NON RUMINANT NUTRITION

Immune system stimulation increases the irreversible loss of cysteine to taurine, but not sulfate, in starter pigs

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Abstract

An isotope tracer study was conducted to evaluate the effects of immune system stimulation (ISS) on the irreversible loss of cysteine (Cys) to taurine (Tau) and sulfate (SO₄), as well as glutathione (GSH) synthesis, during the fed state in pigs. We previously have reported that ISS increases plasma Cys flux and the GSH synthesis rate at the tissue and whole-body levels in growing pigs. Thus, the current article presents the data on the irreversible loss of Cys during ISS in pigs. Ten gilts (BW: 7.0 ± 0.12 kg) were feed restricted a sulfur amino acids (SAA) limiting diet and injected twice with either saline (*n* = 4) or increasing amounts of *E. coli* lipopolysaccharide (*n* = 6). The day after the second injection, a 5-h primed continuous intravenous infusion of ³⁵S-Cys was conducted. ISS reduced plasma Cys and total SAA concentrations (16% and 21%, respectively; *P* < 0.05). However, ISS had no effect on the plasma concentrations of Tau and SO₄, nor did it affect the appearance of ³⁵S in plasma Tau, plasma SO₄, urinary Tau, or urinary SO₄ (*P* > 0.19). On a whole-body basis and including urinary excretion, ISS increased the appearance of ³⁵S in Tau by 67% (*P* < 0.05), but tended to decrease the appearance of ³⁵S in SO₄ by 22% (*P* < 0.09). Overall, the current findings indicate that during ISS, decreased plasma SAA concentrations and increased plasma Cys flux are attributed in part to increased rates of Cys conversion to Tau, but not Cys catabolism to SO₄. Thus, increased utilization of Cys for the synthesis of immune system metabolites, such as GSH and Tau, is likely the main contributor to increased Cys flux during ISS in pigs. In addition, the irreversible loss of Cys during ISS is small and has a minimal impact on the daily SAA requirements of starter pigs.

Key words: cysteine catabolism, immune system stimulation, pig, sulfate, taurine

Introduction

We previously observed that pigs preferentially retain sulfur amino acids (SAA; Met plus cysteine [Cys]) in the body during immune system stimulation (ISS) (Rakhshandeh et al., 2010a).

Cys, the major extracellular thiol, is conditionally essential during ISS due to its increased utilization for the synthesis of proteins and metabolites that are involved in the immune response (Breuillé and Obled, 2000; Rakhshandeh et al., 2014). Some of the Cys-related metabolites include glutathione (GSH),

Abbreviations

CRD	completely randomized design
Cys	cysteine
GIT	gastrointestinal tract
GSH	glutathione
ISS	immune system stimulation
RA	radioactivity
SAA	sulfur amino acids
SO ₄	sulfate
SRA	specific radioactivity
Tau	taurine

sulfate (SO₄), and taurine (Tau), which are central to various detoxification, antioxidant, and bioreduction reactions, as well as regulation of osmotic and electrolyte balance in the body (Stapleton et al., 1998; Stipanuk, 1999; Ripps and Shen, 2012). GSH and Tau are abundant in the intracellular fluid, whereas SO₄ is predominantly present in the extracellular fluid (Huxtable, 1992; Stipanuk, 2004). Several studies have shown an increased utilization of Cys for the synthesis of GSH and acute-phase proteins during ISS in pigs and other species (Young et al., 2010; Rakhshandeh et al., 2019). However, the impact of ISS on the irreversible loss of Cys in individuals fed a SAA limiting diet has not been quantified. Although the incorporation of Cys into GSH is reversible, Cys metabolism to Tau and SO₄ represents an irreversible loss of Cys via Cys sulfinate-dependent and Cys sulfinate-independent pathways (Stipanuk, 2004). The objective of the present study was to determine the influence of ISS on the irreversible catabolism of Cys to Tau and SO₄ in growing pigs fed a SAA limiting diet. We hypothesized that increased Cys flux during ISS would be due, in part, to an increased irreversible loss of Cys to Tau and SO₄.

Materials and methods

The experimental protocol for this study was reviewed and approved by the University of Guelph Animal Care Committee (ACC approval number 09R104). Experimental details including ISS model, methods for evaluation of ISS effectiveness, isotope infusion, animals, housing, diets, feeding, surgical catheterization, and recovery care, as well as measures of growth performance, have been reported previously (Rakhshandeh et al., 2019 and 2014).

Observations and sampling

For determining plasma Cys concentrations and the specific radioactivity (SRA) of Cys metabolites, a 5-mL blood sample was drawn into a heparinized tube (BD Vacutainer, BD Franklin Lakes, NJ) via the right catheter just before the isotope infusion started and at 1-h intervals during the infusion. To determine plasma concentrations of free AA and SO₄, blood samples were collected in EDTA containing tubes (BD Vacutainer) at the end of the infusion period and centrifuged at $1,500 \times g$ for 15 min to separate plasma from packed erythrocytes. Packed erythrocytes and plasma were then stored at -20°C . Urine was quantitatively collected during the 5-h infusion period into tared bottles containing sufficient amounts of 5 N HCl to lower pH below 3. Urine was then stored at 4°C for quantification of urinary AA and SO₄ excretion. At the end of the infusion period, pigs were euthanized and samples of tissues were collected, as described previously (Rakhshandeh et al., 2019).

Chemical analysis

All chemicals and reagents were obtained from Sigma Aldrich (Sigma Aldrich Canada Ltd., Oakville, ON), unless stated

otherwise. All samples were analyzed in duplicate. Erythrocytes, plasma, and tissue-free Cys were isolated and quantified using the high-performance liquid chromatography (HPLC) method of Reid and Jahoor (Reid and Jahoor, 2000), with the minor modifications noted by Rakhshandeh et al. (2019). Tissue-free Cys was also extracted and determined as monobromobimane derivatives, as described previously (Rakhshandeh et al., 2019). The isolation and quantification of Cys, as well as reverse phase separation of Cys, were carried out as previously described (Rakhshandeh et al., 2019).

Quantification and isolation of plasma, urine, and tissue-free Met and Tau, as well as urinary Cys, were carried out according to Pico.Tag procedures for free AA analysis (Cohen et al., 1989), with the use of a Microcon Centrifugal Filter Device YM 50 (Millipore, Bedford, MA) for deproteinizing the plasma and tissue samples. All the chemicals used for free AA analysis, including the derivatization reagent and Pico.Tag elution buffers, were obtained from Waters Canada Ltd (Mississauga, ON, Canada; Waters Chemistry Package). Isolation and quantification of plasma, urine, and tissue SO₄ were performed using the HPLC method of Coloso and Stipanuk (Coloso and Stipanuk, 1989) with modifications.

Cysteine, Tau, and SO₄ peaks were collected over a window of time that started and ended 1 min before and after the elution time of Cys, GSH, Tau, or SO₄, using a Cygnat-ISCO fraction collector (Lincoln, NE). The level of radioactivity (RA) in the collected fractions was determined by liquid scintillation counting using a Beckman LS 6000 liquid scintillation system (Beckman Instruments, Fullerton, CA), after adding 5.0 mL of Ecolite biodegradable scintillation fluid (Fisher Scientific). Erythrocyte Tau concentrations and SRA were not determined due to technical issues and the loss of samples.

Calculations

SRA (dpm/nmol) of Cys, Tau, and SO₄ were calculated from measured RA and their concentrations in either the plasma or tissues according to the methods of Wolfe and Chinkes (Wolfe and Chinkes, 2005; Rakhshandeh et al., 2019).

Due to the hazardous nature of working with large doses of radioactive material, tissue weights were not determined. However, the pool sizes of Tau and SO₄ (PS; $\mu\text{mol/tissue}$) were estimated as the product of tissue weight and the corresponding Tau and SO₄ concentrations, using Pluske et al. (Pluske et al., 2003) and the final BW of each ISS group. It was assumed that ISS increases the weights of the liver, spleen, and small intestine by 11%, 58%, and 10%, respectively, and reduces the skeletal muscle mass by 15% (Malmezat et al., 2000a; Rakhshandeh et al., 2010a).

Recovery of ^{35}S -Tau and ^{35}S SO₄ in the tissues was calculated as a percentage of the total ^{35}S infused, using the following equation:

$$\text{Recovery} = \frac{\text{SRA}_p \times \text{PS}_p}{\text{Total}^{35}\text{S infused in 5-h}} \times 100,$$

where SRA_p and PS_p are specific radio activity (dpm/nmol) and estimated pool size (nmol/tissue) of ^{35}S -Tau or ^{35}S SO₄, respectively.

Statistical analysis

Statistical analyses were carried out using SAS software version 9.1 (SAS institute Inc., Cary, NC), as described previously (Rakhshandeh et al., 2019). Briefly, one-time observations such as the precursor SRA, recovery of RA as Cys metabolites, and plasma concentrations of Met, Tau, and SO₄ were analyzed using a completely randomized design (CRD; PROC MIXED), with ISS as a fixed effect and litter as a random effect. Plasma

Cys SRA, and Cys concentration, were analyzed as repeated measurements in a CRD (PROC MIXED). This model included ISS, time, and ISS $\times$ time as fixed effects, and litter as a random effect. Treatment effects were considered significant at $P \leq 0.05$. A tendency towards a significant difference between treatment means was also considered at $P \leq 0.10$. Values were reported as means $\pm$ standard error (SE).

Results

Plasma kinetics of Cys and its metabolites

Plasma Cys kinetics measurements have been previously reported (Rakhshandeh et al., 2019). Free Cys SRA rose to a plateau within 1 h of starting the infusion and remained steady thereafter in both ISS- and ISS+ pigs ($P = 0.44$). Additional results are presented in Table 1. No interaction effect between plasma concentrations of Cys and time was observed during the infusion period (Figure 1; $P = 0.60$). ISS decreased the plasma-free Cys concentrations ($P < 0.01$), but not Met concentrations ($P > 0.05$), whereas it had no effect on the plasma concentrations of Tau or SO_4 ($P > 0.27$). ISS had no effect on the SRA of free Cys, Tau, and SO_4 in plasma (Table 1; $P > 0.20$). A tendency ($P < 0.09$) for a higher infusion rate was observed for ISS+ pigs when the analyzed infusion rate values were expressed per kg of final body weight (Rakhshandeh et al., 2019).

Tissue free SAA, Tau, and SO_4 concentrations

Results are presented in Table 2. Methionine concentrations were higher in the liver ($P < 0.03$), but lower in the lungs and heart of ISS+ pigs, when compared with ISS- pigs ($P < 0.04$). Concentrations of free Cys were higher in the liver ($P < 0.03$), but

lower in the spleen of ISS+ pigs, when compared with ISS- pigs ($P < 0.05$). In the liver, lung, and muscle, Tau concentrations were increased by ISS ($P < 0.05$). ISS increased the concentrations of free SO_4 in the heart and muscle ($P < 0.05$) and tended to increase these in the lungs ($P < 0.10$). ISS had no effect on urinary excretion of total Met, Cys, and Tau ($P < 0.56$). Over the entire infusion period, cumulative excretion was 2.10 ± 0.47 , 18.8 ± 4.13 , and 22.3 ± 3.62 μmol for Met, Cys, and Tau, respectively. ISS decreased concentrations (ISS- vs. ISS+; 5.95 ± 0.95 vs. 2.87 ± 0.26 mM) and total excretion of SO_4 (0.93 ± 0.14 vs. 0.40 ± 0.03 mmol/5 h of infusion) in the urine ($P < 0.01$).

Recovery of ^{35}S as ^{35}S -Tau and $^{35}\text{SO}_4$ in the tissues

Results, expressed as the total appearance of RA per kg of tissue, are presented in Table 3. ISS increased the recovery of ^{35}S -Tau in the large intestine, lung, and skeletal muscle ($P < 0.05$), whereas it had no effect on the recovery of Tau in other tissues ($P > 0.20$). Recovery of $^{35}\text{SO}_4$ was higher in the lungs and heart ($P < 0.05$) of ISS+ pigs, when compared with ISS- pigs. ISS reduced the recovery of $^{35}\text{SO}_4$ in skeletal muscle, the small intestine, and urine ($P < 0.05$). No effect of ISS on the recovery of $^{35}\text{SO}_4$ was observed in the other tissues that were evaluated ($P > 0.15$). The SRA of urinary Tau and SO_4 was not affected by ISS ($P > 0.41$; 7.47 ± 2.59 and 10.4 ± 2.86 dpm/nmol, respectively).

When recovery data were expressed as a percentage of the total amounts of RA infused (Table 4), recovery of ^{35}S -Tau in the liver, heart, kidney, small intestine, muscle, plasma, and urine was not affected by ISS ($P > 0.27$). RA recovered in Tau was higher in the spleen, large intestine, and lung of ISS+ pigs than those of ISS- pigs ($P < 0.05$). ISS increased and tended to increase the recovery of ^{35}S as SO_4 in the lungs and the spleen, respectively, whereas it substantially decreased the recovery of $^{35}\text{SO}_4$ in skeletal muscle and urine. The recovery of $^{35}\text{SO}_4$ in the liver, heart, kidney, small and large intestines, muscle, erythrocytes, and plasma was not affected by ISS ($P > 0.25$). When the amounts of ^{35}S recovered as ^{35}S -Tau were calculated on a whole tissue basis, about 1% of the total amount of ^{35}S -Tau generated was recovered in the plasma and urine. In contrast, when the same calculation was made for $^{35}\text{SO}_4$, more than 80% of the total amount of $^{35}\text{SO}_4$ was recovered in the plasma and urine. Across all the tissues that were considered, 0.95% and 1.23% of infused ^{35}S was recovered in Tau plus SO_4 in ISS- and ISS+ pigs, respectively, representing a minor increase in the irreversible loss of Cys

Table 1. Impacts of ISS on plasma concentrations of methionine and cysteine kinetics, as well as plasma concentrations and SRA of taurine and sulfate in starter gilts¹

Parameter	ISS-	ISS+	SE	P
n ²	4	6		
Methionine plasma concentration, μM	41.7	31.6	7.82	0.30
Cysteine				
Plasma concentration, μM	67.0	56.1	4.52	0.01
Infusion rate, dpm/kg BW/h $\times 10^5$	432.0	589.2	63.70	0.09
SRA ³ , dpm/nmol	341.9	360.0	54.00	0.63
Flux ³ , $\mu\text{mol/kg BW/h}$	132.7	166.6	14.70	0.01
Taurine				
Plasma concentration, μM	41.1	48.7	11.70	0.62
SRA ³ , dpm/nmol	5.73	7.2	1.920	0.52
Sulfate				
Plasma concentration, μM	880.0	950.0	84.00	0.59
SRA ³ , dpm/nmol	7.8	9.7	1.38	0.28

¹Data are least square mean values $\pm$ largest SE among means observed at the end of a 5 h continuous infusion of ^{35}S -cysteine. Pigs with an initial body weight of 7.0 ± 0.12 kg were injected (i.m.) every 48 h (total of two injections) with either increasing amounts of LPS (ISS+; $n = 6$) or a sterile saline solution (ISS-; $n = 4$). In the morning of the day after the second injection with LPS, pigs were given a primed constant i.v. infusion of ^{35}S -cysteine for 5 h.

²Number of observations (pigs) per mean.

³Plasma SRA data are the average of the last 3 h of infusion when ^{35}S -cysteine SRA was at steady state. Plasma SRA of cysteine, taurine, and sulfate were determined as metabolite radioactivity (dpm)/metabolite concentration (nmol). Plasma cysteine flux was determined as ^{35}S -cysteine infusion rate (dpm/kg BW/h)/SRA of ^{35}S -cysteine at steady state (dpm/nmol).

Figure 1. Impact of ISS on plasma cysteine concentrations during the isotope infusion period. Pigs with an initial body weight of 7.0 ± 0.12 kg were injected (i.m.) every 48 h (total of two injections) with either increasing amounts of LPS (ISS+; $n = 6$) or a sterile saline solution (ISS-; $n = 4$). In the morning of the day after the second injection with LPS, pigs were given a primed constant i.v. infusion of ^{35}S -cysteine for 5 h.

Table 2. Impacts of ISS on the concentrations (mmol/kg) of free methionine, cysteine, taurine, and sulfate in the tissues of growing pigs¹

	Methionine				Cysteine				Taurine				Sulfate			
	ISS–	ISS+	SE	P	ISS–	ISS+	SE	P	ISS–	ISS+	SE	P	ISS–	ISS+	SE	P
Tissue																
Liver	0.12	0.20	0.031	0.03	0.61	0.83	0.070	0.03	2.13	3.36	0.340	0.02	0.21	0.22	0.010	0.38
Small intestine	0.12	0.10	0.010	0.25	0.34	0.27	0.041	0.22	2.20	2.39	0.200	0.49	0.20	0.25	0.054	0.48
Large intestine	0.07	0.06	0.012	0.17	0.07	0.06	0.014	0.69	1.36	1.44	0.364	0.86	0.05	0.09	0.033	0.17
Lung	0.09	0.05	0.010	0.04	0.40	0.46	0.651	0.49	4.74	6.06	0.331	0.01	0.22	0.46	0.140	0.10
Heart	0.15	0.09	0.020	0.02	0.01	0.01	0.001	0.96	7.50	6.83	1.722	0.77	0.13	0.23	0.030	0.04
Spleen	0.11	0.10	0.024	0.69	0.40	0.31	0.030	0.05	3.22	3.53	0.451	0.62	0.14	0.15	0.044	0.32
Kidney	0.09	0.11	0.020	0.33	2.29	2.39	0.680	0.94	2.47	2.51	0.380	0.94	0.28	0.28	0.023	0.71
Muscle ²	0.14	0.14	0.013	0.89	0.01	0.01	0.001	0.58	4.50	7.24	0.970	0.05	0.19	0.36	0.041	0.02
Erythrocytes ³	–	–	–	–	0.36	0.47	0.134	0.48	–	–	–	–	0.53	0.33	0.230	0.52

¹Data are least square means $\pm$ largest standard error (SE) among means observed at the end of a 5-h continuous infusion with ³⁵S-cysteine. Pigs with an initial body weight of 7.0 ± 0.12 kg were injected (i.m.) every 48 h (total of two injections) with either increasing amounts of LPS (ISS+; $n = 6$) or a sterile saline solution (ISS–; $n = 4$). In the morning of the day after the second injection with LPS, pigs were given a primed constant i.v. infusion of ³⁵S-cysteine for 5 h.

²Gastrocnemius.

³mmol/L.

Table 3. Impacts of ISS on the recovery of ³⁵S-³taurine and ³⁵S-sulfate in the tissues¹

	Taurine ² , kBq/kg tissue				Sulfate ² , kBq/kg tissue			
	ISS–	ISS+	SE	P	ISS–	ISS+	SE	P
Tissue								
Liver	116	138	29.7	0.55	17.3	16.0	6.00	0.88
Small intestine	72	56	16.3	0.34	10.2	5.9	2.71	0.05
Large intestine	25	67	10.5	0.02	8.2	12.3	3.16	0.26
Lung	191	275	37.0	0.05	31.6	62.3	13.56	0.05
Heart	86	109	26.5	0.50	2.4	4.2	0.79	0.05
Spleen	146	154	18.2	0.71	7.6	17.4	7.82	0.28
Kidney	174	172	20.8	0.92	6.1	6.3	0.93	0.88
Muscle	35	80	29.6	0.05	2.1	1.5	0.20	0.04
Erythrocytes	–	–	–	–	2.7	3.5	1.11	0.56
Plasma	3.5	4.7	1.25	0.45	112	146	19.2	0.21
Urine	19	19	1.5	0.90	853	584	36.4	0.01

¹Data are least square means $\pm$ largest standard error (SE) among means observed at the end of a 5-h continuous infusion with ³⁵S-cysteine. Pigs with an initial body weight of 7.0 ± 0.12 kg were injected (i.m.) every 48 h (total of two injections) with either increasing amounts of LPS (ISS+; $n = 6$) or a sterile saline solution (ISS–; $n = 4$). In the morning of the day after the second injection with LPS, pigs were given a primed constant i.v. infusion of ³⁵S-cysteine for 5 h. Total RA (kBq/kg or L tissue) of ³⁵S-³taurine and ³⁵SO₄ in the plasma were calculated as [product SRA (dpm/nmol) $\times$ Product concentration (nmol/kg or L tissue)]/[60000].

during a moderate ISS. ISS had no effect on distribution of free ³⁵S-Cys as a percentage of the total amount of RA infused in the tissues ($P > 0.01$).

Discussion

In this study, the impact of ISS on the irreversible loss of Cys to Tau and SO₄ was investigated in starter pigs during the fed state. We measured SRA of Cys and its metabolites in a range of tissues to better understand how plasma Cys is partitioned and catabolized among the tissues during ISS. SRA of Cys and its metabolites in each tissue free metabolite pool reflect the balance between the uptake of the metabolite from the blood, catabolism, and release from degradation. In addition, a constant 5-h infusion of ³⁵S-Cys was utilized to avoid the application of large doses of radioactive Cys and to reduce interference with Cys metabolism (Stipanuk, 2004; Wolfe and Chinkes, 2005). An

effective ISS was achieved using an LPS model in the present study (Rakhshandeh et al., 2019).

In previous studies, administration of ¹³C-Cys and the appearance of ¹³C in expired CO₂ have been used to generate estimates of irreversible Cys loss (Lyons et al., 2001). However, this approach tends to underestimate the irreversible loss of Cys, since it does not account for the accumulation of ¹³C in pyruvate and the incomplete recovery of generated ¹³CO₂ (Weinstein et al., 1988). When using ³⁵S-Cys as the tracer, the rate of catabolism of Cys to Tau and SO₄ is measured directly, providing an alternative and more accurate estimate of irreversible Cys loss. Of course, this method assumes that there is no further metabolism of Tau to products other than SO₄, and that SO₄ is not incorporated into other metabolites (Stipanuk, 2004). We previously reported and discussed the effects of ISS on plasma Cys flux and its association with enhanced GSH turnover at the tissue and whole-animal level. (Rakhshandeh et al., 2019). Clearance from the plasma is through irreversible

Table 4. Impacts of ISS on the distribution of ³⁵S-Cysteine, ³⁵S-aurine, and ³⁵S-sulfate in tissues (% of total amount of ³⁵S infused)^{1,2}

	Tissue weight, g		Cysteine, %				Taurine, %				Sulfate, %			
	ISS–	ISS+	ISS–	ISS+	SE	P	ISS–	ISS+	SE	P	ISS–	ISS+	SE	P
Liver	278	309	0.12	0.19	0.031	0.08	0.07	0.09	0.020	0.36	0.01	0.01	0.003	0.93
Small intes.	322	354	0.09	0.04	0.031	0.24	0.05	0.04	0.010	0.57	0.007	0.004	0.0020	0.22
Large intes.	103	103	0.01	0.01	0.002	0.40	0.005	0.014	0.002	0.02	0.002	0.003	0.0010	0.26
Lung	136	136	0.02	0.03	0.010	0.70	0.051	0.092	0.013	0.05	0.008	0.021	0.0040	0.04
Heart	63	63	0.0009	0.0005	0.00048	0.19	0.011	0.014	0.003	0.49	0.0003	0.0005	0.00011	0.15
Spleen	27	43	0.003	0.004	0.0005	0.03	0.008	0.014	0.002	0.02	0.0004	0.0015	0.00069	0.10
Kidney	70	70	0.058	0.049	0.0156	0.65	0.025	0.025	0.003	0.92	0.0009	0.0009	0.00013	0.71
Muscle	4130	3510	0.037	0.052	0.0170	0.42	0.30	0.58	0.220	0.27	0.018	0.011	0.0018	0.01
RBC	189	189	0.017	0.019	0.0066	0.84	–	–	–	–	0.0011	0.0014	0.00045	0.57
Plasma	531	531	0.42	0.39	0.007	0.60	0.004	0.005	0.0014	0.45	0.124	0.162	0.0211	0.20
Urine	158	140	ND	ND	–	–	0.006	0.005	0.0003	0.56	0.252	0.138	0.0379	0.01

¹Data are least square means $\pm$ the largest standard error (SE) of means observed at the end of a 5-h continuous infusion with ³⁵S-cysteine. Pigs with an initial body weight of 7.0 ± 0.12 kg were injected (i.m.) every 48 h (total of two injections) with either increasing amounts of LPS (ISS+; $n = 6$) or a sterile saline solution (ISS–; $n = 4$). In the morning of the day after the second injection with LPS, pigs were given a primed constant i.v. infusion of ³⁵S-cysteine for 5 h. Small intes. = small intestine. Large intes. = large intestine. RBC = red blood cells.

²Radioactivity was measured for each end product and expressed as the percentage of total radioactivity infused during the 5-h infusion period.

Cys loss and Cys incorporation into protein and GSH (Waterlow et al., 1978; Stipanuk, 1999). In the present study, dietary Cys and SAA intake was identical for ISS– and ISS+ pigs. Therefore, the considerable increase in plasma Cys flux in ISS+ pigs can be attributed largely to increased entry of Cys from protein degradation induced by ISS (Breuillé and Obled, 2000). In addition, increased GSH turnover and increased biosynthesis of Cys from Met (via transsulfuration) may contribute to the elevated plasma Cys flux in ISS+ pigs (Malmezat et al., 2000b; Malmezat et al., 2000a; Lyons et al., 2001; Jahoor et al., 2006). We have shown previously that ISS activates transsulfuration pathways at the transcriptional level in the liver of growing pigs fed SAA limiting diets (Rakhshandeh et al., 2010b). The use of Cys for protein synthesis was not determined in the current experiment. However, studies with rats and humans have suggested that Cys utilization for protein synthesis is either unaffected or reduced during ISS (Malmezat et al., 2000b; Jahoor et al., 2006). Taken together, in the present study, increased Cys flux and decreased plasma concentrations of free Cys in ISS+ pigs can be attributed to enhanced uptake of Cys by the tissues, especially the liver. Increased synthesis of GSH in the liver is likely to be a main contributor to increased Cys uptake in this tissue (Melchior et al., 2004; Schalinske, 2009).

Previous work by others has shown that Tau and SO₄ can be transferred between tissues (Weinstein et al., 1988; Malmezat et al., 1998). In the current study, the presence of ³⁵S-Tau in tissues that are unable to synthesize Tau confirmed that Tau exchange between the tissues is occurring during the 5-h isotope infusion period. Furthermore, only about 1% of the total amount of generated ³⁵S-Tau appeared in the plasma and urine, demonstrating the important role of blood cells in transporting Tau between the tissues (Jacobsen and Smith, 1968; Huxtable, 1992). In contrast, more than 80% of total ³⁵SO₄ that was generated during the 5-h infusion appeared in the plasma and urine. This appearance indicated a rapid loss of SO₄ from the tissues into the plasma, followed by subsequent SO₄ excretion into the urine (Stipanuk, 2004). Because of the high rates of Tau and SO₄ exchange between the tissues, the within-tissue rate of conversion of Cys to its end products could not be estimated and the results are reported as total and cumulative appearances of ³⁵S-Tau and ³⁵SO₄ in individual tissues.

Based on the results in Table 4, ISS increased the overall recovery of ³⁵S in the end products of Cys catabolism from 0.94% to 1.21% of the total amount of ³⁵S infused. This increase occurred mainly due to the enhanced irreversible loss of Cys to Tau in ISS+ pigs, since the overall production of ³⁵SO₄ was reduced in these same pigs. Malmezat et al. (1998) reported a similar observation in septic rats. In sum, our results suggested that in response to ISS, growing pigs modify the irreversible loss of Cys by increasing Tau production and reducing catabolism of Cys to SO₄. However, quantitatively the increase in the irreversible loss of Cys due to ISS is small (~2.5% of dietary SID Cys intake).

In the current study, ISS showed no effect on the appearance of ³⁵S-Tau and ³⁵SO₄ in the plasma. As mentioned earlier, data on the effects of ISS on plasma Tau need to be interpreted with caution because of the rapid between-tissue transfer of Tau and the very low contribution of plasma ³⁵S-Tau to whole-body ³⁵S-Tau (Stipanuk, 2004). In addition, Tau metabolism and homeostasis is regulated largely at the cellular level. Specifically, plasma Tau mainly reflects Tau synthesis in Tau-rich blood cells and is unlikely to reflect the generation of Tau within other tissues since the Tau transport through circulation is carried out mainly by blood cells (Jacobsen and Smith, 1968). In the current study, the accumulation of ³⁵SO₄ in the plasma and urine represents a major fraction (~40% and 50%, respectively) of whole body ³⁵SO₄ generation during the 5-h infusion period. Therefore, evaluating the irreversible loss of Cys to SO₄ in the plasma, as well as urine, is more appropriate. In the current study, the amount of ³⁵S excreted as SO₄ in the urine is equivalent to 0.25% and 0.14% of the total ³⁵S infused in ISS– and ISS+ pigs, respectively. Additionally, Tau excretion in the urine is equivalent to 0.006% of the total amount of ³⁵S infused. These amounts are in addition to the accumulation of ³⁵S in Tau and SO₄ in the various tissues. Collectively, these results show the importance of quantification of irreversible loss of Cys at the tissue level.

During ISS, the liver is the major site for SAA metabolism, and one of the major sites for the synthesis of GSH and Tau (Breuillé et al., 1998; Stipanuk, 1999; Malmezat et al., 2000a; Rakhshandeh et al., 2010b). In the current study, ISS increased the concentrations of free Met and Cys in the liver, which is also consistent with previous observations in rats and pigs (Rakhshandeh et al., 2008; Malmezat et al., 2000b) and may

reflect increased uptake of these AA by the liver from the plasma during ISS (Reeds and Jahoor, 2001). In the liver, ISS also increased free Tau concentrations, whereas it did not affect SO_4 concentrations and the appearance of labeled Tau and SO_4 . This result likely occurred due to the high export rate of these metabolites from the liver (Huxtable, 1992; Malmezat et al., 1998). In sum, our results suggest that ISS increases hepatic utilization of SAA, likely for synthesis of acute phase proteins, GSH and Tau.

The gastrointestinal tract (GIT) is the largest immune organ in a pig's body and serves as an important site for the metabolism of SAA and the synthesis of GSH during oxidative stress (Sansonet, 2004). In the current study, ISS did not affect Tau concentrations and the appearance of ^{35}S -Tau in the small intestine, seemingly because Tau concentrations are maintained largely via uptake from the blood, rejugation of bile acids, and reabsorption of Tau (Benight et al., 2009). ISS decreased the appearance of $^{35}\text{SO}_4$ in the small intestine, suggesting either a higher rate of SO_4 export from the small intestine or a lower rate of Cys catabolism to SO_4 (Waterlow, 2006; Burrin and Stoll, 2007). The latter is more probable, since the overall production of $^{35}\text{SO}_4$ was decreased in ISS+ pigs. Reduced catabolism of Cys in the small intestine of ISS+ pigs likely occurs via partitioning of Cys to GSH, as suggested by other investigators (Jahoor et al., 1995; Malmezat et al., 2000a; Burrin and Stoll, 2007). In the present study, increased accumulation of ^{35}S -Tau in the large intestine of ISS+ pigs can largely be attributed to enhanced uptake from the blood, since no production or reabsorption of Tau takes place in this tissue (Stipanuk, 2004). The biological significance of the accumulation of ^{35}S -Tau in the large intestine is unclear. Metabolism of SAA in the heart and lungs, especially during oxidative stress, is poorly documented. In the present study, ISS reduced Met concentrations in the lungs and the heart, whereas it had no effects on Cys concentrations in these tissues. Lower concentrations of free Met in the lungs and the heart of ISS+ pigs cannot be associated with increased use of Met for biosynthesis of Cys, because cystathionine beta synthase, a key enzyme for Cys synthesis, is absent in these tissues (Stipanuk, 2004; Jhee and Kruger, 2005). Therefore, free Met in these tissues was likely used for protein synthesis or exported from the cell (Breuill et al., 1998; Malmezat et al., 2000a; Waterlow, 2006). In the lungs, ISS increased Tau concentrations and the appearance of ^{35}S -Tau, which can be attributed to both increased Tau production and uptake from the circulation (Stipanuk, 2004). Enhanced Tau concentrations in lung tissue are essential for controlling oxidative damage during inflammation (Banks et al., 1992). In the present study, a tendency towards increased SO_4 concentrations and the appearance of $^{35}\text{SO}_4$ were observed in the lungs of ISS+ pigs, suggesting enhanced catabolism of Cys to SO_4 in this tissue. The latter can likely be associated with the role that SO_4 may play in maintaining lung electrolyte homeostasis, which is usually disturbed during ISS (DuBose, 1998; Stipanuk, 2004). Overall, these results suggest that ISS increases the irreversible loss of Cys in the lungs through both Tau and SO_4 production. In the current study, Tau concentrations and the appearance of ^{35}S -Tau in the heart were not influenced by ISS. This result may be due to the need for stimulating myocardial performance during ISS, through maintaining constant concentrations of Tau via uptake from the blood (Abel, 1989; Stipanuk, 1999; Stipanuk and Ueki, 2011). ISS increased the appearance of $^{35}\text{SO}_4$ and SO_4 concentrations in the heart, suggesting an increase in the catabolism of Cys via the cysteine sulfinate-independent pathway or through higher SO_4 uptake from the plasma (Stipanuk, 1999). The biological significance of this increase

is unclear. Together, these results suggest that during ISS the lungs and the heart maintain their own Cys concentrations through uptake from the circulation and through increasing the irreversible loss of Cys in these tissues. Thus, the quantitative contribution of the lung and heart to the irreversible loss of Cys at the whole-body level warrants further studies.

Unusually high free Cys concentrations are observed in the kidneys. However, little is known about the metabolism of SAA in the kidneys, particularly during ISS (Stipanuk, 2004). Results obtained from the current study showed that the concentrations of Met, Cys, Tau, and SO_4 as well as the appearance of ^{35}S -Tau and $^{35}\text{SO}_4$ in the kidneys were not influenced by ISS. These results suggest that the irreversible loss of Cys in the kidneys was not affected by ISS, probably because the kidneys partition Cys to GSH synthesis rather than to catabolism (Stipanuk, 2004; Rakhshandeh et al., 2019).

In the current study, ISS had no effect on Tau and SO_4 as well as appearance of ^{35}S -Tau and $^{35}\text{SO}_4$ in the spleen. Therefore, ISS-induced decreases in spleen Cys concentrations can mainly be attributed to increased utilization of Cys for enhanced GSH synthesis in this tissue (Malmezat et al., 2000a; Rakhshandeh et al., 2019). Moreover, Cys concentrations in the spleen cannot be from biosynthesis of Cys from Met, since cystathionine beta synthase is absent in this tissue (Jhee and Kruger, 2005). This idea is further supported by our observation in the current study in which Met concentrations in the spleen were not affected by ISS.

During ISS, AA from muscle tissue are important for supporting an immune response (Breuill and Obled, 2000). Indeed, disease and inflammation increase the turnover of GSH, and consequently Cys utilization, in muscle tissue (Malmezat et al., 2000a; Rakhshandeh et al., 2019). In the current study, ISS-induced increases in the concentrations of Tau and the appearance of radioactively labeled Tau in muscle suggest enhanced uptake of Tau by skeletal muscle from the blood (Jacobsen and Smith, 1968). This observation may be due to the important role that Tau plays in intracellular osmoregulation and protection against oxidative agents in skeletal muscle during ISS (Di Leo et al., 2002). In the current study, ISS increased SO_4 concentrations, but reduced the appearance of $^{35}\text{SO}_4$ in muscle, suggesting reduced catabolism of Cys via SO_4 production in this tissue. The biological significance of accumulating SO_4 in the muscles of ISS+ pigs is unclear; however, accumulation of SO_4 in muscle might facilitate muscle proteolysis during ISS, by inducing within tissue acidosis (Kalantar-Zadeh et al., 2004). Taken together, these results indicated that mild ISS decreased the irreversible loss of Cys in skeletal muscle, which may help preserve Cys for immune functions in ISS+ pigs.

It has been suggested that end products of SAA catabolism are almost entirely excreted via the urine (Hou et al., 2003). In the present study, reduced concentrations and total SO_4 excretion in the urine of ISS+ pigs can likely be attributed to reduced SO_4 production in the skeletal muscle and small intestine, and to a lesser extent to increased reabsorption of SO_4 in the proximal tubules of the kidney (Burckhardt and Burckhardt, 2003). It is well established that anion (i.e., SO_4^{2-}) reabsorption increases during ISS to compensate for respiratory and metabolic alkalosis induced by endotoxemia (DuBose, 1998). Despite ISS-induced increases in the concentrations and production of Tau at the whole-body level, urinary Tau excretion and SRA did not differ between ISS- and ISS+ pigs. This result suggests that an increased retention of Tau is occurring in ISS+ pigs. The relatively low urinary Tau excretion rate in the current study is largely attributable to low dietary Tau intake

(0.3–1.1 $\mu\text{mol/d}$) (Stipanuk, 1999). Thus, these results suggest that due to preferential retention of Tau, urinary SO_4 or total sulfur excretion is not a reliable indicator for the short-term evaluation of the irreversible loss of SAA during ISS.

Collectively, these results showed that the contribution of Tau production to the whole-body irreversible loss of Cys is considerably higher than that of SO_4 production during ISS. However, this increase in the irreversible loss of Cys is quantitatively small. Therefore, increased Cys utilization during ISS in growing pigs can be attributed largely to the synthesis of GSH and probably other metabolites that are involved in an immune response (Melchior et al., 2004; Rakhshandeh et al., 2019). These results also suggest that ISS modifies the catabolism of Cys in several tissues, specifically increasing the production of Tau and SO_4 in the visceral tissues while decreasing the catabolism of Cys to SO_4 in skeletal muscle and the small intestine. In addition, these results indicate that growing pigs fed a SAA limiting diet, like mice and unlike rats, catabolize Cys primarily to Tau and not to SO_4 (Weinstein et al., 1988).

Conclusions and implications

The current findings suggest that decreased plasma Met plus Cys (SAA) concentrations and increased plasma Cys flux during LPS-induced ISS are due to increased GSH turnover and, to some extent, increased catabolism of Cys. This increased catabolism results in an increased irreversible loss of Cys to Tau, while at the same time reducing the catabolism of Cys to SO_4 . However, the irreversible loss of Cys is small, overall. Collectively, this study provides further support for our previous findings that in growing pigs, increases in the maintenance requirement for SAA during ISS are due to increased Cys utilization for the synthesis of nonprotein nitrogenous compounds that are involved in the immune response, rather than irreversible Cys losses.

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Conflict of interest statement

The authors have no conflicts of interest.

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