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Protein metabolism during an acute phase response in chickens*

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Summary. Fractional rates of liver, muscle, plasma and acute phase portein synthesis were measured in chickens injected with saline or E. *coli* lipopolysaccharide (LPS). Male Single Comb White Leghorns were infused with a primed constant infusion of ^{15}N-L-methionine and $^{2}H_5$-L-phenylalanine into the portal vein for 2 h. Changes in plasma amino acid enrichment were similar for both amino acids reaching an apparent plateau by the 30 min sampling time. The enrichment of plasma protein-bound amino acid was measurable after 1 h of isotope infusion and increased linearly over 2 h. LPS injection decreased free phenylalanine enrichment in the carotid artery (50%), and reduced tissue free methionine enrichment in the liver, pectoralis, and gastrocnemius by 16, 41, and 31% respectively. Isotopic enrichment of phenylalanine in liver protein, plasma protein and hemopexin increased in LPS injected birds relative to control birds. Fractional rates of muscle protein synthesis were not affected by LPS injection, however, liver protein, plasma protein, and hemopexin fractional synthesis rates increased 141, 161 and 266% respectively compared with untreated animals.

Keywords: Amino acids – Acute phase response – Poultry – Stable isotopes – Hemopexin – Protein synthesis

Introduction

The acute phase response is a series of metabolic changes that occur following tissue injury or an immunological challenge (Kushner and Rzewnicki, 1994).

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While the impact of the acute phase response on performance parameters has received considerable attention, our understanding of the effect on nutrient partitioning is less well understood (Klasing and Barnes, 1988; Beisel, 1984). One important component of the acute phase response is a significant alteration in protein metabolism, specifically the synthesis of acute-phase proteins. Acute phase proteins are presumed to serve some modulatory role in the progression of inflammation and tissue repair. Synthesis of several acute-phase proteins increases dramatically during infection (Kushner, 1982) and may utilize a large proportion of available amino acids (Reeds et al., 1994). For example, during an immune challenge, synthesis of C-reactive protein increases several hundred-fold, representing 250 mg of protein/kg body weight in the human (Kushner, 1982; Reeds et al., 1994). Hemopexin is another acute phase protein that, in the chicken, is developmentally regulated and responsive to a variety of stress stimuli (Grieninger et al., 1986). It has been suggested that the mobilization of muscle protein that occurs during an immune challenge supplies the necessary amino acid precursors for acute-phase protein synthesis (Reeds et al., 1994). A first step in understanding the potential relationship between acute phase protein synthesis and amino acid metabolism is to quantify their contribution to whole body protein synthesis. Measurements of avian acute-phase protein synthesis have not been previously reported despite their potential importance in protein and amino acid metabolism during infection. In this report changes in plasma amino acid enrichment and fractional rates of tissue protein synthesis in response to an immune challenge were measured.

Materials and methods

Reagents

Stable isotopes (^{15}N-L-methionine 98% atom % enrichment, APE and $^{2}H_5$-ring-L-phenylalanine, 98% APE) were obtained from Cambridge Isotope Laboratories (Andover, MA) and analyzed for chemical and isotopic purity by HPLC and mass spectrometry. E. *Coli* lipopolysaccharide (LPS, serotype O55:B5) was purchased from Sigma (St. Louis, MO). All chromatographic reagents were HPLC grade or better. The University of Arkansas Institutional Animal Use and Care Committee approved all experimental protocols.

Analytical system

A Waters Platform LCZ benchtop quadrapole mass spectrometer (MS, Milford, MA) was interfaced with a Waters Alliance 2690 HPLC. The MS was equipped with an electrospray ionization probe and operated in positive ion mode. The MS was operated in scan mode and collected mass data from 100 m/z to 180 m/z with the settings optimized to achieve baseline mass resolution between isotopomers of leucine. Isotopomers of the amino acids of interest (phenylalanine, methionine) were extracted from the total ion current and the peak corresponding to the retention time of the standard was integrated.

Separation of underivatized amino acids was accomplished on a Waters Symmetry C18 column (2.1 × 150 mm, 3.5 μm particle size, Milford, MA) using a 0.1% trifluoroacetic

acid (TFA)/acetonitrile gradient and a 0.2 ml/min flow rate. The relationship between peak area and pmole of amino acid injected was linear for phenylalanine, and methionine for the range 12.5–500 pmole amino acid injected. In addition, preliminary studies showed that the relationship between the amount of amino acid injected and the isotope ratio measurements were constant for the range 100–1,200 pmole injected.

Infusion studies

Animals were prepared for infusion experiments as previously described (Wang et al., 1998). Briefly, 1.5–2.0 kg male Single Comb White Leghorns from the University of Arkansas Poultry Research Farm were anesthetized and catheters placed in the portal vein, hepatic vein and carotid artery. Birds were infused in the portal vein with a priming dose of amino acid (~7 μmole amino acid infused over 1 minute,) followed by a constant infusion of 1.5 and 1.3 μmole/min$*$kg body weight^{-1} of methionine and phenylalanine respectively (~0.1 ml$*$min^{-1}). Blood samples (0.2–0.5 ml/vessel) were drawn into heparinized tubes at timed intervals from the hepatic vein and carotid artery. Levels of an amino acid or metabolite in the carotid artery would be essentially identical to levels in any major artery (e.g. the hepatic artery) due to the rapid transit time of the circulation. Blood was centrifuged to obtain plasma and the plasma mixed 1:1 with ice-cold 15% sulfosalicyclic acid (SSA) to precipitate proteins. Samples were centrifuged at 10,000 g for 10 minutes, the supernatant diluted 1:10 with 0.1% TFA in water and analyzed for plasma-free amino acid enrichment. The protein pellet was washed 3 times with 7.5% SSA, solubilized in 0.5 N NaOH and an aliquot hydrolyzed with 6 N HCL at 110°C under vacuum for 18 hours. Protein hydrolysates were lyophilized and reconstituted in 1.0 ml of 0.1% TFA in water for determination of protein bound amino acid enrichment.

LPS challenge

Three birds each were injected with saline or 1 mg/kg body weight E. *Coli* lipopolysaccharide (LPS, O55:B5) intraperitoneally 12 h prior to surgery. This level of LPS induces a general acute phase response characterized by fever, anorexia, and decreased growth (Klasing et al., 1987). After implanting catheters, the primed constant amino acid infusion (^{15}N-L-methionine and $^{2}H_5$-ring-L-phenylalanine) was initiated and blood samples were every 30 minute for 2 h. Blood was drawn into heparinized tubes and centrifuged to obtain plasma. An aliquot was deproteinized with 15% SSA and the remaining sample frozen for hemopexin purification. A single bird was infused each day with the treatments alternating days. Following the termination of infusion liver, pectoralis, and gastrocnemius were rapidly removed and frozen until analyzed for amino acid enrichment in free and protein bound amino acid compartments. Enrichment of tracer amino acids in the plasma free and plasma protein pools were determined for each blood sample. Aliquots of the initial blood sample (T = 0) and final blood sample (T = 120 min) were pooled for hemopexin isolation and enrichment analysis. Aliquots of liver, pectoralis, and gastrocnemius were homogenized in 10 volumes of ice-cold distilled water. Proteins were precipitated by the addition of an equal volume of 15% SSA. Samples were further processed as described for the plasma free and bound amino acids.

Hemopexin was isolated from plasma by passage through a heme-agarose affinity column (Sigma Chemical Co., St. Louis, MO) and eluted with 1.0 ml succinic acid buffer (50 mM succinic acid, 500 mM NaCl, 50 mM Tris, pH 8.0). Hemopexin concentration was estimated using the extinction coefficient for mammalian hemopexin (OD_{280}/1.69, Damm, 1966).

Calculations

Enrichment was determined based on the equation T/t_a–T/t_b where T/t_a is the ratio of the target isotopomer ion (*m/z* 151 or 171 for methionine and phenylalanine respectively) to the molecular ion at time *t* of the infusion, and T/t_b is the same ratio measured prior to infusion (t = 0). Fractional rates of tissue protein synthesis (FSR) were calculated using the following equation

$$\frac{\left[T/t\right]_{\mathrm{p}}}{\left[T/t\right]_{\mathrm{f}}} * \mathrm{tm} * 100 = \mathrm{FSR}\left(\%/\mathrm{day}\right)$$

Where $[T/t]_{\mathrm{p}}$ is the change in the tracer/tracee ratio of protein bound phenylalanine during the infusion, $[T/t]_{\mathrm{f}}$ is the change in intracellular free phenylalanine enrichment during the infusion, and tm is the incorporation time expressed in days. The enrichment of plasma protein bound amino acid at tm = 0 was assumed to be representative of liver and muscle protein amino acid enrichment at tm = 0.

Amino acid entry (flux, Q in μmole$*$kg$^{-1}*$h^{-1}) was calculated from the tracer-to-tracee ratios of arterial methionine or phenylalanine using the following equation where I is the rate of intravenous amino acid infusion (μmole$*$kg$^{-1}*$h^{-1}).

$$\mathrm{Q} = \mathrm{I} \times \left[\frac{\mathrm{T/t\ infused\ amino\ acid} - 1}{\mathrm{T/t\ plasma\ amino\ acid}}\right]$$

Statistics

Plasma amino acid enrichment data were analyzed using repeated measures analysis of variance. Differences between free and protein bound amino acid enrichments were assessed with a unpaired two-tailed t-test. Probability of $P < 0.10$ was considered statistically significant.

Results

Amino acid enrichment

Figure 1 shows the change in enrichment of plasma methionine and phenylalanine measured during the constant infusion of 1.5 and 1.3 μmole amino acid$*$min$^{-1}*$kg^{-1} respectively in a control animal. The pattern of enrichment was similar for both amino acids although the magnitude of the enrichment differed (due to differences in amino acid pool size). Apparent steady state equilibrium was achieved by 45 min and the plateau sustained through 120 min of infusion (Fig. 1). Comparisons of plasma amino acid enrichment in the carotid artery and hepatic vein showed no differences between the vessels. Figure 2 shows that enrichment of plasma protein bound amino acids was detectable after 30 min of infusion and appeared to increase linearly through the remainder of the experiment, although only the 120 min protein enrichment was significantly greater than the initial enrichment.

To examine the sensitivity of these methods to measure changes in amino acid metabolism we examined the effects of co-infusion of methionine with a potential precursor. Birds were infused for 60 minutes with [methyl-^{13}C]-L-methionine and then infused with [methyl-^{13}C]-L-methionine and 2-hydroxy-

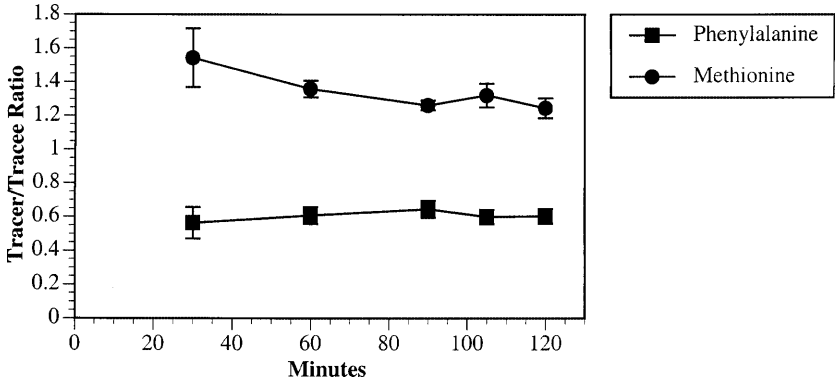
Phenylalanine
Methionine
Tracer/Tracee Ratio
Minutes
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
0
20
40
60
80
100
120

Fig. 1

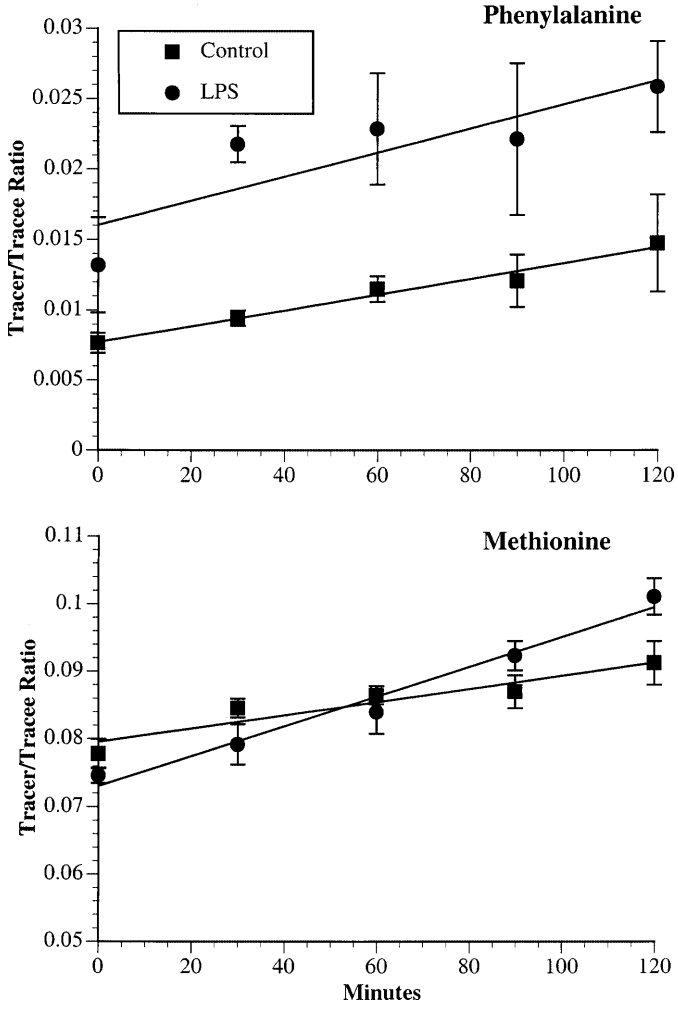
Phenylalanine
Control
LPS
Tracer/Tracee Ratio
0
0.005
0.01
0.015
0.02
0.025
0.03
0
20
40
60
80
100
120
Methionine
Tracer/Tracee Ratio
Minutes
0.05
0.06
0.07
0.08
0.09
0.1
0.11
0
20
40
60
80
100
120

Fig. 2

Table 1. The effects of E. *Coli* O55:B5 lipopolysaccahride (LPS) injection on the enrichment (tracer/tracee ratio) of plasma free and intracellular free amino acids[1]

	Plasma-free[3]		Tissue-free		
	Hepatic	Carotid	Liver	Pectoralis	Gastrocnemius
Phenylalanine[2]					
Control	0.696 ± 0.008	0.731 ± 0.099	0.054 ± 0.001	0.254 ± 0.025	0.204 ± 0.007
LPS	0.635 ± 0.073	0.479 ± 0.032	0.072 ± 0.008	0.193 ± 0.020	0.205 ± 0.022
P-value[4]	NS	0.0005	NS	NS	NS
Methionine[5]					
Control	1.218 ± 0.057	1.246 ± 0.032	0.165 ± 0.007	0.315 ± 0.031	0.359 ± 0.017
LPS	1.314 ± 0.141	1.120 ± 0.084	0.138 ± 0.007	0.187 ± 0.008	0.248 ± 0.018
P-value	NS	NS	0.053	0.016	0.011

[1] Values are means ± SEM; n = 3. [2] Tracer/Tracee ratio of Phe^{171}/Phe^{166}. [3] Plasma enrichment was averaged from samples taken at 60, 90 and 120 min. [4] P-value from unpaired t-test, NS = not significant ($P > 0.1$). [5] Tracer/Tracee ratio of Met^{151}/Met^{150}.

4-methylthiobutanoic acid (HMB, 0.22 mg amino acid$*kg^{-1}*min^{-1}$ of each). HMB is converted to methionine in the avian liver (Dibner and Knight, 1984, Saunderson, 1987) and might be expected to reduce the enrichment of methionine due to they synthesis of endogenous unlabeled amino acid. Plasma methionine enrichment in the hepatic vein decreased from 2.288 ± 0.337 to 1.023 ± 0.116 in the presence of HMB.

LPS challenge

Table 1 shows the changes in free amino acid enrichment due to LPS injection. Enrichment of free methionine or phenylalanine in the hepatic vein were not affected by treatment, however, the carotid phenylalanine enrichment was significantly lower in LPS injected animals compared with saline injected controls ($P < 0.05$, Table 1). Mean (60–120 min) carotid plasma phenylalanine enrichment decreased by 34% with LPS injection. Tissue-free phenylalanine and methionine enrichment were significantly lower than their respective enrichment in plasma (either carotid or hepatic samples). Tissue-free methionine ranged from 11 to 29% of plasma methionine enrichment and phenylalanine enrichment ranged from 8.5 to 40% of plasma phenylalanine enrichment depending on the tissue. Tissue-free methionine was significantly lower in LPS treated animals, decreasing 17, 41 and 31% in the liver, pectoralis and gastrocnemius respectively. Despite the lower plasma phenylalanine enrichment observed with LPS injection there were no differences between treatments in tissue free phenylalanine enrichment (Table 1).

Protein bound amino acid enrichment was determined for phenylalanine in tissue protein, plasma protein and hemopexin, and for methionine in plasma protein (Table 2). Phenylalanine enrichment of muscle protein did not differ significantly between treatments. However, significant differences were

Table 2. The effects of E. *Coli* O55:B5 lipopolysaccahride (LPS) injection on the enrichment (tracer/tracee ratio) of protein bound phenylalanine[1]

	Tissue protein			Plasma protein	Hemopexin
	Liver	Pectoralis	Gastrocnemius		
Control[2]	0.0080 ± 0.0012	0.0039 ± 0.0014	0.0041 ± 0.0006	0.0056 ± 0.0005	0.0133 ± 0.0052
LPS	0.0144 ± 0.0008	0.0045 ± 0.0018	0.0042 ± 0.0010	0.0122 ± 0.0023	0.0457 ± 0.0107
P-value[3]	0.012	NS	NS	0.047	0.053

[1]Values are means ± SEM; n = 3. [2]Tracer/Tracee ratio of Phe^{171}/Phe^{166} at T = 120 min. [3]P-value from unpaired t-test, *NS* not significant (P > 0.1).

Table 3. The effects of E. *Coli* O55:B5 lipopolysaccahride (LPS) injection on the fractional rates of tissue protein, plasma protein and hemopexin synthesis (FSR)[1]

	Liver	Pectoralis	Gastrocnemius	Plasma protein	Hemopexin
			FSR %/day		
Control	176.3 ± 22.3	18.2 ± 5.8	24.4 ± 4.1	123.9 ± 8.6	289.3 ± 107.8
LPS	248.5 ± 38.6	26.9 ± 10.1	23.6 ± 3.8	199.5 ± 19.5	769.3 ± 165.8
P-Value[2]	NS	NS	NS	0.046	0.072

[1]Values are means ± SEM; n = 3. [2]P-value from unpaired t-test, *NS* not significant (P > 0.1).

observed in liver protein, plasma protein and hemopexin phenylalanine enrichment. Liver, plasma protein and hemopexin phenylalanine enrichment increased 180, 217 and 343% respectively in LPS injected birds. Plasma protein phenylalanine enrichment closely approximated that of liver protein. In contrast, hemopexin phenylalanine enrichment was significantly greater than hepatic protein enrichment.

Table 3 shows the effects of LPS injection on fractional rates of protein synthesis of liver protein, muscle protein and hemopexin. Fractional rates of protein synthesis were calculated based on the intracellular free PHE enrichment. LPS injection had no effect on muscle or liver FSR compared with rates in untreated animals. In contrast, LPS significantly increased the fractional rate of plasma protein synthesis (161%, P = 0.046) and increased in hemopexin FSR by 266%-fold (P = 0.072).

Whole body entry of methionine and phenylalanine was estimated from enrichment values and reported in Table 4. The differences between control and LPS treated animals were not significant.

Discussion

Amino acid enrichment

Methionine and phenylalanine rapidly reached an apparent enrichment plateau in both the hepatic vein and carotid artery (Fig. 1) during a primed constant infusion. Studies in swine and sheep report lower enrichment of

Table 4. The effects of E. *Coli* O55:B5 lipopolysaccahride (LPS) injection on the estimated whole body entry rate of phenylalanine and methionine[1]

	Methionine	Phenylalanine
Control	113.4 ± 1.9	202.5 ± 38.2
LPS	125.5 ± 7.6	270.6 ± 23.0

[1] Values are means ± SEM; n = 3.

arterial amino acids compared with pre-hepatic (portal venous) amino acids (Connell et al., 1997; Stoll et al., 1999). In the present experiment, arterial and venous (post-hepatic) enrichments were equal. This is likely due to the site of isotope infusion and blood sampling. For example, we infused amino acids into the portal vein (pre-hepatic) and sampled the hepatic vein (post-hepatic) whereas in the swine and sheep experiments (Connell et al., 1997; Stoll et al., 1999; van Eijk et al., 1999) the label was infused systemically into the jugular vein and sample taken pre-hepatic. Thus, in the birds the infusate was introduced directly into hepatic circulation and sampled immediately after leaving the liver rather than being infused systemically and passing through capillary beds in the peripheral tissues. This approach was chosen due to our interest in using the model for future liver metabolism studies, however, similar measurements could have been made using a simpler catheterization protocol. In contrast to the saline injected birds, the arterial samples from LPS injected animals were lower in amino acid enrichment than hepatic venous amino acids (Table 2). The lower arterial enrichment due to LPS injection may be explained by the mobilization of unlabeled amino acids from peripheral tissue occurring during an immune response (Reeds et al., 1994). In addition to lower arterial amino acid enrichment, muscle free methionine enrichment was significantly lower. Muscle free phenylalanine enrichment tended to be lower and whole body phenylalanine entry higher in LPS injected birds (Table 2 and 4). Since the primary source of unlabelled amino acid available for enrichment dilution are the protein bound amino acids these observations suggest dilution of the tracer with unlabelled amino acids from proteolysis.

The present results also suggest that the source of amino acids for the synthesis of constitutive and secretory hepatic protein differ. Previous reports suggested that amino acid enrichment of hepatic secreted protein would exceed that of the hepatic-free amino acid pool (Stoll et al., 1999) indicating preferential use of extracellular amino acids for the synthesis of secreted proteins. Under our experimental conditions the opposite was observed, the hepatic-free phenylalanine enrichment was approximately 10- and 6-fold higher than the enrichment of total plasma protein in saline and LPS injected birds respectively. Thus the apparent precursor amino acid pool for secretory protein synthesis had a lower enrichment than the intracellular pool. One explanation for this apparent disparity may be the contribution of high enrichment blood to the liver sample. The liver was blotted on paper towels prior to freezing, however, residual blood still present in the liver would be highly

enriched and would result in an overestimation of the hepatic intracellular-free amino aid enrichment. Alternatively, the synthesis of secretory protein may be relatively slow (compared with the infusion time) resulting in non-equilibration of protein bound amino acids with their precursor pool at the time of sampling. The lag time for excreted protein synthesis has been reported to be 30–40 min (Ballmer et al., 1995; Hunter et al., 1995), close to the 60 min it took to see a significant increase in plasma protein enrichment in these experiments (Fig. 2). Thus, total plasma protein enrichment measured at 2 h may represent actual enrichment at the site of synthesis much earlier in the experiment. The slow turnover of plasma proteins combined with a relatively short infusion period could also explain the low protein bound amino acid enrichment compared with the intracellular free amino acid enrichment. This possibility is supported by the closer agreement of hemopexin phenylalanine enrichment with intracellular hepatic amino acid enrichment (Table 1 and 2).

Differences were also observed between the hepatic (total) protein and plasma protein amino acid enrichment. Hemopexin enrichment of phenylalanine exceeded both the plasma protein and the liver protein enrichment by approximately 3-fold. Stoll et al. (1999) reported a similar finding in sheep where secreted ApoB-100 phenylalanine enrichment was significantly greater than hepatic protein bound phenylalanine (10-fold increase). From this and other evidence they concluded that extracellular phenylalanine was the primary source of phenylalanine for secreted protein synthesis (Stoll et al., 1999). The data presented here for the various protein pools indicate differences in amino acid precursor, however additional experiments are necessary to determine the true precursor amino acid pool for a specific protein pool. The low enrichment of all protein pools compared with intracellular amino acid enrichment suggest that secretory and cellular proteins were primarily synthesized from cellular phenylalanine and possibly phenylalanine from proteolysis of cellular proteins. The greater enrichment of hemopexin may indicate increased use of amino acids derived from plasma for its synthesis. Another explanation for the difference between hepatic protein and free amino acid enrichment may be that amino acids derived from peripheral tissue protein degradation were preferentially used for hepatic protein synthesis. This possibility would require some distinction between labeled and unlabelled amino acids within the liver. Such a distinction may be possible since the natural ^{15}N abundance of hepatic nitrogenous compounds has been shown to vary significantly in the rat (Sick et al., 1997). For example, urea and plasma protein can differ by 10% in their natural enrichment of ^{15}N based on partitioning of ^{15}N down various metabolic pathways. Sick et al. (1997) showed that ^{15}N abundance of precursor amino acids is enriched in plasma protein at the expense of isotope in urea. Compartmentalization of amino acids and protein synthesis remains a controversial subject with experimental results supporting most of the possible scenarios (Fern and Garlick, 1976; Hod and Hershko, 1976; Barnes et al., 1995).

Fractional synthesis rates (FSR) of muscle protein reported here agree with values reported in the literature. Hiramoto et al. (1990) measured FSR in

the laying hen using ^{15}N-methionine and reported rates of 91, 22 and 27%/day for the liver, breast muscle and leg muscle respectively. Jones (1990) reported FSR in the same tissues of the broiler chicken to be 45, 15 and 16%/day. Barnes et al. (1995) using the flooding dose method reported FSR for the gastrocnemius and pectoralis to be 15.2 and 11.3%/day respectively. In comparison, the values reported here were 176, 18 and 24%/day for the liver, pectoralis and gastrocnemius (Table 3). In retrospect, we now realize that liver tissue might have been flushed with saline to minimize overestimation of intracellular enrichment values and the corresponding high FSR in the liver.

Acute phase protein synthesis

The liver is a key organ in the regulation of protein and amino acid metabolism. The liver undergoes several significant changes in response to an immune challenge including increased plasma protein synthesis, increased enzyme synthesis and changes in transport processes. In mammals, the increased synthesis of acute phase proteins in response to an immune challenge varies in magnitude from a 50% increase in ceruloplasmin to a several hundred-fold increase in C-reactive protein. The magnitude of the response represents a significant repartitioning of amino acids that may, in part, be responsible for the well-known association of cachexia with infection. The observation that several acute phase proteins have a high percentage of aromatic amino acids lead Reeds et al. (1994) to suggest that the muscle wasting often associated with infection was due to the demand for aromatic amino acid to synthesize acute phase proteins. This possibility is supported by the observation that during an infection, plasma levels of phenylalanine are increased (presumably due to its release from muscle [Kushner, 1982]) and enrichment decreases (Table 2). Reeds et al. (1994) calculated that due to differences in the amino acid composition between muscle protein (the suggested precursor) and acute phase proteins, substantially more muscle protein must be catabolized than the amount of acute phase proteins synthesized. Reeds et al. (1994) estimated that for every mg of acute phase protein synthesized, 2.33 mg of muscle protein would be catabolized to supply the necessary amino acids. Waterlow (1984) calculated that the amount of acute phase proteins produced during an infection in man was ~1.2 g protein$*$kg$^{-1}*$d^{-1} which represents 30% of the total body protein turnover, providing further indication of the importance of the acute phase proteins to whole body protein metabolism. Grieninger and Granick (1978) measured the synthesis and secretion of plasma proteins in chick hepatocytes and found that plasma protein synthesis represented 20% of the total hepatic protein synthesis. The contribution of specific acute phase proteins to total plasma protein synthesis remains uncertain since some plasma proteins increase while others decrease during infection. In later experiments Grieninger et al. (1986) showed that the synthesis of hemopexin reaches a maximum 12-h post induction of stress and that, *in vitro*, hemopexin accounted for 5–10% of total plasma protein synthesis. In the young chicken, plasma hemopexin increases 5-fold in response to an

immune challenge (Korver and Klasing, 1997). In our experiment, fractional rates of hemopexin synthesis increased 2.6-fold during an immune response compared with that measured in saline injected animals.

To estimate the significance of increased hemopexin synthesis on protein metabolism we calculated the potential amount of muscle protein required to support hemopexin synthesis. Plasma hemopexin concentration was approximately 68 μg/ml in LPS inject birds. Assuming blood volume to be 7% of body weight for a 2 kg bird, the total amount of hemopexin would be 9.5 mg. Total daily synthesis of hemopexin is thus (9.5 mg$*$768%/d)/100% or 80 mg of hemopexin/day. Assuming that hemopexin represents 5% of the total acute phase protein synthesis (Grieninger et al., 1986), the male leghorn would synthesize ~0.73 g$*$kg$^{-1}*$d^{-1} of acute phase proteins during an immune challenge. Based on the estimate of 2.3 mg muscle protein$*$mg^{-1} acute phase protein (Reeds et al., 1994) this represents 1.7 g of muscle protein$*$d^{-1} or 8.5 g of muscle mass$*$d^{-1} (muscle 20% protein). Thus 8.5 g of muscle mass$*$d^{-1} may be mobilized to support hemopexin synthesis if dietary amino acids were not available.

In conclusion, the data presented here show that amino acid and protein metabolism are altered during an acute phase immune response. These changes can lead to significant muscle wasting in order to supply precursors for the synthesis of acute-phase proteins. To our knowledge this is the first measurement of fractional rates of avian acute phase protein synthesis. Such information is important in understanding the amino acid requirements of birds during infection.

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