text{Phe } R_a)]$$

where $^2H_4$ and $^2H_5$ Phe are the isotopic enrichments of the representative tracers in plasma, and I_{Phe} is the infusion rate of tracer phenylalanine (mmol/kg/h). The expression Phe $R_a/(I_{Phe}$ +Phe $R_a)$ corrects for the contribution of the phenylalanine tracer infusion to Q_{PT}. Phenylalanine utilization for protein synthesis was calculated by subtracting Q_{PT} from Phe R_a, because phenylalanine is irreversibly lost either by its degradation pathway *via* its conversion to tyrosine, or by incorporation of protein (20,21). Patient data were excluded from analysis if isotopic steady state was not achieved.

Sample size justification. The primary end point to be measured is change in rates of appearance of essential amino acids (reflecting proteolysis). Our previous data indicate a decrease in proteolysis of 10% in the fasting state after a single dose of infliximab in children with Crohn disease with a SD of the difference of 13% (10). When comparing the change from baseline to follow-up in the outcome between pre- and postcorticosteroid treatment groups, a sample size of 16 per group will provide 80% power to detect a 10% difference in the outcome (assuming a 13% SD) using a two-sided, paired *t* test and a 0.05 level of significance.

Statistical analysis. All results are reported as the mean ± SEM. Steady-state tracer enrichment was defined as an insignificant correlation ($p > 0.05$) with time. Comparisons between sample groups were made using the unpaired *t* test. Comparisons within the sample group between pre- and postcorticosteroid measurements were made using the paired *t* test. A $p < 0.05$ was considered statistically significant. This study was approved by the Indiana University–Purdue University Indianapolis (IUPUI) and Clarian Institutional Review Board, and informed assent and consent was obtained from each pediatric subject and parent/guardian.

RESULTS

Clinical and biochemical outcomes. Thirty-one children completed the study (Table 1). All patients were Caucasian. Eighteen patients had a normal endoscopic examination. Thirteen patients were diagnosed with Crohn disease after endoscopic examination. These thirteen patients had a mean PCDAI score of 36 ± 4, indicating moderate disease activity. A summary of clinical characteristics demonstrated no differences in age between the groups, but children with newly diagnosed Crohn disease had higher erythrocyte sedimentation rates (ESR) and lower serum albumin levels than control patients. Gender- and age-based *Z* scores were calculated for height, weight, and BMI, and no significant differences in anthropometric measures were found between controls and patients with newly diagnosed Crohn disease.

Nine of these 13 patients (mean age: 12.9 ± 0.6 y) were placed on oral prednisone (2 mg/kg/d, maximum 60 mg/d) at physician discretion based on severity of disease and returned for a second metabolic study after 2 wk of corticosteroid therapy. The mean ESR decreased significantly from 35 ± 5 to 7 ± 2 mm/h. In eight of these nine patients (data not available in one patient), the mean PCDAI score decreased significantly from 39 ± 3 to 21 ± 2, and the mean serum albumin increased significantly from 2.4 ± 0.2 to 2.8 ± 0.1 mg/dL.

Proteolysis. Isotopic steady state was achieved for [(1-^{13}C)] leucine, [1-^{13}C] KIC, [d_5] phenylalanine, [d_4] tyrosine, [d_2] tyrosine, and [$^{15}N_2$] urea during the study for the control patients and for patients with Crohn disease at both timepoints in most patients (data not shown). Using two independent methods of assessing whole body proteolysis (using phenylalanine and leucine), there was no significant difference in proteolysis (protein breakdown) between control patients (n = 18) and patients with Crohn disease (n = 13) at the time of

Table 1. *Clinical characteristics of control and newly diagnosed Crohn disease patients*

	Control	Crohn disease
Age	13.9 ± 0.6	13.0 ± 2.0
Gender	10 male/8 female	9 male/4 female
ESR (mm/h)	9 ± 4	31 ± 5*
Albumin (mg/dL)	4.1 ± 0.1	2.8 ± 0.2*
Height, *Z* score	0.28 ± 0.25	0.05 ± 0.31
Weight, *Z* score	0.21 ± 0.33	−0.54 ± 0.40
BMI, *Z* score	−0.15 ± 0.42	−0.95 ± 0.57

* $p < 0.01$ compared with control patients.

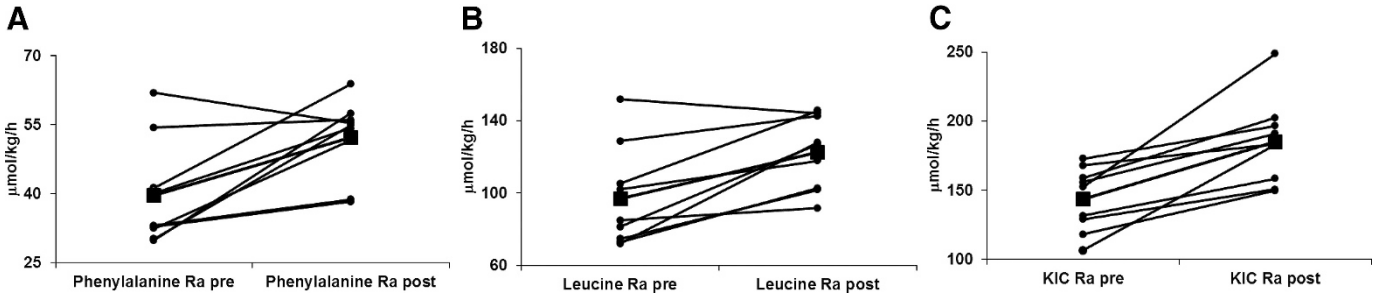

Figure 1. Endogenous rates of appearance (a marker of protein breakdown) of phenylalanine (*A*), leucine (*B*), and KIC (*C*) before and after 2 wk of high-dose corticosteroid therapy in children with newly diagnosed Crohn disease. There was a significant increase ($p < 0.05$) in the rates of appearance of phenylalanine, leucine, and KIC after corticosteroid therapy, indicating increased rates of protein breakdown. *Square boxes* indicate mean values.

diagnosis. Phenylalanine R_a was 40.7 ± 2.5 μmol/kg/h in control patients and 42.7 ± 3.4 in patients with Crohn disease before corticosteroid therapy. The endogenous R_a (reflecting proteolysis) of leucine is presented based on both enrichment of plasma leucine and the enrichment of plasma KIC. Leucine R_a was 104.7 ± 5.1 μmol/kg/h in control patients and 97.1 ± 9.3 in patients with Crohn disease before corticosteroid therapy. On the basis of plasma KIC, the leucine R_a was 143.4 ± 8.4 μmol/kg/h in control patients and 143.7 ± 7.7 μmol/kg/h in patients with Crohn disease before corticosteroid therapy.

After 2 wk of corticosteroid therapy in nine patients with newly diagnosed Crohn disease, significant ($p < 0.05$) increases in the rates of proteolysis were observed using phenylalanine and leucine kinetics. Phenylalanine R_a (Fig. 1*A*) increased 32% from 39.6 ± 3.8 to 52.3 ± 2.8 μmol/kg/h. Leucine R_a (Fig. 1*B*) increased 26% from 97.1 ± 9.3 to 122.4 ± 6.7 μmol/kg/h, and based on plasma KIC (Fig. 1*C*), the leucine R_a increased 29% from 143.7 ± 7.7 to 185.0 ± 10.3 μmol/kg/h.

Protein loss. Urea R_a, a marker of whole body protein loss, was measured in control patients and in patients with newly diagnosed Crohn disease before and after corticosteroid therapy. No significant difference in rates of protein loss was noted between control patients and patients with Crohn disease at the time of diagnosis before corticosteroid therapy. Control patients ($n = 15$) had a urea R_a of 600 ± 53 μmol/kg/h, whereas the patients with newly diagnosed Crohn disease ($n = 10$) had a urea R_a of 429 ± 83 μmol/kg/h. In seven patients with Crohn disease, urea R_a was measured before and after corticosteroid therapy and increased in each patient, with a significant ($p < 0.05$) mean 273% increase (Fig. 2) from 310 ± 41 μmol/kg/h to 1157 ± 191 μmol/kg/h.

Protein synthesis. No difference in phenylalanine utilization for protein synthesis was observed between controls and patients with newly diagnosed Crohn disease. The control patients ($n = 17$) had a utilization rate of 33.2 ± 1.8 μmol/kg/h, whereas the patients with Crohn disease ($n = 8$) had a rate of 39.4 ± 4.4 μmol/kg/h. After corticosteroid therapy, five patients with Crohn disease had no significant increase in the rate of utilization of phenylalanine for protein synthesis (precorticosteroid: 36.6 ± 6.2 μmol/kg/h; and postcorticosteroid: 43.8 ± 1.5 μmol/kg/h).

Phenylalanine hydroxylation. No difference in phenylalanine hydroxylation to tyrosine, a marker of amino acid oxidation, was observed between controls and patients with newly diagnosed Crohn disease. The control patients ($n = 17$) had a hydroxylation rate of 6.5 ± 0.6 μmol/kg/h, whereas the patients with Crohn disease ($n = 8$) had a rate of 8.1 ± 0.6 μmol/kg/h. After corticosteroid therapy, an increase in hydroxylation was observed in all five patients with Crohn disease with available steady-state data with a significant ($p < 0.05$) mean 47% increase (Fig. 3) (precorticosteroid: 7.2 ± 0.7 μmol/kg/h; and postcorticosteroid: 10.6 ± 0.8 μmol/kg/h).

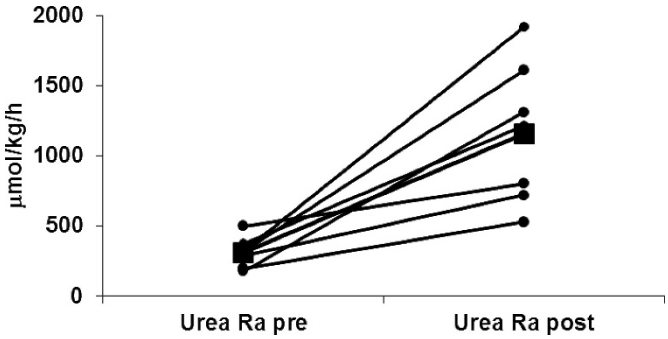

Figure 2. Endogenous rates of appearance of urea (a marker of protein loss) before and after 2 wk of high-dose corticosteroid therapy in children with newly diagnosed Crohn disease. There was a significant increase ($p < 0.05$) in the rate of appearance of urea after corticosteroid therapy, indicating increased rate of protein loss. *Square boxes* indicate mean values.

DISCUSSION

There is a paucity of data on the effects of corticosteroids on protein metabolism in children with inflammatory bowel disease. Despite improvement in clinical symptoms, our study demonstrated that the use of corticosteroids resulted in significant alterations in protein metabolism within 2 wk of their initiation in patients with newly diagnosed Crohn disease. These patients had increased rates of whole body protein breakdown, protein oxidation, and protein loss after corticosteroid therapy. Long-term use of corticosteroids may have adverse effects on lean body mass acquisition in these children, which may results in deficiencies in bone mineral density acquisition and attainment of full potential adult height. Continuing evolvement of strategies to limit corticosteroid exposure should occur, along with studies of the effects of newer therapeutic strategies on similar metabolic outcomes.

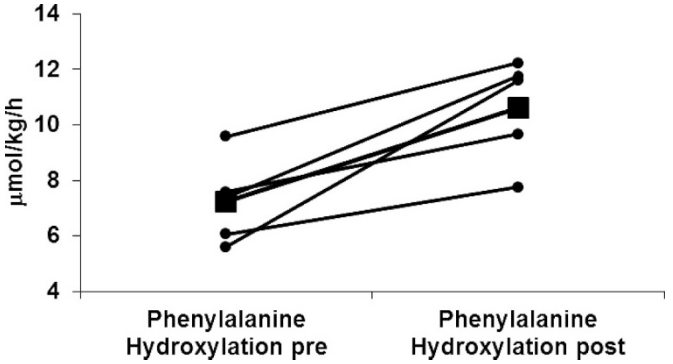

Figure 3. Rates of phenylalanine hydroxylation to tyrosine, a marker of irreversible protein oxidation, before and after 2 wk of high-dose corticosteroid therapy in children with newly diagnosed Crohn disease. There was a significant increase ($p < 0.05$) in the rate of hydroxylation after corticosteroid therapy, indicating increased rate of irreversible protein oxidation. *Square boxes* indicate mean values.

We observed similar rates of whole body protein metabolism in healthy control subjects and patients with newly diagnosed Crohn disease patients before corticosteroid therapy. These similar results may be a result of the opposite effects of malnutrition and inflammation in the latter population. Malnutrition generally results in decreased protein turnover to preserve remaining lean body mass, whereas inflammation usually results in increased protein turnover, and the sum of these effects may net minimal difference from healthy control subjects. Nevertheless, the chronic effects of undiagnosed disease in these children often result in significant lean body mass and linear growth deficits at the time of their diagnosis, perhaps because the adaptations to malnutrition cannot occur in the presence of ongoing inflammation. Although we did not observe significantly different anthropometric measures between healthy control patients and patients with newly diagnosed Crohn disease patients, a larger sample size may have revealed statistically significant differences.

In prior studies, prednisolone therapy for active Crohn disease resulted in a reduction in protein breakdown. Thomas *et al.* (9) administered prednisolone (2 mg/kg/d, maximum 60 mg/d) to four patients with active relapsed Crohn disease. The dose was tapered after 2 wk, and both before therapy and 4 wk after therapy was started, whole body protein turnover was measured using L-[1-^{13}C] leucine. Protein breakdown and protein synthesis both decreased after corticosteroid therapy, although it was not clear what dose of prednisolone the patients were receiving at the 4-wk follow-up. This result did not correlate with our results and the expected effects of corticosteroids, which are known to have adverse effects on protein metabolism. The significantly increased protein breakdown that we observed is consistent with findings from healthy adult volunteers who received short-term corticosteroids. Beaufrere *et al.* (12) demonstrated a 31% increase in the endogenous rate of appearance of leucine (a marker of protein breakdown) when healthy adults received 5 d of high-dose prednisone. In addition, the increased oxidation of leucine observed in their study suggested significant essential amino acid loss, with protein loss observed even in the postabsorptive state. However, enteral nutrition resulted in greater increases in fat free mass than corticosteroid therapy in children with active Crohn disease (22).

Current standards of care, including the use of corticosteroid therapy for the initiation of remission of Crohn disease, have not resulted in improved lean body mass in these children, as demonstrated by Sylvester *et al.* (2). Infliximab did result in improvements in linear growth in patients Tanner stages I-III with chronically active severe Crohn disease (23). In pediatric patients with Crohn disease, Thayu *et al.* (3) examined lean and fat mass at diagnosis, again at 6 and 12 mo, as well as a median of 43 mo after diagnosis. Female patients had persistent lean mass deficits relative to controls at final visit, and overall, significant increases in lean mass relative to height were associated with infliximab therapy. These results suggest that changes in treatment paradigms should be considered if fat-free mass is considered an important outcome. Given its correlation with the acquisition of bone mineral content, the acquisition of fat-free mass should be of critical importance although these children are in an important stage of lean body mass and bone mineral content acquisition.

Protein metabolism in children with Crohn disease is likely affected by the degrees of inflammation and malnutrition, as well as the effects of exogenous medications. The anti-inflammatory effects of medications may be tempered by their adverse consequences on protein metabolism. In the era of top-down *versus* step-up strategies for the treatment of Crohn disease (24), more attention to substrate metabolism and growth as a result of treatment strategies is necessary. Although corticosteroid therapy results in improvements in disease activity, its negative effects on protein metabolism may lead to further deficits in lean body mass. Alternatively, anti-TNF-α therapies may not have such adverse effects on protein metabolism, as we have previously demonstrated (10). Therapeutic decision making in patients who are still actively growing must take into account the metabolic effects of these agents.

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