Effects of wine intake on postprandial plasma amino acid and protein kinetics in type 1 diabetes¹⁻³

Paolo Tessari, Daniela Bruttomesso, Alessandro Pianta, Monica Vettore, Michela Zanetti, Edward Kiwanuka, and Elisabetta Iori

ABSTRACT

Background: Alcohol may impair protein turnover and insulin sensitivity in vivo.

Objective: The acute effects of moderate wine intake on amino acid kinetics and on the fractional synthetic rate (FSR) of albumin and fibrinogen in patients with type 1 diabetes were studied.

Design: Six patients with type 1 diabetes ingested an elementary mixed meal (46 kJ/kg) over 4 h, first without and 3 mo later with 2 glasses of wine. Postprandial glucose concentrations were maintained at <10 mmol/L.

Results: Postprandially, the FSR of fibrinogen was 30% greater (21.5 ± 6.6% compared with 14.1 ± 3.6% of pool/d; P < 0.01) and glucagon concentrations were 40% greater (103 ± 20 compared with 61 ± 13 ng/L; P < 0.015) with wine than without wine. However, the FSR of albumin and the rates of appearance of total and endogenous phenylalanine and leucine were not significantly different between treatments. First-pass splanchnic uptake (in μmol·kg⁻¹·min⁻¹) of dietary phenylalanine (0.22 ± 0.02 compared with 0.19 ± 0.02) and leucine (0.25 ± 0.04 compared with 0.14 ± 0.02) were greater with wine (P < 0.05), whereas dietary phenylalanine oxidation was lower with wine, by 25% (0.10 ± 0.02 compared with 0.14 ± 0.01 μmol·kg⁻¹·min⁻¹; P < 0.05). Selected amino acid concentrations were significantly lower but glutamate concentrations were significantly higher with wine.

Conclusions: In insulin-infused patients with type 1 diabetes, moderate wine intake with a meal resulted in (1) a higher fibrinogen FSR, glucagon concentration, and first-pass splanchnic uptake of leucine and phenylalanine; (2) lower dietary phenylalanine oxidation; (3) selective changes in plasma amino acid concentrations; and (4) no impairment in endogenous protein synthesis and albumin synthesis.

KEY WORDS Alcohol, protein kinetics, phenylalanine oxidation, amino acid concentrations, redox state, type 1 diabetes, wine

INTRODUCTION

Moderate intakes of wine (preferably red) are usually allowed in the diets of patients with type 1 diabetes (1, 2). Thus, knowledge of all the possible metabolic effects of alcohol in diabetes is relevant. Regarding glucose metabolism, although alcohol may affect both insulin action (3, 4) and intermediary metabolism (5), moderate wine intakes do not worsen postprandial glucose homeostasis in patients with type 1 diabetes (6, 7). However, alcohol may also affect amino acid and protein metabolism. In rat liver preparations, ethanol administration decreases protein synthesis (8–10) and α-amino acid transporters. In healthy volunteers that ingestion of a bottle of wine suppresses the physiologic, postprandial increase in albumin synthesis and inhibits leucine oxidation (16, 17). These findings suggest both an inhibition of hepatic protein synthesis and a protein-sparing effect in the whole body. The adverse effect on albumin synthesis was dose dependent because it was less pronounced when ~50% less alcohol (30 g) was ingested (17). Also, changes in hormone secretion and effectiveness may mediate the alcohol-dependent effects on amino acid and protein metabolism (18). Indeed, alcohol impairs the metabolic effects of insulin (3, 4, 15) and increases glucagon secretion (19), which may interfere with protein turnover (20).

Despite these potentially adverse effects, whether a moderate alcohol intake impairs postprandial amino acid and protein turnover in patients with type 1 diabetes has never been investigated. Thus, we studied the acute effects of a moderate intake of wine (~300 mL, or ~2 glasses) with a mixed meal on postprandial amino acid and protein turnover in patients with type 1 diabetes. To avoid any potential variability due to unpredictable insulin absorption with the usual subcutaneous route of therapy, insulin was infused intravenously at similar rates in the 2 studies.
under both fasted and fed conditions. Whole-body and splanchnic amino acid turnover were determined with the use of oral and intravenous isotopic leucine and phenylalanine infusions. The fractional synthetic rates (FSRs) of albumin and fibrinogen were evaluated on the basis of precursor-product relations.

SUBJECTS AND METHODS

Subjects

Six patients with type 1 diabetes [5 men and 1 woman aged 38 ± 5 y (± SEM)] and a normal body mass index (23.8 ± 0.8; in kg/m²) were recruited. The duration of diabetes in the patients was 14 ± 3 y. None of the patients had β cell secretion, as indicated by the lack of a response in C-peptide with intravenous glucagon stimulation. The patients had been treated with intensive subcutaneous insulin therapy, consisting of 3 premeal boluses and a fixed baseline rate, via portable pumps (43 ± 3 U/d) for ≥ 6 mo before the study began. None of the patients had clinical or instrumental evidence of proliferative retinopathy, autonomic neuropathy, overt diabetic nephropathy, or gastroenteric disease; 2 patients were microalbuminuric and 2 had signs of background retinopathy. For ≥30 d before the study began, the patients consumed a weight-maintaining diet (∼8.37 MJ/d) providing ∼50% of energy as carbohydrate, ∼20% as protein, and ∼30% as fat. Two patients usually drank about one glass of wine at lunch and dinner; the remaining 4 patients consumed no wine or other alcoholic beverages at any time of the day. All patients were informed about the aims of the study and gave their consent. The study was approved by the local institutional review board, was performed in compliance with the Helsinki Declaration, and followed the recommendations of the local Radiation Safety Officer.

Isotopes

The stable isotopes L-[5,5,5-2H]leucine ([D₅]leucine) and L-[1-14C]leucine ([1⁴C]leucine), both >98% pure (21, 22), were purchased from Tracer Technologies (Somerville, MA). The radioisotope tracer, tested by HPLC (23), was [3H]phenylalanine (∼82% and that of [1⁴C]phenylalanine was ∼92%. The actual isotope-infusion rates were corrected accordingly. All isotopes were dissolved in sterile saline, filtered through a 0.2-μm filter (Millipore, Molsheim, France), and proven to be sterile and pyrogen-free before use.

Meal preparation

A liquid mixed meal of elementary composition was freshly prepared on the morning of the study. Its energy content (∼46 kJ/kg body wt) was similar to that of each patient’s usual lunch, and it contained 50% of energy as carbohydrate, 18% as crystalline amino acids, and 32% as fat. The carbohydrate content of the meal consisted of sucrose and glucose, added in part to the amino acid solution (see below) to make up a chocolate spread, which consisted of butter (0.42 g/kg body wt) and cocoa powder (∼4 g). The amino acid composition was designed to match the average amino acid content of meat proteins. The amino acids were dissolved in 1020 mL water and were administered in the following amounts (in mg/kg body wt): aspartic acid, 50.7; threonine, 25.3; serine, 23.2; glutamine, 81.3; glycine, 29.6; alanine, 29.6; cysteine, 6.3; methionine, 14.8; tyrosine, 20.1; phenylalanine, 22.2; lysine, 46.4; histidine, 16.9; arginine, 32.7; proline, 24.3; isoleucine, 28.5; leucine, 43.3; valine, 30.6; and tryptophan, 56.9. To this solution, ∼3.0–3.7 MBq [1³C]phenylalanine and ∼50 μmol [1⁴C]leucine/kg body wt were added. On the basis of a previous dosimetry study with [3H]leucine and [1⁴C]leucine (KL Classic, WF Schwenk, and MW Haymond, unpublished observations, 1985), exposure to the phenylalanine radioactive tracer should result in a whole-body absorbed dose of ∼1 mGy because phenylalanine turnover follows that of leucine (ie, that of proteins), and the fractional oxidation of dietary phenylalanine and leucine are approximately the same (22, 24). The natural mole percent enrichment (MPE) of [1⁴C]leucine in the amino acid solution and in the meal enriched with [1⁴C]leucine were measured. The meal was administered in study 1 without wine and in study 2 with red wine (Red Toci, 12% alcohol by vol; G Bruttomesso, Vicenza, Italy); the red wine provided an additional 18.4 kJ/kg body wt (ie, ∼300 mL over 4 h) and accounted for 28% of the total dietary energy.

Experimental design

The patients were admitted to the Metabolic Unit of the Department of Diabetes and Metabolic Diseases (University of Padova, Padova, Italy) 1 d before the study began. The subcutaneous bolus of insulin usually injected by the subjects at 1800, was reduced by ∼50% on the day preceding the study and the basal subcutaneous insulin infusion by the portable pump was discontinued. At 2100, an intravenous catheter was placed in a forearm vein and was used for overnight insulin infusion at a rate of 0.5–1.0 U/h to maintain plasma glucose concentrations between 5 and 10 mmol/L (Figure 1). This glycemic target was chosen to avoid both hypoglycemic and excessive hyperglycemic episodes. At 0700 on the study day, a dorsal hand vein of the forearm opposite the arm with the intravenous catheter was cannulated in a retrograde fashion and placed in a warming box maintained at 55 °C for arterialized venous blood sampling. After collection of baseline sample, primed continuous infusions of [1⁴C]phenylalanine (∼160–170 Bq·kg⁻¹·min⁻¹) and of [D₅]leucine (∼0.05 μmol·kg⁻¹·min⁻¹) were started and maintained throughout the study. The priming doses were equivalent to ∼60 times the continuous infusion rate per minute. Two hours were allowed to achieve steady state substrate and isotope concentrations (Figure 2). During this period, the infusion rate of intravenous insulin was slightly adjusted to further decrease plasma glucose toward near-normal values, but it was kept constant at least between −60 and 0 min (Figure 1). Blood samples were collected at −60, −30, −20, −10, and 0 min for the measurement of substrate concentrations, baseline hormone and amino acid concentrations, the specific activity (SA) of [1⁴C]phenylalanine and of albumin- and fibrinogen-bound phenylalanine, and the MPE of [D₅]leucine and [D₅]α-ketoisocaproate (KIC). Thereafter, the test meal was administered.

Study 1: meal without wine

Study 1 was performed first to titrate in each patient the prandial infusion rate of intravenous insulin. The liquid meal was given as 14 isoenergetic aliquots every 20 min for 260 min to attain steady state plasma substrate, isotope, and hormone...
concentrations. The infusion rate of intravenous insulin was increased empirically in a stepwise fashion during the first 2 h of meal administration, with the target to maintain plasma glucose concentrations <10 mmol/L (Figure 1). In the last hour, the infusion rate of insulin was kept constant (Figure 1). Blood and breath samples were collected at 30, 90, 150, 190, 210, 230, 250, and 270 min, ie, 10 min after the ingestion of each meal aliquot. The total amount of regular insulin (10 ± 4 U) administered intravenously during the meal was not significantly different from the patients' preprandial, usual, subcutaneous insulin bolus (9 ± 1 U).

Study 2: meal with wine

Study 2 was conducted ≥3 mo after the completion of study 1; the procedures used were the same as in study 1. In the weeks preceding the study, the patients' usual daily dose of insulin delivered by pump (41 ± 2 U/d) was similar to that before study 1. Every effort was made to keep the dosage of insulin administered at 1800 and during the night on the day preceding study 2 the same for study 1. Isoenergetic aliquots of wine were given with each of the 14 meals, which were identical to the meals given in study 1. The pattern of the postprandial insulin infusion rate was kept similar to that in study 1, particularly after 170 min, irrespective of the resulting plasma glucose concentrations (Figure 1).

Analytic determinations

After blood withdrawal, the samples were rapidly centrifuged at ≈600 × g for 15 min at 4°C, and the plasma was stored at −20°C until assayed. Plasma concentrations of leucine (25) and phenylalanine (23) and the SA of plasma phenylalanine (23) and tyrosine (26) were measured by HPLC. The MPE of plasma

---

**FIGURE 1.** Mean (±SEM) insulin infusion rates and plasma glucose concentrations at baseline and after a meal with (●) or without (○) wine. n = 6.

**FIGURE 2.** Mean plasma phenylalanine and leucine concentrations, the specific activity (SA) of plasma [3H]phenylalanine and [14C]phenylalanine, the rate of 14CO2 expiration, and the mole percent enrichment (MPE) of [D3]leucine, [D3]-ketoisocaproate (KIC), and [13C]leucine at baseline and after the meal without (empty symbols) or with (filled symbols) wine. n = 6.
leucine and KIC was determined by using gas chromatography–mass spectrometry as t-butyldimethylsilyl derivatives with electron impact ionization (21, 22). Plasma amino acid concentrations were measured by using ion-exchange chromatography with an amino acid analyzer (Beckman, Palo Alto, CA). Plasma insulin, glucagon, and C-peptide concentrations were measured by radioimmunoassay (22). In the patients with type 1 diabetes, plasma free insulin concentrations were measured after precipitation of insulin-antibody complexes with the use of polyethylene glycol. Albumin and fibrinogen were isolated from plasma as described by Horber et al (27) in 5 of the 6 patients (paired data). Hydrolysis was accomplished by adding 4 mL of 4 mol HCl to the isolated protein and by incubating the samples in airtight vials at 110°C for 72 h. The protein-derived free amino acids were further purified through columns containing 3 mL of an Ag 50 × 8 cation-exchange resin, and the SA of [3H]phenylalanine was determined by HPLC (23). Blood alcohol concentrations were measured with the use of standard techniques.

Calculations

All kinetic data, including the FSRs of albumin and fibrinogen, were calculated at steady state conditions of plasma substrate and hormone concentrations, at steady state of the specific activity of phenylalanine, and at steady state of the MPE of leucine and KIC. With the combined use of intravenous and oral amino acid tracers, it was possible to calculate 1) the total rates of appearance (Ra) of leucine and phenylalanine, 2) the rate of entry into the systemic circulation of dietary leucine and phenylalanine postprandially (ie, dietary Ra), 3) the endogenous Ra of amino acids (derived from body protein degradation postprandially), 4) first-pass splanchnic uptake (expressed both as absolute values, ie, μmol·kg⁻¹·min⁻¹, and as a percentage of the oral administration rate, ie, fractional uptake) of dietary leucine and phenylalanine, 5) dietary phenylalanine oxidation (expressed both as absolute values and as a percentage of the oral administration rate), and 6) the FSRs of albumin and fibrinogen, which were calculated with the use of intravenous [3H]phenylalanine as the tracer. The calculations were performed with the use of established models and equations (27–33) and are reported in Appendix A.

Statistical analyses

All data are expressed as means ± SEMs. The paired two-tailed Student’s t test was used to compare the meal against the fasting period within each group and to compare the meal data for the same subjects on the 2 occasions (ie, after ingestion of the meal with and without wine). Bonferroni’s correction was used for multiple comparisons. STATISTICA (version 4; StatSoft Inc, Tulsa, OK) was used for the analyses. A P value <0.05 was considered statistically significant.

RESULTS

Insulin infusion rate and glucose, glycated hemoglobin, substrate, and hormone concentrations

The mean rates of intravenous insulin infusion between –30 and 0 min were not significantly different between studies 1 (1.1 ± 0.4 U/h) and 2 (1.3 ± 0.4 U/h) (Figure 1). These rates closely matched each patients’ usual overnight insulin requirement administered with the subcutaneous pump (data not shown). The resulting plasma free insulin concentrations were not significantly different between the 2 studies (Table 1). Fasting plasma glucose concentrations at −150 min were 9.1 ± 1 mmol/L in study 1 and 8.0 ± 0.1 in study 2 (NS). Between −150 and −30 min, plasma glucose concentrations slowly decreased further to baseline values (ie, between −30 and 0 min) that were not significantly different (Figure 1 and Table 1). Glycated hemoglobin concentrations before study 1 (8.5 ± 0.5%) and study 2 (8.2 ± 0.5%) were not significantly different. Fasting glucagon concentrations were also not significantly different between the 2 studies (Table 1).

After the start of the meal, the increase in intravenous insulin infusion rates to the same extent in both studies (Figure 1) resulted in similar plasma free insulin concentrations in the final 210–270 min (Table 1). Postprandial plasma glucose concentrations did not differ significantly between the 2 studies (Figure 1 and Table 1). Postprandial plasma glucagon concentrations did not increase significantly from baseline in study 1 postprandially, but did increase significantly from baseline (P < 0.01) in

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Meal without wine</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>6.8 ± 0.4</td>
<td>9.8 ± 1.1</td>
</tr>
<tr>
<td>Glycerol (mmol/L)</td>
<td>0.06 ± 0.01</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>Fatty acids (mmol/L)</td>
<td>0.48 ± 0.11</td>
<td>0.10 ± 0.03</td>
</tr>
<tr>
<td>Pyruvate (mmol/L)</td>
<td>0.45 ± 0.03</td>
<td>0.73 ± 0.06</td>
</tr>
<tr>
<td>3-OH (mmol/L)</td>
<td>0.19 ± 0.14</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>AcAc (mmol/L)</td>
<td>0.12 ± 0.02</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>11.8 ± 2.8</td>
<td>12.0 ± 1.9</td>
</tr>
<tr>
<td>Lactate/pyruvate</td>
<td>1.3 ± 0.8</td>
<td>0.3 ± 0.1</td>
</tr>
</tbody>
</table>

Significantly different from baseline, P ≤ 0.05.
Significantly different from meal without wine, P ≤ 0.05.

---

Statistical analyses

All data are expressed as means ± SEMs. The paired two-tailed Student’s t test was used to compare the meal against the fasting period within each group and to compare the meal data for the same subjects on the 2 occasions (ie, after ingestion of the meal with and without wine). Bonferroni’s correction was used for multiple comparisons. STATISTICA (version 4; StatSoft Inc, Tulsa, OK) was used for the analyses. A P value <0.05 was considered statistically significant.

RESULTS

Insulin infusion rate and glucose, glycated hemoglobin, substrate, and hormone concentrations

The mean rates of intravenous insulin infusion between −30 and 0 min were not significantly different between studies 1 (1.1 ± 0.4 U/h) and 2 (1.3 ± 0.4 U/h) (Figure 1). These rates closely matched each patients’ usual overnight insulin requirement administered with the subcutaneous pump (data not shown). The resulting plasma free insulin concentrations were not significantly different between the 2 studies (Table 1). Fasting plasma glucose concentrations at −150 min were 9.1 ± 1 mmol/L in study 1 and 8.0 ± 0.1 in study 2 (NS). Between −150 and −30 min, plasma glucose concentrations slowly decreased further to baseline values (ie, between −30 and 0 min) that were not significantly different (Figure 1 and Table 1). Glycated hemoglobin concentrations before study 1 (8.5 ± 0.5%) and study 2 (8.2 ± 0.5%) were not significantly different. Fasting glucagon concentrations were also not significantly different between the 2 studies (Table 1).

After the start of the meal, the increase in intravenous insulin infusion rates to the same extent in both studies (Figure 1) resulted in similar plasma free insulin concentrations in the final 210–270 min (Table 1). Postprandial plasma glucose concentrations did not differ significantly between the 2 studies (Figure 1 and Table 1). Postprandial plasma glucagon concentrations did not increase significantly from baseline in study 1 postprandially, but did increase significantly from baseline (P < 0.01) in
TABLE 2

Individual and total plasma amino acid concentrations at baseline and in the last hour of a meal with or without wine.

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Meal without wine&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Taurine</td>
<td>55 ± 6</td>
<td>49 ± 6</td>
</tr>
<tr>
<td>Aspartate</td>
<td>25 ± 3</td>
<td>23 ± 3</td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>11 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Threonine</td>
<td>131 ± 8</td>
<td>199 ± 19&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serine</td>
<td>127 ± 11</td>
<td>156 ± 16&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Asparagine</td>
<td>27 ± 6</td>
<td>20 ± 5&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glutamate</td>
<td>230 ± 27</td>
<td>245 ± 33</td>
</tr>
<tr>
<td>Glutamine</td>
<td>218 ± 22</td>
<td>248 ± 21</td>
</tr>
<tr>
<td>Proline</td>
<td>130 ± 13</td>
<td>192 ± 17&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glycine</td>
<td>245 ± 28</td>
<td>320 ± 37&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alanine</td>
<td>232 ± 18</td>
<td>391 ± 37&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Citrulline</td>
<td>29 ± 3</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>Amino butyrate</td>
<td>27 ± 4</td>
<td>29 ± 7</td>
</tr>
<tr>
<td>Valine</td>
<td>218 ± 12</td>
<td>308 ± 18&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methionine</td>
<td>5 ± 2</td>
<td>10 ± 4&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>53 ± 3</td>
<td>108 ± 10&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Leucine</td>
<td>123 ± 8</td>
<td>193 ± 12&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>41 ± 4</td>
<td>59 ± 3&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>50 ± 4</td>
<td>72 ± 2&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>42 ± 4</td>
<td>49 ± 3</td>
</tr>
<tr>
<td>Ornithine</td>
<td>59 ± 4</td>
<td>79 ± 7&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lysine</td>
<td>163 ± 11</td>
<td>265 ± 21&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Histidine</td>
<td>56 ± 3</td>
<td>76 ± 5&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Arginine</td>
<td>53 ± 5</td>
<td>94 ± 7&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total&lt;sup&gt;6&lt;/sup&gt;</td>
<td>24.0 ± 0.1</td>
<td>3.2 ± 0.2&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>x ± SEM; n = 6.
<sup>2</sup>Data from –30 to 0 min.
<sup>3</sup>Data from 190 to 270 min.
<sup>4</sup>Significantly different from meal without wine, P ≤ 0.05.
<sup>5</sup>Significantly different from baseline, P ≤ 0.05.
<sup>6</sup>In mmol/L.

study 2, to a value significantly greater (P < 0.015) than that in study 1 (Table 1).

Meal ingestion resulted in the suppression of lipolysis, as indicated by the decrease from baseline in both fatty acid and glycerol concentrations (Table 1). The suppression of fatty acids was slightly, although not significantly, lower in study 2 than in study 1. Blood lactate increased postprandially in both studies, significantly more so in study 2. In contrast, blood pyruvate and acetocetate concentrations did not change significantly after the meal in either study and were not significantly different between the 2 studies. However, the ratio of lactate to pyruvate postprandially in study 2 was significantly greater than that in study 1. The concentration of 3-hydroxybutyrate decreased significantly from baseline after the meal in study 1 but not in study 2. However, the resulting ratio of 3-hydroxybutyrate to acetocetate postprandially was significantly greater in study 2 than in study 1. In study 2, postprandial blood ethanol was undetectable in all but one subject, in whom it was just above the detection limits of the assay (data not shown).

Plasma amino acid concentrations at baseline were not significantly different between studies 1 and 2 (Table 2). Meal ingestion resulted in significant increases in the branched-chain and aromatic amino acids and in threonine, serine, proline, glycine, alanine, methionine, ornithine, lysine, histidine, and arginine in study 1. After the meal in study 2, concentrations of aspartate, hydroxyproline, threonine, serine, glycine, alanine, phenylalanine, lysine, and arginine were significantly lower than after the meal in study 1. In contrast, glutamate concentrations did not change significantly after the meal in study 1 but increased significantly after the meal in study 2, resulting in 30% greater concentrations than in study 1.

Amino acid and protein kinetics

The time pattern of plasma phenylalanine and leucine concentrations, the SA of [1H]phenylalanine and [13C]phenylalanine, of expired 14CO2, and of the MPE of [13C]leucine, [1H]KIC, and [13C]leucine are reported in Figure 2. Steady state was nearly achieved for all variables in both the fasting period and in the last 80 min of meal administration. The isotopic intravenous and oral infusion rates of the isotopes, the MPE of [13C]leucine, and the specific activity of [13C]phenylalanine are shown in Table 3. The steady state plasma MPE of [1H]leucine, [13C]KIC, [13C]leucine, and [13C]KIC; the SA of [1H]phenylalanine and [13C]phenylalanine; and expired 14CO2 are shown in Table 4.

The kinetics of leucine and phenylalanine are shown in Table 5. In the fasting state, no significant differences in the Ra of leucine or phenylalanine were observed between the 2 studies. The total Ra of leucine and phenylalanine increased significantly from baseline after the meal in studies 1 and 2 to roughly similar values in both studies, although the relative increase in the leucine Ra (primary pool) tended to be lower (NS) in study 2 than in study 1.

The first-pass splanchnic uptake of both leucine and phenylalanine was significantly greater in study 2 than in study 1 (Table 5, Figure 3). Conversely, the dietary Ra of both amino acids was significantly lower in study 2 than in study 1 (Table 5). The endogenous Ra of leucine decreased significantly from baseline in both studies, whereas the changes in endogenous phenylalanine Ra were not significant in either study (Table 5). The postprandial endogenous Ra of both amino acids was not significantly different between the 2 studies.

Dietary phenylalanine oxidation was significantly lower after the meal in study 2 than in study 1, when expressed as absolute values (Table 5) and as a percentage of the amount ingested (Figure 3). The ratios of the SA of [1H]tyrosine to [1H]phenylalanine, [1H]tyrosine to [13C]phenylalanine, and [14C]phenylalanine to [14C]phenylalanine are shown in Table 6.

TABLE 3

Intravenous and oral infusion rates of leucine and phenylalanine isotopes, the mole percent enrichment (MPE) of [13C]leucine, and the specific activity (SA) of [13C]phenylalanine in the meal

<table>
<thead>
<tr>
<th></th>
<th>Study 1 (without wine)</th>
<th>Study 2 (with wine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous isotope infusion</td>
<td>[14C]Phenylalanine (Bq · kg&lt;sup&gt;−1&lt;/sup&gt; · min&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Oral isotope infusion</td>
<td>[13C]Phenylalanine (μmol · kg&lt;sup&gt;−1&lt;/sup&gt; · min&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>0.123 ± 0.001</td>
</tr>
<tr>
<td>Meal</td>
<td>[14C]Phenylalanine (MPE)</td>
<td>116 ± 8</td>
</tr>
<tr>
<td></td>
<td>SA of [13C]Phenylalanine (mBq/mol)</td>
<td>256 ± 18</td>
</tr>
</tbody>
</table>

<sup>1</sup>x ± SEM; n = 6. There were no significant differences between groups.
which are an indirect index of phenylalanine flux through the hydroxylation pathway (34), were not significantly different at baseline (study 1: 0.10 ± 0.06; study 2: 0.10 ± 0.04) or postprandially (study 1: 0.12 ± 0.06; study 2: 0.12 ± 0.04) (n = 4).

The FSR of albumin was not significantly different between the 2 studies in the postabsorptive state, and it was stimulated to similar amounts after meal ingestion (Figure 4). The postprandial FSR of albumin was not significantly different between the 2 studies when calculated with the oral $^{14}$Cphenylalanine tracer (study 1: 26.2 ± 3.2% of pool/d; study 2: 29.7 ± 5.2% of pool/d). The FSR of fibrinogen was not significantly different at baseline (study 2: 3.2% of pool/d; study 2: 3.2% of pool/d). Also, with the oral $^{14}$Cphenylalanine tracer, the postprandial FSR of fibrinogen was significantly greater in study 2 (60 ± 5.2% of pool/d) than in study 1 (45.2 ± 1.5% of pool/d). The postprandial FSRs of both proteins calculated with the oral $^{14}$Ctracer were significantly greater (by 2–3 fold, P < 0.002) than those calculated with the intravenous $^{3}$Htracer.

**DISCUSSION**

This study showed that the ingestion of a moderate amount of red wine with a standard mixed meal over 4 h increased postprandial fibrinogen synthesis, glucagon concentrations, and the first-pass splanchnic uptake of ingested leucine and phenylalanine in insulin-infused patients with type 1 diabetes. Wine intake decreased the postprandial concentrations of some amino acids but increased glutamate. In contrast, wine intake did not affect postprandial plasma glucose concentrations, endogenous protein breakdown, or albumin synthesis. Thus, alcohol did not impair the inhibitory effect of insulin on endogenous proteolysis (18) or counteract the insulin-mediated stimulation of albumin synthesis (35).

**TABLE 4**

Steady state plasma mole percent enrichment (MPE) of leucine and $\alpha$-ketoisocaproate (KIC) isotopes, the specific activity (SA) of phenylalanine isotopes, and expired $^{14}$CO$_2$ at baseline and in the last hour of a meal with or without wine.

<table>
<thead>
<tr>
<th></th>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Meal without wine</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$[^{3}$H$]_{[^{1}}$Leucine (MPE)</td>
<td>$2.61 \pm 0.12^{[a]}$</td>
<td>$4.01 \pm 0.14$</td>
</tr>
<tr>
<td>$[^{14}$C$]_{[^{1}}$KIC (MPE)</td>
<td>$1.88 \pm 0.04^{[a]}$</td>
<td>$2.34 \pm 0.11$</td>
</tr>
<tr>
<td>$[^{13}$C$]$Leucine (MPE)</td>
<td>$5.39 \pm 0.32$</td>
<td>—</td>
</tr>
<tr>
<td>$[^{12}$C$]$KIC (MPE)</td>
<td>$5.30 \pm 0.24$</td>
<td>—</td>
</tr>
<tr>
<td>SA of $[^{3}$H$]$phenylalanine (mBq/nmol)</td>
<td>$248 \pm 41$</td>
<td>$191 \pm 30^{[a]}$</td>
</tr>
<tr>
<td>SA of $[^{14}$C$]$phenylalanine (mBq/nmol)</td>
<td>$62 \pm 8$</td>
<td>—</td>
</tr>
<tr>
<td>Expired $^{14}$CO$_2$ (Bq kg$^{-1}$ min$^{-1}$)</td>
<td>$37 \pm 2$</td>
<td>—</td>
</tr>
</tbody>
</table>

$^{[a]}$SEM; n = 6.
$^{[b]}$Data from 190 to 270 min.
$^{[c]}$Significantly different from baseline, $P \leq 0.05$.
$^{[d]}$Significantly different from meal without wine, $P \leq 0.05$.

**TABLE 5**

Leucine and phenylalanine kinetics at baseline and in the last hour of a meal with or without wine.

<table>
<thead>
<tr>
<th></th>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Meal without wine</td>
</tr>
<tr>
<td><strong>Leucine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ra in primary pool</td>
<td>$1.29 \pm 0.09$</td>
<td>$2.04 \pm 0.10^{[a]}$</td>
</tr>
<tr>
<td>Ra in reciprocal pool</td>
<td>$2.14 \pm 0.07$</td>
<td>$2.87 \pm 0.13^{[a]}$</td>
</tr>
<tr>
<td>Dietary Ra</td>
<td>—</td>
<td>$0.97 \pm 0.02$</td>
</tr>
<tr>
<td>Endogenous Ra in primary pool</td>
<td>$1.29 \pm 0.09$</td>
<td>$1.07 \pm 0.09^{[a]}$</td>
</tr>
<tr>
<td>Endogenous Ra in reciprocal pool</td>
<td>$2.14 \pm 0.07$</td>
<td>$1.50 \pm 0.10^{[a]}$</td>
</tr>
<tr>
<td>First-pass splanchnic uptake</td>
<td>—</td>
<td>$0.12 \pm 0.02$</td>
</tr>
<tr>
<td><strong>Phenylalanine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ra</td>
<td>$0.80 \pm 0.13$</td>
<td>$0.98 \pm 0.10^{[a]}$</td>
</tr>
<tr>
<td>Dietary Ra</td>
<td>—</td>
<td>$0.23 \pm 0.02$</td>
</tr>
<tr>
<td>Endogenous Ra</td>
<td>$0.80 \pm 0.13$</td>
<td>$0.75 \pm 0.09$</td>
</tr>
<tr>
<td>First-pass splanchnic uptake</td>
<td>—</td>
<td>$0.19 \pm 0.02$</td>
</tr>
<tr>
<td>Dietary oxidation</td>
<td>—</td>
<td>$0.14 \pm 0.01$</td>
</tr>
</tbody>
</table>

$^{[a]}$SEM; n = 6. Ra, rate of appearance.
$^{[b]}$Data from 30 to 0 min.
$^{[c]}$Data from 190 to 270 min.
$^{[d]}$Significantly different from baseline, $P \leq 0.05$.
$^{[e]}$Significantly different from meal without wine, $P \leq 0.05$. 

$^{[f]}$Data from 190 to 270 min.
amount of alcohol used in the present study was moderate, resulting in virtually undetectable ethanol concentrations in peripheral blood (6, 7), consistent with nearly complete ethanol oxidation by liver alcohol dehydrogenase (36). Nevertheless, alcohol induced a shift to the reduced state of the redox equilibrium, as indicated by the ratio of lactate to pyruvate and of 3-hydroxybutyrate to acetoacetate (37).

The increase in the apparent first-pass splanchnic uptake of leucine and phenylalanine after wine intake may have been due to a true increase in amino acid extraction by splanchnic tissues (ie, the gut and liver) or to a decrease in intestinal absorption, uptake, or both. The techniques used in the present study did not allow us to distinguish between these 2 possibilities; therefore, a decrease in intestinal amino acid absorption after wine intake cannot be entirely excluded (8, 13, 38–41). However, inhibition of hepatic amino acid uptake would result in an increase (not a decrease as observed in the present study) in the posthepatic delivery of dietary amino acids. Despite the increase in splanchnic uptake of leucine and phenylalanine, the concentrations and total Ra of leucine and phenylalanine were not significantly affected by wine intake, probably because the dietary Ra of the amino acids represents a minor fraction of the total postprandial Ra of amino acids. The lower postprandial concentrations of aspartate, hydroxyproline, threonine, serine, glycine, alanine, phenylalanine, lysine, and arginine and increase in glutamate after wine intake agrees with the findings of previous reports (42–46). These effects, apart from possible changes in intestinal absorption, might be largely due to alcohol-induced modifications in the metabolic pathways of amino acids (47, 48) resulting from changes in the redox state (decreased ratio of NAD to NADH), in the limited availability of pyruvate, and in the increase in the ratio of lactate to pyruvate (37).

The greater postprandial FSR of fibrinogen after wine intake may have been due to the ethanol-induced activation of the pancreatic α cell (19) and to postprandial hyperglucagonemia. Indeed, glucagon was shown to stimulate fibrinogen synthesis acutely (31). The finding of greater FSRs after oral [14C]phenylalanine than after intravenous [3H]phenylalanine tracers agrees with the findings of a previous report (49), suggesting a preferential use of dietary rather than endogenously derived amino acids for visceral protein synthesis in the postprandial state. Alternatively, the greater values with the oral tracer may reflect an underestimation of the correct hepatic precursor pool in peripheral plasma samples. Indeed, sampling of both ingested tracer and tracee in the portal vein, ie, at the site where they are channeled to the liver after absorption, is not feasible in humans.

In contrast with our findings, previous studies showed that ingestion of a higher dose of ethanol (750 mL wine) than that used in our study by healthy volunteers results in lower postprandial fibrinogen FSRs than does ingestion of a lower dose of wine (300 mL) or no wine (16, 50). The reason for this discrepancy is not clear. It may be possible that healthy subjects respond differently to wine intake than do patients with type 1 diabetes and that the amount of ethanol ingested is a factor.

Whether the extra energy ingested because of the addition of wine to the meal was responsible for the greater postprandial FSRs of fibrinogen and for the other differences observed in study 2 could not be determined from the results of our study. In previous studies, ingestion of amino acid–complete or amino acid–deficient meals resulted in no significant changes from
baseline in the FSRs of fibrinogen in healthy subjects (16, 50, 51), suggesting no role of energy derived from dietary sources on the observed differences. On the other hand, a relative increase in the ingestion of amino acids, carbohydrates, or lipids—alone or in combination—by a theoretically energy-matched control group might result in significant changes in the metabolic kinetics of amino acids or protein, sometimes in opposite or unpredictable directions (52–56). Nevertheless, the effect of increased energy intakes on the FSR of fibrinogen needs to be investigated further.

The acute stimulatory effect of alcohol on fibrinogen synthesis in type 1 diabetes might appear in contrast with the observation that chronic alcohol intake decreases fibrinogen concentrations (57, 58). Note, however, that in the present study we tested the effects of alcohol intake in relatively acute conditions, ie, over 4 h. In addition, the test was performed in diabetic subjects who, for the most part, were not accustomed to drinking alcoholic beverages. Furthermore, whether patients with type 1 diabetes respond differently from healthy control subjects to alcohol intake needs to be investigated further. Notably, the FSRs of albumin and fibrinogen were not significantly different between insulin-infused patients with type 1 diabetes and healthy subjects in response to a mixed meal (59).

The known increased redox state with wine ingestion (60, 61) may have accounted for the inhibition of phenylalanine oxidation (ie, decarboxylation of carbon in the 1-position) observed in the present study, in agreement with previous reports in healthy subjects in which a leucine tracer was used (16, 17). This effect must have largely occurred within the liver, which is the major site of phenylalanine decarboxylation (62). Such an amino acid–sparring effect of alcohol could also have counterbalanced a possible impairment in phenylalanine intestinal absorption, helping to maintain a normal postprandial albumin synthesis. Although we did not actually measure the first irreversible step of phenylalanine catabolism (ie, hydroxylation to tyrosine), which precedes oxidation (62), phenylalanine flux through the hydroxylation step was apparently not modified by wine nor did it move in a direction opposite to that of oxidation. Thus, the observed inhibition of oxidation should indicate a true amino acid–sparring effect. Interestingly, the decrease in phenylalanine oxidation occurred despite postprandial hyperglycemia, which was previously shown to stimulate phenylalanine oxidation (26). Thus, a larger suppressive direct effect by alcohol on oxidation might have been blunted by concurrent hyperglycemia.

In conclusion, intake of ~300 mL (~2 glasses) red wine with a mixed meal by insulin-infused patients with type 1 diabetes increased the FSR of fibrinogen, glucagon and glutamate concentrations, and intrasplanchic dietary amino acid retention, whereas it decreased phenylalanine oxidation and concentrations of selected amino acids. In contrast, wine intake with a meal did not impair postprandial glucose concentrations, albumin synthesis, or endogenous proteolysis. These effects should be considered in the dietary recommendations for patients with type 1 diabetes.

We thank Noemi Dussini (Pediatric Department, University of Padova) for the amino acid analyses and Antonio Piccoli for help with the statistical analysis.

REFERENCES


APPENDIX A

The total rate of appearance (Ra) of leucine was calculated from the [D₃]leucine tracer with conventional formulas (1) by using both the primary pool model (also known as the Ra of plasma leucine) and the reciprocal pool model (also known as the Ra of intracellular leucine). The total Ra of plasma phenylalanine was calculated from the intravenously infused [³H]phenylalanine tracer by using conventional steady state formulas (2).

The rate of entry into the systemic circulation of the oral [¹³C]leucine tracer (defined as dietary [¹³C]leucine Ra) (3) was calculated as follows:

\[ \text{Dietary [¹³C]leucine Ra} = \frac{\text{[inavenous [D₃]leucine infusion \times \text{plasma [¹³C]leucine MPE}}]}{\text{plasma [D₃]leucine MPE}} \quad (A1) \]

where the infusion of [D₃]leucine is in \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\) and MPE is the mole percent enrichment. Similarly, the rate of entry into the systemic circulation of the oral [¹³C]phenylalanine tracer (defined as dietary [¹³C]phenylalanine Ra) was calculated as follows (3, 4):

\[ \text{Dietary [¹⁴C]phenylalanine Ra} = \frac{\text{[inavenous [³H]phenylalanine infusion \times [¹⁴C]phenylalanine SA}}{\text{[³H]phenylalanine SA}} \quad (A2) \]

where the infusion of [³H]phenylalanine is in \(\text{mBq} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\) and the SA (specific activity) of [¹⁴C]phenylalanine and [³H]phenylalanine is in \(\text{mBq/nmol}\). The first-pass fractional splanchnic uptakes (as a percentage) of the oral tracers were then calculated as follows (3):

\[ \text{[¹³C]Leucine fractional uptake} = \left[ 1 - \frac{\text{[¹³C]leucine Ra}}{\text{[¹³C]leucine infusion}} \right] \times 100 \quad (A3) \]

and

\[ \text{[¹⁴C]Phenylalanine fractional uptake} = \left[ 1 - \frac{\text{[¹⁴C]phenylalanine Ra}}{\text{[¹⁴C]phenylalanine infusion}} \right] \times 100 \quad (A4) \]

The first-pass fractional splanchnic uptakes (in \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\)) of dietary leucine and phenylalanine were then calculated by multiplying the infusion rates of leucine and phenylalanine in the meal by the fractional uptakes of leucine and phenylalanine (Equations A3 and A4). This approach assumes that the splanchnic bed behaves as a single compartment, ie, includes both the gut and liver (3), and that the entire dietary amino acid load was absorbed.

The systemic Ra of dietary leucine and phenylalanine (in \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\)) was calculated by dividing the Ra of the isotope (Equations A1 and A2) by the corresponding MPE of leucine or the SA of phenylalanine in the meal.

The endogenous Ra of leucine was calculated by subtracting the dietary leucine Ra, calculated either with the primary (Equation A1) or the reciprocal pool model (Equation A5), from the total postprandial Ra of leucine, also calculated with the corresponding model.

\[ \text{Dietary leucine Ra}_{dp} = \frac{\text{[D₃]leucine infusion \times [¹³C]KIC MPE}}{\text{(D₃)KIC MPE \times meal \ [¹³C]leucine MPE}} \quad (A5) \]

As discussed by Biolo et al (3), the use of the Ra of dietary leucine calculated by using the reciprocal pool (Ra rp) model [ie, using the ratio between plasma MPEs of [D₃]KIC and [¹³C]KIC] is preferable because it accounts for the intracellular appearance of endogenous whole-body leucine, ie, it provides a more precise estimate of leucine release from endogenous proteolysis. In contrast, the use of a primary pool model to calculate the systemic Ra of dietary leucine (Equation A1) is justified by the fact that leucine (but not KIC) is contained in the meal (3).

Then

\[ \text{Endogenous leucine Ra}_{dp} = \text{total leucine Ra}_{dp} - \text{dietary leucine Ra}_{dp} \quad (A6) \]

The endogenous Ra of phenylalanine was calculated by subtracting the dietary Ra of phenylalanine from the total Ra of postprandial phenylalanine:

\[ \text{Endogenous phenylalanine Ra} = \text{total phenylalanine Ra} - \text{dietary phenylalanine Ra} \quad (A7) \]

The fractional synthetic rates (FSRs) of albumin and fibrinogen were calculated with the use of a standard precursor-product linear relation (5–8) by using the intravenous phenylalanine tracer incorporation into the proteins from samples taken after the steady state SA of [³H]phenylalanine was achieved at baseline (ie, at −30, −20, −10, and 0 min) and after the meal periods (ie, at 190, 210, 230, 250, and 270 min).

\[ \text{FSR (% of pool synthesized/d)} = \frac{[\text{SA}_{2} - \text{SA}_{1}]/(t_{2} - t_{1})}{[³H]phenylalanine SA} \times 1440 \times 100 \quad (A8) \]

where \(\text{SA}_{2}\) and \(\text{SA}_{1}\) are protein-bound leucine SAs (in mBq/nmol) at time points \(t_{2}\) and \(t_{1}\), respectively; [³H]phenylalanine SA is in mBq/nmol; the factor 1440 is used to convert the data from minutes to 1 d; and 100 is used to convert the FSR to a percentage. The term \((\text{SA}_{2} - \text{SA}_{1})/(t_{2} - t_{1})\) of Equation A8 actually corresponds to the slope of the change in phenylalanine-bound SA versus time (calculated by using 4–5 time points). The coefficients of linear regression of the slopes were >0.9 at baseline and after the meal periods in all subjects in both groups. The FSRs of fibrinogen and albumin were also calculated postprandially with the use of orally administered [¹⁴C]phenylalanine tracer and Equation A8.

Note that this commonly used index of protein synthesis, FSR, is actually a measurement of the rate at which the newly synthesized proteins are reversed into the circulation (5–8). Therefore, the FSR indicates new secretion but not necessarily new synthesis, should the newly synthesized molecule be retained within the hepatic cell but not secreted. Although the SA of plasma phenylalanine may not correspond to the true intrahepatic precursor pool for protein synthesis, the equilibration between plasma and intrahepatic phenylalanine enrichment should be extensive because of the small intrahepatic phenylalanine pool (9). Indeed, the phenylalanine tracer method was previously used to measure plasma protein synthesis (8, 10).

REFERENCES


