Impaired muscle protein anabolic response to insulin and amino acids in heart failure patients: relationship with markers of immune activation

Michael J. TOTH*, Martin M. LEWINTER*, Philip A. ADES* and Dwight E. MATTHEWS*†

*Department of Medicine, University of Vermont College of Medicine, Burlington, VT 05405, U.S.A., and †Department of Chemistry, University of Vermont College, Burlington, VT 05405, U.S.A.

ABSTRACT

Patients with chronic HF (heart failure) experience muscle atrophy during the course of the disease. The mechanisms underlying muscle atrophy in HF, however, are not understood. Thus we evaluated leg phenylalanine balance and kinetics in HF patients and controls following a brief fast (24 h) and under euglycaemic–hyperinsulinaemic–hyperaminoacidaemic conditions to determine whether HF increases muscle protein catabolism in response to nutritional deprivation and/or diminishes the anabolic response to meal-related stimuli (insulin and amino acids) and whether alterations in protein metabolism correlate to circulating cytokine levels. No differences in phenylalanine balance, rate of appearance or rate of disappearance were found between patients and controls under fasting conditions. However, the anabolic response to hyperinsulinaemia–hyperaminoacidaemia was reduced by more than 50% in patients compared with controls. The diminished anabolic response was due to reduced suppression of the leg phenylalanine appearance rate, an index of protein breakdown, in HF patients; whereas no group difference was found in the increase in the leg phenylalanine disappearance rate, an index of protein synthesis. The diminished responses of both phenylalanine balance and appearance rate to hyperinsulinaemia–hyperaminoacidaemia were related to greater circulating IL-6 (interleukin-6) levels. Our results suggest that, following a brief period of nutritional deprivation, HF patients demonstrate an impaired muscle protein anabolic response to meal-related stimuli, due to an inability to suppress muscle proteolysis, and that this diminished protein anabolic response correlates with markers of immune activation. The inability to stimulate muscle protein anabolism following periods of nutritional deficiency may contribute to muscle wasting in HF patients.

INTRODUCTION

Patients with chronic HF (heart failure) lose skeletal muscle tissue during the course of the disease. Muscle atrophy can lead to functional disability, as it is a primary determinant of both muscle strength [1] and endurance [1,2], and may contribute to poor prognosis [3]. Despite these important clinical consequences, the mechanisms underlying atrophy in HF patients are not well understood.

Because protein is the primary structural and functional macromolecular component of skeletal muscle, tissue size and functionality is dictated by the balance between protein synthesis and breakdown. Muscle...
protein is catabolized during periods of nutrient deficiency (i.e. fasting) to provide energy and gluconeogenic substrates for other tissues, and is replenished following subsequent nutrient intake [4]. In this context, the response of muscle protein metabolism to periods of nutrient deficiency and intake is a primary determinant of muscle size and alterations in these normal responses could promote muscle atrophy. That an impaired muscle protein metabolic response to nutritional stimuli might contribute to muscle atrophy in HF is made more likely by the fact that patients frequently experience weight loss during the course of the disease [3]. During these periods of energy imbalance, an increased protein catabolic response to energy deficiency, an impaired anabolic response to subsequent nutrient intake or a combination of these two factors could promote muscle atrophy. To our knowledge, however, no study has examined how HF affects the muscle protein metabolic response to nutrient deficiency or the anabolic response to meal-related protein anabolic factors (i.e. insulin and amino acids).

Inflammatory cytokines are elevated in HF patients [5] and several correlative studies have suggested that cytokine levels are related to skeletal muscle atrophy [6,7]. Although the ability of cytokines to promote muscle atrophy has been firmly established in animal models [8], there is question whether they have similar catabolic effects on skeletal muscle protein metabolic processes in humans [9,10]. In HF patients, cytokines and inflammatory markers are negatively related to myofibrillar gene expression and synthesis under postabsorptive conditions [11,12]. Whether inflammatory cytokines relate to the protein catabolic response to fasting or the anabolic response to meal-related stimuli, however, has not been examined.

The primary aim of the present study was to evaluate the muscle protein metabolic response to nutritional deprivation and repletion in HF patients and controls. To accomplish this goal, leg phenylalanine balance and kinetics were measured using a combination of arteriovenous balance and stable isotope tracer methodologies following a brief (24 h) fast and during infusion of amino acids and insulin to simulate meal-related muscle protein anabolic stimuli. For the meal-related anabolic stimuli, we chose to infuse insulin and amino acids intravenously because, compared with oral feeding, this approach affords greater control over hormone and substrate levels. A secondary goal of these studies was to evaluate whether the protein metabolic response to fasting or to hyperinsulinaemia–hyperaminoacidaemia was related to circulating levels of pro-inflammatory cytokines, their soluble receptors or inflammatory markers. Additionally, because HF is an insulin-resistant syndrome [13], we also evaluated the relationship of insulin-stimulated glucose disposal, the criterion method for assessing tissue insulin sensitivity, to the anabolic response to insulin/amino acid infusion. We hypothesized that HF patients would demonstrate a more pronounced catabolic response to fasting and diminished anabolic response to insulin/amino acid infusion and that both defects would be related to greater circulating levels of inflammatory cytokines markers.

**MATERIALS AND METHODS**

**Subjects**
A total of nine volunteers were studied. Four male patients with chronic HF were recruited from the Heart Failure Clinic of the Cardiology Unit at the University of Vermont. The population consisted of patients with systolic dysfunction (left ventricular ejection fraction <40%; mean ± S.E.M., 27.0 ± 5.2%). The average New York Heart Association functional class was 2.25 ± 0.25, with three class II patients and one class III patient. The aetiology of HF was ischaemic in three patients, as defined by a history of myocardial infarction and/or multi-vessel coronary obstructions upon cardiac catheterization, and idiopathic dilated cardiomyopathy in one patient. Two patients had Type 2 diabetes mellitus and were being treated with oral hypoglycaemic agents. All patients were non-smokers, clinically stable and had not been hospitalized for 6 months prior to testing. None had signs or symptoms of severe hepatic (i.e. cirrhosis) or renal disease (i.e. plasma creatinine >3), peripheral vascular disease or an active neoplastic process. Upon physical examination, there was no evidence of peripheral oedema during screening or at the time of testing. All patients were on stable doses of HF medication, including ACEIs (angiotensin-converting enzyme inhibitors)/ARBs (angiotensin receptor blockers) (100%), β-blockers (100%) and diuretics (100%), and none were taking testosterone replacement therapy.

Controls (five men) were recruited, who were non-smokers and had a stable body weight (± 2 kg) for 6 months prior to testing. They had no signs or symptoms of HF, coronary heart disease or diabetes (fasting blood glucose <126 mg/dl and 2-h glucose following 75 g oral glucose load of <140 mg/dl), normal blood counts and biochemistry values, and were not taking testosterone replacement therapy.

Written informed consent was obtained from each volunteer, and the protocol was approved by the Committees on Human Research at the University of Vermont.

**Experimental protocol**
Volunteers were tested during outpatient and inpatient visits to the research centre. Following an outpatient screening visit to determine eligibility and perform aerobic capacity measurements, patients were studied during an overnight visit. For 3 days prior to admission, all subjects were provided with a weight-maintenance diet (60% carbohydrate, 25% fat and 15% protein). Each volunteer...
was admitted to the research centre in the early morning and the last meal of the standardized diet was consumed by 10:00 hours. Thereafter all volunteers fasted and were only allowed water and medication until the completion of testing the following day. After completion of the meal, body composition measurements were performed and a catheter was placed in the antecubital vein for infusions. Upon waking the following morning, the volunteer was allowed to void urine, catheters were placed in the radial artery of the arm contralateral to the infusion catheter and in the femoral vein of the right leg for sampling of arterial and venous blood respectively. Leg phenylalanine balance and kinetics were measured first over a 180 min period, which represented leg protein metabolism under fasting conditions, and during a second 180 min period of infusion with insulin, glucose and amino acids meant to simulate meal-related anabolic stimuli (i.e. hyperinsulinaemia–hyperaminoacidemia). Following collection of baseline blood samples for background isotope levels and inflammatory cytokines/markers, a primed (37.5 μmol/kg of body weight) and constant (37.5 μmol·kg⁻¹·h⁻¹ of body weight·h⁻¹) infusion of [ring-²H₅]phenylalanine was started (zero time) and maintained throughout the study. Blood for arterial and venous phenylalanine concentrations and enrichment were drawn at 135, 150, 165 and 180 min into tubes containing perchloric acid (1:1). Directly following each blood draw, leg blood flow was measured by venous occlusion plethysmography, as described previously [14]. Five separate measures were taken at each time point and were averaged. At 180 min, a euglycaemic–hyperinsulinaemic clamp was begun. Briefly, during the constant infusion of insulin (40 m-units · m⁻² · min⁻¹), euglycaemia was maintained by a continuous infusion of 20 % (v/v) dextrose. Plasma glucose was monitored every 5 min and the dextrose infusion was adjusted to maintain euglycaemia [15], as described previously [16]. Concomitant with the start of the glucose clamp, an infusion of 10 % (v/v) Aminosyn II (66 mg · kg⁻¹ · body weight · h⁻¹; Hospira) was started. [ring-²H₅]Phenylalanine was added to the amino acid infusate to minimize alterations in steady-state levels of the tracer. The goal of the amino acid and insulin infusions was to achieve circulating levels of insulin and amino acids that are generally comparable with what is observed in the postprandial condition. Blood for arterial and venous phenylalanine concentrations and enrichment were drawn at 315, 330, 345 and 360 min into tubes containing perchloric acid (1:1), followed by measurement of leg blood flow. All infusions were stopped at 360 min, except for the dextrose infusion, which was continued and tapered until no longer required to maintain normal glycaemia.

**Total and regional body composition**

Body mass was measured on a digital scale (ScaleTronix). Total and regional fat mass and fat-free mass were measured by dual energy X-ray absorptiometry using a GE Lunar Prodigy densitometer. Appendicular skeletal muscle mass was measured as described by Heymsfeld et al. [17].

**Peak VO₂ (oxygen consumption)**

Peak VO₂ was determined as described previously [11], and was defined as the highest 30 s average VO₂ value during the final 2 min of the test.

**Hormone and substrate measurements**

Serum insulin was measured by ELISA (ALPCO). Plasma glucose concentrations were measured by a glucose analyser (Yellow Springs Instruments). Plasma amino acid levels were measured by cation-exchange HPLC with fluorescent detection (Dionex) by the Yale General Clinical Research Center core laboratory, with inter-assay CVs (coefficient of variations) of between 5 and 10 %. CRP (C-reactive protein) levels were measured by ELISA [18] with an inter-assay CV of 2–4 %. TNF-α (tumour necrosis factor-α) and IL-6 (interleukin-6) levels and their soluble receptors [TNF-RII (TNF-α receptor II) and sIL-6R (soluble IL-6 receptor) respectively] were measured by ultra-sensitive ELISA (R&D Systems) with inter-assay CVs of 6–16 %.

**MS measurements**

Arterial and venous [ring-²H₅]phenylalanine enrichment and concentration were measured by positive-chemical-ionization GC/MS, as described previously [19]. Prior to measurement, [²H₂]phenylalanine was added to each blood sample to serve as an internal standard, amino acids were isolated and then converted into their HFBP (N-heptafluorobutyl, n-propyl) derivative [20]. HFBP-amino acid derivatives were injected into the GC/MS (model 5973; Hewlett-Packard) and ions at a m/z of 404, 406 and 409 were monitored for unlabelled, [²H₂]phenylalanine and [ring-²H₅]phenylalanine respectively. Ion current ratios derived from these species were used to calculate enrichment and concentration values, as described previously [21].

**Calculations**

The net balance of phenylalanine across the leg was calculated from arterial (A) and venous (V) whole-blood phenylalanine concentrations and leg blood flow (F) as:

\[
\text{Phenylalanine net balance} = (A - V) \times F
\]

Whole-blood data were used to avoid assumptions regarding the equilibration of amino acids between plasma and red blood cells [22] in the event that red blood cells participate in the inter-organ exchange of phenylalanine.
The leg tissue disposal rate \((Rd)\) (rate of disappearance) of phenylalanine was calculated as:

\[
Rd = f_{phe} \times A \times F
\]

where \(f_{phe}\) is the fractional extraction of the phenylalanine tracer, calculated as the arteriovenous difference in tracer enrichment divided by arterial enrichment.

\(Ra\) (rate of appearance) of phenylalanine from leg tissues was calculated as:

\[
Ra = FV \times (1 - Ev/Ea)
\]

where \(Ev\) and \(Ea\) are the enrichment of the phenylalanine tracer in venous and arterial blood respectively. Because phenylalanine is neither synthesized nor metabolized in muscle [23], the kinetic processes defined by \(Rd\) and \(Ra\) should reflect the rate of incorporation (i.e. synthesis) and release (i.e. breakdown) of phenylalanine respectively, from tissue protein. The rate of whole-body phenylalanine flux was calculated from arterial phenylalanine enrichment under fasting conditions using standard equations [20] and under hyperinsulinaemic–hyperaminoacidic conditions using equations that consider the input of tracer from two exogenous sources (i.e. tracer infusion and amino acid infusion), as described previously [16].

**Insulin sensitivity**

Insulin-stimulated glucose disposal, an index of tissue insulin sensitivity, was derived from the euglycaemic–hyperinsulinaemic clamp, as described previously [16]. The average rate of glucose infusion during the last 30 min of the clamp, expressed relative to fat-free tissue mass \((i.e. \text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})\) of fat-free mass \(\text{kg}^{-1}\), was used as an estimate of insulin-stimulated glucose disposal, assuming complete suppression of endogenous glucose production. This assumption is reasonable in HF patients given the suppression of endogenous glucose production in muscle [23], the kinetic processes defined by \(Rd\) and \(Ra\) should reflect the rate of incorporation (i.e. synthesis) and release (i.e. breakdown) of phenylalanine respectively, from tissue protein. The rate of whole-body phenylalanine flux was calculated from arterial phenylalanine enrichment under fasting conditions using standard equations [20] and under hyperinsulinaemic–hyperaminoacidic conditions using equations that consider the input of tracer from two exogenous sources (i.e. tracer infusion and amino acid infusion), as described previously [16].

**Statistics**

All values are reported as means \(\pm\) S.E.M. Unpaired Student’s \(t\) tests were used to compare groups for those measurements performed under fasting conditions. Examination of the response of phenylalanine balance to insulin and amino acid infusions was made using a 2 \(\times\) 2 repeated measures ANOVA (control compared with HF) as the between-subject factor and infusion (no infusion (fasting) compared with insulin/amino acid infusion) as the within-subject factor. 95% CIs (confidence intervals) associated with the difference between groups under fasting conditions or the group by infusion interaction effects for the insulin/amino acid infusion are provided.

**RESULTS**

Physical characteristics of HF patients and controls are shown in Table 1. Groups were similar for age, body mass, total and regional body composition, although peak \(\dot{V}O_2\) was lower \((P < 0.01)\) in HF patients, as expected. Although not significant \((P = 0.31\) and 0.25 respectively), the greater body mass and fat mass in the controls was due to one control volunteer with a body mass of 125 kg and fat mass of 43.4 kg [height, 194 cm; BMI (body mass index), 33.2 kg/m\(^2\)].

Insulin and amino acid levels under fasting and hyperinsulinaemic–hyperaminoacidic conditions are shown in Table 2. There were no differences between patients and controls in fasting insulin \((P = 0.51)\) or amino acid levels (range of \(P\) values, 0.13 to 0.98). The insulin infusion increased plasma insulin levels in both groups similarly \((i.e. P < 0.01\) infusion effect; \(P = 0.98\) group by infusion interaction effect). Levels of most amino acids were increased \((P < 0.05)\) by the infusion, with the exception of asparagine, glutamine and tyrosine, which were decreased \((P < 0.05)\), and methionine, valine and cysteine, which did not change \((P = 0.13, 0.69\) and 0.64 respectively). There were no differences observed between groups in the increase, decrease or lack of change in amino acid levels with the infusion (i.e. no group by infusion interaction effects).

Both arterial and venous phenylalanine concentrations were in steady state during the final 45 min of both fasting and hyperinsulinaemic–hyperaminoacidic conditions (i.e. slope between the phenylalanine concentration
Table 2 Serum insulin and plasma amino acid levels in controls and HF patients under fasting conditions and in response to insulin/amino acid (Ins/AA) infusions

Values are means ± S.E.M. *Significant effect (P < 0.05) of infusion.

<table>
<thead>
<tr>
<th>Plasma level</th>
<th>Controls</th>
<th></th>
<th></th>
<th>HF patients</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fasting</td>
<td>Ins/AA</td>
<td>Fasting</td>
<td>Ins/AA</td>
<td></td>
</tr>
<tr>
<td>Insulin (με-units/ml)</td>
<td>6.3 ± 1.8</td>
<td>70.4 ± 7.5</td>
<td>4.4 ± 2.2</td>
<td>68.8 ± 9.5*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-essential amino acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>70.2 ± 4.9</td>
<td>129.5 ± 8.0</td>
<td>72.4 ± 4.9</td>
<td>125.9 ± 5.2*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asparagine</td>
<td>48.8 ± 4.4</td>
<td>28.6 ± 3.0</td>
<td>43.8 ± 3.4</td>
<td>28.2 ± 1.2*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartate</td>
<td>3.5 ± 0.8</td>
<td>21.1 ± 1.4</td>
<td>3.8 ± 0.2</td>
<td>22.4 ± 0.6*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td>32.4 ± 3.0</td>
<td>29.8 ± 5.5</td>
<td>35.9 ± 2.8</td>
<td>34.8 ± 5.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamate</td>
<td>48.5 ± 7.4</td>
<td>73.0 ± 7.5</td>
<td>69.0 ± 12.0</td>
<td>100.9 ± 9.1*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamine</td>
<td>404.5 ± 30.3</td>
<td>361.8 ± 23.1</td>
<td>433.5 ± 41.0</td>
<td>365.9 ± 20.7*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>168.5 ± 21.4</td>
<td>224.8 ± 21.1</td>
<td>167.7 ± 28.7</td>
<td>217.8 ± 24.4*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>65.6 ± 3.3</td>
<td>77.8 ± 2.6</td>
<td>77.2 ± 6.6</td>
<td>86.8 ± 3.9*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>74.9 ± 5.7</td>
<td>95.9 ± 3.8</td>
<td>79.2 ± 4.0</td>
<td>99.7 ± 2.7*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>47.7 ± 5.2</td>
<td>33.2 ± 2.3</td>
<td>48.2 ± 2.1</td>
<td>39.4 ± 2.3*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Essential amino acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>63.2 ± 6.8</td>
<td>88.2 ± 6.3</td>
<td>61.8 ± 3.8</td>
<td>93.9 ± 8.1*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>134.7 ± 6.7</td>
<td>149.8 ± 9.8</td>
<td>139.3 ± 4.5</td>
<td>164.8 ± 13.4*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>132.3 ± 7.0</td>
<td>220.3 ± 8.5</td>
<td>152.0 ± 8.0</td>
<td>229.8 ± 11.2*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>21.3 ± 2.3</td>
<td>24.3 ± 1.7</td>
<td>20.6 ± 0.8</td>
<td>22.3 ± 1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>60.0 ± 5.2</td>
<td>68.5 ± 2.8</td>
<td>63.2 ± 4.9</td>
<td>70.5 ± 8.8*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>78.4 ± 4.5</td>
<td>91.3 ± 3.7</td>
<td>66.2 ± 6.0</td>
<td>80.6 ± 3.8*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>30.7 ± 5.6</td>
<td>64.1 ± 5.8</td>
<td>36.4 ± 3.2</td>
<td>57.2 ± 2.5*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>212.9 ± 12.5</td>
<td>207.0 ± 12.4</td>
<td>220.0 ± 10.0</td>
<td>229.9 ± 16.2*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

and time was not different from zero). Leg phenylalanine balance under fasting and hyperinsulinaemic–hyper-aminoacidaemic conditions and its change between these two conditions are shown in Figure 1. There was no difference between groups in phenylalanine balance in the fasting condition (15.46 ± 4.36 compared with −8.89 ± 0.92 nmol · 100 g⁻¹ of body weight · min⁻¹ in the controls and HF patients respectively; P = 0.23; 95% CIs, −18.41 to 5.27). As expected, the insulin/amino acid infusion promoted net protein anabolism, as indicated by a more positive leg phenylalanine balance (4.36 ± 1.37 compared with −0.36 ± 1.73 nmol · 100 g⁻¹ of body weight · min⁻¹ in the controls and HF patients respectively; P < 0.01 infusion effect). The response of phenylalanine balance to insulin/amino acid infusion showed a strong trend (P = 0.06 group by time interaction effect) towards a greater anabolic response in controls (change in phenylalanine balance, 19.82 ± 4.29 compared with 8.53 ± 1.71 nmol · 100 g⁻¹ of body weight · min⁻¹ in the controls and HF patients respectively; 95% CI, −0.76 to 23.33). The sample sizes in our present study would be sufficient to detect the aforementioned changes in phenylalanine balance with insulin/amino acid infusion with a Type I error rate of 0.05 and a Type II error rate of 0.18.

Figure 1 Leg phenylalanine net balance in controls and HF patients under fasting and hyperinsulinaemic–hyper-aminoacidaemic conditions and the change between these two conditions

Values are means ± S.E.M. *P = 0.06. Filled bars, controls; open bars, HF patients. Fast, fasting; Ins + AA, insulin/amino acids (hyperinsulinaemic–hyper-aminoacidaemic conditions).

To evaluate the basis for the diminished anabolic response to insulin/amino acid infusion in HF patients, leg phenylalanine Ra and Rd were measured using [3H₃]phenylalanine kinetics (Figure 2). Both
arterial and venous phenylalanine enrichments were in steady state during the final 45 min of both fasting and hyperinsulinaemic–hyperaminoacidemic conditions (i.e. the slope between phenylalanine enrichment and time was not different from zero; results not shown).

No group differences were found in phenylalanine Rd in the fasting condition (15.67 ± 4.02 compared with 10.20 ± 2.28 nmol · 100 g⁻¹ · min⁻¹ in the controls and HF patients respectively; P = 0.31; 95 % CI, 6.31 to 17.25). As expected, insulin/amino acid infusion increased phenylalanine Rd (21.27 ± 4.61 compared with 17.09 ± 4.50 nmol · 100 g⁻¹ · min⁻¹ in the controls and HF patients respectively; P < 0.01 infusion effect). This increase in phenylalanine Rd did not differ between groups (change in Rd, 5.6 ± 2.41 compared with 6.89 ± 2.51 nmol · 100 g⁻¹ · min⁻¹ in the controls and HF patients respectively; P = 0.72; 95 % CI, −9.60 to 7.01). The Ra of phenylalanine did not differ between groups in the fasting condition (31.13 ± 8.01 compared with 19.09 ± 3.04 nmol · 100 g⁻¹ · min⁻¹ in the controls and HF patients respectively; P = 0.24; 95 % CI, −10.34 to 34.43). As expected, the insulin/amino acid infusions promoted a reduction in phenylalanine Ra (16.91 ± 3.88 compared with 17.45 ± 5.15 nmol · 100 g⁻¹ · min⁻¹ of body weight · min⁻¹ in the controls and HF patients respectively; P < 0.05 infusion effect). This effect of insulin/amino acid infusion to diminish phenylalanine Ra was primarily driven by the response in controls as there was a strong trend (P = 0.07 group by time interaction effect) towards a greater reduction in phenylalanine Ra in controls compared with HF patients (change in Ra, 14.22 ± 4.88 and 1.64 ± 2.51 nmol · 100 g⁻¹ · min⁻¹ of body weight · min⁻¹ respectively; 95 % CI, −1.51 to 26.67). Finally, no group differences in leg blood flow were observed under fasting conditions (P = 0.26), in the response to insulin/amino acid infusion (P = 0.70 infusion effect) or any difference between groups in the response to infusion (P = 0.66 group by infusion interaction effect; results not shown).

Whole-body phenylalanine Ra results are shown in Figure 3. Whole-body phenylalanine Ra did not differ between controls and HF patients in the fasting condition (44.5 ± 2.2 and 62.7 ± 11.3 μmol · kg⁻¹ · h⁻¹ of free-fat mass · h⁻¹ respectively; P = 0.12; 95 % CI, −44.54 to 1.38). As expected, the insulin/amino acid infusion reduced (P < 0.01 infusion effect) whole-body phenylalanine Ra (38.0 ± 1.6 compared with 44.2 ± 6.6 μmol · kg⁻¹ of free-fat mass · h⁻¹ in the control and HF patients respectively). The reduction in whole-body protein breakdown was greater (P < 0.05 group by infusion interaction effect) in HF patients compared with controls (change in whole-body Ra: 6.5 ± 0.9 compared with 18.4 ± 5.0 μmol · kg⁻¹ of free-fat mass · h⁻¹ in the control and HF patients respectively; 95 % CI, −25.33 to −5.15).

In an attempt to identify factors that might contribute to impaired anabolic response to insulin/amino acid infusion, we evaluated the relationship of circulating cytokines and insulin-stimulated glucose disposal to the change in leg phenylalanine balance and Ra.
average results for plasma inflammatory markers and insulin-stimulated glucose uptake are shown in Table 3. No differences were found between groups in these potential correlates. Table 4 shows correlation coefficients for the relationship of these variables to phenylalanine balance and $R_a$. We found negative correlations between IL-6 and the change in phenylalanine balance and $R_a$. Scatterplots for these relationships are shown in Figure 4. Insulin-stimulated glucose disposal, an index of tissue insulin sensitivity, was not related to either variable ($P = 0.11$ and $0.31$ respectively).

### DISCUSSION

In the present study, we observed that HF patients had a similar protein catabolic response to short-term (24 h) dietary energy deficiency compared with controls. This finding was somewhat unexpected given the elevated circulating levels of catabolic and reduced levels of anabolic hormones in these patients [26]. However, the absence of an exaggerated protein catabolic response to short-term energy deprivation is not unprecedented in clinical conditions characterized by muscle atrophy. In weightlosing cancer patients, for example, leg protein balance following an overnight fast (12 h) is similar to weightstable controls [27]. Moreover, previous studies from our laboratory have shown that postabsorptive muscle protein synthesis rates and indices of protein breakdown do not differ between clinically stable HF patients and controls [11,28]. Our results differ from those of Morrison et al. [29], who found more negative leg tyrosine balance following an overnight fast in HF patients who had experienced weight loss (i.e. cachectic) compared with controls, suggesting enhanced protein catabolism. Differing results between studies may relate to the patient population investigated (i.e. weight-stable compared with cachectic) and the timing of the measurements. Regarding the latter, the patients studied by Morrison et al. [29] were evaluated directly following inpatient clinical management for their HF. Thus their greater negative protein balance may be explained by the exacerbation of their

### Table 3 Group differences in circulating hormones and insulin sensitivity variables used as potential correlates of changes in phenylalanine balance and kinetics

Values are means ± S.E.M. No significant differences were found between groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls</th>
<th>HF patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP ($\mu$g/$\mu$l)</td>
<td>2.23 ± 1.1</td>
<td>6.4 ± 2.2</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>1.69 ± 0.23</td>
<td>2.41 ± 0.71</td>
</tr>
<tr>
<td>TNF-RII (pg/ml)</td>
<td>2805 ± 192</td>
<td>3933 ± 588</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>1.89 ± 0.20</td>
<td>3.88 ± 0.93</td>
</tr>
<tr>
<td>sIL-6R (ng/ml)</td>
<td>31.4 ± 2.6</td>
<td>42.3 ± 0.8</td>
</tr>
<tr>
<td>Insulin-stimulated glucose disposal</td>
<td>4.52 ± 0.94</td>
<td>2.84 ± 0.68</td>
</tr>
</tbody>
</table>

(mg · kg$^{-1}$ of fat-free mass · min$^{-1}$)

### Table 4 Correlation coefficients for the relationship of circulating cytokine levels to the change in leg phenylalanine balance and phenylalanine $R_a$ from fasting to hyperinsulinaemic–hyperaminoacidemia conditions

Values are the Pearson correlation coefficients for $n = 9$. $^*P < 0.05$. ISGU, insulin-stimulated glucose uptake.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CRP</th>
<th>TNF-α</th>
<th>TNF-RII</th>
<th>IL-6</th>
<th>sIL-6R</th>
<th>ISGU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in leg phenylalanine balance</td>
<td>−0.574</td>
<td>0.022</td>
<td>−0.440</td>
<td>−0.690$^*$</td>
<td>−0.599</td>
<td>0.556</td>
</tr>
<tr>
<td>Change in leg phenylalanine $R_a$</td>
<td>−0.366</td>
<td>−0.330</td>
<td>−0.600</td>
<td>−0.720$^*$</td>
<td>−0.468</td>
<td>0.381</td>
</tr>
</tbody>
</table>
HF, as acute illness is known to promote greater fasting protein catabolism [30,31]. We acknowledge that our small sample of patients precludes definitive conclusions from being drawn; however, considering that, if anything, HF patients exhibited a diminished protein catabolic response to fasting, it is unlikely that the addition of more patients would substantially alter our conclusions. Taken together with our present and prior results [11,28], these findings suggest that an enhanced catabolic response to short-term fasting is not an intrinsic feature of the HF syndrome (i.e. not present in clinically stable patients), but may occur during or directly following periods of disease exacerbation and hospitalization [29].

Our present results showed a strong trend toward a diminished protein anabolic response (i.e. more negative net leg phenylalanine balance) to insulin/amino acid infusion in HF patients compared with controls (Figure 1). Practically speaking, net balance was positive in controls, indicating leg protein accretion, whereas it remained slightly negative in HF patients, signifying continued protein loss. Although this difference did not reach statistical significance, in part due to our small sample size, it is similar in direction and of greater magnitude when compared with protein imbalances found in other physiological/pathophysiological conditions that show a diminished protein anabolic response to meal-related stimuli, such as aging and cancer [27,32]. Additionally, our present findings are congruent with a growing body of results suggesting that a reduced anabolic response to meal-related anabolic stimuli is a characteristic of a number of physiological and pathophysiological conditions that promote muscle wasting in humans [33,34]. Collectively, these results suggest a common mechanism whereby muscle protein may be lost in healthy and diseased elderly.

The blunted anabolic response in patients was explained by the trend towards a diminished suppression of leg phenylalanine Ra, an index of protein breakdown, whereas the stimulation of phenylalanine Rd, an index of protein synthesis, was similar in patients and controls (Figure 2). Group differences in phenylalanine Ra are not likely to be related to variation in hormone or substrate levels, as the degree of hyperinsulinaemia and hyperaminoacidemia were comparable between the groups. Because the suppression of muscle proteolysis is due primarily to insulin [35], it is reasonable to posit that tissue insulin resistance in HF patients [13,24,36] might explain our results. In particular, it could be argued that inclusion of two patients with Type 2 diabetes mellitus made our cohort excessively insulin-resistant. However, the degree of tissue insulin resistance observed in our cohort (−37%), as measured by the criterion approach for estimating insulin action (i.e. euglycaemic–hyperinsulinaemic clamp), is less than that reported in larger cohorts of non-diabetic HF patients (−58%; [13]). Moreover, insulin sensitivity was not related to the change in phenylalanine Ra from fasting to hyperinsulinaemic–hyperaminoacidaemic conditions. Although we clearly cannot discount a role for muscle insulin resistance in the diminished suppression of protein breakdown in HF patients, our present results suggest other possible mediators.

IL-6 was negatively related to both the change in leg phenylalanine balance and Ra from fasting to hyperinsulinaemic–hyperaminoacidaemic conditions (Figure 4). Although the increased circulating IL-6 level in HF patients (+100%) did not reach significance, it was similar in magnitude to that observed in larger cohorts of HF patients [37]. These relationships are in keeping with previous work from our laboratory in HF patients showing negative relationships of IL-6 and CRP levels to myofibrillar gene expression and protein synthesis rates respectively [11,12] and the generally acknowledged effect of cytokines to promote muscle protein catabolism [38,39]. We interpret these findings broadly as evidence of a potential role for immune activation in the regulation of skeletal muscle protein metabolism through their effects to antagonize the protein anabolic response to insulin.

The reduction in whole-body phenylalanine Ra with insulin/amino acid infusion, an index of whole-body protein breakdown, was greater in HF patients (Figure 3), contrary to leg phenylalanine Ra. Considering that liver and skeletal muscle together constitute the majority of whole-body protein turnover [40] and are the two main organ systems responsive to meal-related stimuli, group differences in the whole-body response are most likely to be due to differential effects of insulin and/or amino acids on liver protein metabolism. Because protein breakdown in the splanchnic bed in humans is primarily affected by amino acids [41], not insulin, the differential effect of insulin/amino acid infusion on the whole-body phenylalanine Ra is likely to be due to the ability of amino acids to reduce hepatic proteolysis to a greater extent in HF patients. The reason for such an effect in HF patients is unclear, but may relate to altered hepatic protein metabolism secondary to circulatory deficiencies [42].

Several caveats to our present study should be acknowledged. First, the phenylalanine balance and kinetic measurements across the leg cannot be attributed solely to muscle, since skin, bone and adipose tissue also contribute. However, because the large majority (85–90%) of metabolism of phenylalanine in the leg occurs in skeletal muscle [43] and the fact that there were no differences between groups in leg fat and fat-free mass (Table 1), our present results are reasonable estimates of muscle protein balance and kinetics. Secondly, regarding protein kinetic measurements, the Rd and Ra of phenylalanine across the leg are not direct measures of protein synthesis and breakdown respectively, because phenylalanine must transit the intramuscular amino acid pool prior to entering protein (via protein synthesis) or the circulation (from protein breakdown). Thus the intracellular concentration
and enrichment of phenylalanine must be constant for our kinetic estimates to reflect protein synthesis and breakdown. Of the amino acids typically used for limb balance measurements, phenylalanine is likely to give the most reliable estimates of these metabolic processes, given that it is not synthesized or metabolized in muscle [23] and that it has a small intramuscular pool and rapid transmembrane transport rate [44]. Finally, our conclusions are limited somewhat by the small number of volunteers studied. Lending support to the significance of our results, however, is the fact that the magnitude of differences in the protein anabolic response to meal-related stimuli was comparable with or greater than that observed in prior studies of healthy and diseased elderly [30,32]. Considering the assertion that smaller differences in leg protein balance have effects in other muscle-wasting conditions [33,34], it appears reasonable that the impaired protein anabolic responsiveness observed in our present study is physiological relevant.

In summary, our results suggest that HF patients are characterized by an impaired anabolic response to meal-related stimuli, particularly the effect of insulin to reduce protein breakdown. Moreover, the diminished anabolic response was related to increased circulating IL-6 levels. The fact that we studied clinically stable patients with mild-to-moderate disease and without a history of cachexia suggests that our results are not merely a consequence of end-stage disease or a metabolic adaptation to the cachectic state. Our results therefore define a potential mechanism whereby muscle protein could be lost in HF patients during bouts of negative energy imbalance and the possible involvement of inflammatory cytokines in this defect.

AUTHOR CONTRIBUTION

All authors contributed to the design of the study. Michael Toth and Dwight Matthews were primarily responsible for data collection and sample analysis; Michael Toth performed the statistical analysis of the data and drafted the manuscript; and Dwight Matthews, Martin LeWinter and Philip Ades all reviewed and revised the manuscript.

ACKNOWLEDGEMENTS

We thank all the volunteers who dedicated their valuable time to these studies.

FUNDING

This study was funded the National Institutes of Health [grant numbers AM-02125, AG-17949 and RR-00109].

REFERENCES

Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. Clin. Chem. 43, 52–58
Effect of epinephrine on amino acid and energy metabolism in humans. Am. J. Physiol. 258, E948–E956
20 Matthews, D. E., Marano, M. A. and Campbell, R. G.
Use of aromatic amino acids as monitors of protein turnover. Am. J. Physiol. 249, E677–E681
24 Paolisso, G., De Riu, S., Marrazzo, G., Verza, M.,
25 Paolisso, G., Gambarella, A., Marrazzo, G., Verza, M.,
26 Anker, S. D., Chua, T. P., Ponikowski, P., Harrington, D.,
27 Lundholm, K., Bennegard, K., Edén, E., Svaninger, G.,
28 Miller, M. S., VanBuren, P., LeWinter, M. M., Lecker, S. H.,
30 Bennegard, K., Lindmark, L., Eden, E., Svaninger, G. and
32 Wilkes, E. A., Selby, A. L., Atherton, P. J., Patel, R.,
36 Kemppainen, J., Tsuchida, H., Stolen, K., Karlsson, H.,
Björnholm, M., Heinonen, O. J., Nautila, P., Krok, A.,
39 Vary, T. C., Dardevet, D., Grizard, J., Voisin, L., Buffiere,
43 Biolo, G., Gastaldelli, A., Zhang, X. J. and Wolfe, R. R.
44 Biolo, G., Fleming, R. Y., Maggi, S. P. and Wolfe, R. R.