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Serum L-arginine and endogenous methylarginine concentrations predict irritable bowel syndrome in adults: A nested case-control study

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Abstract

Background & Aims: Nitric oxide, a major inhibitory nonadrenergic, noncholinergic neurotransmitter that relaxes smooth muscle, may be implicated in the pathophysiology of visceral hypersensitivity in irritable bowel syndrome (IBS). Impaired bioavailability of the nitric oxide precursor molecule L-arginine and higher concentrations of methylarginines (endogenous inhibitors of nitric oxide synthesis) are known to impair nitric oxide synthesis in numerous gastrointestinal cell types. We therefore examined serum concentrations of L-arginine and the methylarginines in a nested case-control study, to assess whether these factors are associated with adult IBS.

Methods: Data on clinical characteristics, methylarginines, and L-arginine (measured using LC-MS/MS) were collected from a random population-based cohort of Australian adults (median age = 64 years; IQR = 60–70). Cases of IBS, defined according to Rome III criteria ($N = 156$), and controls ($N = 332$) were identified from within the cohort at the 5-year follow-up.

Results: In adjusted logistic regression analyses, L-arginine, asymmetric dimethylarginine, symmetric dimethylarginine, L-arginine/asymmetric dimethylarginine ratio, and Kessler-10 psychological distress scores were significantly associated with IBS ($p > 0.05$). Similar results were found for IBS subtypes. Higher serum L-arginine concentration had the strongest association with IBS diagnosis, with an odds ratio of 9.03 for those with serum L-arginine at the 75th (84 μmol/L) versus 25th (46 μmol/L) percentile (95% CI: 5.99–13.62). L-arginine had the best discriminative ability with a bias-adjusted area under the receiver operator characteristic curve of 0.859.

Conclusions: Higher serum concentrations of L-arginine and endogenous methylarginines are strongly associated with IBS in adults.

KEYWORDS
irritable bowel syndrome, L-arginine, methylarginine, older adults

INTRODUCTION

Irritable bowel syndrome (IBS) is a common, debilitating, functional gastrointestinal (GI) disorder of unknown etiology.[1,2] Worldwide, the prevalence of IBS ranges from 2.1% to 22% depending on which diagnostic criterion is used.[3,4] The chronic abdominal pain characteristic of IBS is associated with significant morbidity[5] including decreased quality of life[6] and depression[7] and frequent visits to health care providers[8] with subsequent increases in diagnostic testing and hospitalization.[9]

The proposed pathogenesis of IBS is complex. The most prominent features of the condition include impaired GI motility, visceral hypersensitivity, abnormal secretion and absorption and an altered intestinal microbiome.[1,10] There is emerging evidence that low grade inflammation in the GI mucosa and immune activation may be responsible for the motor and sensory alterations of IBS in a subset possibly triggered by infection, food antigens or stress.[11,12] Indeed there is a clinical overlap between inflammatory bowel disease (IBD) and IBS, with IBS-like symptoms frequently reported in patients before the diagnosis of IBD, and a higher than expected prevalence of IBS symptoms in patients in remission from established IBD.[13]

Nitric oxide (NO) is a major inhibitory nonadrenergic, noncholinergic neurotransmitter in the GI tract.[14] Motility of the GI tract is directly controlled by enteric inhibitory and excitatory motor neurons that innervate the smooth muscle layers. NO released in response to nerve stimulation of the myenteric plexus causes relaxation of the smooth muscle and has been shown to play an important role in esophageal, gastric, and intestinal motility regulation.[15] The other major role of NO in the GI tract is the maintenance of GI mucosa integrity *via* modulation of gastric mucosal blood flow, epithelial mucus and fluid secretion, and barrier function. Within the myenteric plexus NO is primarily synthesized from the amino acid L-arginine by the constitutive enzyme neuronal nitric oxide synthase (nNOS) producing L-citrulline in the process (Figure 1).[16] The similar process in the endothelium of GI blood vessels is catalyzed by the constitutive enzyme endothelial nitric oxide synthase (eNOS).[17] An inducible nitric oxide synthase (iNOS) is also expressed in large amounts in the enteric nervous system and GI tract in response to inflammation, bacterial and viral infection, or trauma.[18]

Subsets of patients with IBS have inflammatory changes in their gut mucosa.[19] A prominent feature is the increase in the number of mast cells and intraepithelial lymphocytes within the small and large intestines in IBS.[20] Furthermore, mast cell concentrations and their distance from enteric neurons are positively associated with IBS pain.[21]

Evidence from animal studies supports an inhibitory action of NO on mast cell activation and mast cell-dependent inflammatory processes *in vivo*[22] This is consistent with work in rodent gut[23,24] and blood vessels,[25] and pig[26] and rabbit lungs.[27] Overall, this evidence suggests that inhibition of NOS and a corresponding decrease in NO synthesis is likely to activate mast cells within the GI tract and impair NO-dependent regulatory processes resulting in disturbed GI motility, secretion, permeability, and the development of inflammatory changes within the GI tract.

Given the available evidence in support of the hypothesis that impaired NO synthesis leads to disturbed GI function and potentially the development of IBS, it is important to identify new potentially modifiable factors responsible for decreased synthesis of NO within

Key summary

Scientific knowledge on the subject

- Nitric oxide is a major inhibitory nonadrenergic, noncholinergic neurotransmitter that relaxes smooth muscle and may be implicated in the pathophysiology of visceral hypersensitivity in irritable bowel syndrome.
- Impaired bioavailability of the nitric oxide precursor molecule L-arginine and higher concentrations of methylarginines (endogenous inhibitors of nitric oxide synthesis) are known to impair nitric oxide synthesis in numerous gastrointestinal cell types.
- L-arginine and the nitric oxide synthesis inhibitor asymmetric dimethylarginine have been implicated in the pathogenesis and/or pathophysiology of several conditions including inflammatory bowel disease but have not previously been examined for their association with IBS in humans.

What this study adds to the field

- This is the first study to examine the association of serum L-arginine and methylarginines with IBS diagnosis in humans.
- These well-characterized endogenous molecules are potentially modifiable new risk factors for IBS.
- This molecular epidemiological study, conducted within an Australian population-based cohort of men and women, is the first study to identify serum L-arginine and methylarginines as predictors of incident IBS in humans.
- Given that serum L-arginine and methylarginines can be modified pharmacologically, confirmation that these molecules are associated with IBS in further studies might pave the way for strategies aimed at preventing or treating this condition.

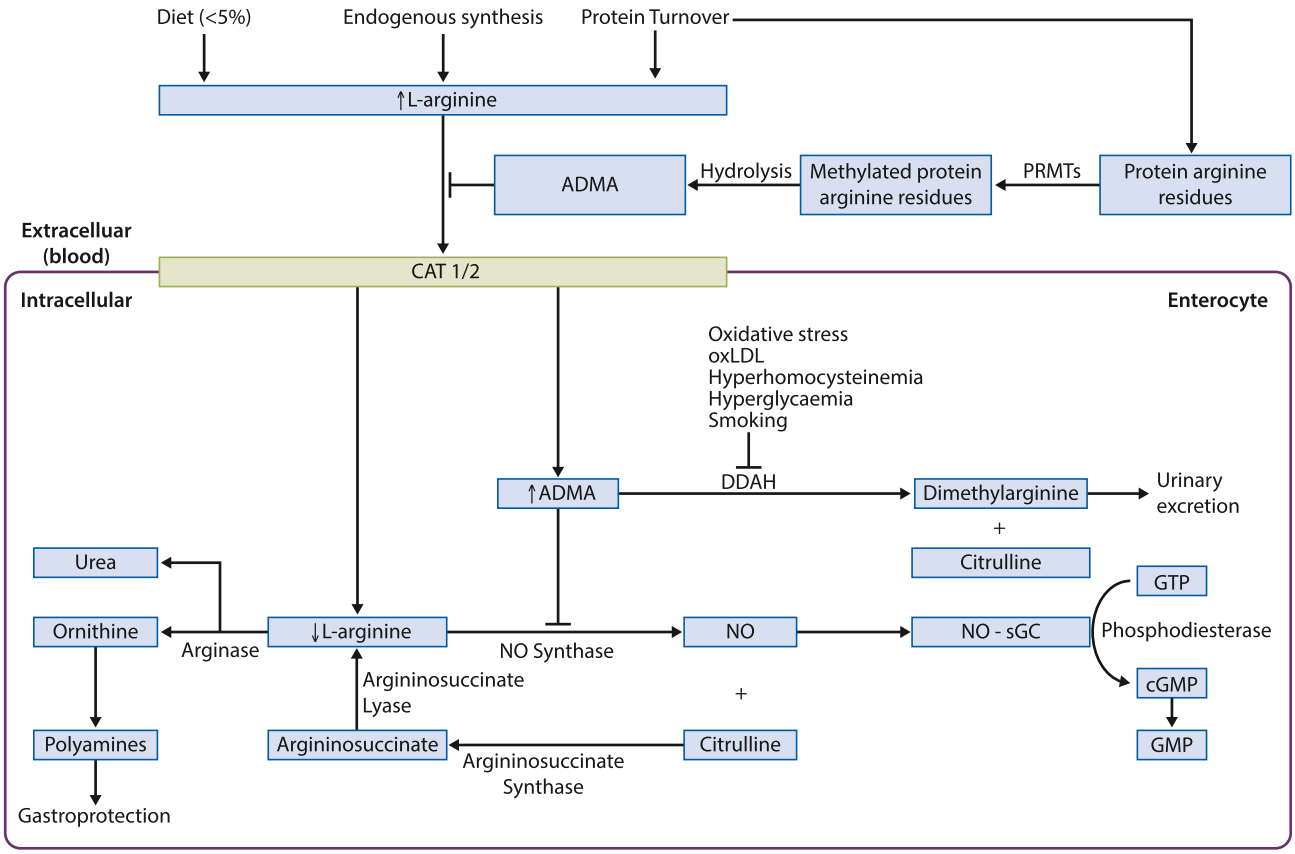

FIGURE 1 Nitric oxide (NO) released in response to nerve stimulation of the myenteric plexus causes relaxation of the smooth muscle and has been shown to play an important role in esophageal, gastric, and intestinal motility regulation. The other major role of NO in the gastrointestinal (GI) tract is the maintenance of GI mucosa integrity *via* modulation of gastric mucosal blood flow, epithelial mucus and fluid secretion, and barrier function. Within the myenteric plexus NO is primarily synthesized from the amino acid L-arginine by the constitutive enzyme neuronal nitric oxide synthase producing L-citrulline in the process. The similar process in the endothelium of GI blood vessels is catalyzed by the constitutive enzyme endothelial nitric oxide synthase. An inducible nitric oxide synthase is also expressed in large amounts in the enteric nervous system and GI tract in response to inflammation, bacterial and viral infection, or trauma. Nitric oxide synthesis is dependent on the intracellular availability of L-arginine which is supplied to the cell by the cationic amino acid membrane transporters CAT1 and CAT2. L-arginine is supplied to the cell through endogenous production from Citrulline and Argininosuccinate and by cellular protein breakdown. Less than 5% of cellular L-arginine is supplied from diet. However, in disease states, there is a greater need for L-arginine, and this may be impaired by competitive inhibition with other amino acid derivatives such as Asymmetric Dimethylarginine (ADMA). Cellular ADMA concentrations increase in various disease states and during oxidative stress. ADMA is a competitive inhibitor of NOS which attenuates the synthesis of NO. ADMA is also known to compete with L-arginine for transport into intestinal cells. Hence, there may be a functional deficiency of intracellular L-arginine that impairs NO synthesis resulting in intestinal dysmotility, epithelial permeability, increased production of proinflammatory cytokines, and visceral hypersensitivity in irritable bowel syndrome

the GI tract. Asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor of all NOS isoforms is one potential factor that has received a great deal of attention in the area of cardiovascular medicine. ADMA competes with L-arginine for eNOS and nNOS and has been shown to reduce NO synthesis in isolated human blood vessels and numerous cell types.[28–32] There is substantial evidence that excessive serum ADMA concentrations inhibit NOS activity and cause sustained endothelial dysfunction and a proinflammatory state.[33] Given that L-arginine is the substrate for the synthesis of NO, a depletion of L-arginine and/or reduced intracellular bioavailability of L-arginine may also be an important factor in the development of IBS.

The primary aim of this research was to determine if serum concentrations of L-arginine and the methylarginines ADMA and Symmetric dimethylarginine (SDMA), another methylated arginine with indirect inhibitory effects on NO synthesis,[34] are independenly associated with IBS in a representative sample of IBS cases and controls nested within a cohort of community-dwelling adults.

METHODS

Subject recruitment

Participants for the current investigation were drawn from an existing population-based Australian prospective cohort study known as the Hunter Community Study (HCS).[35] Briefly, between 2005 and 2007 the study randomly recruited 3253 Hunter men and women aged between 55 and 85 years from the electoral roll. Written

informed consent was obtained from each study participant. Ethical approval for this research was granted by the Hunter New England Research ethics committee (2011). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Follow-up of the cohort occurred in 2011; 3115 participants were sent a follow-up postal questionnaire that included a validated IBS questionnaire. Of these, 2297 returned the questionnaire. By follow-up, the study team was notified of 132 deaths (4%), 169 people actively withdrew (5%) and 767 (23%) were lost to follow-up with unknown reasons, leaving 2250 who completed follow-up questionnaires.

Case and control ascertainment

A validated GI functional disorder questionnaire was used to ascertain all HCS 5-year incident cases of IBS according to Rome III criteria.[2] This is sufficient to form an IBS diagnosis as these symptoms are much more likely to be functional than due to organic disease. Those subjects with known diagnosis of IBS at baseline were excluded from the study. Of the 2250 that completed the questionnaire, 2137 had sufficiently completed the IBS questionnaire to be classified as a Rome III criteria IBS case or not. The IBS cases for the current investigation were all cases with serum available for the determination of L-arginine and methylated arginine concentrations ($N = 156$). Due to the nature of the nested case-control design, controls were a random sample of the remaining cohort who were not IBS cases at follow-up ($N = 322$).

Measurement of L-arginine and dimethylarginines

The primary predictor variables examined in this study were serum concentrations of L-arginine and the methylarginines ADMA and SDMA. Blood was collected in EDTA tubes and centrifuged at 4°C and 3000 g for 10 min to separate serum, which was stored for 4–6 years at −80°C before analysis. There were no previous freeze-thaw cycles. Arginine and its di-methylated forms (ADMA and SDMA) were measured in serum by hydrophilic-interaction liquid chromatography and isotope dilution tandem mass spectrometry at the Advanced Mass Spectrometry unit within the School of Biomedical Sciences at the University of Aberdeen, Scotland.[36] The intra and inter-assay coefficient of variances for arginine, ADMA and SDMA were all less than 15%.

Potential confounding variables

Baseline characteristics were chosen a priori to control for confounding when assessing the association between the biomarkers and IBS diagnosis. These were selected based on previously published association with IBS and included the participant's age, gender, self-reported asthma (clinician diagnosis), Centre for Epidemiologic Studies Depression Scale depressive symptom score (CESD score),[37] and Kessler-10 Psychological Distress Score (K10 score).[38] Cardiovascular risk factors such as serum LDL-cholesterol, serum HDL-cholesterol, triglycerides, hypertension, and type-2 diabetes were not considered confounders as they are known to be associated with serum ADMA concentration but are not risk factors for IBS.

Statistical analysis

Baseline characteristics were compared between IBS cases and controls using Students *t*-test for continuous measures and chi-squared tests for categorical measures. Associations between serum concentrations of L-arginine, ADMA, SDMA, and L-arginine/ADMA ratio, another clinically relevant marker of arginine availability for NO synthesis,[39] with IBS diagnosis were assessed using logistic regressions adjusting for known confounders age, gender, asthma, K10 score, and CESD score. Predictor effects were presented as odds ratios with 95% confidence intervals, corresponding to a 1 unit increase for L-arginine, a 10 unit increase for L-arginine/ADMA ratio, and 0.1 unit increase for ADMA and SDMA. The fit of each multivariable logistic regression model was assessed using the Le Cessie—van Houwelingen—Copas-Hosmer unweighted sum of squares test and Akaike's information criterion (AIC) was used to compare models. The area under the receiver operator characteristic curve (AUC) was used to assess the discriminative ability of the model. We performed bootstrapping using 200 samples to provide bias-adjusted AUCs (internal validation).[40] The Youden index was used to determine suitable cut-off points for the best performing models. The sensitivity and specificity of each model was then calculated using this cut-off. Linearity was assessed by graphical inspection of estimated coefficients from categorized versions of the continuous measures. All models were fitted using the rms (R package version 3.6-3) library with the R (v3.0.1) statistical computing system.[41] Significance was declared at the conventional 0.05 level.

RESULTS

At the HCS 5-year follow-up in 2011 there were 228 IBS cases identified according to Rome III criteria. Of these, 156 cases had serum available for measurement of L-arginine and methylated arginines. According to Rome III definitions of IBS subtypes, 35% ($N = 55$) were classified as diarrhea-predominant (IBS-D), 15% ($N = 24$) were constipation-predominant (IBS-C), 42% ($N = 65$) were a mixed phenotype, and the remainder were unclassified. Demographic characteristics are described in Table 1. Significantly more IBS cases were female compared with controls ($p = 0.049$); however, there was no difference in age ($p = 0.57$). Mean K10 and CESD scores were significantly higher in IBS cases than controls ($p < 0.001$). The mean serum concentrations of ADMA, SDMA, L-arginine, and L-arginine/ADMA ratio were statistically significantly higher in IBS cases than controls ($p < 0.001$).

TABLE 1 Case and control demographic characteristics, established and potential risk factors along with unadjusted and adjusted logistic regression analyses of L-arginine, ADMA, SDMA, and L-arginine/ADMA ratio with IBS

Predictor	IBS cases (*N* = 156)	Population Controls (*N* = 332)	*p*-value	OR (95%CI) Unadjusted	Adjusted L-arginine mode OR (95%CI) P (LR test)	Adjusted ADMA model OR (95%CI) P (LR test)	Adjusted SDMA model OR (95%CI) P (LR test)	Adjusted L-arginine/ ADMA ratio model OR (95%CI) P (LR test)
Age (mean ± SD)	64.5 (6.5)	65.2 (7.1)	0.57	0.98 (0.96–1.01)	0.99 (0.95–1.02)	0.97 (0.94–1.0)	1.01 (0.98–1.05)	1.01 (0.97–1.04)
					0.519	0.091	0.408	0.785
Gender; F versus M (%)	62% (96)	50% (166)	0.049	1.6 (1.09–2.36)	**1.74 (1.03–2.94)**	1.48 (0.98–2.25)	**1.53 (1.01–2.32)**	**1.96 (1.18–3.26)**
					0.038	0.063	**0.045**	**0.009**
K10 score (mean ± SD)	16.0 (6.0)	12.7 (4.6)	<0.001	1.12 (1.08–1.17)	**1.11 (1.05–1.17)**	**1.1 (1.05–1.15)**	**1.12 (1.07–1.17)**	**1.12 (1.06–1.18)**
					<0.001	**<0.001**	**<0.001**	**<0.001**
CESD score (mean ± SD)	9.7 (9.7)	6.0 (7.2)	<0.001	1.05 (1.03–1.08)	1.02 (0.99–1.06)	1.03 (1.0–1.05)	1.02 (0.99–1.05)	1.03 (1.0–1.06)
					0.167	0.058	0.134	0.088
Asthma (yes) (%)	13% (21)	13% (43)	0.36	0.96 (0.55–1.68)	1.31 (0.58–3.0)	1.4 (0.75–2.63)	1.26 (0.67–2.37)	1.34 (0.6–2.98)
					0.512	0.286	0.465	0.475
L-arginine (mean ± SD)	98 (34)	56 (19)	<0.001	1.06 (1.05–1.07)	**1.06 (1.05–1.07)**	X	X	X
					<0.001			
ADMA (mean ± SD)	0.582 (0.098)	0.544 (0.074)	0.002	1.71 (1.32–2.21)	X	**1.60 (1.21–2.12)**	X	X
						<0.001		
SDMA (mean ± SD)	0.65 (0.18)	0.72 (0.16)	<0.001	0.74 (0.65–0.85)	X	X	**0.74 (0.64–0.85)**	X
							<0.001	
L-arginine/ADMA ratio (mean ± SD)	170 (61)	103 (34)	<0.001	1.34 (1.27–1.42)	X	X	X	**1.36 (1.28–1.44)**
								<0.001
AIC	NA	NA	-	NA	387.33	559.71	557.53	408.79
Unadjusted AUC	NA	NA	-	NA	0.862	0.720	0.723	0.856
Adjusted AUC	NA	NA	-	NA	0.859	0.710	0.707	0.850

Abbreviations: ADMA, asymmetric dimethylarginine; AIC, Akaike's information criterion; AUC, area under the receiver operator characteristic curve; CESD, Centre for Epidemiologic Studies Depression Scale depressive symptom; SDMA, symmetric dimethylarginine; X, variable not included in adjusted model.

TABLE 2 Model performance for the association of each primary predictor with IBS-D and IBS-C subtypes

Marker	IBS-D ($N = 55$)		IBS-C ($N = 24$)	
	Unadjusted AUC	Adjusted AUC (Bootstrap cross validation)	Unadjusted AUC	Adjusted AUC (Bootstrap cross validation)
L-arginine/ADMA ratio	0.764	0.725	0.764	0.725
L-Arginine	0.753	0.699	0.753	0.699
ADMA	0.631	0.607	0.631	0.607
SDMA	0.702	0.662	0.702	0.662

Abbreviations: ADMA, asymmetric dimethylarginine; AUC, Area under the receiver operator characteristic curve; IBS, irritable bowel syndrome; IBS-C, IBS-Constipation; IBS-D, IBS-Diarrhoea; SDMA, symmetric dimethylarginine.

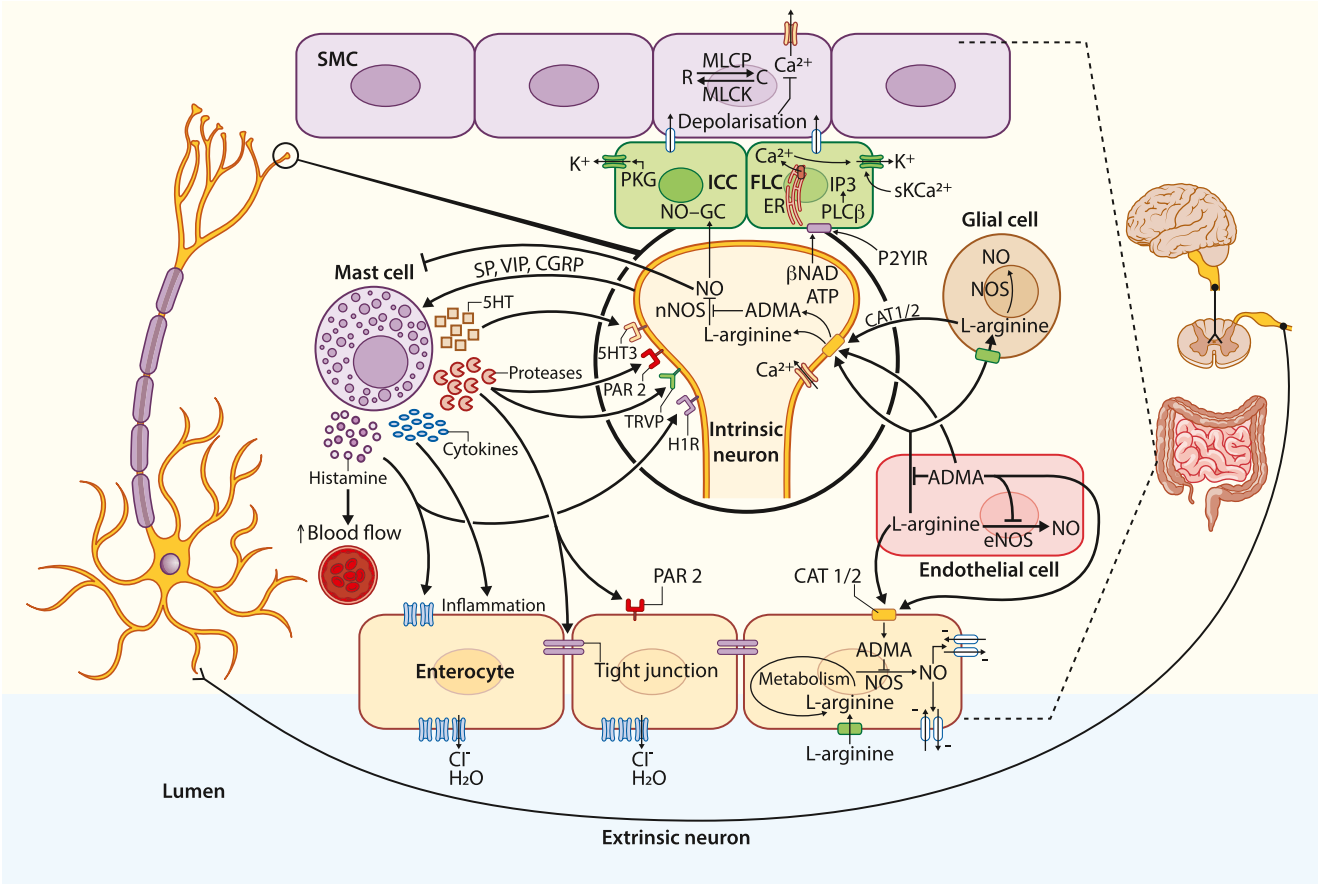

FIGURE 2 Smooth muscle cells (SMCs) form gap junctions with Interstitial cells of Cajal (ICC) and fibroblast-like cells (FLCs), creating an electrical syncytium that together regulate SMC function. Intrinsic motor neurons, with cell bodies located in the myenteric plexus, innervate this syncytium. Information is sent by sensory neurons from the gut to the enteric ganglia, the CNS, and to autonomic ganglia (not shown) and extrinsic neurons transmit information from CNS to enteric ganglia. As described by Gallego D et al. (2016), during high frequencies neuronal action potentials, calcium entry to the neuron activates neuronal nitric oxide synthase (nNOS), which converts L-arginine (L-arg) to NO. NO activates guanylyl cyclase (Gc) in FLCs which turns guanosine triphosphate into cyclic guanosine monophosphate, activating protein kinase G. The latter relaxes the cell via two mechanisms: potassium channel activation and myosin light chain phosphatase (MLCP) activation, which uncouples actin from myosin, the opposite from contraction due to myosin light chain kinase (MLCK) activation. Subsequently, hyperpolarization is transmitted to smooth muscle cells through gap junctions. During low-frequency neuronal action potentials Adenosine triphosphate (ATP) and Nicotinamide Adenine Dinucleotide (βNAD) facilitate polarization through the inositol 1,4,5-trisphosphate (IP3) pathway. Asymmetric dimethylarginine (ADMA), produced in response to oxidative stress and aging, reaches the gut through the circulation and is delivered to intrinsic neurons, via cationic amino acid transporter 1/2 (CAT1/2) proteins. ADMA competes with L-arg for entry into neurons, and once inside acts as an inhibitor of nNOS, disrupting the synthesis of NO. The reduced synthesis of NO impairs smooth muscle contraction via the ICC nitrergic pathway. ADMA is also taken up by enterocytes where it interferes with L-arg metabolism and inhibits NO synthesis. NO is necessary for mucus production and solute transport, and impaired synthesis of NO disrupts this protective effect.

Unadjusted and adjusted logistic regression analyses of L-arginine, ADMA, SDMA, and L-arginine/ADMA ratio with IBS are also described in Table 1. In both unadjusted and adjusted logistic regression analyses, L-arginine, ADMA, SDMA, and L-arginine/ADMA ratio were all statistically significantly associated with IBS. The AUC and AIC values indicate the models with L-arginine and the L-arginine/ADMA ratio were the best fitting models.

The odds of IBS increased by 6% for a one unit increase (i.e., 1 μmol/L) in serum L-arginine concentration and this effect was unchanged by controlling for confounders (OR = 1.06; 95% CI: 1.05–1.07). This effect corresponds to a ~ninefold increase in odds of IBS (OR = 9.03 [95% CI: 5.99–13.62]) for those in the 75th percentile (84 μmol/L) compared to those in the 25th percentile (46 μmol/L). For the association between L-arginine/ADMA ratio with IBS, a 10 unit increase in L-arginine/ADMA ratio is associated with a 36% increase in odds of IBS diagnosis (OR = 1.36; 95% CI: 1.28–1.44). This corresponds to a ~sevenfold increase in odds of IBS (OR = 7.38 [95% CI: 5.01–10.86]) for those in the 75th percentile (151 μmol/L) compared to those in the 25th percentile (86 μmol/L).

For the L-arginine-IBS model a suitable serum L-arginine concentration cut-off identified using the Youden Index was 77.81 μmol/L. Using this cut-off, the model has a sensitivity of 77.6% (95% CI: 70.7–83.5) and specificity of 89.5% (95% CI: 85.7–92.6). For the L-arginine/ADMA ratio-IBS model a suitable serum L-arginine/ADMA concentration cut-off identified using the Youden Index was 143.53 μmol/L. Using this cut-off the model has a sensitivity of 71.3% (95% CI: 63.9–77.9) and specificity of 88.9% (95% CI: 85.1–92.1).

We also investigated the discriminative ability of the models using two subtypes of IBS (IBS-Diarrhoea and IBS-Constipation) as outcomes and found that the model describing the association between L-arginine/ADMA ratio and each subtype performed equally well (IBS-Diarrhoea and IBS-Constipation Adjusted AUC = 0.725; see Table 2).

DISCUSSION

This population-based nested case-control study examined the association of plasma L-arginine and the endogenous methylarginines, ADMA, and SDMA with IBS defined by Rome III criteria. In adjusted analyses L-arginine, ADMA, SDMA, L-arginine/ADMA, and K10 score were statistically significant independent predictors of IBS diagnosis. Model discrimination was strongest for the model with L-arginine (Adjusted AUC = 0.859) and L-arginine/ADMA ratio (Adjusted AUC = 0.850). At their optimal cut points, L-arginine and L-arginine/ADMA ratio showed moderate sensitivity (71%–77%) and high specificity (approximately 90%) yielding positive likelihood ratios in the range of seven to eight, suggesting that these biomarkers may be useful for IBS diagnosis in a clinical setting, This research provides evidence to support the hypothesis that higher serum concentrations of L-arginine and the endogenous methylarginines are associated with IBS in adults. Interestingly, this finding applied to all subtypes of IBS suggesting it is not bowel related but more likely represents some CNS effect. Corsetti et al. performed a small randomized crossover trial (n = 10) of the effects of the NOS inhibitor, N-monomethyl-L-arginine, on the motility and sensitivity of the distal colon in healthy humans.[42] This study showed that inhibition of NO synthase did not affect the colonic motor responses whereas it increased the sensitivity to colonic distension. In another double-blind randomized crossover trial (n = 22), N-monomethyl-L-arginine significantly increased the threshold for pain in IBS patients compared with healthy volunteers, while rectal compliance was not affected by N-monomethyl-L-arginine.[43] These findings support our hypothesis that NOS inhibitors are involved in the pathophysiology of visceral hypersensitivity in IBS.

The association of increased serum ADMA concentration and IBS observed in this study is similar to that observed in a recent study

Furthermore, in gastric and duodenal mucosa when NO is available it mediates the release of calcitonin gene-related peptide (CGRP) from neurons, and this results in immediate dilation of submucosal arterioles facilitating the dilution and buffering of back-diffused acid. Given that NOS inhibitors have been shown to abolish the reactive hyperemic response, it is likely that ADMA results in a marked increase in the susceptibility of the mucosa to damage. ADMA may also interfere with NO synthesis in enterocytes and neurons through availability of substrate L-arg, which is supplied to these cells through diet, endogenous synthesis, and Endothelial or Glial cells. As outlined in Figure 1 this may result in a functional deficiency of intracellular L-arg. In irritable bowel syndrome, there is an increase in the number and/or activation of mast cells and intraepithelial lymphocytes within the small and large intestines. ADMA-mediated inhibition of NOS and a corresponding decrease in NO synthesis activates mast cells. Mast cells and neurons communicate bidirectionally. Activated mast cells release bioactive substances preformed in granules (i.e., histamine, serotonin, and enzymes) and cytokines de novo, which act on neuron receptors (e.g., Transient receptor potential cation channel subfamily V member 1 receptor [TRVPR], 5-hydroxytryptamine 3 receptor [5HT3R], protease activated receptor 2 [PAR2], Histamine 1 receptor [H1R]), and result in visceral hypersensitivity and impaired smooth muscle contraction. Furthermore, histamine and mast cell tryptase are also known to stimulate chloride ion secretion via activating H1R and PAR2, which is expressed in both basolateral and apical membranes of enterocytes. Intrinsic and extrinsic neurons respond to a variety of stimuli and release a high number of neuropeptides, such as substance P (SP), vasoactive intestinal polypeptide (VIP), and CGRP, which in turn regulate the activation of mast cells. Mucosal mast cells also influence intestinal permeability, and mast cell-derived tryptase has been identified as an important contributor in the disruption of the intestinal barrier. Mast cell tryptase cleaves PAR2 on colonocytes to increase paracellular permeability by acting on intercellular apical junction complex, which mainly consists of the tight junctions such as claudins and the adherens junction such as E-cadherin. Activated mast cells also release proinflammatory mediators that further contribute to epithelial barrier disruption through recruitment of other immune cells (e.g., TNF-α recruit neutrophils), while interleukins 3, 5, 13, and granulocyte macrophage colony-stimulating factor recruit eosinophils and basophils. Although impaired NO synthesis leads to mast cell activation in the GI tract it is important to note that commensal bacteria and products, food antigens, allergens, toxins, and psychological distress also play key roles in regulating mast cell activation and secretion. Created with Biorender.com

that examined the association of serum concentrations of ADMA and SDMA with IBD.[44] In a case-control study involving 31 consecutive patients with ulcerative colitis (UC) and 32 with Crohn's disease, ADMA, and SDMA concentrations were increased in the IBD group as compared to an age and sex-matched control group.[44] In a preliminary study of IBS (n = 18) versus IBD (n = 100) and controls (n = 40), significant alterations of L-arginine pathway metabolites were observed in both IBS and IBD.[45] By comparison the effects of various NOS inhibitors in animal models of experimental colitis have been conflicting. Some synthetic NOS inhibitors have attenuated disease activity while others have exacerbated the disease.[46] These conflicting findings are likely explained by the fact that some NOS inhibitors lack specificity and inhibit all NOS isoforms, potentially also inhibiting NOS-mediated tissue repair.

Research suggests that iNOS may be the primary NOS implicated in the pathophysiology of IBD and there is some evidence for a corresponding role in IBS. Activation of iNOS produces potentially damaging micromolar amounts of NO compared with the constitutive nanomolar amounts produced by eNOS and nNOS. The high concentration of iNOS-derived NO contributes to an inflammatory response and leads to cellular damage by reaction with superoxide anions (O^{2-}), forming reactive nitrogen species such as peroxynitrite (ONOO-) which mediate this effect.[47] Research indicates that iNOS-derived NO produced within colonic epithelial cells and granulocytes participates in the inflammatory process of IBD[48] and IBS.[49]

The finding of increased serum L-arginine concentration with IBS has not been seen previously. By comparison, a recent study of 14 normal controls and 22 UC patients with pancolitis of moderate or severe activity determined by histopathology score observed that L-arginine concentrations were increased in subjects with severe colitis when compared to those with moderate colitis or normal mucosa.[50] However, relative arginine availability, as calculated by the Arginine Availability Index, was not increased in these subjects suggesting a functional deficiency of intracellular L-arginine availability in UC patients.

Given the evidence for low-grade inflammation and activation of mast cells in IBS and the clinical overlap with IBD it is reasonable to speculate that the elevated serum L-arginine observed in the current investigation is the result of decreased cationic amino acid transporter (CAT)-mediated L-arginine transport into intestinal cells. Hence, as described in Figure 1, there may be a functional deficiency of intracellular L-arginine that impairs NO synthesis. Figure 2 describes the effects of increased ADMA on NO synthesis and the proposed pathophysiology of intestinal dysmotility, epithelial permeability, increased production of proinflammatory cytokines, and visceral hypersensitivity in IBS. The increased serum L-arginine observed in the DSS colitis model in mice[51] (and the corresponding decreased intracellular L-arginine availability) may be the direct result of defective CAT-mediated L-arginine transport as CAT2 expression is up-regulated in UC subjects compared to control tissues, and deletion of CAT2 results in increased serum L-arginine and exacerbation of DSS colitis.[52] As speculated by Hong et al., this may be the result of competitive inhibition of CAT-mediated L-arginine transport into cells by other amino acids such as L-ornithine and L-lysine. One further possibility is that increased serum ADMA may also compete with L-arginine for transport into intestinal cells as methylarginine transport into cells is also facilitated by CATs. Indeed one study has shown that increased ADMA and SDMA inhibited CAT1-mediated uptake of L-arginine in Human embryonic kidney cells (HEK293) stably overexpressing CAT1 (HEK-CAT1) and vector-transfected control cells (HEK-VC).[53]

This study has a number of strengths. The research was conducted in a relatively large population-based sample of community-dwelling older adults and cases of IBS were incident cases selected from within this cohort. Second, controls are population-based controls (without IBS) selected from the same cohort. Third, serum L-arginine, ADMA, and SDMA were all measured using hydrophilic-interaction liquid chromatography and isotope dilution tandem mass spectrometry, currently the gold standard for methylarginine measurement. Fourth, the bias-adjusted AUC estimates virtually did not change from the crude estimate and this suggests that the findings are robust. Finally, IBS cases were ascertained using a well-validated self-reported questionnaire according to Rome III criteria.

Limitations of our study include the use of a subjective self-reported questionnaire tool rather than a physician diagnosis of IBS. There were also no measures of NO metabolites to determine if these levels were altered in the presence of elevated L-arginine or methylarginines. Another limitation is the absence of dietary L-arginine intake measures and the inability to control for its potential confounding effects. Although there is likely to be variation in dietary L-arginine intake in the population, it is known that less than 5% of bioavailable L-arginine comes from dietary intake. Most is derived from endogenous synthesis from citrulline. However, there is evidence that increasing L-arginine intake can reduce ADMA concentrations and hence it is possible that dietary arginine may confound the observed association. Finally, the calculated fit and discrimination of the models may be overstated given the artificial dichotomy of those with IBS and those without, rather than an undifferentiated clinical population with symptoms; nevertheless, these results are encouraging and warrant further investigation.

In conclusion, higher serum concentrations of L-arginine and endogenous methylarginines are strong predictors of IBS in adults. These findings are potentially clinically relevant as L-arginine cellular uptake may be enhanced through L-arginine supplementation and ADMA concentrations may be lowered using several available medications. Longitudinal research is needed to determine if elevated concentrations of L-arginine or ADMA are a cause or a consequence of IBS. It is important to determine this causal direction as lowering their concentrations may lead to treatment. However, if it is the result of IBS, it is unlikely to have benefit. Furthermore, serum L-arginine concentration may be a useful clinical biomarker of IBS diagnosis. Further longitudinal research is needed to validate these findings.

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CONFLICTS OF INTEREST

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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