Acute responses of muscle protein metabolism to reduced blood flow reflect metabolic priorities for homeostasis

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solutions for calculation of Phe concentrations in the blood and muscle.

Experimental design. There were three groups of a total of 20 rabbits. The rabbits in group 1 (n = 9) were used to compare the femoral artery flow (measured from the ultrasonic flowmeter), total leg flow (measured from the dye dilution method), and muscle capillary flow (measured from the microsphere method). The rabbits in groups 2 (n = 5) and 3 (n = 6) were used to investigate the responses of muscle protein kinetics to reduced blood flow by either partial clamp (group 2) or full clamp (group 3) of the femoral artery. In group 2, the arteriovenous (A-V) model (1) was used to determine the protein kinetics in leg muscle, and the tracer incorporation (20) and tracee release (23) methods were used to measure protein fractional synthetic rate (FSR) and fractional breakdown rate (FBR) in the adductor femoris. In group 3, the tracer incorporation and tracee release methods were used to measure protein FSR and FBR in the gastrocnemius muscle as a representative tissue; the A-V model was not used because of full clamp on one femoral artery.

Experimental procedures. After an overnight fast with free access to water, the rabbits were anesthetized with intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg) followed by continuous infusion at various rates to maintain a sufficient depth of anesthesia (25). A tracheal tube was placed via tracheotomy. Further procedures varied to complete the measurements in each group. In four rabbits in group 1, catheters were inserted in a carotid artery and a jugular vein. The arterial line was used for blood collection and monitoring of blood pressure and heart rate; the venous line was used for infusion of anesthetics. The femoral artery was isolated in the experimental leg (either side) at the inguinal level. A flow probe (model 1.5RB; Transonic Systems, Ithaca, NY) was placed on the artery, which was connected to an ultrasonic small animal blood flowmeter (T106; Transonic Systems) for measurement of femoral artery flow rate. A 4-0 silk tie was placed with four loops around the femoral artery at the level above the flow probe, so that when the tie was gently pulled tight the blood flow in the artery was reduced to desired rates. Intravascular Over-the-Needle catheters (24G; Baxter Health Care, Deerfield, IL) were inserted in the femoral artery and vein below the level where the flow probe was placed. The femoral artery line was used for infusion of a dye solution, and the venous line was used for blood withdrawal to determine the dye dilution.

After blood (4 ml) was collected for background measurement, indocyanine green (Akorn, Decatur, IL) in 0.9% saline (0.5 mg/ml) was infused in the femoral artery at 15 ml/h. During the dye infusion, the femoral artery was either not clamped or clamped at 50, 33, or 0% (complete occlusion) of the basal rate. After the start of the dye infusion (10 min), blood samples (1.2 ml each) were drawn simultaneously from the femoral vein and jugular vein. Additional 0.2 ml of blood was drawn from the arterial line for measurement of hematocrit. During the blood collection, blood flow rate in the femoral artery was recorded from the flowmeter. After two to three times blood collection at intervals of 10 min, the dye infusion was discontinued, and the rabbit was allowed 30–60 min to recover from blood loss. The femoral artery flow rate was then changed by adjusting the silk loops on the artery, and the dye solution was infused again. In each rabbit, the total blood collection was limited to <30 ml, which was estimated to be <10% of total blood volume, and hematocrit was kept >20%.

The remaining five rabbits in group 1 were used to determine muscle capillary flow with or without complete clamp of the femoral artery. In these rabbits, catheters were inserted in the femoral artery and vein of one leg (either side) through a groin incision that completely occluded the femoral artery flow. The arterial catheter was used for collection and monitoring of arterial blood pressure and heart rate, and the venous catheter was used for infusion of the tracer and anesthetics. Because we completely clamped the femoral artery in one leg, the A-V model was not applied. This is because, if the dye dilution method had been used for the A-V model, it would have required blood withdrawal for both the blood flow measurement and A-V Phe enrichment and concentration measurement so that the blood loss would be too much for a rabbit. Thus protein FSR and FBR were measured in the gastrocnemius muscle in both legs using the sampling schedule described for group 2.

In groups 2 and 3, the blood samples were collected in tubes with heparin and put in an ice-water bath until the end of the infusion. The muscle samples were immediately frozen in liquid nitrogen and stored at −80°C for later processing. In all three groups, mean arterial blood pressure, heart rate, and rectal temperature were monitored continuously and were maintained relatively constant by adjusting the dose of anesthesia, infusion rate of saline, and heating lamps. These vital signs were recorded every 30 min.

Sample analysis. Indocyanine green concentration in serum was measured on a spectrophotometer (Spectronic 1001; Bausch & Lomb, Rochester, NY) at λ of 805 nm. Blood hematocrit was measured on an automated Hematology Analyzer (model JT3; Coulter, Hialeah, FL). To determine Phe enrichment and concentration in the blood, blood was transferred to tubes containing sulfosalicylic acid and 1-[ring-13C]Phe as an internal standard (30 μmol/l). After deproteinization, the supernatant was processed to make the N-acetyl-L-n-propyl ester (NAP) derivatives of amino acids (20). Muscle samples were homog-
enized in 5% (wt/vol) perchloric acid at 4°C. Except for the background sample, a tissue internal standard solution (6 μmol/l of l-[ring-13C6]Phe) was added to the muscle samples (1, 23). Muscle supernatant was processed to make the t-butylidimethylsilyl (TBDM) derivative of Phe (14). The protein pellets were washed thoroughly to remove free amino acids and lipids and dried in an oven at 80°C (24). The wet and dry weights were recorded to calculate water content in the muscle. The dry protein was hydrolyzed in 6 N HCl at 110°C for 24 h and was prepared for the N-heptafluorobutyl-n-propyl ester (HFBBp) derivatives (13).

**Mass spectrometry analysis.** Isotopic enrichment in the blood prepared as the NAP derivatives was measured on a Hewlett-Packard 5985 gas chromatograph–mass spectrometer (GC-MS) (Hewlett-Packard) with chemical ionization. Ions were selectively monitored at mass-to-charge (m/z) ratios of 250, 251, 255, and 256 for Phe. Isotopic enrichment in the muscle supernatant prepared as the TBDM derivatives was determined on a Fison MD 800 GC-MS (Beverly, MA); ions were selectively monitored at m/z ratios of 234, 235, 239, and 240 for Phe. The protein hydrolysate in HFBBp derivatives was analyzed for l-[ring-13C6]Phe enrichment using the method previously described (13). Thus the ratio of m + 6/ m + 4 ions was measured on the Fison MD 800 GC-MS and converted to the true enrichment of l-[ring-13C6]Phe by means of a standard curve.

The isotopic enrichment was expressed as molar percent excess for the A-V model and FSR calculations and as tracer-to-trace ratio for FBR calculations, which were required by the methods. The l-[ring-13C6]Phe enrichment was corrected for the contribution of the abundance of isotopomers of lower weight to the apparent enrichment of isotopomers with larger weight, and also a skew correction factor (15).

**Calculations and statistics.** The rate of leg blood flow was calculated from the dye dilution method: rate = ([infusate OD × 15 ml/h × 51]/[Δserum OD × (1 – Hct)], where infusate OD is the optical absorption in the indocyanine green infusion solution, which was infused at 15 ml/h, and 51 is the times of dilution; Δserum OD is the difference of optical absorption between femoral venous and jugular venous serum. The measured blood flow rate (ml·leg−1·min−1) was divided by the weight of muscle in the leg to convert to the unit of milliliters per 100 g per minute, where the weight of leg muscle was calculated by 4.8% × body weight since the data of leg dissection in our previous experiment indicated that the muscle weight in a hindlimb was 4.8% of the body weight (22). The flow rate in the femoral artery was directly measured by the flowmeter and converted to the same unit as for the leg flow.

The rate of capillary flow was calculated from the microsphere data as follows: capillary flow rate in ml·capillary·min−1·g−1 = (total tissue spheres)/[(tissue wt in grams) × (reference spheres in ml−1/min)] (24).

Protein and Phe kinetics in leg muscle were calculated by the three-pool model (Fig. 1) (1). The equations are as follows.

- **Inflow** = CA × BF
- **Inward transport** = (Eₐ − Eᵥ) × (Eₐ − Eᵥ) × CA × CV + CA × BF
- **A-V shunting** = [(Eₐ − Eᵥ)/Eₐ − Eᵥ] × CV × BF
- **Outward transport** = [(Eₐ − Eᵥ)/Eₐ − Eᵥ] × CV × BF
- **Outflow** = CA × BF
- **NB** = (CA − CV) × BF
- **Synthesis** = [(Eₐ × CA − Eᵥ × CV)/Eₐ] × BF
- **Breakdown** = synthesis − NB

where Eₐ, Eᵥ, and Eₐ are Phe enrichment in the arterial blood, femoral venous blood, and muscle free amino acid pool, respectively. CA and CV are Phe concentration in the arterial blood and femoral venous blood, respectively. BF is the blood flow rate in the hindlimb.

NB is net Phe balance. Inflow is the rate of Phe entering the limb via artery; inward transport is the rate of delivery from artery to the muscle free pool; A-V shunting is the rate of delivery directly from artery to vein; outward transport is the rate of delivery from the muscle free pool to vein; and outflow is the rate of exit via vein. Because Phe is neither synthesized nor degraded in muscle, its disappearance reflects protein synthesis, and its appearance reflects protein breakdown.

The FSR of muscle protein was calculated by the tracer incorporation method (20), and the equation is

FSR = Eₐ(t₂ − t₁) × (t₂ − t₁) / (Eₐ(t₂) − Eₐ(t₁))

where Eₐ(t) is the average free Phe enrichment in the muscle protein-bound pool from time period 1 (t₁) to time period 2 (t₂) and Eₐ(t₂) − t₁ is the average free Phe enrichment in muscle from t₁ to t₂.

FBR of muscle protein was calculated from the trace release method (23). The equation used to calculate FBR is

FBR = ∫ₐ(t₂ − t₁)dt / (Qₐ/T) (10)

where Eₐ(t) is the muscle free Phe enrichment at time t, Eₐ(t) is the arterial Phe enrichment at time t, Qₐ/T is the ratio of free to protein-bound Phe content in muscle, and P = Eₐ(Eₐ − Eₐ), where Eₐ and Eₐ are the free Phe enrichments in the muscle and arterial blood at isotopic steady state.

Data are expressed as means ± SE in the text and Tables 1–5 and as means ± SE in Figs. 1–4. Paired t-test was used to test the differences between the clamped and nonclamped legs in the same rabbits, and nonpaired t-test was used to test the differences between different groups. A P value <0.05 was considered as statistically significant.
RESULTS

There were no significant differences in the body weight (average 4.5–4.8 kg), mean arterial blood pressure (average 70–76 mmHg), heart rate (average 187–195 beats/min), or rectal temperature (average 38.6–38.9°C) among groups. In group 1, four rabbits were used to estimate the leg flow (measured by dye dilution) from the femoral artery flow (measured by the flowmeter). The paired values of femoral artery and leg flow were pooled according to the clamping of the femoral artery. Thirteen paired measurements were completed without clamping; the femoral artery flow and leg flow were 5.9 ± 1.2 and 9.1 ± 1.6 ml·100 g⁻¹·min⁻¹, respectively. Thus, without clamping, the femoral artery flow accounted for 65 ± 4% of the leg flow. When the flow in the femoral artery was clamped at 3.55 ± 0.20 (4 measurements) and 1.95 ± 0.13 (9 measurements) ml·leg⁻¹·min⁻¹, which were 40 and 67% reductions in the femoral artery flow, respectively, the percent contributions of femoral artery flow to leg flow were 53 ± 7 and 33 ± 2%, respectively (Table 1). There was a linear correlation (r = 0.93; P < 0.001) between the femoral artery flow and the ratio of femoral flow to leg flow: (ratio of femoral flow to leg flow) = 0.1230 × (femoral flow) + 0.0892, where the flow is expressed in milliliters per 100 grams per minute. When the femoral artery was fully clamped (2 measurements), the leg flow was 5.3 ml·100 g⁻¹·min⁻¹ (5.0 and 5.6 ml·100 g⁻¹·min⁻¹), which was a 42% reduction in basal leg flow when compared with the contralateral nonclamp leg.

In group 1, the colored microspheres were injected in five rabbits for measurement of the capillary flow in leg muscles. The measured capillary flow rates in the right and left kidney were 54% in the gastrocnemius muscle from 9.99 ± 3.10 to 4.09 ± 2.71 ml·100 g⁻¹·min⁻¹ and in the adductor muscle from 7.83 ± 3.85 to 3.19 ± 1.03 ml·100 g⁻¹·min⁻¹.

Group 2 was used to investigate the effect of a 50% reduction of the femoral artery flow on protein kinetics in leg muscle. During the isotope infusion, the femoral artery flow rate in the control leg was 6.11 ± 1.41 ml·100 g⁻¹·min⁻¹. The leg flow rate was estimated to be 9.41 ± 2.17 ml·100 g⁻¹·min⁻¹, which was calculated by dividing the femoral flow rate by 65%. In the partial clamp leg, according to the results in group 1, the femoral artery flow was no longer 65% of leg flow but followed the equation: (ratio of femoral flow to leg flow) = 0.1230 × femoral flow + 0.0892. Thus the leg flow rate in the partial clamp leg was estimated to be 6.52 ± 0.29 ml·100 g⁻¹·min⁻¹ (Table 1). The Phe enrichment and concentration in the arterial and venous blood and Phe enrichment in the muscle free amino acid pool (data not presented here) were used to calculate protein kinetics and Phe transport rates (Table 2) using the three-pool model (1). Whereas in the partial clamp leg the rate of inward transport decreased significantly, the rates of protein synthesis, breakdown, and net balance were not significantly different from those in the control leg (Table 2). The synthetic rate of muscle protein in the partially clamped leg tended to be lower than that in the control leg (Table 2), although statistical significance was not achieved (P = 0.18). This could be explained by the fact that the A-V model calculates protein metabolism not only in the muscle but also in bone and skin. The convincing data are the identical (P = 0.50) protein FSR in the adductor muscle between the control and partial clamp legs (Table 3), which confirmed that muscle protein synthesis was not changed by the 50% reduction in femoral artery flow.

Group 3 was used to investigate the effect of full clamp of the femoral artery on protein kinetics in leg muscle. Full clamp of the femoral artery resulted in a decrease (P = 0.003) of FSR in the gastrocnemius muscle compared with that in the control leg (Table 3). FBR were measured in both groups 2 and 3. During the 120–180 min of tracer infusion, Phe enrichment in the arterial blood reached isotopic plateau. After stopping the tracer infusion, the enrichment decay in the arterial blood followed the same pattern in the two groups (Fig. 2). The percentages of protein in wet muscle in the adductor muscle (group 2) and in the gastrocnemius muscle (group 3) were 23 ± 1 and 21 ± 2% by weight, respectively. We previously reported that 1 g of dry muscle protein contained 250 μmol Phe (22). Thus the contents of protein-bound Phe in 1 g of adductor and gastrocnemius muscle were 57.5 and 52.5 μmol, respectively. The femoral artery clamp, either partial or full, did not change (P =

### Table 1. Comparisons between femoral artery flow rate and leg flow rate (group 1)

<table>
<thead>
<tr>
<th></th>
<th>Dyel Dilution, ml·100 g⁻¹·min⁻¹</th>
<th>Flowmeter, ml·100 g⁻¹·min⁻¹</th>
<th>Flowmeter/Dye Dilution, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonclamp</td>
<td>13</td>
<td>9.14 ± 1.57</td>
<td>5.92 ± 1.16</td>
</tr>
<tr>
<td>Clamp 1</td>
<td>4</td>
<td>6.75 ± 0.80</td>
<td>3.55 ± 0.20</td>
</tr>
<tr>
<td>Clamp 2</td>
<td>9</td>
<td>5.96 ± 0.70</td>
<td>1.95 ± 0.13</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of experiments. Clamp 1 and Clamp 2 were designed to reduce the femoral artery flow to 50 and 33% of the basal rate, respectively. The dye dilution measures the total leg flow rate, and flowmeter measures the femoral artery flow rate.

### Table 2. Protein kinetics and Phe transport in leg muscle (group 2)

<table>
<thead>
<tr>
<th></th>
<th>Synthesis</th>
<th>Breakdown</th>
<th>NB</th>
<th>Inflow</th>
<th>Inward Transport</th>
<th>Outward Transport</th>
<th>A-V Shunt</th>
<th>Total Free Phe Rₜ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.6 ± 4.1</td>
<td>14.3 ± 2.5</td>
<td>-3.8 ± 2.3</td>
<td>35.9 ± 9.8</td>
<td>21.5 ± 10.0</td>
<td>25.3 ± 9.2</td>
<td>14.4 ± 7.1</td>
<td>35.8 ± 9.0</td>
</tr>
<tr>
<td>Partial clamp</td>
<td>8.3 ± 1.8</td>
<td>13.4 ± 2.7</td>
<td>-4.0 ± 1.3</td>
<td>25.0 ± 5.9</td>
<td>15.9 ± 9.0</td>
<td>19.9 ± 9.7</td>
<td>9.1 ± 4.0</td>
<td>28.3 ± 10.9</td>
</tr>
<tr>
<td>P value</td>
<td>0.18</td>
<td>0.21</td>
<td>0.36</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Values are means ± SD in μmol·100 g⁻¹·h⁻¹. NB, net balance; inflow, rate of Phe entering the leg via artery; inward transport, rate of delivery from artery to the free pool in muscle; outward transport, rate of delivery from muscle free pool to femoral venous blood; A-V shunt, rate of delivery from artery to vein; total free Phe Rₜ, total rate of appearance in the free amino acid pool in muscle, which is the sum of inward transport and breakdown. The P values are calculated from paired t-test.
concentration in the adductor muscle was lower (group 3 with their corresponding control values (Fig. 4). When compared to the control leg, the rates of muscle protein synthesis and breakdown both decreased, indicating that the reduction of femoral artery, the blood flow was reduced by 42% by fully clamping the leg (group 3). Muscle protein FSR (groups 2 and 3).

Table 3. Muscle protein FSR (groups 2 and 3)

<table>
<thead>
<tr>
<th></th>
<th>Group 2 (n = 5)</th>
<th>Group 3 (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Free Pool</td>
<td>Protein-Bound Pool</td>
</tr>
<tr>
<td>Control</td>
<td>6.38 ± 1.17</td>
<td>0.0247 ± 0.0077</td>
</tr>
<tr>
<td>Partial clamp</td>
<td>5.85 ± 0.86</td>
<td>0.0226 ± 0.0083</td>
</tr>
<tr>
<td>Control</td>
<td>4.32 ± 0.98</td>
<td>0.0339 ± 0.0030</td>
</tr>
<tr>
<td>Complete clamp</td>
<td>3.50 ± 0.38*</td>
<td>0.0148 ± 0.0027*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of experiments. FSR, fractional synthetic rate. *P < 0.05 vs. control within group by paired t-test. †P < 0.05 vs. corresponding values between groups by nonpaired t-test.

DISCUSSION

We found that, in the basal state, the femoral flow accounted for 65% of total leg flow, and the remaining was derived from collateral flow. A 50% reduction of femoral flow resulted in a 28% decrease in leg blood flow, which did not significantly change protein turnover rate in leg muscle. This finding indicates that the muscle protein metabolism can compensate for an ~30% reduction of blood flow. When the leg blood flow was reduced by 42% by fully clamping the femoral artery, the rates of muscle protein synthesis and breakdown both decreased, indicating that the reduction of blood flow exceeded the compensation capacity. Therefore, in this animal model, the flow threshold to cause a fall of protein turnover was between 30 and 40% reduction in total leg flow.

The responses of muscle protein kinetics to the reductions in blood flow may reflect the mechanisms of muscle homeostasis. When the reduction in blood flow was <30% of the basal flow, the synthetic and breakdown rates of muscle protein were not changed (Tables 2–4). According to the definition of the three-pool model, the inflow of amino acids in the leg muscle from the artery is the sum of the inward transport and A–V shunting (Fig. 1). Thus the inward transport is analogous to the nutritive flow, and the A–V shunting is nonnutritive. The 28% reduction in leg flow, which resulted from 50% clamp of femoral artery flow, was associated with a 26% reduction in inward transport (Table 2). Thus the response to the reduction in flow cannot be explained by blood redistribution between the nutritive and nonnutritive routes as was proposed to explain increases in metabolic activities induced by insulin or exercise (2, 6, 17). A reasonable explanation would be that, in the basal state, the nutritive flow delivers amino acids to the muscle at the rate that exceeds the minimal requirement for the basal rate of protein synthesis. A relatively small decrease of the nutritive flow, such as a 26% decrease of inward transport, did not cause a deficiency of precursors for protein synthesis.

When the flow in the leg was reduced by 42% by fully clamping the femoral artery, both the synthetic and breakdown rates of muscle protein were decreased significantly (Tables 3 and 4). However, the difference in net balance (FSR – FBR) was not only insignificant but also minor, indicating maintenance of muscle mass (Fig. 4). In other words, when the reduction in blood flow reached the flow threshold, the muscle homeostasis was preserved at the expense of decreasing its turnover rate. Because the A–V model was not applied with full clamp of the femoral artery, the amino acid transport data were not available. Therefore, we measured the capillary flow in five rabbits in group 1 to examine the changes in nutritive flow. Because the injected colored microspheres have a diameter of 15 µm, entrapment in the tissue reflects capillary flow that is considered to be nutritive. In the nonclamp leg, the capillary flow rates were 9.99 ± 3.10 and 7.83 ± 3.8 ml·100 g–1·min–1 in the gastrocnemius and adductor muscles. These values were close to the leg flow rates of 9.14 ± 1.57 and 9.41 ± 2.17 ml·100 g–1·min–1 in groups 1 and 2 measured from the dye dilution method. With the full clamp, the leg flow was reduced by 42%, and the capillary flow was reduced by 59%.
reduction in the capillary flow appeared to be greater than that of leg flow. Therefore, the results do not support blood redistribution from the nonnutritive to nutritive route.

In the partial and full clamp legs, the net protein balance was 0.01%/h more or less than the corresponding control legs (Fig. 4). A reverse-power analysis indicates that 567 rabbits are needed to achieve a power of 0.80. Because the differences in net protein balance were not only insignificant but also minor, the conclusion of muscle homeostasis is considered to be valid without a further statistical analysis after increasing numbers in each group. According to our previous publication, the muscle mass in the hindlimb of rabbits is 4.8% × body weight (22), which is equivalent to 216 g of leg muscle in a 4.5-kg body weight rabbit. If the muscle contains 20% of dry protein, there is 43 g of muscle protein in a hindlimb. The difference of 0.01%/h equals 0.0043 g muscle protein/h. Even if there was such an additional loss (or gain) and the loss (or gain) lasted for 24 h, the clamp leg would have lost (or gained) 0.1 g more muscle protein than the control leg. However, such a minor change, if it turns out to be true from statistical analysis of a large number of subjects, could lead to physiological importance when it persists for a prolonged period of time. Therefore, the findings from the present study cannot be extrapolated to more chronic disturbances of blood flow without further experimental validation.

The muscle homeostasis was reflected by not only a constant protein mass but also a constant intracellular amino acid concentration (Fig. 3). This finding is consistent with a previous study in which hemodialysis significantly decreased plasma amino acid concentrations and muscle protein synthesis, but the intracellular essential amino acid concentrations in the muscle were maintained at the basal level (3, 12). The results support the notion that maintenance of intracellular essential amino acid concentrations and protein mass are the metabolic priorities superior to the maintenance of protein turnover rate. By clamping the femoral artery, the maximal reduction in leg flow was 42% of the basal rate. Therefore, it is not known how muscle would respond to a flow reduction that is greater than 42% of the basal rate. Furthermore, the present study was conducted in the basal state, so the findings may not be extrapolated to other conditions such as during exercise or insulin administration.

When comparison is made between the two control groups in groups 2 and 3, the basal rates of protein synthesis and breakdown in the gastrocnemius muscle were greater (P < 0.01–0.05) than those in the adductor muscle (Table 4). Consistent with the difference in protein turnover rate, basal concentration of free Phe in the gastrocnemius muscle was greater (P < 0.05) than that in the adductor muscle. The metabolic heterogeneity of leg muscles might be related to their physiological functions and fiber composition, but the mechanism is not clear.

In summary, with 50% clamp of the femoral artery, the nutritive flow was reduced by 26%, and the leg flow was reduced by 28%, which did not change muscle protein turnover rate in the leg. Full clamp of the femoral artery resulted in 59% reduction in nutritive flow and 42% reduction in leg flow; the maintenance of muscle mass was accomplished by parallel decreases of both synthesis and breakdown. Therefore, the flow threshold that caused a decrease in muscle protein turnover rate was a 30–40% reduction of the basal flow. The clamp of the femoral artery, either partial or full, did not decrease muscle free amino acid concentration. Thus the acute responses of leg muscle protein metabolism to the reductions in blood flow by femoral artery clamp reflected the metabolism priorities for homeostasis.

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