Corticosteroids increase glutamine utilization in human splanchnic bed

Ronan Thibault,1,2,3 Susan Welch,4 Nelly Mauras,4 Brenda Sager,4 Astride Altmare,4 Morey Haymond,4,5 and Dominique Darmaun1,2,3,4

1Institut National de la Recherche Agronomique, Unité mixte de recherche 1280, Physiologie des Adaptations Nutritionnelles, Université de Nantes; 2Department of Gastroenterology and Nutritional Support, Centre Hospitalier Universitaire Hôtel Dieu; 3Centre de Recherche en Nutrition Humaine, Nantes, and Institut des Maladies de l’Appareil Digestif, Nantes, France; 4Nemours Children’s Clinic, Jacksonville, Florida; and 5US Department of Agriculture Children’s Nutrition Research Center at Baylor College of Medicine, Houston, Texas

Submitted 5 October 2007; accepted in final form 26 December 2007

Glutamine plays an important role in the maintenance of intestinal function (39, 43, 46). Glutamine indeed is both a preferred fuel (9, 47) for enterocytes and a precursor for the intestinal synthesis of purines, pyrimidines (9), and glutathione (37), and it may regulate gut protein synthesis (21, 30, 32). In addition, glutamine is a major donor of carbon for gluconeogenesis (19). This explains why glutamine is extensively extracted in the splanchnic tissues in several animal species (39, 47).

In humans, the cotransaminating infusion of two tracers of glutamine (one infused intravenously, the other one enterally) can be used to assess the extraction of glutamine by the splanchnic bed. By using this technique, ~50 to 75% of enterally administered glutamine undergoes first-pass extraction within the splanchnic bed, presumably by the gut and liver, in healthy humans (4, 17, 18, 34). Additionally, the major fate of splanchnic glutamine after enteral administration was oxidation, since 83% of the total tracer extracted was oxidized and only 10% was used for gluconeogenesis (17).

A dramatic rise in the rate of glutamine uptake in splanchnic tissues has been documented in animal models of stress or after administration of glucocorticoids, which are believed to mediate some of the protein catabolic response to stress (2, 27, 38, 40, 42). It is well known that glucocorticoids mimic some of the alterations in amino acid metabolism observed in stress-induced protein wasting (5, 24, 35). In humans, little is known about the effect of stress or glucocorticoids on glutamine uptake in splanchnic tissues. A single study showed that postoperative stress following coronary bypass surgery was associated with an increased splanchnic extraction of glutamine (41). To our knowledge, the effects of glucocorticoids on splanchnic glutamine metabolism have not been studied in humans. The aim of this study was to determine, in healthy adult volunteers, whether a 6-day treatment with prednisone alters first-pass glutamine extraction in the splanchnic bed and glutamine oxidation, as assessed with isotopic methods.

MATERIALS AND METHODS

Materials. L-[1-14C]leucine (>55 mCi/mmol; New England Nuclear, Boston, MA) and L-[1-13C]glutamine (99% 13C) and L-[2H5]glutamine (98% 2H5) (both stable isotopes from Cambridge Isotope Laboratories, Woburn, MA) were mixed with 0.9% saline and tested for chemical, isotopic, and optical purity by high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GCMS) for radioactive and stable isotope tracers, respectively, and verified to be sterile (plate culture) and pyrogen free (Limulus lysate assay). Solutions were passed through a 0.22-μm Millipore filter and stored in sterile containers at 4 °C for <24 h prior to use.

Experimental design. This protocol was reviewed and approved by the Nemours Children’s Clinic Research Committee and the Institutional Review Committee at Baptist Medical Center, Jacksonville, FL. Subjects were judged to be free of any chronic or acute illness based on detailed medical history, physical exam, and routine blood chemistry. Women had to have a negative pregnancy test within 48 h of study and could not be breastfeeding. Both groups of healthy volun-
PREDNISONE AND SPLANCHNIC GLUTAMINE UPTAKE

G549

...teers received detailed information on the purpose and potential risks of the study and were enrolled after signing a written consent form. A dietary history was obtained, and subjects were instructed to maintain their usual intake and their regular level of activity for 6 days prior to isotope infusion. Subjects were enrolled into either a control group (group 1; n = 6) or a prednisone group (group 2; n = 8). Subjects enrolled into group 2 received a 6-day course of treatment with prednisone (0.8 mg·kg⁻¹·day⁻¹, with a maximum dose set at 60 mg/day) split in three daily doses; in contrast, subjects enrolled in group 1 (controls) did not receive any medication prior to the isotope infusion. Data concerning glutamine splanchnic uptake were obtained in five of six control subjects, and data concerning glutamine oxidation were available in seven of eight prednisone-treated subjects.

Isotope infusion protocol. For each protocol, the night before each infusion study day, each subject ate dinner at 1800 and then remained fasting (with the exception of water and calorie-free, caffeine-free drinks) until completion of the infusion study at 1300 the following day. On the morning at 0700, each subject was studied as an outpatient in the Baptist Medical Center/Wolfson Children’s Hospital Clinical Investigation Unit. Two short catheters were inserted, one in a forearm vein for isotope infusion and the other one in a superficial vein of the contralateral hand; the hand was placed in a warming pad at ~60°C to obtain arterialized venous blood samples (1). Five-hour continuous infusions of tracers were administered in the postabsorptive state between 0800 and 1300, as follows: 1) a primed, continuous infusion of 1-[1-⁴¹⁴C]leucine (0.08 µCi/kg·h·[⁴¹⁴C]leucine) and 1-[⁴⁻¹⁴C]glutamine (8 µmol·kg⁻¹·h⁻¹) was administered intravenously; and 2) 1-[⁴⁻¹⁴C]glutamine (8 µmol·kg⁻¹·h⁻¹) was administered orally as q. 20 min sips for 5 h, simultaneously with the intravenous infusion of 1-[1-⁴¹⁴C]leucine and 1-[⁴⁻¹⁴C]glutamine.

Arterialized venous blood samples were obtained 30 and 15 min before the start and at 30-min intervals between the second and the fifth hour of isotope infusion. Plasma was analyzed for the isotopic enrichment of glutamine and specific activity of plasma ^13C-glutamine (dpm/min) was determined by obtaining timed 2-min collections of expired air being trapped by slowly bubbling the expired air into a solution of ethanol-water (1:1). The CO₂ in the collected air was then quantitatively analyzed for carbon dioxide by mass spectrometry (Isochrom III, VG, Ipswich, UK). Plasma [¹³C]glycine enrichment was estimated as dietary [¹³C]glycine Ra, calculated as dietary [¹³C]glycine Ra = DGLN/G549 = 0.423 mol glutamine/kg·h.

Glutamine appearance into the plasma compartment (RaGLN; pmol/kg·h) was calculated as RaGLN = iGLN/GLN × [(Eid⁵-GLN + Eps⁵-GLN) - 1], where iGLN is the rate of [⁵²H]glutamine infusion and Eps⁵-GLN and Eid⁵-GLN are the [⁵²H]glutamine enrichments (mole percent excess) in the infused tracer solution and plasma at steady state, respectively. RaGLN is an index of interorgan glutamine exchange between tissues (12). Glutamine release from protein breakdown. Because glutamine is a nonessential amino acid, both glutamine release from protein breakdown (BGLN) and glutamine de novo synthesis (DGLN) contribute to glutamine Ra. Although body protein is known to contain 1.39 g of glutamine + glutamate per 100 g protein, the exact contribution of glutamine per se to that total amount is not precisely known. Recently, by using a novel analytical tool, human skeletal muscle protein was, however, shown to contain only 4.32 g glutamine per 100 g of bound amino acid residues. BGLN was therefore estimated as 0.423 × RaLEU (28). This approach assumes that 1) the release of an amino acid from proteolysis is proportional to its abundance in body protein; 2) 100 g of body protein contain 9,180 mg leucine, i.e., 70.1 mmol leucine (as 1 mmol leucine = 131 mg, and 9,180/131 = 70.1); and ~4,320 mg glutamine (= 29.6 mmol glutamine per 100 g of bound amino acid residues). BGLN was therefore estimated as 0.423 × RaLEU (28). This approach assumes that 1) the release of an amino acid from proteolysis is proportional to its abundance in body protein; 2) 100 g of body protein contain 9,180 mg leucine, i.e., 70.1 mmol leucine (as 1 mmol leucine = 131 mg, and 9,180/131 = 70.1); and ~4,320 mg glutamine (= 29.6 mmol glutamine per 100 g of bound amino acid residues). BGLN was therefore estimated as 0.423 × RaLEU (28). This approach assumes that 1) the release of an amino acid from proteolysis is proportional to its abundance in body protein; 2) 100 g of body protein contain 9,180 mg leucine, i.e., 70.1 mmol leucine (as 1 mmol leucine = 131 mg, and 9,180/131 = 70.1); and ~4,320 mg glutamine (= 29.6 mmol glutamine per 100 g of bound amino acid residues). This approach assumes that 1) the release of an amino acid from proteolysis is proportional to its abundance in body protein; 2) 100 g of body protein contain 9,180 mg leucine, i.e., 70.1 mmol leucine (as 1 mmol leucine = 131 mg, and 9,180/131 = 70.1); and ~4,320 mg glutamine (= 29.6 mmol glutamine per 100 g of bound amino acid residues). This approach assumes that 1) the release of an amino acid from proteolysis is proportional to its abundance in body protein; 2) 100 g of body protein contain 9,180 mg leucine, i.e., 70.1 mmol leucine (as 1 mmol leucine = 131 mg, and 9,180/131 = 70.1); and ~4,320 mg glutamine (= 29.6 mmol glutamine per 100 g of bound amino acid residues). This approach assumes that 1) the release of an amino acid from proteolysis is proportional to its abundance in body protein; 2) 100 g of body protein contain 9,180 mg leucine, i.e., 70.1 mmol leucine (as 1 mmol leucine = 131 mg, and 9,180/131 = 70.1); and ~4,320 mg glutamine (= 29.6 mmol glutamine per 100 g of bound amino acid residues). This approach assumes that 1) the release of an amino acid from proteolysis is proportional to its abundance in body protein; 2) 100 g of body protein contain 9,180 mg leucine, i.e., 70.1 mmol leucine (as 1 mmol leucine = 131 mg, and 9,180/131 = 70.1); and ~4,320 mg glutamine (= 29.6 mmol glutamine per 100 g of bound amino acid residues). This approach assumes that 1) the release of a...
groups by Student’s t-test. If nonnormally distributed, continuous variables were reported as medians ± interquartiles and compared between prednisone-treated and control groups by Mann-Whitney U-test. Categorical variables (sex) were reported as number of patients and compared by Fisher exact test. P values <0.05 were considered to be statistically significant.

RESULTS

Subjects characteristics. The clinical characteristics of the subjects studied are summarized in Table 1. No differences were observed between the two groups (e.g., sex, age, height, weight, and body mass index).

Leucine metabolism. Results of leucine kinetics are reported in Table 2. Plasma leucine and glutamine concentrations were near steady state during the 5 h of each isotope infusion study. Leucine concentration was significantly higher (P = 0.03) in prednisone-treated patients than in controls. No differences were observed in leucine Ra, leucine oxidation, and nonoxidative leucine disposal between the two groups (Fig. 1).

Glutamine metabolism. As expected, glutamine concentrations were higher (P = 0.02) in the prednisone group. Prednisone treatment increased glutamine Ra by ~37%, compared with the control group (medians ± interquartiles, 477.2 ± 60.2 vs. 346.8 ± 198.5 μmol·kg⁻¹·h⁻¹, P = 0.003) (Table 3 and Fig. 2). The increase in glutamine Ra was mostly due to an ~47% rise in glutamine de novo synthesis in the prednisone group (medians ± interquartiles, 412.0 ± 61.3 vs. 279.9 ± 190.4 μmol·kg⁻¹·h⁻¹, P = 0.003), since glutamine release from protein breakdown did not differ between groups (mean ± SE, 65.2 ± 2.0 vs. 59.6 ± 4.3 μmol·kg⁻¹·h⁻¹, NS) (Fig. 2).

Glutamine uptake and oxidation. Results of glutamine splanchnic uptake and glutamine oxidation are reported in Tables 4 and 5, respectively. Dietary [¹³C]glutamine Ra and splanchnic extraction rate of [¹³C]glutamine were ~68 and ~525% higher, respectively, in the prednisone group, compared with controls. Thus prednisone resulted in a 50% increase in the splanchnic extraction of glutamine (Table 4 and Fig. 3). In contrast, although breath [¹³C]CO₂ excretion and breath

Table 1. Physical characteristics of subjects enrolled

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 6)</th>
<th>Prednisone (n = 8)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F), n</td>
<td>3/3</td>
<td>4/4</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Age, yr</td>
<td>26.0±7.7</td>
<td>25.1±4.9</td>
<td>0.80</td>
</tr>
<tr>
<td>Height, cm</td>
<td>170.2±4.1</td>
<td>171.3±6.8</td>
<td>0.73</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>64.6±6.9</td>
<td>64.8±7.9</td>
<td>0.96</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.3±2.3</td>
<td>22.1±2.1</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Values are means ± SD, except for sex. BMI, body mass index.

13C enrichment were higher (increased by 216 and 236%, respectively, P < 0.01) in the prednisone group, the fraction of glutamine oxidized (as calculated from steady-state [¹³C]CO₂ in expired air) was not statistically different between the prednisone and control groups (Table 5 and Fig. 3). The increased rate of glutamine splanchnic uptake observed in prednisone-treated healthy volunteers therefore was not associated with an increased rate of glutamine oxidized.

DISCUSSION

Using stable isotope methodology in volunteers receiving prednisone treatment, a model of mild, stress-induced protein wasting, the present study provides evidence for an increased rate of splanchnic extraction of glutamine. Since prednisone treatment did not increase glutamine oxidation, the present findings demonstrate that high-dose glucocorticosteroids enhance the rate of glutamine utilization via nonoxidative pathways.

Prednisone increased plasma leucine concentration but had no effect on leucine Ra, which is in contrast to our previous studies. We believe that this was the result of intersubject variability that precluded statistical significance. Treatment

Table 2. Leucine kinetics in healthy volunteers receiving prednisone or not (control)

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>Prednisone (n = 8)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leu concentration, μmol/l</td>
<td>121.3±9.7</td>
<td>144.5±6.2</td>
<td>0.03</td>
</tr>
<tr>
<td>¹⁴C excreted, % of infused</td>
<td>14.6±1.6</td>
<td>13.2±1.6</td>
<td>0.56</td>
</tr>
<tr>
<td>RaLeu, μmol·kg⁻¹·h⁻¹</td>
<td>141.0±10.1</td>
<td>154.1±4.8</td>
<td>0.11</td>
</tr>
<tr>
<td>OxLeu, μmol·kg⁻¹·h⁻¹</td>
<td>31.4±3.1</td>
<td>33.9±4.4</td>
<td>0.33</td>
</tr>
<tr>
<td>NOLD, μmol·kg⁻¹·h⁻¹</td>
<td>109.6±8.1</td>
<td>120.2±6.7</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Values are means ± SE. Leu, leucine; Ra, rate of appearance; Ox, oxidation; NOLD, nonoxidative leucine disposal.

Table 3. Glutamine kinetics in healthy volunteers receiving prednisone treatment (prednisone group) or not (control group)

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>Prednisone (n = 8)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Gln concentration, μmol/l</td>
<td>565.3±93.0</td>
<td>658.5±97.0</td>
<td>0.02</td>
</tr>
<tr>
<td>RaGln, μmol·kg⁻¹·h⁻¹</td>
<td>346.8±198.5</td>
<td>477.2±60.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Bgln, μmol·kg⁻¹·h⁻¹</td>
<td>59.6±4.3</td>
<td>65.2±2.0</td>
<td>0.11</td>
</tr>
<tr>
<td>Dgln, μmol·kg⁻¹·h⁻¹</td>
<td>279.9±190.4</td>
<td>412.0±61.3</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are expressed as medians ± interquartiles (*) or means ± SE (†). Gln, glutamine; B, breakdown; D, de novo synthesis.
with glucocorticosteroids nevertheless increased glutamine Ra and de novo synthesis, as observed in earlier reports (6, 11).

Glucocorticosteroids indeed enhance glutamine de novo synthesis in rats (14), dogs (24), and humans (11). As already reported in hypercatabolic or corticosteroid-treated patients (5, 16, 26), the de novo synthesis of glutamine most likely occurs in peripheral tissues.

Nevertheless, a large body of evidence from earlier studies suggest that, in a clinical setting of stress-induced hypercatabolism, i.e., in patients admitted to intensive care unit (ICU) or patients with severe burn, depletion in plasma glutamine concentration occurs (16, 26). Therefore, glutamine is considered by many as a conditionally essential amino acid in the ICU (29). The specific mechanisms responsible for glutamine depletion in stress continue to be debated. From a theoretical standpoint, glutamine depletion can arise either from an insufficient production rate, or from an increased rate of glutamine utilization. The failure of de novo glutamine synthesis to rise in situations associated with stress (16, 26), contributes to glutamine depletion. Yet since glutamine production rate (Ra) was found to be increased in stress, increased rate of glutamine utilization must play a role as well. The primary event leading to glutamine depletion under stress conditions or corticosteroid treatment remains unclear.

The splanchnic bed is a “prime suspect” as a site of increased glutamine utilization during stress. In several animal models of sepsis, increased rates of glutamine splanchnic extraction have been reported (27, 40, 41), but little is known in humans. In the present paper, the fate of splanchnic glutamine was examined by using the concomitant infusion of [1-13C]glutamine and oral administration of [1-13C]glutamine. We and others (4, 17) have used [1-13C]glutamine by both routes over two consecutive 3-h periods. Because the kinetics of [2H5]- and [13C]-labeled glutamine were found to be similar when infused intravenously (18), the choice of tracer is unlikely to account for the observed changes in glutamine kinetics associated with prednisone. Accordingly, the 42% fractional extraction in the first pass in healthy controls under baseline conditions in the present report is consistent with the 50–74% extraction of enterally administered glutamine documented in earlier studies (4, 17, 18, 33). Consistent with previous reports, oxidation was the main fate of glutamine, as

### Table 4. Splanchnic glutamine uptake in healthy volunteers receiving prednisone or not (control)

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 5)</th>
<th>Prednisone (n = 8)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral infused [13C]Gln, μmol·kg⁻¹·h⁻¹</td>
<td>6.0±0.1</td>
<td>18.1±0.9</td>
<td>0.004</td>
</tr>
<tr>
<td>Ep, [13C]Gln, MPE†</td>
<td>1.1±0.08</td>
<td>1.5±0.2</td>
<td>0.33</td>
</tr>
<tr>
<td>Dietary [13C]Gln Ra, μmol·kg⁻¹·h⁻¹</td>
<td>4.4±2.9</td>
<td>7.4±1.6</td>
<td>0.02</td>
</tr>
<tr>
<td>SUGln, μmol·kg⁻¹·h⁻¹</td>
<td>1.6±2.9</td>
<td>10.0±2.7</td>
<td>0.004</td>
</tr>
<tr>
<td>Glu splanchnic uptake, % of oral dose of [13C]Gln†</td>
<td>42.3±12.1</td>
<td>64.1±5.9</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Values are expressed as medians ± interquartiles (*) or means ± SE (†). Gln, glutamine; Ep, plasma enrichment; MPE, mole percent excess; SUGln, glutamine splanchnic extraction rate.

### Table 5. Glutamine oxidation in healthy volunteers receiving prednisone or not (control)

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>Prednisone (n = 7)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breath [13C]CO₂, MPE×1,000†</td>
<td>38.7±7.8</td>
<td>150.2±34.2</td>
<td>0.0045</td>
</tr>
<tr>
<td>CO₂ production rate, ml/min†</td>
<td>197.6±16.4</td>
<td>180.9±8.7</td>
<td>0.18</td>
</tr>
<tr>
<td>Breath [13C]CO₂ excretion, μmol·kg⁻¹·h⁻¹</td>
<td>4.4±1.0</td>
<td>13.9±6.0</td>
<td>0.0045</td>
</tr>
<tr>
<td>Breath [13C]CO₂ excretion, % of infused [13C]†</td>
<td>76.7±4.2</td>
<td>75.4±3.6</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Values are expressed as medians ± interquartiles (*) or means ± SE (†). MPE, mole percent excess.
oxidation accounted for 75% of glutamine flux, compared with 69 and 83% in earlier studies (17, 18). In addition, Haisch et al. (17) showed that 53% of the glutamine was oxidized specifically upon its first pass in the splanchnic bed.

The primary observation of the present study is that prednisone increased the splanchnic extraction of glutamine by ~50%, without altering the fraction of glutamine undergoing oxidation. This result is consistent with those of Suojaranta-Ylinen et al. (41), who showed that 2 h after a coronary bypass the splanchnic extraction of glutamine rose by 56%, along with that of alanine, serine, and threonine. In sepsis, glutamine uptake by the gut is decreased (27). In contrast, endotoxemia was found to be associated with a dramatic rise in glutamine uptake by the liver (2). We demonstrated that prednisone increased significantly the splanchnic uptake of glutamine. This is consistent with the view that the increase of glutamine splanchnic uptake may be an early event in catabolic states and a major contributor to glutamine depletion under these conditions.

From a therapeutic standpoint, the fact that splanchnic glutamine extraction rate increased under stress conditions suggests that the enteral route may not be optimal to increase glutamine availability in peripheral tissues in acutely ill patients. Moreover the effect of intestinal inflammation on glutamine utilization is unknown. In patients with quiescent Crohn’s disease, glutamine splanchnic extraction and oxidation were found unchanged compared with healthy controls (4), but the effects of acute intestinal inflammation, and, with glucocorticosteroids, on glutamine uptake have yet to be determined.

Despite increased extraction in the splanchnic bed, the rate of glutamine oxidation failed to increase in our prednisone-treated healthy subjects. The ultimate fate of the glutamine extracted in the splanchnic bed remains to be elucidated. Glutamine is known to play a pivotal role in the gut, because glutamine not only serves as a preferred fuel for the enterocytes but also helps maintain rates of intestinal protein synthesis (8, 18) and increases ubiquitin mRNA level in human gut mucosa. Enteral glutamine stimulates protein synthesis and decreases ubiquitin mRNA level in human gut mucosa. Am J Physiol Endocrinol Metab 285: E366 –E373, 2003.

In summary, we confirm that oral prednisone administration, even to healthy volunteers, increases glutamine Ra because of an increased glutamine de novo synthesis. In addition, we show that a 6-day prednisone treatment induces an increased rate of glutamine extraction in the splanchnic bed, without any change in glutamine oxidation. Although the fate of nonoxidized glutamine in splanchnic bed remains to be ascertained, the increased rate of glutamine splanchnic extraction may serve to maintain intestinal mucosal protein synthesis or immune function or help maintain the enhanced supply of glutathione or arginine that are in increased demand during critical illness. These data further suggest that glutamine supplementation may be important to maintain the metabolic needs of the gut and splanchnic tissues during catabolic stress. The latter deserves further study.

ACKNOWLEDGMENTS

We are indebted to Bernice Rutledge, RN, and her nursing team at Wolfson Children’s Hospital for the care of our subjects. We acknowledge the technical help of Lynda Everline for amino acid analysis.

GRANTS

This work was supported in part by National Institute of Diabetes and Digestive and Kidney Disease Grant RO1 DK-51477 and by a grant from the Nemours Foundation, Jacksonville, FL.

REFERENCES


