In vivo rabbit hindquarter model for assessment of regional burn hypermetabolism

JI XU, ZHEWEI FEI, YONG-MING YU, WENYIN XU, ANDREW RHODES, RONALD G. TOMPKINS, AND JOHN T. SCHULZ.

In vivo rabbit hindquarter model for assessment of regional burn hypermetabolism. J Appl Physiol 94: 135–140, 2003. First published September 27, 2002; 10.1152/japplphysiol.00513.2002.—Severe burn injury evokes hypermetabolism and muscle wasting, despite nominally adequate nutrition. Although there is much information on whole organism and isolated tissue metabolism after burn injury, data examining regional burn hypermetabolism in vivo are lacking. Using surgically implanted (general anesthesia) regional vascular catheters and primed constant infusion of L-[1-13C]phenylalanine tracer, we have determined in vivo burn-induced alterations in rabbit hindquarter protein and energy metabolism. Burn injury evokes increased whole body resting energy expenditure and phenylalanine turnover, accompanied by significantly increased hindquarter proteolysis, creating a negative protein balance in burned rabbit hindquarter. Hindquarter oxygen consumption showed an increase after burn injury, but it did not reach statistical significance. Burn-induced changes in hindquarter protein turnover account for approximately one-third of the whole animal hypermetabolism. This model offers a system for regional manipulation of postburn hypermetabolism.

SEVERE BURN INJURY RESULTS in a hypermetabolic and catabolic state characterized by increased energy expenditure, diminished capacity to resist infections, and erosion of muscle protein mass associated with muscle weakness (7, 9, 12, 24). Although nutritional support is partially successful in restoring nitrogen equilibrium, the net protein wasting continues for a prolonged period (9), causing debility and impeding prompt rehabilitation of the convalescent burn patient. Anabolic steroids have been employed in attempts to interdict muscle wasting after burn injury. The merits of this type of therapy await wide clinical confirmation.

The relationship between burn-induced changes in energy and protein metabolism has been studied in multiple animal models. Although at least one pathway implicated in stress-related proteolysis is ATP dependent (8), the precise relationship between burn-induced hypermetabolism and protein catabolism remains poorly defined (27, 28).

Both radioactive and stable isotope tracers are used to measure the rate of muscle protein turnover in various animal models. Large-animal models are not suitable for study of burn-induced metabolic changes, because induction of significant burn injury in larger mammals is ethically unacceptable. In smaller animals, such as rodents, muscle protein kinetics have mostly been quantified by postmortem analyses of muscle samples (6, 18) and by ex vivo muscle incubation or hindquarter perfusion (19). These studies have provided substantial quantitative data on myoskeletal protein and substrate dynamics under various pathophysiological conditions. However, the influence of a burn injury on regional energy consumption and amino acid metabolism remains poorly defined because accurate measurements cannot be accomplished in ex vivo perfusion models or in muscle biopsy specimens.

Having developed a rabbit model for whole animal metabolic studies after burn injury (11), we turned again to the rabbit in seeking a model for study of burn injury’s effect on the in vivo relationship between energy and protein metabolism in skeletal muscle. We expected the model to fulfill these criteria: 1) large enough to permit surgery for implantation of sampling devices, 2) small enough for easy care (including limited analgesic needs) after a full-thickness burn, and 3) possessing an assayable compartment made largely of muscle and preferably a large percentage of the whole animal muscle.

In this paper, we describe the development of a burned rabbit hindquarter model that satisfies these criteria, allowing the simultaneous quantitative evaluation of both energy and protein metabolism in vivo in a specific region with a large (by weight) skeletal muscle component.

MATERIALS AND METHODS

The studies were carried out in 14 male New Zealand white rabbits (weight 3–3.5 kg). They were arbitrarily divided into sham burn (n = 8) and burn (n = 6) groups. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: J. T. Schulz, Burn and Trauma Division, Dept. of Surgery, GRB 1302, 55 Fruit St., Boston, MA 02114 (E-mail: jschulz@partners.org).
animals were purchased through a commercial animal supplier (Millbrook Breeding Labs, Amherst, MA). On arrival, they were habituated to the environment for at least 48 h before use. All animals were kept in the Animal Farm of the Massachusetts General Hospital, under the care of veterinary staff. Water and food (Prolab Hi-FI pellets, PMI Nutritional International, Brentwood, MO) were fed ad libitum. The study protocol was approved by the Subcommittee on Research Animal Care of Massachusetts General Hospital.

Surgical preparation of the hindquarter model. On the day of surgery, hair was clipped around the neck and the abdomen. All animals were procured under general anesthesia induced with intramuscular injection of a mixture of ketamine-xylazine-acepromazine (30, 10, and 0.5 mg/kg body wt, respectively). After induction of anesthesia, the animal was placed on the operating table in the supine position, and anesthesia was maintained by a continuous inhalation of halothane (0.5%–1%). Catheters (PE 90 with 3 cm of Silastic tip) were implanted into the left jugular vein and left carotid artery by use of aseptic procedures. Details of this surgical procedure have been described in our laboratory’s previous publications (11, 13). Postoperatively, the animals were monitored and allowed to recover for 3 days. They were monitored daily for general conditions.

On the third postoperative day, the animals were reanesthetized as described above. They were positioned supine atop a small roll to lift up the loin. A right paramedian incision (4–6 cm) was made. The retroperitoneum and the right kidney were exposed. The ureter was ligated and severed. The right renal vein was encircled with a silk tie and severed. The right kidney was removed. The ends of the ureter were ligated. Atop a small roll to lift up the loin, a right paramedian skin incision in the neck, where they were secured with silk sutures. The right kidney was removed. The ends of the renal vein and artery were ligated with a silver wire and flushed with 1 ml saline-heparin solution (1 ml saline: 100 IU heparin) daily. Postoperative pain was treated with buprenorphine (0.02 mg/kg every 12 h as needed).

Burn rabbit model. A full-thickness burn injury was induced ~60 h after right nephrectomy and implantation of aortic and IVC catheters, according to the method described in detail recently (11). Briefly, the animal was anesthetized as described above and then subjected to a full-thickness burn by immersing its dorsum along the mark made from an oval template (~25% total body surface area) into boiling water for 15 s, followed by fluid resuscitation and careful observation. Control (sham burn) animals were anesthetized but not burned. Details of anesthesia, the induction of thermal injury, the postburn care, and the metabolic characterization of the injured animals have been described before (11).

Tracer studies. Tracer studies were conducted on the third postburn (or sham burn) day after overnight fasting. Each animal received a primed constant infusion of L-[1-13C]phenylalanine tracer with a targeted infusion rate of 0.182 μmol·kg⁻¹·min⁻¹ (0.03 mg·kg⁻¹·min⁻¹), and a priming dose of 11 μmol/kg for 6 h. Five pairs of blood samples, 2.5 ml each, were taken from the catheters placed in the carotid artery and IVC (via the right renal vein) before the tracer infusion was started (0 min), then at 300, 320, 340, and 360 min after the commencement of the tracer infusion. During the same period of time, para-aminohippurate (PAH, Merck Sharp, Dohme, West Point, PA) 1.5% was infused at 0.16 ml/min into the catheters in the abdominal aorta for the measurement of blood flow in the hindquarter by using the dilution principle (13, 29). For each blood sample, 0.1 ml of whole blood was immediately mixed with 1.4 ml of 10% trichloroacetic acid solution. After centrifugation, the supernatant was preserved at −20°C until the analysis. The remaining blood in each sample was immediately cryopreserved at −70°C until analysis. During the study, three pairs of arterial and venous blood gas were also measured by use of a blood-gas analyzer. The total energy expenditure of the animal was calculated on the basis of oxygen consumption as described (11, 22).

Sample treatment and analysis. The blood enrichment of L-[1-13C]phenylalanine was determined by gas chromatography mass spectrometry (GC-MS, Hewlett-Packard, 5985B), and the concentration of blood phenylalanine was also determined simultaneously by using L-[ring-2H₅]phenylalanine (M+5 phenylalanine) as internal standard, following the principles described before (2). Briefly, phenylalanine in blood was separated via ion-exchange column (Bio-Rad cation ion exchange resin) and derivatized to form a trifluoroacetyl methyl ester. The analysis was carried out by using the electron impact ionization technique. The enrichments (%) of L-phenylalanine, L-[1-13C]phenylalanine, and L-[ring-2H₅]-phenylalanine were monitored on a molecular mass (protons + neutrons)-to-protons ratio of 162.2 (nonisotopic phenylalanine), 163.2 (L-[1-13C]phenylalanine), and 167.2 (L-[ring-2H₅]-phenylalanine). Blood PAH concentrations were determined spectrophotometrically at λ = 530 nm on a microplate reader (THERMO max, Molecular Devices) against a standard curve. This method is a slight modification from that described before by Katz and Bergman (13).

Calculation of whole body and hindquarter blood flow rate, phenylalanine kinetics, and oxygen consumptions. The calculations of protein and amino acid metabolism in whole body and hindlimb were based on arterial-venous (A-V) differences of both tracer and tracee in whole blood. Details of the method have been described before (10, 26, 29).

Briefly, whole body phenylalanine turnover rate (QPHE) is measured by using the steady-state isotope tracer dilution approach (23)

\[ Q_{PHE} = \frac{i_{PHE}}{E_{P}} (E_{P} - 1) \]

where \( i_{PHE} \) is the infusion rate of phenylalanine tracer, \( E_{P} \) is the isotopic enrichment of L-[1-13C]phenylalanine in the infused, \( E_{P} \) is the plateau level plasma L-[1-13C]phenylalanine enrichment. In steady state, \( Q_{PHE} = i_{PHE} - B_{PHE} \), where \( B_{PHE} \) is the rate of phenylalanine intake from whole body proteinolysis. In fasting state, \( i_{PHE} = 0 \); then, \( B_{PHE} = Q_{PHE} \).

Because phenylalanine is not metabolized in the hindquarter, its only mode of incorporation into the hindquarter is as

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Because phenylalanine is not metabolized in the hindquarter, its only mode of incorporation into the hindquarter is as
phenylalanine residues in newly synthesized protein. Similarly, because phenylalanine is not synthesized in muscle, it can only be produced from muscle by proteolysis. Consequently, at isotopic steady state, the metabolic uptake (or disappearance) of the arterial blood phenylalanine tracer in passing through the hindquarter represents the rate of its utilization for protein synthesis. On the other hand, the release of the unlabeled phenylalanine from muscle, which utilizes for protein synthesis. Simultaneously, because phenylalanine is not synthesized in muscle, it produces a significant alteration in serum creatinine and BUN levels (Fig. 1). Three days after burn injury, hemoglobin (sham burn vs. burn, means ± SE: 12.8 ± 0.5 vs. 12.3 ± 0.5 mg/dl, P > 0.1) and arterial oxygen saturation (0.99 vs. 0.99) are not significantly different between burned and sham burned rabbits (Table 1). In both sham and burned animals, the IVC and arterial hemoglobin concentration are the same. The oxygen saturation in IVC blood averaged 0.86 ± 0.05 in the sham rabbits and 0.84 ± 0.04 in the burned rabbits (P > 0.1). The whole body oxygen consumption shows a higher value 3 days after burn injury, in agreement with our previous observations in rabbits with the same total body surface area burn (11). The stable isotope enrichment reaches plateau level between 300 and 360 min (Fig. 2). On the basis of the data in Fig. 2, the kinetics of whole body and hindquarter protein metabolism were calculated by assuming isotopic steady-state conditions.

**Burn injury induced accelerated whole animal protein catabolism.** The metabolic parameters measured in the burn (n = 6) and the control (n = 8) animals are shown in Table 1. The resting energy expenditure measured in burned animal was ~26% higher than the control animals on the third postburn day (P < 0.05). Whole body phenylalanine turnover, which is an indicator of whole body protein breakdown, was signif-

**RESULTS**

Rabbits tolerate the surgical and experimental protocol fairly well. The daily food intake for healthy rabbit was ~45 ± 3 g/kg, which contains protein 7.5 g/kg and total energy ~70 kcal/kg per day. Experimental rabbits began eating and drinking within 24 h of recovery from anesthesia and reached normal intake by ~48 h after induction of burn injury (24 h before the tracer experiments). Unilateral nephrectomy did not produce a significant alteration in serum creatinine and BUN levels (Fig. 1). Three days after burn injury, hemoglobin (sham burn vs. burn, means ± SE: 12.8 ± 0.5 vs. 12.3 ± 0.5 mg/dl, P > 0.1) and arterial oxygen saturation (0.99 vs. 0.99) are not significantly different between burned and sham burned rabbits (Table 1). In both sham and burned animals, the IVC and arterial hemoglobin concentration are the same. The oxygen saturation in IVC blood averaged 0.86 ± 0.05 in the sham rabbits and 0.84 ± 0.04 in the burned rabbits (P > 0.1). The whole body oxygen consumption shows a higher value 3 days after burn injury, in agreement with our previous observations in rabbits with the same total body surface area burn (11). The stable isotope enrichment reaches plateau level between 300 and 360 min (Fig. 2). On the basis of the data in Fig. 2, the kinetics of whole body and hindquarter protein metabolism were calculated by assuming isotopic steady-state conditions.

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**Table 1. Whole body energy expenditure and phenylalanine metabolism in sham burn and burn animals**

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Sham Burn</th>
<th>Burn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>2.80 ± 0.10*</td>
<td>2.92 ± 0.09</td>
</tr>
<tr>
<td>Energy expenditure, kcal·kg⁻¹·day⁻¹</td>
<td>49.3 ± 6.4</td>
<td>62.3 ± 11.0*</td>
</tr>
<tr>
<td>Tracer infusion rate, g/kg</td>
<td>10.8 ± 0.7</td>
<td>11.0 ± 0.2</td>
</tr>
<tr>
<td>Whole body PHE turnover rate, µmol·kg⁻¹·h⁻¹</td>
<td>138.0 ± 12.4</td>
<td>229.4 ± 36.3*</td>
</tr>
<tr>
<td>Whole body proteolysis, g/kg·d⁻¹</td>
<td>10.8 ± 1.0</td>
<td>18.0 ± 2.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 for sham burn group and n = 6 for burn group. PHE, phenylalanine. *Significantly different from sham burn group; P < 0.05, unpaired t-test (unequal variance).
As a result, the net protein balance, as indicated by the difference between the rates of phenylalanine incorporated into and released from the hindquarter proteins, showed a significantly negative value in the burned group compared with the sham burn group. In addition, the data in Tables 1 and 2 indicate that the hindquarter accounts for approximately one-third of whole body protein turnover as well as about one-third of the whole body energy expenditure in rabbits.

**DISCUSSION**

Burn injury continues to exact a high toll in morbidity and mortality, both of which are exacerbated by the intense metabolic storm that follows a large burn. Hallmark features of the postburn illness are altered energy and protein metabolism. Although there is much information on whole organism metabolism and isolated tissue metabolism after burn injury, the quantitative data on burn-induced changes in regional protein and energy metabolism in vivo are quite limited. In this paper, we have demonstrated a refinement of a burned-rabbit model that allows in vivo measurement of both whole body and regional (hindquarter) protein and energy metabolism. We have found that hindquarter accounts for approximately one-third of protein turnover. Burn injury produces a significant change in protein catabolism in hindquarter, a change that may

### Table 2. In vivo measurements of hindquarter phenylalanine metabolism in sham burn and burned animals

<table>
<thead>
<tr>
<th>Metabolic Parameters</th>
<th>Sham Burn</th>
<th>Burn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting PHE concentration, μM</td>
<td>87.5 ± 5.1</td>
<td>86.3 ± 2.5</td>
</tr>
<tr>
<td>Arterial blood [ArPHE]</td>
<td>86.5 ± 5.0</td>
<td>95.7 ± 2.3</td>
</tr>
<tr>
<td>Enrichments of [13C] PHE (MPE)</td>
<td>7.8 ± 0.8</td>
<td>5.2 ± 1.1 *</td>
</tr>
<tr>
<td>Arterial blood [EA]</td>
<td>6.9 ± 0.7</td>
<td>4.2 ± 0.9 *</td>
</tr>
<tr>
<td>Hindquarter blood flow rate, ml·kg⁻¹·min⁻¹</td>
<td>46.6 ± 6.1</td>
<td>42.7 ± 4.8</td>
</tr>
<tr>
<td>Total arterial delivery of PHE to the hindquarter, μmol·kg⁻¹·h⁻¹</td>
<td>237.5 ± 25.3</td>
<td>221.3 ± 24.9</td>
</tr>
<tr>
<td>Fraction of total delivered PHE extracted by the hindquarter (εₑₓₚ), %</td>
<td>15.7 ± 2.7</td>
<td>22.1 ± 4.7</td>
</tr>
<tr>
<td>PHE balance, μmol·kg⁻¹·h⁻¹</td>
<td>2.0 ± 3.8</td>
<td>−23.4 ± 3.2 †</td>
</tr>
<tr>
<td>Rate of PHE to protein in hindquarter (Sₚₑₓₚ), μmol·kg⁻¹·h⁻¹</td>
<td>35.4 ± 5.1</td>
<td>48.5 ± 12.7</td>
</tr>
<tr>
<td>Rate of PHE released via proteolysis (Bₑₓₚ), μmol·kg⁻¹·h⁻¹</td>
<td>33.5 ± 5.8</td>
<td>71.9 ± 14.7</td>
</tr>
<tr>
<td>Hindquarter protein synthesis, g·kg⁻¹·day⁻¹</td>
<td>2.8 ± 0.4</td>
<td>3.8 ± 1.0</td>
</tr>
<tr>
<td>Hindquarter protein breakdown, g·kg⁻¹·day⁻¹</td>
<td>2.6 ± 0.5</td>
<td>5.6 ± 1.2 *</td>
</tr>
<tr>
<td>Hindquarter protein balance, g·kg⁻¹·day⁻¹</td>
<td>0.2 ± 0.3</td>
<td>−1.8 ± 0.2 †</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 for sham burn group and n = 6 for burn group. MPE, molar ratio percent excess. *Significantly different from sham burn group; P < 0.05, unpaired t-test (unequal variance). †Significantly different from sham burn group; P < 0.01, unpaired t-test (equal variance).

**Fig. 2.** Isotopic plateau level [13C]phenylalanine enrichments in whole blood in burn and sham burn animals.
be proportionately larger than that seen in the whole animal.

A number of animal models have been established for exploring muscle protein metabolism in healthy and in diseased conditions. Ruderman et al. (19) first developed the ex vivo rat hindquarter perfusion model to study the metabolism of various substrates and the impact of various humoral factors on substrate metabolism. Because the hindquarter is mostly composed of muscle, this model has significantly contributed to the understanding of muscle metabolism. The ex vivo hindquarter perfusion model also provides information on the rate of protein turnover, but its relevance to in vivo protein metabolism is questionable and cannot be used to determine the metabolic effect of a distant injury (such as a torso burn) on hindquarter protein metabolism. In addition, an ex vivo preparation prevents an accurate assessment of regional energy expenditure.

Assessment of protein metabolism in rodent models has also depended on the analysis of tissue samples taken after death (6, 18). Information on proteolysis and energy metabolism is limited in these studies. Furthermore, the relationship between protein metabolism and energy metabolism in these postmortem tissues is of questionable relevance to the living organism. This is especially true after a burn injury, when all the inflammatory mediators and the altered hormonal milieu are available only in vivo. Many previous in vivo animal models for studying protein metabolism in specific regions with stable or radioactive isotope tracers have been described. In these models, the measurement of muscle protein metabolism has been based on muscle biopsy (5, 15) and/or the measurements of A-V difference model without the added stress of another anesthesia and muscle biopsy. Consequently, our approach in calculating hindquarter metabolism does not include recycled phenylalanine that is released by proteolysis and reincorporated before it can be released into circulation. To the extent that this occurs, our model underestimates absolute synthetic and proteolytic rates.

The present rabbit model with catheters implanted into the inferior vena cava and abdominal aorta allows us to quantify both energy and protein metabolism in vivo in the hindquarter region of a living awake animal. However, unlike the ex vivo eviscerated hindquarter model (14), in a living animal, the major metabolically active components of the “hindquarter” include visceral organs, skin, muscle, and skeleton. To estimate the relative contributions of these components, especially the muscle tissues, to our measured total hindquarter metabolism, we have taken into consideration the following points: 1) Position of the catheter tips and the A-V different measurements. We conducted autopsies in four animals weighing an average 2.35 ± 0.5 kg. The average distance between the right renal artery and the iliac bifurcation is ~6 cm. There is only one branch (the right testicular artery) between these two points. Because the catheters are inserted ~3 cm into the aorta, the area perfused by the inferior mesenteric artery can be excluded from our A-V difference calculations. Hence the areas possibly included in this A-V difference measurements are lumbar and leg muscle, lumbar and leg skin, testes, urinary bladder, and partial rectum. 2) The relative mass of the hindquarter tissues. We further estimated the relative weight of the metabolically active tissues in hindquarter at autopsy. According to the transverse section along the umbilicus in four animals, the hindquarters weigh 1.190 ± 0.57 g. The weights of components are muscle 650 ± 15 g, skin 181 ± 23 g, testis and penis 7.3 ± 0.4 g, urinary bladder 3.24 ± 0.15 g. By mass, therefore, the major metabolically active components of the hindquarter compartment are skin and muscle. 3) The relative contributions of skin and muscle to whole hindquarter protein metabolism. Skin and muscle contributions can be further estimated as follows: in four animals, after freeze drying overnight, the average dry-wet weight of the skin was found to be 19.6 ± 0.2%; further treatment with hexane (or pretreatment with hexane before freeze drying) did not significantly alter this ratio. By the same procedures, we found that the dry/wet ratio of muscle samples averages 23.9 ± 0.2%. Assuming 1) the dry weight represents protein content; 2) the fractional synthesis rates (FSR) are 0.30%/h for skin (30) and 0.214%/h for muscle (30), then the estimated relative contributions of muscle vs. skin protein synthesis is ~1:0.25. The above estimate on relative contributions suggests that ~80% of the in vivo hindquarter protein metabolism occurs in muscle and 20% occurs in skin.

It is worth emphasizing that our estimate remains an approximation. We have used the following assumptions in our calculations: 1) the value of muscle FSR in anesthetized dog is taken for the calculation in rabbit muscle; 2) FSR in hindquarter skin tissue is similar to that measured in ear skin; 3) an FSR obtained from an anesthetized rabbit is the same as that obtained from an awake rabbit; and 4) hindquarter skin blood supply comes from the same major branches as hindquarter muscle tissue.

In summary, the animal model described above provides a chance to evaluate in vivo the burn-induced changes in protein turnover and energy metabolism in a compartment comprised mostly of muscle. Reasonable assumptions and empirical measurement of relative tissue mass in the analyzed compartment suggest that myoskeletal tissue accounts for 80% of the protein metabolism seen in the hindquarter model. In addition, this model can potentially be used to test the regional effect of metabolic modulators (e.g., cytokines, ATPase inhibitors) on protein metabolism and oxygen con-
sumption in a muscular compartment both with and without burn injury.

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