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Physiology

Splanchnic metabolism of dietary arginine in relation to nitric oxide synthesis in normal adult man

(nitrate/metabolism/diet)

L. CASTILLO, T. C. DEROJAS, T. E. CHAPMAN, J. VOGT, J. F. BURKE, S. R. TANNENBAUM, AND VERNON R. YOUNG*

Laboratory of Human Nutrition, Clinical Research Center and Division of Toxicology, Massachusetts Institute of Technology, Cambridge, MA 02142; and Shriners Burns Institute, and Department of Surgery, Massachusetts General Hospital, Boston, MA 02114

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ABSTRACT **Urinary nitrate (NO_3) is the stable end product of nitric oxide, which is formed, in turn, from a guanidino nitrogen of arginine. We have conducted two experiments, each in four healthy adult men receiving a low nitrate diet for 7–10 days, to investigate the *in vivo* conversion of arginine to nitrate. In the first study [*guanidino*-$^{15}N_2$, 5,5-$^2H_2$]arginine was given on day 7 via a primed continuous intravenous infusion for 8 h. In the second study, the labeled arginine was given for 8 h by the intragastric route on day 7 and by the intravenous route on day 10. Measurement of $^{15}NO_3$ output in urine collected for 24 h beginning at the time of the arginine tracer infusion revealed a more extensive transfer of ^{15}N when the arginine tracer was given intragastricly. From the comparative labeling of $^{15}NO_3$ after administration of the tracer arginine via the intragastric and intravenous routes, we estimate that 16% ± 2% of the daily production of nitrate arises from the metabolism of dietary arginine that is taken up during its "first pass" in the splanchnic region. Hence, nitric oxide production occurs, to a measurable extent, in this area in healthy subjects, raising the question as to how various pathophysiological states might alter the relations between exogenous and endogenous sources of arginine as precursors of NO· and the relative contributions made by various organs to whole body (NO·) NO_3 formation. These results also raise important questions about the use of nitric oxide synthase inhibitors in animal and human studies.**

The endogenous synthesis of nitrate by mammals (1, 2) was demonstrated to occur via oxidation of trivalent nitrogen (i.e., amino nitrogen) and to be greatly stimulated by an endotoxin challenge (3). Studies by Marletta and coworkers (4, 5) with murine macrophages disclosed an enzymatic pathway that involved the oxidation of a guanidino nitrogen of L-arginine to nitric oxide (NO·) and its subsequent oxidation and excretion as urinary nitrate (e.g., refs. 6 and 7). Numerous other investigations concerning problems of cardiovascular physiology, neuronal signaling, and endotoxic shock have resulted in the discovery of a large family of NO· synthase enzymes (NOS; EC 1.14.23.-). These NOS include both constitutive and inducible forms and may be membrane bound or cytosolic depending on cell type; the physiological roles of these enzymes have been recently reviewed (8–11). In addition, reports have appeared recently on the isolation and cloning of some of the key genes encoding NOS (12–14), which revealed close sequence similarity to cytochrome P450 reductase (15) and also with functional characteristics of a P450-type hemoprotein (16). Thus, given the apparently large number of isoforms of NOS, their varying distribution in human tissues, and their multiple mechanisms of activation, it is of importance to define the *in vivo* relationships between arginine metabolism and urinary nitrate in man.

In an earlier study, Leaf *et al.* (6) gave a large oral bolus dose (85 mg·kg^{-1} of body weight) of [^{15}N]arginine to two human volunteers. Less than 0.1% of the administered dose was recovered in the urinary nitrate excreted during the subsequent 24-h period. Since the dose of labeled arginine given was relatively large, in comparison with a normal dietary intake of 50–80 mg·kg^{-1}·day^{-1}, this raised a number of questions concerning the physiologic fate of arginine and the quantitative importance of various organs or tissues responsible for the conversion of arginine to nitric oxide and excretion as its stable end product, nitrate. Hence, additional tracer studies have now been conducted to explore the physiological aspects of NO· production, measured here as nitrate formation. In our first study, labeled arginine was given by the intravenous route, using a primed constant-infusion approach. We also carried out studies to compare the labeling of urinary nitrate when the tracer arginine was given, on separate occasions to the same subject, by the intravenous and intragastric routes. We believed that such a design would determine whether there was significant site-specific splanchnic organ conversion of arginine to NO· and nitrate, possibly by hepatocytes and Kupffer cells (17).

MATERIALS AND METHODS

Subjects and Experimental Design. Eight healthy, adult males [age = 21 ± 3 years (mean ± SD); body weight = 78 ± 8 kg] participated in the study. They were investigated at the Clinical Research Center (CRC) of the Massachusetts Institute of Technology (MIT). All were in good health, as established by medical history, physical examination, analysis of blood cell count, routine blood biochemical profile, and urinalysis. Their daily energy intake was calculated to maintain body weight, based on a dietary history and an estimate of the subject's usual level of physical activity. The subjects were encouraged to maintain their customary levels of physical activity but did not participate in competitive sports.

The purpose of the study and the potential risks involved were fully explained to each subject. Written consent was obtained according to the protocol approved by the MIT Committee on the Use of Humans as Experimental Subjects and the Executive and Policy Committee of the MIT CRC. All subjects received financial compensation for their participation in the experiments and remained healthy throughout.

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Abbreviations: NOS, nitric oxide synthase(s); KIC, ketoisocaproate; CRC, Clinical Research Center; [$^{15}N_2$]arginine, L-[*guanidino*-$^{15}N_2$,5,5-$^2H_2$]arginine; [$^2H_3$]leucine, L-[5,5,5-$^2H_3$]leucine.

*To whom reprint requests should be addressed at: Massachusetts Institute of Technology, Room E18-613, Cambridge, MA 02142.

Four subjects participated in the first experiment, which consisted of a 6-day, low nitrate experimental diet period followed by an intravenous arginine tracer-infusion study (see below) on day 7; the other four subjects took part in the second study during which they consumed the experimental diet for 10 days. In this experiment three subjects were studied with an intragastric tracer infusion conducted on day 7 and then again with an intravenous route of tracer infusion on day 10. The fourth subject received the different arginine tracer routes in the reverse order.

In a separate pilot study, two young men received an intravenous infusion of labeled arginine over 3 h, to determine the rate at which the plasma arginine isotope enrichment returned to pretracer infusion levels. These subjects received the experimental diet for 2 days. They were then fasted overnight prior to the tracer infusion; during the infusion they received small meals every 30 min, to supply per hour about 1/12th of their usual daily intake.

Diet. Each subject received a complete, low nitrate diet (based on an egg white and whole egg powder), which provided the equivalent of 1 g of protein per kg of body weight per day. The major energy source was provided in the form of protein-free, wheat starch cookies. The diet supplied a mean of 220 μmol of nitrate daily, with a range among the subjects of from 200 to 249 μmol. The total daily intake prior to the infusion studies was consumed as three separate meals at 0800, 1200, and 1700 h. Two of these meals were eaten in the CRC under the supervision of the dietary staff.

Tracer Infusion Studies. The isotope-infusion periods lasted a total of 8 h. For the first 3 h, subjects remained in the postabsorptive state, following an overnight fast, and this was followed by a 5-h fed state. Details of general procedures followed immediately prior to and during the infusion protocol have been described (18).

In the first study, priming doses of L-[*guanidino*-$^{15}N_2$, 5,5-$^2H_2$]arginine ([$^{15}N_2$]arginine) (5.3 μmol·kg^{-1}) and L-[5,5,5-$^2H_3$]leucine ([$^2H_3$]leucine) (4 μmol·kg^{-1}) were administered over 3 min. These doses were immediately followed by constant, known intravenous infusions of [$^{15}N_2$]arginine (5.3 μmol·kg^{-1}·h^{-1}) and [$^2H_3$]leucine (4 μmol·kg^{-1}·h^{-1}) tracers. Beginning at 180 min, the subjects were fed six small isocaloric isonitrogenous meals at hourly intervals, as a liquid formulation. The hourly intake was equivalent to 1/12th of the total daily intake provided by the experimental diet during the preceding diet period.

Blood samples were drawn at 20-min intervals during the last 2 h of the 3-h fast and then again during the last 2 h of the fed phase. These were kept stored on ice until centrifuged, and plasma was then withdrawn and stored at −20° until analyzed.

For the second study, priming and constant tracer doses of [$^{15}N_2$]arginine and [$^2H_3$]leucine were given intravenously, as described above, on day 10. On day 7 the tracers were given via an intragastric tube during the 8-h infusion protocol. Priming doses of arginine and leucine were 5.9 and 4 μmol·kg^{-1}; constant infusions were about 5.9 and 4 μmol·kg^{-1}·h^{-1}, respectively. In each tracer study, at 180 min a continuous feeding, via the nasogastric tube, began, which lasted until the end of the tracer-infusion period. About 250 ml of the dietary formula was supplied per h, which provided about 1/12th of the total protein and energy intake. The amino acid tracers were simultaneously infused through the nasogastric tube, using a two-way stopcock.

Pilot Study. A pilot study with two subjects was performed to examine plasma [^{15}N]arginine decay. Each subject received a 3-h constant intravenous infusion (5.9 μmol·kg^{-1}) of [$^{15}N_2$]arginine. Immediately after the end of the tracer period, unlabeled arginine was infused for 260 min at the same rate as the earlier labeled tracer.

Infusates of tracers were prepared from sterile, pyrogen-free powders of high chemical purity and high isotopic enrichment. For all studies, the tracers [$^{15}N_2$]arginine (99 atom percent excess) and [$^2H_3$]leucine (95 atom percent excess) were purchased from Tracer Technologies (Somerville, MA). It might be noted that we infused a tetralabeled arginine in this study, but [*guanidino*-^{15}N]arginine would have been equally suitable for the present purpose.

Analysis of Enrichment of Plasma Arginine, Leucine, and Ketoisocaproate (KIC). To determine the plasma isotopic enrichment of [$^{15}N_2$]arginine, 300 μl of plasma was extracted by the procedure of Adams (19). Derivatization of arginine was performed essentially according to Nissim *et al.* (20). The trifluoroacetyl derivative was analyzed by GC/MS (Hewlett–Packard HP5988A mass spectrometer with an HP5890, series II gas chromatograph), using selected ion monitoring at m/z 375 $[M - 69]^+$ for unlabeled arginine and m/z 379 for the labeled arginine.

Measurement of the isotope enrichment of plasma KIC was carried out by using the extraction and quinoxalinol derivatization procedures of Rocchiccioli *et al.* (21). A *t*-butyldimethylsilyl derivative was prepared according to Langenbeck *et al.* (22). The quinoxalinol-*t*-butyldimethylsilyl derivative was analyzed by using selected ion monitoring at m/z 259 $[M - 57]^+$ for unlabeled KIC and at m/z 262 for the $^2H_3$-labeled species.

Plasma leucine enrichments were measured in samples extracted as for arginine. A *t*-butyldimethylsilyl derivative was prepared according to Chaves das Naves and Vasconcelos (23). The derivative was analyzed by using selected ion monitoring at m/z 302 $[M - 57]^+$ for unlabeled leucine and m/z 305 for $^2H_3$-labeled species.

All enrichments were determined against calibration standards, and values were expressed as mole fractions above the value for blood drawn prior to tracer administration.

Urine Collection and Analysis. A complete 24-h urine collection was obtained for [^{15}N]nitrate and total nitrate determination, beginning with each tracer infusion. Urine was collected in dark polyethylene bottles containing sodium hydroxide. It was stored at −20°C until analyzed. The ^{15}N enrichment of urinary nitrate was measured by converting nitrate to nitrobenzene (6). A solution of nitrobenzene in ethyl acetate (10 μg/ml) was used to determine the contribution of the ^{13}C natural abundance in nitrobenzene to the abundance of the ion monitored at m/z 124. The theoretical value for this contribution is 6.6%, whereas we measured a slightly lower value, averaging 6.4%. Our experimentally determined values were used in all calculations. There were no significant ions at M − 1 (m/z 122), so atom percent excess ^{15}N was determined by correcting the measured abundance at m/z 124 for 6.4% of the corresponding abundance in the m/z 123 chromatogram and then calculating:

$$\%\ \text{excess}\ ^{15}N = 100\,\frac{\text{area}_{124} - (0.064 \cdot \text{area}_{123})}{\text{area}_{123} + \text{area}_{124} - (0.064 \cdot \text{area}_{123})}.$$

Urinary nitrate was measured utilizing an automated procedure previously described (24).

Calculations and Data Evaluation. Plasma leucine and arginine fluxes were calculated from isotopic data for the last 1.3–2 h of the fast and fed phases of the tracer period, using steady-state isotope dilution equations and a simplified single pool model (18). The splanchnic uptake of dietary arginine and leucine was estimated as described (18), from dose-corrected plasma enrichment ratios of the intragastric and intravenous administered tracers.

For determination of the conversion of arginine to NO· during its "first pass" uptake within the splanchnic region, we used a two-compartment model [(*i*) splanchnic bed and (*ii*)

peripheral pool, which served as the sampling site]. Details of the model can be obtained from the authors but, in brief, the elements are as follows. Intragastric tracer infusion directly reaches the splanchnic bed. Intravenous and intragastric tracer infusion rates are denoted as *IV* and *IG*. Total arginine disappearance from the splanchnic bed and that due to NO· production are denoted as Q_{sp} and N_{sp}, respectively, and as Q_{pr} and N_{pr} for the peripheral region. Arginine transfer between the two compartments is denoted as $T_{s\rightarrow p}$ and $T_{p\rightarrow s}$. Intragastric tracer-derived arginine enrichments are M^G_{sp} and M^G_{pr}, for the splanchnic bed and peripheral tissue (blood), respectively. Similarly, intravenous tracer-derived enrichments are M^V_{sp} and M^V_{pr}. The mass balances for an intragastric tracer are $Q_{sp}M^G_{sp} = T_{p\rightarrow s}M^G_{pr} + IG$ (splanchnic) and $Q_{pr}M^G_{pr} = T_{s\rightarrow p}M^G_{sp}$ (peripheral). Similar equations can be defined for an intravenous tracer. These equations can be solved to express splanchnic and peripheral tracer enrichment as

$$M^G_{sp} = \frac{Q_{pr}IG}{X};\ M^G_{pr} = \frac{T_{s\rightarrow p}IG}{X};\ M^v_{sp} = \frac{T_{p\rightarrow s}IV}{X};\ M^V_{pr} = \frac{Q_{sp}IV}{X},$$

where $X = Q_{sp}Q_{pr} - T_{s\rightarrow p}T_{p\rightarrow s}$. The $^{15}NO\cdot$ production can be estimated as $N_{sp}M^G_{sp} + N_{pr}M^G_{pr}$, for an intragastric tracer and as $N_{sp}M^V_{sp} + N_{pr}M^V_{pr}$ for an intravenous tracer. Dividing the $^{15}NO\cdot$ production over the corresponding plasma arginine enrichment, we obtain $N_{pr} + N_{sp}Q_{pr}/T_{s\rightarrow p}$ for an intragastric tracer and $N_{pr} + N_{sp}T_{p\rightarrow s}/Q_{sp}$ for an intravenous tracer. From the difference between these ratios for intragastric and intravenous tracer-derived $^{15}NO\cdot$ production one obtains, after rearrangement, the first pass arginine to NO· production:

$$\frac{N_{sp}}{Q_{sp}} = \left(\frac{\text{}^{15}\text{NO}\cdot \text{ production from IG tracer}}{M^G_{pr}} - \frac{\text{}^{15}\text{NO}\cdot \text{ production from IV tracer}}{M^V_{pr}} \right) \frac{M^G_{pr}}{IG}. \quad [1]$$

The time-weighted average plasma arginine enrichments, for the 3-h fast and 5-h fed states, were used in the above equation. The dietary arginine-derived NO· production was estimated as total dietary arginine intake times first-pass NO· production (Eq. 1), then related to the endogenous NO· production. The latter is estimated from the daily urinary nitrate production, corrected for dietary nitrate intake, assuming a 60% recovery of nitrate in the urine (2).

For comparisons of interest we used a paired *t* test, with a $P < 0.05$ being considered significant.

RESULTS

In the first study subjects received [$^{15}N_2$]arginine (and [$^2H_3$]leucine) via a primed constant intravenous infusion. In Fig. 1 the enrichment level of the tracers (arginine and leucine) in the plasma compartment is shown for the four subjects during the 8-h period of study. In the pilot experiment with two subjects, we examined the change in enrichment of plasma arginine for 3 h during and then for 240 min after termination of a constant infusion of the label. The results (data not shown) revealed that within about 2 h of tracer withdrawal, the labeling of plasma arginine had declined to 15% or less of the earlier isotopic plateau value.

The plateau levels of isotopic enrichment in plasma KIC and arginine during the fasting and fed phases of the infusion period in the first study are summarized in Table 1. From these isotope data, plasma leucine and arginine fluxes were calculated, and these values are also provided. Mean arginine fluxes were 63 and 72 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ for the fast and fed states, respectively. The plasma leucine fluxes, based on plasma KIC enrichment, were 107 $\mu mol\ kg^{-1} \cdot h^{-1}$.

Complete 24-h urine samples, commencing at the start of the tracer infusion, contained nitrate (Table 1) that was

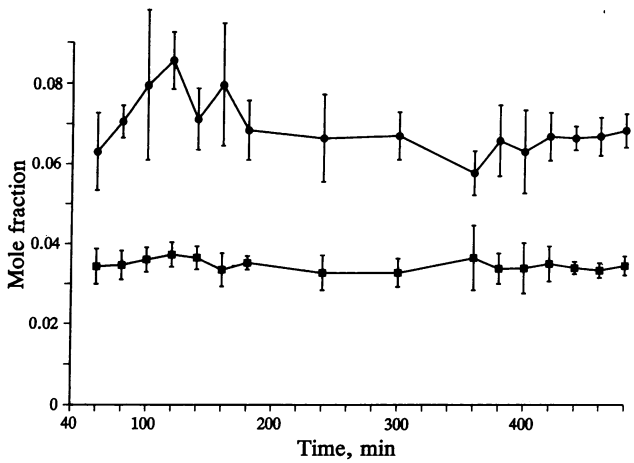

FIG. 1. Plasma enrichment of [^{15}N]arginine (●) and of [$^2H_3$]KIC (■) during a constant intravenous infusion of labeled arginine and leucine in four young men. Each point is the mean value ± SD. Enrichment is expressed as the mole fraction above the baseline value.

mainly, although not entirely, produced from endogenous synthesis, since the subjects had consumed a low nitrate diet for 1 week prior to urine collection. The mean level of $^{15}NO_3$ in the 24-h collection was 0.93%, with about 8 μmol of $^{15}NO_3$ excreted during this period.

In the second study, plasma leucine fluxes based on plasma leucine enrichment and on the intravenous and intragastric routes of tracer administration for the fast and fed states were 89 ± 18, 105 ± 10, 119 ± 10, and 116 ± 10 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ (mean ± SD), respectively. Arginine enrichment values in plasma and arginine fluxes are given in Table 2. After the intragastric infusion of labeled arginine, the plateau level of isotope enrichment was lower ($P < 0.03$) than that achieved during the intravenous route of administration (Table 2). Hence, plasma arginine flux values (Table 2) were higher when based on data obtained with the intragastric tracer. This difference reflects uptake of the arginine tracer during its first pass through the splanchnic region.

The fractions of the intragastric leucine and arginine tracers that were taken up by the splanchnic region, based on dose-corrected plasma fluxes (18), were estimated as follows: the mean uptake (± SD) of arginine tracer during the fed period was 33% ± 9% of the dose, compared with a mean of 10% ± 6% for leucine. For the fasted period, the values for arginine and leucine were 48% ± 11% and 25% ± 14%, respectively.

Results for the labeling of ^{15}N in the urinary nitrate pool are shown in Table 3. The nitrate excreted over the 24-h period, beginning with the start of the tracer infusion, was labeled to 0.6% ^{15}N when [^{15}N]arginine was given by vein. In contrast, the ^{15}N enrichment of nitrate reached levels of 1.8–2.1%, or 2–5 times greater within a given subject, when labeled arginine was supplied via the intragastric route. Mean $^{15}NO_3$ output was 15 μmol during the 24-h period for the intragastric tracer and 3.7 μmol for the intravenous tracer period. These tracer results demonstrate a formation of nitrate from the ^{15}N tracer arginine within the splanchnic region. From our model we estimate, as summarized in Table 3, that 0.34% ± 0.09% of dietary arginine was used for NO· synthesis within the splanchnic region (N_{sp}/Q_{sp}) and that 16% ± 1.5% of the daily endogenous nitrate output was formed from dietary arginine taken up during its first pass within the splanchnic region.

DISCUSSION

The tissue sources and metabolic factors responsible for modulating the rates of endogenous nitrate (and NO·) syn-

Table 1. Plasma arginine and KIC isotopic enrichment, fluxes, and urinary nitrate excretion in young men receiving intravenous [$^{15}N_2$]arginine

	Plasma [$^2H_3$]KIC				Plasma [$^{15}N_2$]arginine				Urine		
	Mole fraction*		Flux, μmol·kg^{-1}·h^{-1}		Mole fraction*		Flux, μmol·kg^{-1}·h^{-1}				
Subject no.	Fast	Fed	Fast	Fed	Fast	Fed	Fast	Fed	$^{15}NO_3$,† %	$^{15}NO_3$,‡ μmol	Total NO_3,§ μmol
1a	0.032	0.034	119	111	0.072	0.062	66	78	0.2	1.3	637
2a	0.038	0.033	99	115	0.078	0.069	62	70	1.5	14	941
3a	0.036	0.041	109	94	0.076	0.073	63	66	1.1	8.8	797
4a	0.037	0.035	101	108	0.080	0.068	59	71	0.9	7.6	839
Mean ± SD	0.036 ± 0.002	0.036 ± 0.003	107 ± 8	107 ± 8	0.076 ± 0.003	0.068 ± 0.004	63 ± 3	72 ± 4	0.93 ± 0.5	8 ± 5	804 ± 110

*Mean mole fraction enrichment during the plateau period.
†% ^{15}N enrichment of NO_3.
‡Cumulative excretion of $^{15}NO_3$ (24 h).
§Total NO_3 excreted per 24 h.

thesis and excretion in human subjects have not been explored extensively. However, these are of interest for a number of reasons: first, reduction of nitrate to nitrite has important implications for the synthesis of nitrosamines, which are potent rodent carcinogens (25). Second, nitrate arises from the oxidation of NO·, which is derived from a guanidino nitrogen of arginine (4, 5, 26–30). Third, NO· plays important roles in signal transduction, cell–cell communication, and host defense, where it is cytotoxic or cytostatic for tumor cells and invasive organisms (8–10).

In a previous study (6), we gave two human volunteers a large oral dose of [^{15}N]arginine and showed that it was a precursor of nitrate. The transfer of the label to nitrate accounted for about 0.07% of the administered dose. The present experiments were conducted to confirm and extend these earlier findings. We now demonstrate that nitrate synthesis in the healthy adult human occurs within the splanchnic region, since equivalent tracer infusions given by vein and via the intragastric route resulted in a distinctly different degree of labeling of urinary NO_3. From the second study, the level of ^{15}N enrichment in urinary nitrate labeled by arginine given as a continuous intravenous infusion was about 30% of that achieved following the intragastric route of administration. The percentage of the ^{15}N dose given via the intragastric route that was recovered as $^{15}NO_3$ was about 0.4%. This represents an ≈5-fold greater recovery of label from the tracer arginine, as compared with that obtained following a large oral bolus of the labeled amino acid (6). Furthermore, this higher relative transfer of the *guanidino*-^{15}N label to NO_3 was achieved with a lower total dose of [^{15}N]arginine. In the present case, the total dose was about 1/10th, or 8.2 mg of arginine per kg per subject, as compared with 85 mg per kg of arginine in the earlier investigation (6). This indicates that a continuous infusion of arginine is a more effective means of maintaining the level of label within the arginine–NO· precursor pool. Our pilot study with two subjects supports this interpretation, since we observed a rapid and marked decline in the plasma enrichment of [^{15}N]arginine followed termination of the [^{15}N]arginine tracer infusion. Assuming this change in plasma isotope enrichment reflects that occurring in the precursor pool, then a significant level of ^{15}N labeling within this pool following a large oral bolus would occur for only a relatively brief period; in consequence, it is to be predicted that a relatively low output of $^{15}NO_3$ would be obtained when a tracer is given as a large single bolus.

From these findings, it is possible, as discussed in *Materials and Methods*, to arrive at an approximation of the quantitative extent to which the first pass uptake of dietary arginine within the splanchnic region contributes to daily urinary nitrate output. Hence, from the comparisons of plasma arginine enrichment and urinary $^{15}NO_3$ excretion following the intravenous and intragastric tracers, we estimate the percentage of dietary arginine that was taken up during a first pass within the splanchnic region and then used for NO· synthesis is about 0.34%, under these conditions. Because the dietary arginine intake provided during the 7- to 10-day diet period was 28 ± 4 mmol daily for our subjects, a 0.34% conversion of the absorbed arginine would give rise to 95 μmol of NO_3. If endogenous nitrate excretion is assumed to be the total output minus 60% of the dietary nitrate intake (3), then mean endogenous excretion in the second study was 599 μmol daily. Thus, the 95 μmol of NO_3 formed from the metabolism of *dietary* arginine within the splanchnic region amounts to 16% ± 1.5% of the daily endogenous nitrate output.

These calculations indicate that first-pass disappearance of intestinally derived arginine accounts for a small, but measurable, proportion of the arginine used as a precursor for total body (NO·) NO_3 synthesis. Hence, it would be of interest to understand how the flow of arginine from the intestine and that from plasma and endogenous sources (protein degradation and *de novo* synthesis) is directed toward the site of NO· formation within the splanchnic organs.

Table 2. Plasma arginine enrichment and flux in young men given labeled arginine via the intravenous and intragastric routes

	Intravenous				Intragastric			
	Fast		Fed		Fast		Fed	
Subject no.	[^{15}N]Arg	Flux, μmol·kg^{-1}·h^{-1}	[^{15}N]Arg	Flux, μmol·kg^{-1}·h^{-1}	[^{15}N]Arg	Flux,* μmol·kg^{-1}·h^{-1}	[^{15}N]Arg	Flux,* μmol·kg^{-1}·h^{-1}
1	0.097	54	0.075	72	0.037	151	0.058	94
2	0.075	64	0.062	78	0.042	132	0.045	123
3	0.062	85	0.063	83	0.044	127	0.049	114
4	0.088	60	0.068	79	0.051	108	0.037	151
Mean ± SD	0.081 ± 0.013	66 ± 11	0.067 ± 0.005	78 ± 4	0.044 ± 0.005	130 ± 15	0.047 ± 0.008	120 ± 20

The [^{15}N]Arg values are the mean mole fractions of [^{15}N]arginine enrichment during the plateau.
*Apparent flux with intragastric tracer (see *Materials and Methods*).

Table 3. $^{15}NO_3$ and total NO_3 output in young men receiving [$^{15}N_2$]arginine by intravenous and intragastric routes and splanchnic metabolism of dietary arginine

	Intravenous			Intragastric				
Subject no.	$^{15}NO_3$,* %	$^{15}NO_3$,† μmol	Total NO_3,‡ μmol	$^{15}NO_3$,* %	$^{15}NO_3$,† μmol	Total NO_3,‡ μmol	N_{sp}/Q_{sp} × 100	Daily NO· from first-pass Arg uptake, %
1	0.8	4.6	581	1.8	14.2	789	0.260	15
2	0.5	4.1	815	2.0	19.6	979	0.448	16
3	0.4	2.4	592	2.1	14.1	702	0.398	17
4	0.5	3.7	738	1.9	12.6	661	0.266	14
Mean ± SD	0.6 ± 0.2	3.7 ± 1	681 ± 99	1.95 ± 0.07	15.1 ± 2.6	783 ± 122	0.343 ± 0.09	16 ± 1.5

*% ^{15}N enrichment of NO_3.
†Cumulative excretion of $^{15}NO_3$ (24 h).
‡Total NO_3 per 24 h.

This is also relevant and important because of the recently described (31) hepatic endotoxin-induced NO· synthase that is stimulated by calmodulin and that is distinct from that in macrophages.

Finally, because intravenous infusion of [$^{15}N_2$]arginine labeled urinary NO_3 to a lower extent than intragastric administration of tracer, this indicates a significant compartmentation of arginine metabolism within the whole body. The uptake of arginine by the liver, from the systemic circulation, is relatively poor due to the low activity of the y^+ transport system in hepatocytes (32), which is responsible for the transmembrane passage of the cationic amino acids arginine, lysine, and ornithine (33). Indeed, the transport of arginine into hepatocytes is believed to be rate limiting for arginine metabolism (32), and the near absence of the y^+ system in hepatocytes may effectively serve as a barrier between the plasma and hepatic arginine pools. There is additional compartmentation of arginine metabolism within the liver, related to the zonal distribution of urea cycle enzymes (34, 35), as well as to the intracellular channeling of arginine and its precursors within complexes of sequential metabolic enzymes (36–38). It will be important to understand more completely the functional importance of the compartmentation of arginine metabolism, in reference to the *in vivo* regulation and activity of the arginine–NO· (NO_3) pathway within the splanchnic tissues.

In conclusion, we have shown that the output of urinary NO_3 in healthy adult humans is derived, in part, via a conversion of arginine within the splanchnic region. For our conditions, dietary arginine entering this region following absorption from the intestinal lumen accounts for ≈16% of daily NO_3 formation (NO· synthesis). It follows that the efficacy of inhibitors of NOS, which include several arginine analogues, may be similarly affected by the influence of compartmentation on their pharmacokinetics.

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