ORIGINAL RESEARCH

Increased Bronchial Hyperresponsiveness and Higher Asymmetric Dimethylarginine Levels after Fetal Growth Restriction

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Abstract

Bronchial hyperresponsiveness (BHR), a feature of asthma, is observed in preterm-born children and has been linked to intrauterine growth restriction. BHR is mediated via airway smooth muscle tone and is modulated by the autonomic nervous system, nitric oxide, and airway inflammation. Interactions among these factors are insufficiently understood. Methacholine-induced BHR (Met-BHR), fractional exhaled NO, and systemic soluble markers of nitric oxide metabolism and inflammation were determined in a population-based sample of 57 eleven-year-old children born extremely preterm (gestational age [GA] $<$ 28 wk) or with extremely low birth weight ($<$1,000 g), and in a matched normal–birth weight term-born control group (n = 54). Bronchopulmonary dysplasia (BPD) was defined as the need for oxygen treatment at a GA of 36 weeks. In preterm-born children, birth weight below the 10th percentile for GA was associated with increased Met-BHR and higher plasma levels of asymmetric dimethylarginine (ADMA), with an increased odds ratio for being in the upper tertile of Met-BHR (11.8; 95% confidence interval, 3.3–42.4) and of ADMA (5.2; 95% confidence interval, 1.3–20.3). Met-BHR was correlated to ADMA level (r = 0.27, P = 0.007). There were no significant differences in Met-BHR, fractional exhaled NO, or z-FEV_1 according to BPD status. No associations with systemic soluble markers of inflammation were observed for Met-BHR, birth, or BPD status. Intrauterine growth restriction in preterm-born children was associated with substantially increased Met-BHR and higher ADMA levels, suggesting altered nitric oxide regulation. These findings contribute to the understanding of the consequences from an adverse fetal environment; they should also be tested in term-born children.

Keywords: methacholine; bronchial hyperresponsiveness; nitric oxide; asymmetric dimethylarginine; intrauterine growth restriction

Intrauterine growth restriction (IUGR) affects fetal programming and is associated with both short- and long-term disorders affecting many organs, including the lungs (1). Bronchial hyperresponsiveness (BHR) is an essential characteristic of asthma (2) and is also observed after preterm birth (3, 4).

Recently, BHR has been linked to IUGR in both humans and animals (5). BHR is defined as excessive bronchoconstriction in response to an inhaled stimulus and is mediated by airway smooth muscle (ASM) tone. It is not known whether the observed ASM dysfunction in preterm-born children is an inherited or an acquired trait. Thus, children might be born with abnormal ASM tone caused by disrupted fetal airway development (6), or ASM abnormalities may be caused by postnatal adverse exposures of immature airways or by other airway disease processes acquired later (e.g.,

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Clinical Relevance

A history of intrauterine growth restriction was associated with increased methacholine-induced bronchial hyperresponsiveness and higher levels of asymmetric dimethylarginine, an inhibitor of NO production, with no associations with markers of inflammation or bronchopulmonary dysplasia. The findings suggest that early fetal growth restriction might be an important factor in the development of bronchial hyperresponsiveness in preterm-born children.

inflammation or remodeling) (7, 8). The first view gains support from studies reporting that childhood and even infant BHR can precede the development of respiratory symptoms by many years (9–13).

ASM tone is modulated by the autonomic nervous system and by nitric oxide (NO), with poorly understood regulatory mechanisms (14). NO is considered a marker of airway inflammation in atopic asthma, but it also decreases ASM tone (15). It is synthesized from arginine by the enzyme NO synthase, which is inhibited by the methylated arginine metabolite asymmetric dimethylarginine (ADMA) and by low arginine bioavailability (16). Low NO production in ASM has been reported to contribute to BHR (17). Methacholine is a bronchoconstrictor that acts via parasympathetic muscarinic M3 receptors, and it is widely used to quantify BHR.

We hypothesized that early fetal adaptation to an adverse intrauterine environment could have long-lasting functional consequences, potentially leading to lung disease. Thus, aiming to better understand the relation between IUGR and later lung function, we studied methacholine-induced BHR (Met-BHR), arginine metabolites, and systemic soluble markers of inflammation in 11-year-old children born extremely preterm (EP) or with an extremely low birth weight (ELBW).

Materials and Methods

Study Population

Preterm infants born at a gestational age (GA) of <28 weeks (EP) or with birth weight (BW) <1,000 g (ELBW) in 1999 and 2000 and admitted to one of the only two neonatal intensive care units within the Western Norway Health Authority were eligible for this population-based prospective cohort study.

Small for gestational age (SGA) was defined as BW below the10th percentile for GA according to Norwegian growth curves (18). A diagnosis of neonatal bronchopulmonary dysplasia (BPD) was assigned to infants who still required treatment with supplemental oxygen at a GA of 36 weeks (19).

Data Collection and Analysis

In 2010–2011, 57 EP-born and 54 control children were assessed at 11 years of age at the University Hospitals in Bergen or Stavanger, Norway. For each preterm-born participant, the next-born child of the same sex with GA >37 weeks and BW >3,000 g were identified from birth protocols in the maternity ward and invited as control subjects.

Lung Function Parameters

Spirometry measurements were taken with Vmax equipment, applying standard quality criteria, with data standardized for age, height, and sex (20). Fractional exhaled nitric oxide (F_{ENO}) was measured with an Exhalyzer CLD-88 (Eco Medics, Duernten, Switzerland) (21).

Methacholine provocation was performed with an inhalation-synchronized dosimetric nebulizer, providing baseline FEV_1 ⩾65% predicted (22, 23). The test continued until a fall of ⩾20% compared with baseline FEV_1, or until the maximal cumulative dose of 11.5 μmol methacholine was reached. The dose–response slope from the methacholine challenge (DRS-Met) was calculated as the ratio of maximal percentage decline in FEV_1 from baseline to cumulative administered dose of methacholine (%/μmol) (24). DRS-Met was used as a measure of BHR and was available in 106 of the 111 children (95%).

Biochemical Parameters

Blood samples from 96 of the 111 children (86%) were obtained by antecubital venipuncture. C-reactive protein, neopterin, and the ratio of kynurenine to tryptophan (Kyn/Trp ratio or KTR) were chosen as inflammation markers; neopterin and KTR are systemic indicators of cell-mediated immune activation (25). Inflammation markers, plasma levels of arginine, and the methylated arginine metabolites ADMA and its biologically inactive, symmetrical stereoisomer, symmetric dimethylarginine (SDMA) (26) were analyzed by established methods at the laboratory at Bevital (www.bevital.no).

Statistical Analysis

Results are presented as means and SD and were compared by analysis of variance or Student's *t* test, or as medians and interquartile range (IQR) and compared by the Kruskal–Wallis or the Mann–Whitney test, as appropriate. Differences in categorical variables were tested by Pearson chi-square test. *P* for trend was assessed by linear regression. Spearman was used for correlation. Multiple linear regressions were used to assess associations of lung function parameters with preterm status and growth parameters. Logistic regression was used to assess the odds ratio (OR) with a 95% confidence interval (CI) of having BHR and ADMA levels in the highest tertile. In the statistical models, DRS-Met was log-transformed to obtain normally distributed data, and the values reported are geometric means. Graphical illustration of the unadjusted dose–response relationship between ADMA levels and DRS-Met was obtained by generalized additive models (*see* online supplement).

Results

Anthropometric Features

With a survival rate of 81%, 61 infants were discharged alive from the neonatal unit. At inclusion at 11 years, two could not be traced and two were excluded because of cerebral palsy, leaving 57 participants (93%) available for the study; all but three were white. In the preterm group, GA varied from 24 to 31 weeks, 46 of the 57 children (81%) were born at a GA of <28 weeks, and 11 of the 57 (19%) were included on the basis of BW <1,000 g (ELBW). BW varied from 450 to 1,250 g, and 49 of the 57 children (86%) were born with ELBW. Twenty (35%) of the 57 preterm-born children had a BW below the 10th percentile for their GA, and these children had a 200-g lower mean BW ($P < 0.001$) than the appropriate-for-gestational-age (AGA) preterms, despite 2 weeks' longer GA ($P < 0.001$) (Table 1). BPD was reported in 31 of

Table 1. Demographic Characteristics in Term-Born and Preterm-Born Children ($n = 111$)

Variables	Term-Born AGA Children ($n = 54$)	Preterm-Born Children		P Value	P for Trend*
		AGA ($n = 37$)	SGA ($n = 20$)		
At birth					
Gestational age, wk, mean (SD)		26.1 (1.1)	28.0 (1.7)	<0.001†	
Weight, g, mean (SD)	3,701 (434)	918 (152)	724 (143)	<0.001†	
Male sex, No. (%)	29 (54)	16 (43)	13 (65)	0.27‡	
BPD, No. (%)	0	23 (62)	8 (40)	0.11‡	
At study inclusion					
Age, yr, median (IQR)	11.7 (11.2–12.0)	11.4 (11.1–11.8)	11.3 (11.0–11.8)	0.08§	
Weight, g, mean (SD)	41.3 (8.6)	40.1 (7.7)	36.2 (8.9)	0.07†	0.03
Length, cm, mean (SD)	151.5 (8.4)	149.2 (7.8)	142.6 (6.0)	<0.001†	<0.001
BMI, mean (SD)	17.8 (2.8)	17.8 (2.3)	17.6 (3.1)	0.95†	0.81

Definition of abbreviations: AGA, appropriate for gestational age; BMI, body mass index; BPD, bronchopulmonary dysplasia; IQR, interquartile range; SGA, small for gestational age.
*P for trend by linear regression (term-born AGA, preterm AGA, and preterm SGA).
†Comparison between the three groups by analysis of variance.
‡Comparison between the three groups by Pearson chi-square test.
§Comparison between the three groups by Kruskal–Wallis test.

the 57 children (54%); 23 of 31 (74%) were preterm AGA, and 19 of 31 (61%) were male (Table 1). In the control group, the GA ranged from 37 to 42 weeks, with mean (SD) BW of 3,701 (434) g and range, 3,000–4,500 g; the BW of 14 subjects > 4,000 g.

At age 11 years, the preterm group had a lower mean height than did the term control group (146.9 cm [SD, 7.8] versus 151.2 cm [SD, 8.4], $P = 0.003$), but there were no differences in weight ($P = 0.11$) or body mass index ($P = 0.90$). In the preterm group, SGA children were shorter ($P = 0.001$) than the AGA children, with no weight differences ($P = 0.09$) (Table 1).

Lung Function

DRS-Met was available in 106 of the 111 children (95%) and was significantly higher in the preterm-born compared with the term-born group ($P < 0.001$), with the highest levels seen in preterm SGA (Table 2). Adjusting the linear regression model for FEV_1, age, sex, and height did not change the results essentially (data not shown). The OR (95% CI) for being in the highest DRS-Met tertile was 11.8 (3.3–42.4) ($P < 0.001$) for preterm SGA, and 3.1 (1.1–8.5) ($P = 0.03$) for preterm AGA, in a logistic regression model that included age and sex. z-FEV_1 was lower in preterm than in term-born children, with the lowest levels seen in preterm SGA, whereas no differences were observed for F_{ENO} (Table 2).

In the preterm-born group, DRS-Met was correlated to z-FEV_1 ($r = -0.43$, $P = 0.001$), but not to F_{ENO} ($r = 0.26$, $P = 0.07$), whereas no significant correlations were seen in the term-born group ($P > 0.07$). There were no differences in DRS-Met ($P = 0.6$), F_{ENO} ($P = 0.5$), or z-FEV_1 ($P = 0.5$) according to BPD status.

Table 2. Lung Function in Term and Preterm Children ($n = 111$)

Variables	Term-Born AGA Children ($n = 54$)	Preterm-Born Children		P Value	P for Trend*
		AGA ($n = 37$)	SGA ($n = 20$)		
Methacholine slope, geometric mean (SD)	3.5 (5)	8.8 (6.2)	28.7 (4.3)	<0.001†	<0.001
z-FEV_1, mean (SD)	−0.31 (0.97)	−0.49 (0.89)	−0.96 (0.96)	0.03†	0.01
F_{ENO}, ppb, median (IQR)	9.7 (7.4–15.8)	9.8 (5.7–14.4)	9.4 (7.0–14.3)	0.57‡	0.13

Definition of abbreviations: AGA, appropriate for gestational age; F_{ENO}, fractional exhaled nitric oxide; IQR, interquartile range; ppb, parts per billion; SGA, small for gestational age; z-FEV_1, z-score (standard deviation score) for forced expiratory volume in 1 second.
*P for trend by linear regression (term-born AGA, preterm AGA, and preterm SGA).
†Comparison between the three groups by analysis of variance.
‡Comparison between the three groups by Kruskal–Wallis test.

Systemic Soluble Inflammation Markers

There were no significant differences in the systemic soluble inflammation markers (C-reactive protein, neopterin, and KTR) according to birth status (preterm versus term born) (Table 3), or in BPD status, or between DRS-Met tertiles.

Arginine, ADMA, and SDMA

Arginine and ADMA levels were higher in preterm-born compared with term-born children ($P = 0.02$ and $P = 0.03$, respectively), but no significant differences were seen for SDMA and the arginine/ADMA ratio ($P = 0.09$ and $P = 0.21$, respectively). The highest arginine, ADMA, and SDMA levels were seen in preterm SGA (Table 4). The OR (95% CI) for being in the highest ADMA tertile was 5.2 (1.3–20.3) ($P = 0.02$) for preterm SGA and 1.5 (0.5–4.1) ($P = 0.42$) for preterm AGA in a logistic regression model that also included age, sex, length, and weight.

Table 3. Inflammation Parameters in Term and Preterm Children ($n = 95$)

Variables	Term AGA Children ($n = 46$)	Preterm Children		P Value*	P for Trend†
		AGA ($n = 33$)	SGA ($n = 17$)		
CRP, mg/L	0.4 (0.3–0.7)	0.4 (0.2–0.7)	0.5 (0.2–0.8)	0.86	0.27
Neopterin, μmol/L	9.9 (7.8–11.1)	9.4 (7.9–12.7)	9.3 (6.4–10.3)	0.16	0.30
Kynurenine/tryptophan ratio	20.8 (17.7–25.6)	24.6 (18.2–26.8)	25.2 (20.9–27.2)	0.16	0.04

Definition of abbreviations: AGA, appropriate for gestational age; CRP, C-reactive protein; SGA, small for gestational age.
Data are presented as median (interquartile range).
*Comparison between the three groups by Kruskal–Wallis test.
†P for trend by linear regression (term-born AGA, preterm AGA, and preterm SGA).

DRS-Met was positively correlated to ADMA ($r = 0.27$, $P = 0.007$) but not to arginine, SDMA, or the arginine/ADMA ratio. The linear relation between change in DRS-Met versus ADMA levels is visualized by generalized additive models in Figure 1. Seven of the 16 preterm-born SGA children (44%) had both high DRS-Met (tertile 3) and high ADMA levels (tertile 3), whereas 6 of the 33 in the preterm-born AGA group (18%) and 4 of the 46 in the control group (9%) ($P = 0.01$) had both high DRS-Met and high ADMA levels.

No correlations were observed for F_{ENO} and z-FEV_1 to arginine, ADMA, or SDMA, apart from a negative correlation between z-FEV_1 and SDMA ($r = -0.23$, $P = 0.03$). There were no differences in ADMA, arginine, or SDMA levels according to BPD status.

Discussion

In this study including a population-based cohort of 11-year-old children born EP or with ELBW and a term-born AGA control group, both Met-BHR and ADMA levels were higher in the preterm-born children with IUGR, independent of group differences in FEV_1 and height. Preterm birth, IUGR, and Met-BHR were not associated with higher levels of systemic soluble inflammation markers. Neonatal BPD was unrelated to BHR, markers of inflammation, or arginine and its metabolites. These findings imply that unfavorable intrauterine conditions may adversely affect airway development and cause later respiratory disease.

Growth and Body Composition in Relation to Prematurity, Low Birth Weight, and SGA

SGA refers to a BW below the 10th percentile for GA, which may be caused by genetic factors defining a constitutional small size, or to fetal growth restriction. In infants born at very low GA, SGA status is more likely to represent IUGR (27). All preterm-born participants were born either EP or with ELBW, with the majority having both characteristics. Apart from a shorter height, their growth parameters at age 11 years equaled those of term-born children.

BHR

The physiological basis of BHR is largely unknown, but it is presumed to be related to airway remodeling and bronchial inflammation (28). BHR may be triggered by different stimuli causing different responses according to patient category, presumably reflecting a diverse pathophysiology of airway dysfunction (29, 30). Bronchoconstriction induced by indirect stimuli such as exercise or adenosine is mediated by factors released from a variety of inflammatory and epithelial cells (30). After preterm birth and BPD, BHR has been found to be triggered mainly by direct stimuli, making an inflammatory basis for bronchoconstriction less likely (31, 32). Our data support these observations, because no differences in markers of inflammation according to birth status (preterm versus term), BPD status, or Met-BHR were observed.

Methacholine-induced bronchoconstriction is mediated by muscarinic acetylcholine subtype M3 receptors through a direct effect on ASM. Muscarinic receptor stimulation promotes smooth muscle contraction, but recent

Table 4. Arginine, ADMA, and SDMA in Term and Preterm Children ($n = 95$)

Variables	Term AGA Children ($n = 46$)	Preterm Children		P Value*	P for Trend†
		AGA ($n = 33$)	SGA ($n = 17$)		
Arginine, μmol/L	90.3 (15.6)	97.8 (18.4)	103.0 (23.5)	0.03	0.009
ADMA, μmol/L	0.65 (0.07)	0.66 (0.07)	0.71 (0.07)	0.003	0.001
SDMA, μmol/L	0.55 (0.08)	0.56 (0.07)	0.61 (0.09)	0.01	0.009
Arginine/ADMA ratio	140.9 (24.1)	147.9 (23.1)	143.8 (23.5)	0.46	0.45

Definition of abbreviations: ADMA, asymmetric dimethylarginine; AGA, appropriate for gestational age; SDMA, symmetric dimethylarginine; SGA, small for gestational age.
Data are presented as mean (SD).
*Comparison between the three groups by analysis of variance.
†P for trend by linear regression (term-born AGA, preterm AGA, and preterm SGA).

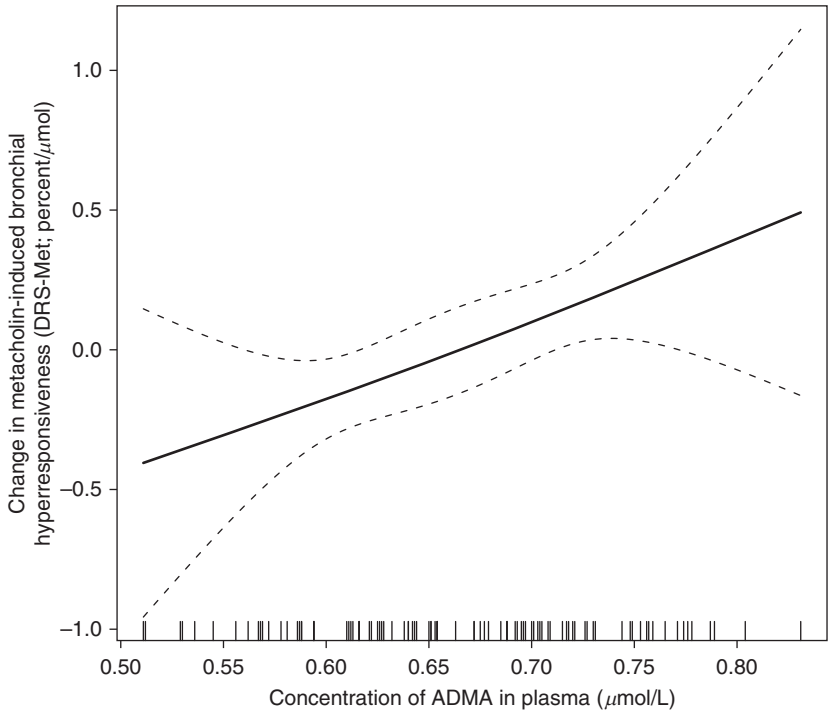

Figure 1. Association of methacholine-induced bronchial hyperresponsiveness (DRS-Met) with plasma asymmetric dimethylarginine (ADMA) levels by generalized additive models reflecting the change in DRS-Met with increasing ADMA levels.

findings show that it also contributes to airway remodeling associated with smooth muscle growth, airway fibrosis, and inflammation (33). We observed that Met-BHR was substantially more pronounced in preterm-born SGA children compared with preterm-born AGA and term-born control children, indicating a possible influence of early fetal growth restriction on later methacholine-induced ASM responses. Pike and colleagues observed BHR after maternal dietary restriction in an animal model and found an inverse association between abdominal circumference in gestational week 11–19 and BHR at 6 years of age in humans (5). Fetal developmental adaptions to adverse intrauterine environments are known to permanently change the structure and function of several organs (34). One example is the change in function of the M3 receptors in pancreatic β-cell islets and reduced insulin secretion caused by maternal protein restriction during lactation (35). Adaptions in muscarinic receptors, widespread in the smooth muscle and epithelial lining of the bronchial as well as the cardiovascular, digestive, and urinary systems, caused by a suboptimal intrauterine environment may be a common denominator for the development of the long-term disorders associated with IUGR.

Nitric Oxide Regulation and Inflammation

In this study, the preterm-born SGA children were more likely to have higher levels of arginine, ADMA, and SDMA, but there were no differences in the arginine/ADMA ratio. NO is an important mediator of smooth muscle dilatation in airways and blood vessels, and dysfunctional NO regulation has been proposed as a mechanism in asthma (36), as well as in the metabolic syndrome (37). Asthma is not a single entity, and allergic asthma is characterized by high F_{ENO} (38). Intracellular arginine levels are reported to be three-fold higher in individuals with asthma compared with healthy control subjects (39), and published data show that the asthmatic epithelium synthesizes arginine through a cell-autonomous citrulline-arginine-NO cycle, at levels sufficient to sustain NO production over prolonged periods (38). This enhanced arginine metabolism has been reported to modulate cell function by increasing oxidative metabolism and reducing inflammation (38).

Low NO production may be caused by low levels of the substrate arginine, or by high levels of the inhibitor ADMA (16). Higher levels of ADMA are reported in children with asthma (40), and negative correlations to GA and BW have been reported in young adults born with ELBW (41), a finding that is supported by our data. ADMA levels are also reported to be higher in obese individuals and in those with the metabolic syndrome (42, 43), and ADMA has been suggested as the link between asthma and the metabolic syndrome (44).

We observed no differences in systemic inflammatory status or F_{ENO} in relation to preterm birth, IUGR, or BPD. Asthma phenotypes independent of inflammation appear to exist (45) (e.g., the obese-asthma phenotype, characterized by low exhaled NO), reflecting an imbalance between arginine and ADMA (46). A higher ADMA and increased oxo-nitrative stress have been a suggested mechanism for airway hyperresponsiveness, unrelated to any classical immune response (47). Exhaled NO is positively correlated to airway inflammation (48, 49), and BHR has been related to increased exhaled NO only in atopic very-low-birth-weight children (50). Low levels of airway NO are observed in patients with chronic lung disease (51) and in preterm children with BPD (52).

Potential Implications

Met-BHR is reported regularly in individuals without respiratory symptoms, even in infants (9), and can precede the development of asthma by many years. Pike and colleagues (5) have suggested a causal role for fetal growth restriction in the development of BHR, and the current findings support this notion. Met-BHR may be an inborn trait and may be causally related to the development of asthma.

Recent studies have linked preterm birth to later metabolic syndrome (53). The current study suggests that early IUGR might play a role in this scenario, possibly by altering M3 receptor activity and NO metabolism. These issues must be explored in term-born cohorts.

Strength and Limitations

The population-based design with almost complete attendance is a strength of this study. The recruitment of the control groups

followed a strict algorithm that was based on the principle of "next-born-subject," minimizing the risk of selection bias. However, the low number of preterm SGA children reduced the statistical power of the analyses. The study design involves inclusion of all subjects born EP, but also of those with ELBW irrespective of GA; thus, the results cannot be generalized to preterm-born children in general, or to growth-restricted groups born at term. In addition, there are obvious mechanistic limitations to lung function research, particularly in children, and systemic soluble markers of NO metabolism and inflammation may not necessarily reflect local airway processes properly.

Conclusions

In 11-year-old children born EP or with ELBW, a history of IUGR was associated with increased Met-BHR and higher levels of ADMA, an inhibitor of NO production, with no associations to markers of inflammation or to BPD. The findings suggest that early fetal growth restriction might be an important factor in the development of BHR in preterm-born children. These issues should also be explored according to fetal growth restriction in term-born children. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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