Kinetics of the utilization of dietary arginine for nitric oxide and urea synthesis: insight into the arginine–nitric oxide metabolic system in humans

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ABSTRACT

Background: The systemic availability of oral/dietary arginine and its utilization for nitric oxide (NO) synthesis remains unknown and may be related to a competitive hydrolysis of arginine into urea in the splanchnic area and systemic circulation.

Objectives: We investigated the kinetics and dose-dependency of dietary arginine utilization for NO compared with urea synthesis and studied the characteristics of the arginine-NO metabolic system in healthy humans.

Design: We traced the metabolic fate and analyzed the utilization dynamics of dietary arginine after its ingestion at 2 nutritional amounts in healthy humans (n = 9) in a crossover design by using [15N-15N-(guanido)]-arginine, isotope ratio mass spectrometry techniques, and data analysis with a compartmental modeling approach.

Results: Whatever the amount of dietary arginine, 60 ± 3% (±SEM) was converted to urea, with kinetics indicative of a first-pass splanchnic phenomenon. Despite this dramatic extraction, intact dietary arginine made a major contribution to the postprandial increase in plasma arginine. However, the model identified that the plasma compartment was a very minor (~2%) precursor for the conversion of dietary arginine into NO, which, in any case, was small (<0.1% of the dose). The whole-body and plasma kinetics of arginine metabolism were consistent with the suggested competitive metabolism by the arginine and NO syntheses pathways.

Conclusions: The conversion of oral/dietary arginine into NO is not limited by the systemic availability of arginine but by a tight metabolic compartmentation at the systemic level. We propose an organization of the arginine metabolic system that explains the daily maintenance of NO homeostasis in healthy humans. Am J Clin Nutr 2013;97:972–9.

INTRODUCTION

Nitric oxide (NO)⁴ is a widespread signaling molecule that has been implicated in virtually all bodily functions (1). More particularly, NO plays a critical and pivotal role in vascular homeostasis (2, 3). The impairment of NO synthesis is considered to be a central feature in the initiation of metabolic syndrome and atherosclerosis (2–4).

Some nutrients can upregulate or downregulate endothelial or whole-body NO synthesis (5, 6) as early as during the postprandial phase (7). As the substrate for NO synthesis, arginine has naturally attracted considerable interest for many years. Oral l-arginine supplementation has widely been shown to modulate many NO-related physiologic functions (8–11), which lends credence to the hypothesis that arginine intake modulates NO synthesis. Likewise, although most studies have resorted to supranutritional doses, arginine intake within the normal dietary range is thought to be an “NO donor” (12), and the current dominant paradigm is that oral/dietary arginine dose-dependently modulates the availability of arginine, which acts as a substrate and promotes and sustains NO synthesis (10, 12–14).

However, there is no direct evidence for this effect because the study of the arginine-NO pathway in humans is technically challenging (15–17). Furthermore, the paradigm has been challenged on 2 different grounds.

First, the systemic bioavailability of oral/dietary arginine remains poorly known. Arginine has been suspected of being largely converted to urea during first-pass splanchnic metabolism (18). However, because the pharmacokinetics and dynamics of arginine have been little explored (19, 20), the availability of arginine relative to the ingested dose is still unknown.

Second, the relation between systemic arginine availability and its utilization for NO synthesis has remained puzzling. On biochemical grounds, this is because arginine concentrations are far higher than the Michaelis constant of NO synthase, and thus, arginine availability should not affect NO synthesis (10, 21). This conflict with background biochemistry is referred to as the...
“arginine paradox” (1, 22), which a number of theories have
tried to resolve (6, 21, 23–25). The common point of these
theories is that arginine metabolism is highly compartmented,
with a possible differential channeling of exogenous arginine to
NO synthesis in subcellular microdomains of endothelial cells
(18, 22, 24, 26). Here again, at the subcellular level, arginase
activity is thought to regulate the availability of arginine for NO
synthesis (27–30). Therefore, it remains unknown to what extent
oral/dietary arginine that reaches the systemic circulation is
eventually directed to NO synthesis (31).

Finally, the bioavailability of dietary arginine and its dose-
dependent utilization for NO synthesis, compared with its con-
version into urea, are still important gaps in our knowledge. During
the current study, we traced the fate of [15N2-(guanidino)]-labeled
dietary arginine under postprandial, non–steady state conditions.
We used isotope ratio mass spectrometry techniques to identify
the low enrichment in dietary arginine metabolites and
isotopic dilution equations to estimate the appearance of di-
etary arginine and its metabolites in different body and urinary
pools. With the use of these data, we resorted to a compart-
mental modeling approach to estimate the postprandial kinetics
dietary arginine conversion and infer the properties of the
arginine-NO/urea system with respect to the utilization of di-
etary arginine.

SUBJECTS AND METHODS

Participants and study design

Healthy, young, nonsmoking men [n = 9; age range: 18–40 y; 
BMI (in kg/m2): 23.1 ± 2.3] were recruited and completed the
study. The initial recruitment date was February 2004. Exclusion
criteria included abnormal blood pressure, any established dis-
ease, or regular medication use. The study was approved by the
Institutional Review Board of Saint-Germain-en-Laye Hospital
and the French Ministry for Health. All participants gave their
written informed consent before enrollment. The study used a
2-period, randomized crossover design. Each period consisted
of a postprandial study separated by ~8 wk, and subjects were
not aware of the (randomized) order of the arginine amount
being tested. After an overnight fast, participants ingested the
meal (in ~5 min) and were studied for 8 h thereafter.

Each liquid meal consisted of a 50-g amino acid mixture (food
grade; Ajinomoto) that was based on the composition of milk
protein, including 1.73 g arginine (32) and further supplemented
(high amount of dietary arginine (High-Arg) meal) or not (low
amount of dietary arginine (Low-Arg) meal) with 3 g l-arginine.
Amino acids were suspended in 500 mL H2O (Cristalline), and
100 mg (Low-Arg) or 170 mg (High-Arg) [15N2-(guanidino)]-
arginine:HCl (Eurisotop) was added to the liquid meal. Partic-
ipants also drank 100 mL H2O (Cristalline) immediately after
ingesting the meal and every hour starting 2.5 h after ingestion
to facilitate urine collection.

Data collection

Blood was drawn from a catheter inserted into a superficial
hand vein before the meal and every hour for 8 h after meal
ingestion. Urine was collected before the meal and every 2 h for
8 h after meal ingestion in polypropylene bottles, each of which
contained 5 mL of 5 mol/L NaOH to prevent the reduction of
nitrate.

Standard analytic methods

Plasma urea and urinary creatinine were determined by using
standard clinical analyzers. Urinary nitrate concentrations were
determined according to a fluorimetric method, and amino acids
were determined by using ion-exchange chromatography with
postcolumn ninhydrine detection (Amino-System 2500; Bio-
Tek) as previously described (32).

Mass spectrometry analytic methods

Arginine and citrulline 15N enrichment

We developed a specific gas chromatography combustion
isotope ratio mass spectrometry method (see Supplemental
material S1 under “Supplemental data” in the online issue for
a full description). Briefly, amino acids were extracted from the
plasma by using an ion-exchange column, dried, and derivatized
with trifluoroacetic acid. Methyl ester trifluoroacetic acid amino
acid derivatives of arginine and citrulline were separated, and
isotopic enrichment was determined by using gas chromatog-
raphy combustion isotope ratio mass spectrometry (Finnigan).

Urine and plasma urea 15N enrichment

Urea was isolated from urine and plasma samples, and its 15N
enrichment was determined by using elemental analysis–isotope
ratio mass spectrometry as previously described (33).

Urine 15NO3 enrichment

Nitrate was extracted from urine by adapting earlier methods,
and its 15N enrichment was determined by using isotope ratio
mass spectrometry as previously reported (32) according to the
method described in full by Forte et al (34).

Calculations

For all compartments, the specific fraction of the substance that
originated from dietary arginine was calculated by using a
standard isotopic dilution equation as

\[ Q(t) \times |E(t) - E(0)| \div E_{\text{meal}} \]

where \( Q(t) \) is the total amount of the substance in the com-
partment at time \( t \), \( E(t) \) is the 15N enrichment (in atom percent
excess) of the substance in the compartment sampled at time
\( t \), and \( E_{\text{meal}} \) is the 15N enrichment of the guanidino moiety
dietary arginine. \( Q \) was calculated as the concentration of the
substance in the sample multiplied by the volume of the com-
partment. Body urea was estimated as previously described (33),
and the plasma volume was calculated by using the equation of
Retzlaff et al (35). \( E(t) \) was calculated by multiplying the raw
(measured) enrichment by the ratio of the total number of ni-
trogen atoms in the molecule to the number of nitrogen atoms
that can originate from the guanidino nitrogen moiety of argi-
nine. This ratio was 2 for arginine, 3 for citrulline, and 1 for urea
and nitrate. This calculation takes advantage of the fact that the
amount of [15N-14N]-arginine is negligible when compared with
the amount of [15N-15N]-arginine, because [15N-14N]-arginine
aggregation to reduce its complexity to a manageable level and, thus, prevent a model that could not be identified numerically from the data in hand (36). Therefore, only important phenomena that were necessary and sufficient to account for transfers between sampled pools were retained in the selected, minimal model.

Thus, the model depicts the different processes as follows. Dietary arginine that enters the body or, specifically, the plasma compartment (via fluxes \( f_\text{in} \) and \( f_1 \), respectively; Figure 1) is used for urea and NO production either in the plasma [fluxes of urea production from the plasma (\( U_P \)) and of NO production from plasma (\( \text{NOP}_P \)), respectively] or elsewhere in the body [fluxes of urea production from elsewhere than in plasma (\( U_{\text{PB}} \)) and of NO production from elsewhere than in plasma (\( \text{NOP}_{\text{PB}} \), respectively] as well as being used for other purposes (fluxes \( f_2 \) and \( f_{11} \) for arginine located in plasma or elsewhere in the body, respectively). Thus, the urea and NO produced are disposed of by urinary excretion (fluxes \( f_7 \) or \( f_9 \), respectively) and by alternate pathways (fluxes \( f_6 \) or \( f_{10} \), respectively). The conversion of arginine into NO is accompanied by a concomitant formation of citrulline either in the plasma (flux \( f_3 \)) or elsewhere in the body (flux \( f_5 \) for the citrulline that is transferred to the plasma and a part of \( f_{11} \) for citrulline not transferred in the plasma). Thus, the plasma citrulline produced disappears for plasma arginine resynthesis and other purposes (fluxes \( f_4 \) and \( f_6 \), respectively). Open fluxes \( f_2 \), \( f_6 \), and \( f_{11} \), which lead into body pools outside the delimited system (ie, their sink-compartments are not represented explicitly in the model on reductionism grounds), aggregate different utilizations of the guanidino nitrogen from dietary arginine incorporated into plasma arginine (its transfer to the body and utilization other than by \( \text{NOP}_B \), \( U_P \), and \( f_3 \)), into plasma citrulline (its transfer to the body and utilization other than by \( f_4 \)), and into body arginine other than plasma arginine (its utilization other than by \( \text{NOP}_B \), \( U_P \), \( f_1 \), and \( f_3 \)), respectively.

This model is based on the following laws and assumptions: 1) all fluxes are governed by the law of mass action, 2) \( \text{NOP}_P \) and \( f_3 \) fluxes are equal because of the 1:1 stoichiometry of NO and citrulline formation from dietary arginine in plasma, and 3) the nonurinary disposal of body NOx (\( f_{10} \)) is supposed to be equal to two-thirds of its urinary disposal (\( f_9 \)) according to previous findings and practices in the literature (16, 37, 38) and the value of the urinary disposal rate (\( k_9 \)) taken from Avogaro et al (16).
The interaction between the amount of arginine and time, order of treatment (ie, which arginine amount was received first), and number of the visit (ie, first or second) were also introduced into the model. When the amount of arginine or the interaction between the amount of arginine and time were significant, comparisons between arginine amounts at different time points were made by using ad hoc contrasts under the mixed model with Bonferroni’s correction. The effect of arginine amounts on AUCs were tested by using a paired Student’s t test.

RESULTS

With the use of raw data on concentrations, urinary outputs, and $^{15}$N enrichments of arginine and/or related products (see Figure S1 under “Supplemental data” in the online issue), we first calculated postprandial concentrations of plasma arginine that originated directly from dietary arginine as traced by using $[^{15}$N$_2$-$(guanidino)$]-arginine, and plasma concentrations and urinary outputs of related products of dietary arginine metabolic utilization. We analyzed these data with a compartment-based model to identify the kinetics of utilization of dietary arginine for urea or NO synthesis and draw insight into the organization of the arginine metabolic system.

Postprandial kinetics of appearance of dietary arginine in plasma

The postprandial appearance of dietary arginine in plasma is presented in Figure 2. Whatever the dietary arginine amount, the concentration of dietary arginine in plasma rose rapidly (time effect: $P < 0.0001$) and reached its highest values from 1 to 1.5 h after ingestion. Compared with a baseline (fasted) concentration of arginine of 65 ± 63 μmol/L, the mean peak plasma concentrations of dietary arginine were 50 ± 5 and 142 ± 12 μmol/L after Low-Arg and High-Arg, respectively. The increase in the amount of dietary arginine resulted in markedly higher plasma dietary arginine concentrations (arginine-amount effect: $P < 0.0001$; arginine amount × time interaction: $P < 0.0001$) with 12-h AUCs of 127 ± 11 and 400 ± 38 μmol·h/L after Low-Arg and High-Arg, respectively. The increase in the plasma AUC of dietary arginine between meals (High-Arg compared with Low-Arg) was 3.2 ± 0.3-fold ($P < 0.0001$), which was significantly higher than the 2.7-fold increase in the dietary arginine amount ($P < 0.05$).

Increases in postprandial plasma dietary arginine closely paralleled those of total (ie, dietary plus endogenous) arginine (Figure 2). The postprandial contribution of dietary arginine to total arginine in plasma increased from 57 ± 4% (Low-Arg) to 70 ± 2% (High-Arg). The dietary arginine as calculated refers to the fraction of plasma arginine that has conserved intact the original guanidino nitrogen moiety of dietary arginine (ie, arginine that originated in its intact form from dietary arginine). Other contributors to the postprandial increase in plasma arginine were dietary arginine that has lost its original guanidino moiety (ie, recycled arginine) and arginine synthesized from other dietary amino acids (ie, neosynthesized arginine).

Postprandial kinetics of synthesis of NO and urea from dietary arginine

The compartmental model that we developed (see Subjects and Methods; also, see Supplemental material S2 under “Supplemental data” in the online issue) is shown in Figure 1, and the model-predicted postprandial fluxes of dietary arginine utilization for urea and NO synthesis are shown in Figure 3.

Whatever the amount of dietary arginine, we showed that the whole-body conversion of dietary arginine into urea (Figure 3A)
occurred at a very early stage and reached its maximum value at 30 min postingestion and rapidly decreased thereafter so that 90 ± 2% of the urea produced from dietary arginine was produced during the first 2 h. As shown by the 12-h AUC, the postprandial increase in the utilization of dietary arginine for urea synthesis was 2.8 ± 0.1-fold (High-Arg compared with Low-Arg), which was very similar to the (2.7-fold) increase in the arginine content of the meal (Table 1). Over the postprandial period, the fraction of dietary arginine that was converted into urea was 60 ± 3%, regardless of the dietary arginine amount (Table 1).

Whole-body NO synthesis from dietary arginine (Figure 3B) also occurred early (maximum value at 40 min and 90 ± 3% of NO produced from dietary arginine during the first 4 h, whatever the amount of dietary arginine). The kinetics were markedly higher after High-Arg ($P < 0.01$ compared with Low-Arg), which resulted in a doubling ($2.1 ± 0.3$) of the amount of NO synthesized from dietary arginine. However, this increase was significantly smaller than the (2.7-fold) increase in the arginine content of the meal (Table 1). Finally, the fractions of dietary arginine entering the NO pathway were 0.087 ± 0.015 (Low-Arg) and 0.068 ± 0.017% (High-Arg), which showed a 22 ± 10% decrease ($P < 0.05$) in the relative utilization of dietary arginine for NO synthesis (Table 1).

Utilization of dietary arginine from the plasma compartment (compared with the whole body) for urea and NO synthesis

As expected, fluxes of urea and NO synthesis from dietary arginine that occurred in plasma (Figure 3, C and D, respectively) had shapes at variance with those of whole-body fluxes (Figure 3, A and B, respectively) and displayed much lower values and delayed kinetics (peak values between 1 and 1.5 h after both...
arginine was respectively). Although low, urea synthesis from plasma dietary fluxes of dietary arginine conversion into urea and NO (col-

The conversion of dietary arginine into urea during first-pass recycling (41). In this regard, our observation suggested that part of the high rate of urea synthesis in the liver, it has been argued that arginine could be dramatically converted to urea by the liver during the first pass (43). However, seminal metabolic studies by Castillo et al (44) and Yu et al (45) showed that urea synthesis in the liver is little related to arginine availability because there is probably little exchange between extracellular arginine and arginine within the urea cycle. More recently, Van de Poll et al (46) estimated that, in surgical patients, only a small quantity (~11%) of arginine is extracted by the liver during the first pass. Therefore, the high rate of arginine conversion into urea reported in the current study should be ascribed to the intestine. Intestinal arginase activity is high in adult animals and presumably high in adult humans (18, 30, 47). The intestinal hydrolysis of arginine may be a means to limit the challenge of a high intake of arginine. In turn, the fate of ornithine that originates from this high rate of arginine hydrolysis remains unclear and deserves additional study.

Our finding that the majority of dietary arginine was hydrolyzed during the first pass was expected to imply that little arginine is made available to the systemic circulation. However, this was not the case, as shown by the dramatic rise in dietary arginine in the plasma after ingestion. The fraction of dietary arginine that was hydrolyzed was the same whatever the dietary arginine amount (60 ± 3%; SEM). Accordingly, the amount of dietary arginine shown in the plasma was also closely related to the dose. Indeed, plasma dietary arginine even increased slightly more than the increase in the arginine dose (3.2 ± 0.3- compared with 2.7-fold; P < 0.05), which evidenced an excellent incremental availability of arginine. Furthermore, the postprandial increase in plasma total arginine was higher than the plasma appearance of (intact) dietary arginine for both doses (Figure 2), which showed an important contribution of arginine synthesized from other dietary amino acids [glutamine, glutamate, and proline (48)] or dietary arginine recycled after conversion to ornithine. Because the amount of plasma arginine that did not directly originate from intact dietary arginine increased in line with the arginine content in the meal, the recycling of dietary arginine should be considered an important contributor to the total postprandial increase in plasma arginine. This effect is in line with a previous report that the steady state plasma arginine flux is noticeably contributed to by arginine recycling (41). In this regard, our observation suggested that part

One of the first important findings of this study was that the conversion of dietary arginine into urea was very high (~60% of the dose) and occurred almost entirely during its first pass. This estimate was clearly higher than that of the total first-pass extraction of arginine (which ranged from 31% to 52%) reported by Castillo et al (39, 40) and Beaumier et al (41). One of the many possible reasons for this apparent discrepancy could have been the use of a steady state approach (with the slow, continuous intragastric administration of arginine that mimicked a fed state) by Castillo et al (39, 40) and Beaumier et al (41), whereas the current study was performed in the dynamic postprandial phase. Compared with continuous feeding, a bolus meal induces a more abrupt influx of absorption, which is known to stimulate the splanchnic extraction of dietary amino acid nitrogen through its preferential orientation toward oxidative disposal pathways (42).

The conversion of dietary arginine into urea during first-pass metabolism has generally been considered to be high, although on the basis of indirect and fragmented data in animals. Because of the high rate of urea synthesis in the liver, it has been argued that arginine could be dramatically converted to urea by the liver during the first pass (43). However, seminal metabolic studies by Castillo et al (44) and Yu et al (45) showed that urea synthesis in the liver is little related to arginine availability because there is probably little exchange between extracellular arginine and arginine within the urea cycle. More recently, Van de Poll et al (46) estimated that, in surgical patients, only a small quantity (~11%) of arginine is extracted by the liver during the first pass. Therefore, the high rate of arginine conversion into urea reported in the current study should be ascribed to the intestine. Intestinal arginase activity is high in adult animals and presumably high in adult humans (18, 30, 47). The intestinal hydrolysis of arginine may be a means to limit the challenge of a high intake of arginine. In turn, the fate of ornithine that originates from this high rate of arginine hydrolysis remains unclear and deserves additional study.

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of the large quantity of ornithine produced in the first pass during arginine hydrolysis may be directed rapidly toward the resynthesis of arginine.

**Utilization of dietary arginine for NO synthesis**

A second important finding is that dietary arginine is used for NO synthesis in pools that are kinetically separated from the plasma pool. We showed that the relative participation of the plasma pool in total NO synthesis from dietary arginine was only 2.1%. With the use of an intravenous bolus of labeled arginine, Avogaro et al (16) have reported that NO synthesis from plasma arginine accounted for 2.8% of total NO synthesis in the fasted state. Therefore, our data and those of Avogaro et al (16) concur as to the view that the plasma arginine pool contributes little to NO synthesis. Thus, our data provide evidence for the high degree of compartmentation of arginine utilization for NO synthesis in the systemic area and explain why dietary arginine, which reaches the plasma compartment in a high amount, is not readily used for NO synthesis. It is possible that dietary arginine is significantly converted to NO during its first pass, as was suggested by the early conversion flux in the current study, and in line with the pioneering work by Castillo et al (40). In this case, the high rate of dietary arginine hydrolysis in the splanchnic area during the first pass was consistent with the possible direct competition with utilization for NO synthesis, as suggested by the parallel kinetics during the first 30 min.

In the plasma pool, the conversion of dietary arginine into urea, although very low compared with the first-pass conversion, was >200-fold higher than its conversion into NO. Furthermore, urea and NO synthesis from the plasma pool were closely parallel. Therefore, systemic arginine hydrolysis, although contributing little to plasma arginine disposal (37), could limit the utilization of plasma arginine for NO synthesis. This result is in line with the body of in vitro and ex vivo evidence that constitutive levels of arginase activity in endothelial cells limit NO synthesis (27, 29).

In the literature, estimates of the basal NO synthesis rate in healthy humans vary and range from 13 to 65 μmol/h (15, 49). On the basis of an average of these figures, the NO synthesis flux from dietary arginine (which averaged 1.1 and 2.3 μmol/h during the 8h after ingestion with the Low-Arg and High-Arg meals, respectively) represented 2.7% and 5.7%, respectively, of the daily basal total NO synthesis. As may be expected on theoretical grounds because of the critical importance of NO homeostasis, such nutritional doses of arginine (ie, arginine in the quantities naturally available to humans) would not challenge the tight regulation of NO synthesis in healthy humans. Therefore, because of the high systemic bioavailability of dietary arginine, we conclude that the homeostasis of NO is mostly permitted by the high degree of compartmentation that almost disconnects plasma arginine from precursor compartments for NO synthesis, as documented in the current study.

In most studies that reported a physiologic impact of arginine, its supplementation was within the (daily) 9–21 g range. Because our study was able to directly evidence a dose-dependent relation between dietary arginine intake and its utilization for NO synthesis, it is tempting to speculate that such higher (supranutritional) doses of arginine might have a real impact on total NO synthesis, even though little is still known about quantitative changes in NO synthesis in health and disease (6, 17). Furthermore, the dose-response relation can be expected to be higher in subjects with a lower total basal NO synthesis in many pathophysiologic situations (12). Likewise, in a recent meta-analysis, Bai et al (50) showed that improvements to flow-mediated dilation by arginine supplementation are only evident when the baseline function is impaired. Additional investigations of the Arginine-NO dose response in pathophysiologic situations are warranted, and the methodologic approach developed in the current study could be of particular interest.

In conclusion, we have revealed that most dietary arginine is converted to urea during the first-pass metabolism, but contrary to the classic view, the efficiency of dietary arginine utilization for NO synthesis is not limited by the extrasplanchnic availability of arginine but by a tight metabolic compartmentation that dramatically restricts the use of the plasma pool for NO synthesis. We propose that the organization of the metabolic system that we depicted ensures the homeostasis of NO in healthy humans in the context of normal arginine challenges. Our results propose a fundamental insight into the dynamics of the arginine metabolic system in relation to the homeostasis of NO and call for additional studies on the modulation of this nutritional system in the various pathophysiologic situations in which normal NO homeostasis is altered.

The authors’ responsibilities were as follows—FM, JFH, and DB: designed the research; FM, IS, and DB: conducted the research; HF, KJP, and FM: contributed to new methods; FM, KJP, HF, JFH, and CB: analyzed data; FM and HF: wrote the manuscript; FM: had primary responsibility for the final content of the manuscript; and all authors: read and approved the final manuscript. None of the authors had a conflict of interest.

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