Biological Sensitivity to Context

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ABSTRACT—*Conventional views suggest that exaggerated biological reactivity to stress is a harmful vestige of an evolutionary past in which threats to survival were more prevalent and severe. Recent evidence, however, indicates that effects of high reactivity on behavior and health are bivalent rather than univalent in character, exerting both risk-augmenting and risk-protective effects depending on the context. These observations suggest that heightened stress reactivity may reflect increased biological sensitivity to context, with potential for negative health effects under conditions of adversity and for positive effects under conditions of support. From an evolutionary perspective, the developmental plasticity of the stress-response systems, along with their structured, context-dependent effects, suggests that variation in these systems has been adaptively patterned to increase the capacity of children to match their stress-response profiles to anticipated developmental environments. Taken together, these theoretical perspectives generate a novel hypothesis: that there is a curvilinear, U-shaped relation between early exposures to adversity and the development of stress-reactive profiles, with high-reactivity phenotypes disproportionately emerging within both highly stressful and highly protected early social environments.*

KEYWORDS—*stress reactivity; evolutionary psychology; developmental psychobiology*

Developmental psychologists frequently consider the effects of life experience on development but rarely consider how these effects have been structured by natural selection. Despite this oversight, the burgeoning field of evolutionary-developmental biology has exciting and profound implications for the study of human development (see especially West-Eberhard, 2003). Over the last two decades, theory and research in the field has come to acknowledge that, in most species, single "best" strategies for survival and reproduction are unlikely to evolve. This is because the optimal strategy varies as a function of the physical, economic, and socioemotional parameters of one's specific environment (Crawford & Anderson, 1989), and thus a strategy that promotes success in some environmental contexts may lead to failure in others. Selection pressures therefore tend to favor *adaptive phenotypic plasticity*, the capacity of a single genotype to support a range of phenotypes in response to particular ecological conditions that recurrently influenced fitness during a species' evolutionary history. Importantly, the development of alternative phenotypes is a nonrandom process; that is, it is the outcome of structured transactions between genes and environments that were shaped by natural selection to increase the capacity and tendency of individuals to track their developmental environments and adjust their phenotypes accordingly.

We have recently proposed a developmental model of adaptive phenotypic plasticity in the human stress-response systems (see Boyce & Ellis, 2005). The model articulates the precepts and rationale for a new claim about the nature of relations between early life experience and stress reactivity, a claim that we have also explored empirically (Ellis, Essex, & Boyce, 2005). We contend that heightened stress reactivity may reflect not simply exaggerated arousal under challenge but, rather, a form of enhanced, neurobiologically mediated sensitivity to context, or *biological sensitivity to context* (BSC).

The logic of our argument can be summarized in the following way. Biological reactivity to psychological stressors comprises a complex, integrated system of central neural and peripheral neuroendocrine responses designed to prepare the organism for challenge or threat. Developmental experience plays a role, along with heritable variation, in calibrating the response dynamics of this system. Individual differences in such stress reactivity are thought to underlie broad variability in associations between stress and illness and to reflect constitutional variation in susceptibility to stressful challenge. Highly reactive phenotypes, in which affected individuals mount vigorous or sustained autonomic, adrenocortical (cortisol), or other biological responses to stressors, have been viewed as an atavistic health risk factor, a legacy of physiological responses more commensurate with the perils of prehistoric human environments. Often overlooked in such accounts is a body of anomalous observations revealing oppositional, counter-regulatory processes within the

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stress-response circuitry itself and, even more compellingly, bidirectional effects of reactivity on biomedical and psychiatric outcomes. Highly reactive children sustain disproportionate rates of morbidity when raised in adverse environments but unusually low rates when raised in low-stress, highly supportive settings (Boyce & Ellis, 2005).

Such bidirectional, environment-contingent health effects suggest that BSC is the core, defining feature of highly reactive phenotypes. These observations call into question the presumably unitary pathogenic effects of high reactivity and suggest that its protective effects within specific developmental ecologies might explain the conservation of such phenotypic variation over evolutionary history. BSC reflects sensitivity to both harmful and protective contextual effects. Indeed, the subset of children with highly reactive biological profiles reveals a unique sensitivity or "permeability" to the influence of environmental conditions (Boyce & Ellis, 2005). Further, although a substantial literature documents the capacity of early developmental trauma to predispose individuals toward high biological reactivity, an evolutionary formulation of recent findings suggests a different and novel hypothesis: that the association between early adversity and reactivity is curvilinear in character, with both highly stressful and highly protective environments yielding disproportionate numbers of highly reactive children (Boyce & Ellis, 2005).

THE DANDELION AND THE ORCHID

A Swedish idiomatic expression, *maskrosbarn* or "dandelion child," refers to the capacity of some children—not unlike those with low-reactive phenotypes—to survive and even thrive in whatever circumstances they encounter, in much the same way that dandelions seem to prosper irrespective of soil, sun, drought, or rain. Observations of such children have generated, for example, an extensive developmental literature on the phenomenon of resilience, the capacity for positive adaptation despite experiences of significant adversity (e.g., Luthar, 2006; Masten, 2007). A contrasting Swedish neologism, *orkidebarn* or "orchid child," might better describe the context-sensitive individual, whose survival and flourishing is intimately tied, like that of the orchid, to the nurturant or neglectful character of the environment. In conditions of neglect, the orchid promptly declines, while in conditions of support and nurture, it is a flower of unusual beauty.

The metaphorical invocation of highly reactive children as *orkidebarn* is consistent with a growing number of studies revealing that high-reactivity phenotypes under specific environmental conditions may be associated with protective, rather than harmful, effects and generate normative or improved health outcomes. Such bivalent effects of BSC on human and primate morbidities have thematically characterized a series of studies reported by Boyce and colleagues. In examining cardiovascular and immunologic reactivity in two samples of 3- to 5-year-old children, for example, significant interactions with environmental stressors were detected (Fig. 1A) in the prediction of respiratory illness incidence over the ensuing several months (Boyce et al., 1995). Specifically, the noted interactions suggested bidirectional effects of reactivity on illness incidence: Highly reactive children in high-stress families or childcare centers sustained significantly higher rates of respiratory illness than their low-reactive peers did, but equally reactive children in low-stress settings were the healthiest of all children in the samples. By contrast, the respiratory-illness incidence of low-reactivity children was unresponsive to environmental stress levels, showing approximately the same, mid-level illness rates in both low- and high-stress conditions. Similarly significant interactions were found for injury incidence (Boyce, 1996).

Even though they were prospective in design, both of these studies were observational in nature and lacked experimental data on the incidence of illnesses or injuries among the same group of highly reactive children in both low- and high-stress conditions. In a subsequent study of semi-free-ranging rhesus

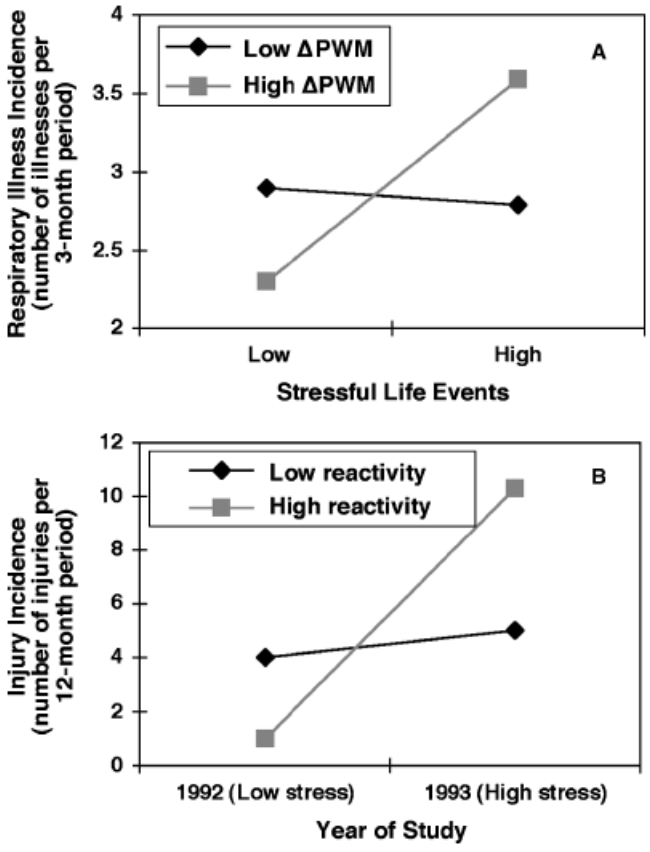

Fig. 1. Variation in effects of BSC on physical health across socioecological conditions. The top graph (A) shows a cross-over interaction between immune reactivity (changes in pokeweed mitogen response [ΔPWM]) and family stressful events in prediction of respiratory illness incidence in kindergartners ($N = 99$; redrawn from Boyce et al., 1995). The bottom graph (B) shows a cross-over interaction between biobehavioral reactivity and confinement stress in prediction of injury rates in a troop of semi-free-ranging rhesus monkeys ($N = 36$; redrawn from Boyce, O'Neill-Wagner, Price, Haines, & Suomi, 1998).

macaques, however, such quasiexperimental conditions were satisfied (Boyce, O'Neill-Wagner, Price, Haines, & Suomi, 1998). The troop of macaques, which had been previously assessed for levels of BSC (degree of biobehavioral reactivity to novel or challenging stimuli), lived in a 6-acre wooded habitat in rural Maryland. In 1993, the troop encountered a 6-month period of protective confinement to a small, 1,000-square-foot building, during a construction project on the habitat grounds. The confinement proved highly stressful, and the incidence of violent injuries increased fivefold during the 6-month period. Blinded ascertainment of medically attended injury rates from veterinary records produced evidence for a significant interaction between reactivity status and confinement stress, plotted in Fig. 1B. As with the prior studies of children, low-reactivity individuals showed little effect of the confinement, while those with high reactivity showed dramatically higher rates of violent injuries in the high-stress situation but lower rates in the low-stress condition.

These findings complement research on the bidirectional, context-dependent effects of high intelligence (or high ego development): Whereas highly intelligent, introspective people tend to flourish under relatively benign life conditions, they also tend to be more reactive than others to distress (see Luthar, 2006). Thus, just as high reactivity to stress has generally been considered pathogenic but can be protective in supportive environmental contexts, high intelligence/introspection has generally been thought of as beneficial, but can be harmful if surrounding forces are negative.

DEVELOPMENT OF BSC: AN EVOLUTIONARY APPROACH

Adaptive phenotypic plasticity enables children to match their biological and behavioral systems to the parameters of their early (and predicted future) developmental environments. Given past evidence that early trauma can increase stress reactivity and new evidence that high reactivity can be protective in highly supportive settings, we (Boyce & Ellis, 2005) postulated a curvilinear, U-shaped relation (shown in Fig. 2) between levels of early adversity and the magnitude of biological response dispositions. Specifically, we hypothesized (a) that exposure to acutely stressful childhood environments up-regulates stress reactivity, increasing the capacity and tendency of individuals to detect and respond to environmental dangers and threats; and (b) that exposure to exceptionally supportive childhood environments also up-regulates stress reactivity, increasing susceptibility to social resources and ambient support. Both of these proposed functions of BSC converge with theory and data indicating that temporary, moderate increases in stress hormones and associated neurotransmitters enhance mental activities in localized domains, focus attention, and prime memory storage and thus improve cognitive processes for dealing with environmental opportunities and threats (Flinn, 2006). By contrast, and

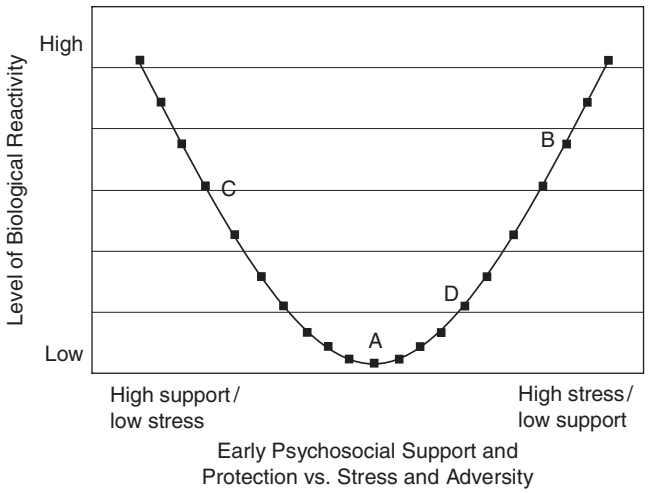

Fig. 2. Hypothesized curvilinear relation between biologic reactivity to stress and experiences of support and protection versus stress and adversity in early environments. Comparisons of subjects at points A and B would lead to the conclusion that low support/high stress results in heightened stress reactivity. Comparisons at points C and D, on the other hand, would generate the inference that low support/high stress produces diminished reactivity (adapted from Boyce & Ellis, 2005).

typically for the large majority of children, (c) exposure to childhood environments that are not extreme in either direction down-regulates stress reactivity, buffering individuals against the chronic stressors encountered in a world that is neither highly threatening nor consistently safe. Although the cellular mechanisms that calibrate such response dispositions are currently unknown in humans, recent work in animal models suggests that epigenetic modifications resulting in differential gene expression may well play an important role (Meaney, Szyf, & Seckl, 2007).

Although the theory predicts up-regulation of stress-response systems in both highly supportive and stressful environments (the U-shaped curve), high stress reactivity may translate into different behavioral phenotypes in supportive versus stressful contexts. Reactive, sensitive children have been found to be more reflective and perhaps more conscious of self and environment; to be more able to delay gratification in pursuit of goals; and to perform better on neuropsychological measures of inhibitory control, executive function, and self-regulation (e.g., Aron & Aron, 1997; Blair, Granger, & Razza, 2005). Up-regulated stress-response systems in children may therefore interact with protective, beneficial developmental environments to produce relatively high levels of cognitive and social competence. Conversely, interactions between high stress reactivity and risky, threatening developmental environments may result in lower thresholds for anticipating threat in ambiguous or unfamiliar situations (e.g., elevated sensitivity to threat cues such as angry faces) and support greater vigilance and wariness in children.

Although the U-shaped curve depicted in Figure 2 specifies environmental sources of variation in BSC, genetic sources of

variation and gene–environment interactions are also important and need to be addressed in a comprehensive theory of BSC (see Ellis, Jackson, & Boyce, 2006). Reaction norms are genetically based "bookends" that constrain the range of phenotypes that can develop within varying environmental contexts. Importantly, children differ in the location of these bookends along phenotypic dimensions. For example, children whose reaction norms are located on the upper end of the BSC spectrum have higher starting points for stress reactivity than do children whose reaction norms are on the lower end. These differences can generally be expected to maintain variation in BSC, even if children have equivalent life experiences. In addition, children differ in how widely their bookends are placed. That is, some children have broad reaction norms and display high levels of plasticity in response to developmental experience, whereas others have more narrow reaction norms and display more fixed developmental trajectories (see Belsky, 2005). The current theory should be more successful in accounting for developmental variation in BSC among children with wider reaction norms.

EMPIRICAL EXPLORATIONS OF BSC THEORY

We have initially investigated our curvilinear, U-shaped model of the development of BSC in two studies comprising 249 children and their families (Ellis et al., 2005). In the first study, 3- to 5-year-old children were concurrently assessed on levels of support and adversity in home and preschool environments and on cardiovascular reactivity to laboratory challenges. Because the early environments of these children ranged from exceptionally stable and supportive to moderately stressful, the sample only provided a window into a portion of the proposed U-shaped association between support/adversity and BSC (i.e., the left half of the curve shown in Fig. 2). Within this range, the theory posits that higher stress will be associated with reduced biological responsivity to stressors. In the second study, children were prospectively assessed on family stress in both infancy and preschool and on autonomic and adrenocortical reactivity to laboratory challenges at age 7. This second study sampled the broad range of variation in early childhood environments that is needed to fully explore the curvilinearity hypothesis. Within this range, both highly protected and highly stressful environments should promote heightened BSC.

We found in both studies that a disproportionate number of children in supportive, low-stress environments displayed high autonomic reactivity. Conversely, in the second study a relatively high proportion of children in very stressful environments showed evidence of heightened sympathetic and adrenocortical reactivity. Consistent with our evolutionary-developmental theory, these exploratory analyses also suggested that relations between levels of childhood support/adversity and the magnitude of stress reactivity are curvilinear, with children from moderately stressful environments displaying the lowest reactivity levels in both studies.

CONCLUSIONS AND FUTURE DIRECTIONS

The proposed U-shaped curve can potentially reconcile important contradictions in the existing literature on the origins and consequences of stress reactivity in children. Investigators comparing individuals from points A and B in Figure 2, for example, would conclude, as many researchers in this area have (e.g., De Bellis et al., 1999), that experiences of family and environmental stress are associated with up-regulatory calibrations in biological reactivity systems. Yet studies comparing individuals from points C and D would find, as those reviewed by Gunnar and Vazquez (2001) have, that early stressors are rather associated with down-regulatory changes in salient biological responses. The current theory, which posits two oppositionally distinctive ontogenies for BSC, explains both of these regulatory effects.

A guiding assumption of our work on stress reactivity is that developmental mechanisms have been organized by natural selection to produce enhanced BSC when it is advantageous to the developing person—in both acutely stressful and exceptionally supportive childhood environments. In shaping intervention strategies to prevent developmental psychopathology and other early morbidities, we may do well to consider this conceptualization of individual differences in stress reactivity. Under some circumstances, highly sensitive children may be usefully targeted for interventions involving the provision of ancillary supportive services, while in other circumstances, the needs of highly sensitive children might define the minimum standards of provision for an entire population of children. In still other settings, ascertainment of reactivity status might simply prevent the adoption of a "one size fits all" approach, facilitating the design of strategies and policies specifically tailored to the needs of children with different biological response phenotypes. Whatever the future direct utility of the theory and research described here, it is our hope that this work will advance collective understanding of the sources of individual differences and their implications for the rearing and well-being of children.

Recommended Reading

Aron, E.N., & Aron, A. (1997). (See References). An insightful empirical paper on sensory-processing sensitivity, which has interesting parallels with BSC.

Belsky (2005). (See References). A very thoughtful presentation of a complementary evolutionary theory of differential susceptibility to environmental influence.

Boyce, W.T., & Ellis, B.J. (2005). (See References). The original, thorough, and more far-reaching theoretical analysis of BSC.

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Biological sensitivity to context: I. An evolutionary–developmental theory of the origins and functions of stress reactivity

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Abstract

Biological reactivity to psychological stressors comprises a complex, integrated, and highly conserved repertoire of central neural and peripheral neuroendocrine responses designed to prepare the organism for challenge or threat. Developmental experience plays a role, along with heritable, polygenic variation, in calibrating the response dynamics of these systems, with early adversity biasing their combined effects toward a profile of heightened or prolonged reactivity. Conventional views of such high reactivity suggest that it is an atavistic and pathogenic legacy of an evolutionary past in which threats to survival were more prevalent and severe. Recent evidence, however, indicates that (a) stress reactivity is not a unitary process, but rather incorporates counterregulatory circuits serving to modify or temper physiological arousal, and (b) the effects of high reactivity phenotypes on psychiatric and biomedical outcomes are bivalent, rather than univalent, in character, exerting both risk-augmenting and risk-protective effects in a context-dependent manner. These observations suggest that heightened stress reactivity may reflect, not simply exaggerated arousal under challenge, but rather an increased *biological sensitivity to context*, with potential for negative health effects under conditions of adversity and positive effects under conditions of support and protection. From an evolutionary perspective, the developmental plasticity of the stress response systems, along with their structured, context-dependent effects, suggests that these systems may constitute *conditional adaptations*: evolved psychobiological mechanisms that monitor specific features of childhood environments as a basis for calibrating the development of stress response systems to adaptively match those environments. Taken together, these theoretical perspectives generate a novel hypothesis: that there is a curvilinear, U-shaped relation between early exposures to adversity and the development of stress-reactive profiles, with high reactivity phenotypes disproportionately emerging within *both* highly stressful and highly protected early social environments.

Biological reactivity to environmental stressors is now widely implicated in the processes linking psychological adversity to psychiatric and biomedical disorder. The neuroendocrine changes that reliably accompany stressful events, in humans and other species, are the physiological, homeostatic means by which survival under threat is protected, but are also among the dysregulatory pathways by which psychological trauma is transmuted into pathogenic biological processes. Individual differences in such "stress reactivity" are thought to underlie the broad variability in stress–illness associations and to reflect constitutional variation in susceptibility to stressful challenge. Highly reactive phenotypes, in which affected individuals mount vigorous and/or persistent

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autonomic, adrenocortical, or other biological responses to stressors, have been viewed as an atavistic health risk factor, a legacy of physiological responses more commensurate with the perils of prehistoric human environments. As such, exaggerated stress reactivity is generally viewed as a maladaptive, monotonically harmful heritage of an ancient preparedness for endangerment. High reactivity, so the argument goes, is a heritable response disposition, often unmasked by traumatic experiences in early life, which places individuals at heightened risk for disorders of mental and physical health. It is the central claim of this two-part series that this view, that high reactivity phenotypes are uniformly harmful psychobiological reversions to primitive and maladaptive modes of response, is mistaken.

Rather, an evolutionary reinterpretation of evidence regarding reactivity and health suggests that highly reactive phenotypes can be more usefully viewed as reflecting heightened *biological sensitivity to context* (BSC), an attribute that may have conferred selective advantages in certain social and ecological contexts during human evolution. Further, although a substantial literature documents the capacity of early developmental trauma to predispose an individual toward high biological reactivity, an evolutionary formulation of recent findings suggests a different and novel hypothesis: that the association between early adversity and reactivity is curvilinear in character, with *both* highly stressful and highly protective environments yielding disproportionate numbers of highly reactive children. In this paper, the theoretical and evidential grounds for this new hypothesis are presented, and in the second paper, exploratory data analyses in two studies of early development and psychopathology are used to generate empirical observations commensurate with the same hypothesis. Together, the papers present both theoretical and empirical lines of argument that converge upon a single thesis: that highly reactive phenotypes are forms of enhanced, neurobiologically mediated sensitivity to context, which have been favored by natural selection due to their fitness-enhancing effects in both minimally and maximally stressful environments. This evolutionary paradox, of reactive phenotypes being selected within two oppositional categories of environments, can be resolved, it will be argued, by the supposition that BSC increases adaptive competence in highly *stressful* environments by augmenting vigilance to threats and dangers and in highly *protective* environments by increasing susceptibility to social resources and ambient support. In the sections that follow, we (a) describe the structure and phylogeny of the neural circuits implicated in stress reactivity, (b) delineate genetic and environmental contributions to the calibration of such reactivity, (c) review past and recent findings linking stress reactivity and health, and (d) conclude that phenotypic expressions of unusually heightened reactivity reflect an underlying biological sensitivity to contextual signals. Finally, exploring this reconceptualization of exaggerated reactivity as context sensitivity, we construct an evolutionary–developmental theory of the origins and functions of the human stress response and formulate new hypotheses characterizing the relation between early adversity and the magnitude of biological responses to stress. This theory and derivative hypotheses are founded on the concept of conditional adaptation, in which a single genotype supports a range of environmentally contingent phenotypic expressions, enabling an adaptive correspondence between the developing organism and its environment.

The Structure and Phylogeny of the Human Stress Response

Environmental events signaling threats to survival or well being produce a set of complex, highly orchestrated responses within the neural circuitry of the brain and peripheral neuroendocrine pathways regulating metabolic, immunologic, and other physiological functions. As first described in the work of Claude Bernard on homeostasis (Bernard, 1878) and the subsequent research of 20th century stress physiologists Walter Cannon (1929) and Hans Selye (1950), this elaborate and tightly integrated repertoire of responses results in an immediate, relatively automatic

shift to a state of biological and behavioral preparedness, involving increases in heart rate and blood pressure, metabolic mobilization of cellular nutrients, preferential redirection of energy resources and perfusion to the brain, and the induction of behavioral vigilance and fear. Comprehensively detailed in the writings of neuroscientists such as Chrousos (1998), Meaney (2001), and McEwen (1998), the neural substrate for the organism's stress response comprises two anatomically distinct but functionally integrated circuits, the corticotropin-releasing hormone (CRH) system and the locus coeruleus–norepinephrine (LC-NE) system. The coactivation of the these two systems, along with their linkages to limbic structures, such as the amygdala and anterior cingulate, as well as the mesolimbic dopaminergic system and the medial prefrontal cortex, produce the coordinated biobehavioral changes associated with the stress response in mammalian species.

The CRH system actually comprises two distinguishable subsystems, one centered in the paraventricular nucleus (PVN) of the hypothalamus and involved in the homeostatic regulation of the hypothalamic–pituitary–adrenocortical (HPA) axis, and the other involved in the corticolimbic circuitry of the amygdala and its connections. Within the former subsystem, CRH is released into the portal blood supply of the pituitary in a pulsatile (and, under normative conditions, circadian) fashion by neurons in the PVN and serves, along with synergistic effects of arginine vasopressin (AVP), as the primary secretagogue for expression of pro-opiomelanocortin (POMC) polypeptide by the anterior pituitary. In the second subsystem, CRH cell bodies are more widely represented in loosely related, extrahypothalamic locations, including the amygdala, the substantia innominata, the bed nucleus of the stria terminalis, and in the prefrontal, insular, and cingulate regions of the cortex (Gold & Chrousos, 2002; Owens & Nemeroff, 1991). Two or more types of CRH receptors have been elucidated, with species variation in the expression of specific receptor types. CRH_1 receptors are found in the anterior pituitary and other brain regions and are involved in generating fear-related behavior, and CRH_2 receptors, found mostly in the periphery, may play a counterregulatory role in anxiogenesis. POMC is cleaved into its component proteins, corticotropin (ACTH) and β-endorphin (Smith et al., 1998), and ACTH is transported in plasma to the zona fasciculata of the adrenal cortex, triggering secretion of cortisol, the principal human glucocorticoid regulating blood pressure, glucose metabolism, and immune competence. Glucocorticoids also inhibit those neuroendocrine axes promoting growth and reproduction (Gold, Goodwin, & Chrousos, 1988).

The intracellular actions of cortisol are mediated through binding to a widely distributed cytoplasmic receptor, translocation to the nucleus of the target cell, and subsequent direct effects on gene transcription and inhibition of other, proregulatory transcription factors such as c-jun/c-fos, and NF-kB (van der Saag, Caldenhoven, & van de Stolpe, 1996). Although such actions acutely facilitate essential biological responses to stress and threat, chronic glucocorticoid secretion is associated with a variety of pathogenic processes and disease states, including major depression, insulin resistance and diabetes, hypertension and atherosclerosis, bone loss, and disorders related to diminished immune functions (Gold & Chrousos, 1999; McEwen, 1998). The hippocampus, a brain region closely involved in memory and learning, is particularly susceptible to the effects of glucocorticoids, showing decreased dendritic branching and neuronal loss in the CA3 area, as well as changes in synaptic terminal structure and inhibition of neuron regeneration (Bremner & Vermetten, 2001; Sapolsky, 1996). Under more normative circumstances, circulating cortisol therefore adaptively regulates the activation level of the HPA axis through a process of feedback inhibition at the hypothalamus, the pituitary, and extrahypothalamic centers such as the hippocampus and frontal cortex (Dallman, Akana, Cascio, Darlington, Jacobson, & Levin, 1987).

The LC-NE system comprises the noradrenergic cells of the medulla and dorsal pons and their projections to the amygdala, hippocampus, mesolimbic dopamine system, and the medial prefrontal cortex (Aston–Jones,

Rajkowski, Kubiak, Valentino, & Shipley, 1996). LC activation of hypothalamic centers also contributes to activation and regulation of the autonomic nervous system (ANS), initiating the so-called fight or flight responses to challenge. The ANS, comprising sympathetic, parasympathetic, and enteric branches, modulates physiologic arousal and recovery in the periphery and produces the familiar biological concomitants of severely stressful encounters, including heart rate and respiratory rate acceleration, sweat production, dry mouth, and, if sufficiently severe, loss of urinary or fecal continence. These biological responses are mediated both by direct autonomic innervation of target organs by postganglionic neurons and by secretion of epinephrine and norepinephrine by the adrenal medulla. Immune regulatory effects of the catecholamines, as well as those of CRH and the glucocorticoids, appear due to differential effects on T-helper-1/T-helper-2 cells and type 1/type 2 cytokine production (Habib, Gold, & Chrousos, 2001). Through such direct effects on immune cells, experiences of severe or prolonged stress may influence susceptibility to a variety of infectious, autoimmune/inflammatory, or neoplastic diseases (Elenkov & Chrousos, 1999). NE from the LC may also contribute to vulnerability to stress-related symptoms by facilitating emotional memory retention in the hippocampus and striatum (Introini–Collison, Dalmaz, & McGaugh, 1996).

Although anatomically distinct, the neural functioning of the CRH and LC-NE systems is highly integrated and cross-regulatory. CRH-expressing neurons in the central nucleus of the amygdala, for example, project directly to the LC, escalating the firing rate of LC neurons, enhancing NE release within the ascending noradrenergic system, and producing many of the fear-related behaviors associated with stressful experience (Meaney, 2001; Valentino, Curtis, Page, Pavcovich, & Florin–Lechner, 1998). These CRH-mediated pathways from the amygdala to the LC may also serve as the neural substrate for many of the symptoms of anxiety disorders, such as increased acoustic startle responses, vigilance, symptoms of avoidance, and recurrent emotional memories. Reciprocally, activation of NE secreting neurons in the LC has been shown to increase CRH production in the PVN (Habib et al., 2001). This cross-regulatory process is only one of several ways in which the LC-NE and CRH are functionally interactive (Gold & Chrousos, 2002; Viau, 2002), and together constitute a primary integrative pathway by which psychologically and emotionally relevant environmental signals are transmuted into the behavioral, autonomic, and immunologic manifestations of human pathology (Cacioppo, Berntson, Malarkey, Kiecolt–Glaser, Sheridan, Poehlmann, Burleson, Ernst, Hawkley, & Glaser, 1998; Heilig, Koob, Ekman, & Britton, 1994; McEwen & Stellar, 1993). Dysregulated activation has been implicated, as well, in the genesis and presentation of the major neuropsychiatric disorders (Bloom & Kupfer, 1995), and the experimental administration of neurohormonal products from the CRH and LC-NE systems produces many of the physiological and behavioral symptoms that characterize affective and anxiety disorders (Dunn & Berridge, 1990; Heilig et al., 1994).

Both stress response systems, as well as their central and peripheral components, appear early in phylogeny and have been extensively conserved in the evolutionary history of vertebrate and mammalian species. Steroid hormones are derived from cholesterol and occur widely in both animal and plant species, exhibiting chemical activities reminiscent even of the mammalian glucocorticoids (Bentley, 1998). Different forms of C_{18}, C_{19}, and C_{21} adrenal corticosteroids appear in all the major vertebrate groups, with both cortisol and corticosterone found in primitive, cartilaginous fish and aldosterone and corticosterone identified in the higher fish, amphibians, and reptiles. The glucocorticoid receptors of mammalian species are thought to have evolved from a 500 million year old ancestral gene, such as that coding for the single steroid receptor expressed in teleost fish. Such fish corticosteroid receptors exhibit a 97% homology in their DNA binding region to the amino acid sequence of the human glucocorticoid receptor. Even pituitary hormones similar to those found in the vertebrate HPA axis are present in some invertebrates, including mollusks and insects.

Catecholamine hormones of the LC-NE and ANS have a similarly long phylogeny. They are present in many invertebrates, such as the chordate *Amphioxus* arrow worm, and even in some ciliated protozoans, where they function metabolically in a manner remarkably similar to their roles in higher animal species. Like steroid receptors, catecholamine receptors are also represented in primitive species and have transmembrane domains that have been highly conserved. The complexity of the ANS appears to have increased phylogenetically with that of the CNS, attaining approximately similar levels of organization and complexity within the amniote classes of reptiles, birds, and mammals.

Taken together, these observations from the human and infrahuman neurosciences suggest the following interim observations. First, homeostatic systems protecting survival and stability under conditions of stress are phylogenetically ancient, showing both genetic expression and comparable biological functions in animal species from invertebrates to primates. Second, the CRH and LC-NE systems subserve a complex, highly interactive repertoire of central and peripheral stress responses, which mobilize neurobiological and behavioral resources in defense of the organism's integrity and well being. Third, although these neurobiological responses are protective and essential in acutely stressful conditions, they can become themselves pathogenic when persistently activated under circumstances of chronic or overwhelming stress and adversity.

Genes, Environments, and Reactive Phenotypes

Reactivity has been defined (Matthews, 1986) as "the deviation of a physiological response parameter from a comparison or control value that results from an individual's response to a discrete, environmental stimulus." Broad individual variation in reactivity to psychological stressors has been documented in human adults (Cacioppo et al., 1998), human children (Alkon, Goldstein, Smider, Essex, Kupfer, & Boyce, 2003; Allen & Matthews, 1997), and both young and mature laboratory animals (Meaney, 2001; Suomi, 1987a). Although the origins of such individual differences in reactivity, which is the central focus of the present papers, remain incompletely understood, there is wide acknowledgement that both the genome and early experience account for some share of the variance in phenotypic stress responses.

Within *rodent and other subprimate mammalian models* of reactivity developed by Meaney and colleagues (Liu & Meaney, 1997; Meaney, 2001) and others (Reis & Golanov, 1997), there is evidence that individual differences are determined by strain-related genetic variations, by aspects of early maternal–infant experience, and by interactions among gene expression and experiential factors. On the one hand, clear biobehavioral differences exist between strains of mice and rats on dimensions such as behavioral and adrenocortical reactivity to stressors. BALBc mice, for example, are inherently more fearful and show more vigorous glucocorticoid responses to stressors than do C57 mice (Zaharia, Kulczycki, Shanks, Meaney, & Anisman, 1996), and comparable differences exist between Fisher 344 and Long–Evans rats (Dhabhar, McEwen, & Spencer, 1993). Such biobehavioral differences in strains are likely due, at least in part, to heritable variation in the alleles that regulate stress responsive biological systems in these animals.

On the other hand, Meaney and others have shown that perturbations in early experience resulting in changed maternal–infant behavior can also have profound regulatory effects on the calibration of biological systems, including the CRH system and HPA axis (Hofer, 1994; Meaney, 2001; Plotsky & Meaney, 1993). An experimental procedure known as "handling," in which rodent pups are separated from their mothers for 3–15 min each day over the first several weeks of life, results in permanent downregulatory changes in the CRH system at the level of the PVN and central nucleus of the amygdala and, as a consequence, produces a decreased exposure to the adrenocortical and autonomic effects of stressful events. Such downregulatory effects have been shown to result from increased glucocorticoid receptor expression following changes in mothering behavior, that is, the intensity of licking and grooming and other characteristic maternal behaviors, upon the pups' return to the nest. Further, handling can override the genetic propen-

sities shared with a fearful, highly reactive mother by inducing maternal behaviors that produce long term underarousal in the infants' adrenocortical and autonomic response systems (Champagne & Meaney, 2001). It remains unclear at present whether the regulatory effects of these maternal behaviors are constrained to a "critical," early period of development or are capable of recalibrating response systems later in infancy or beyond.

When early maternal–infant separations are more prolonged, however, in a regimen involving true deprivation of maternal care for as long as 180 min, the effect on biological stress response systems is exactly opposite that of handling (Meaney, 2001). Separated rodent pups develop chronically upregulated CRH activity in the HPA axis, the amygdala, bed nucleus of the stria terminalis, and the LC, as well as behavioral changes consistent with fearfulness and inhibition under conditions of novelty (Sanchez, Ladd, & Plotsky, 2001). There is also evidence for effects of prenatal stress-induced maternal glucocorticoids on fetal, and later infant, physiology and development. Maternal glucocorticoid exposures are associated, for example, with elevations of CRH (Cratty, Ward, Johnson, Azzaro, & Birkle, 1995) and CRH receptors (Ward, Johnson, Salm, & Birkle, 2000) in the amygdala of offspring, and with downregulation of 11β-hydroxysteroid dehydrogenase, the placental enzyme that inactivates fetal steroidal effects (Benediktsson, Lindsay, Noble, Seckl, & Edwards, 1993). Both down- and upregulatory alterations in CRH system regulation (those produced by handling and by maternal separation, respectively) can have detrimental effects on disease and survival, depending upon the kinds of exposures that the animals later sustain. Handled animals have been shown to be more susceptible, for example, to immune-mediated disorders, such as experimental allergic encephalomyelitis (Laban, Dimitrijevic, von Hoersten, Markovic, & Jankovic, 1995), while those experiencing extended maternal separations have shown increased vulnerability to stress-related hippocampal damage and deficits in learning or memory (Issa, Rowe, Gauthier, & Meaney, 1990; Sapolsky, 1996). These studies suggest both heritable and experiential influences on the expression of stress reactivity in rodent models and reveal influences that are bivalently regulatory in their effects on psychobiological response parameters.

Studies of *nonhuman primates* have similarly contributed important evidence to an understanding of constitutional and contextual determinants of stress reactivity. Suomi and colleagues (Byrne & Suomi, 2002; Champoux & Suomi, 1994; Suomi, 1987b), for example, have increasingly documented the influence of heritable genetic factors on the neurobiological systems that underpin temperamental differences in behavior. One study comparing neurobiological differences between Indian origin and Chinese hybrid rhesus monkeys found significantly lower cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid levels (a metabolite of serotonin) in Chinese hybrid monkeys beginning at 6 months of age, suggesting "strain" differences in the magnitude of central serotonergic activity (Champoux et al., 1997). Serotonergic neurons are implicated in the neural circuitry of the stress response and in brain structures involved in the processing of emotional information (Rolls, 1999). Another study by Lyons, Yang, Sawyer–Glover, Moseley, and Schatzberg (2001) showed that stress-related hippocampal atrophy is partially heritable, raising questions regarding an attribution of hippocampal volume variation to purely experiential factors. Further, primate studies by Higley and others have demonstrated heritable variation in central serotonergic drive, which is in turn related to aspects of behavioral reactivity (Higley & Linnoila, 1997), and have identified both genetic and environmental contributions to differences in serotonin and dopamine metabolites in the cerebrospinal fluid of young macaques (Higley, Thompson, Champoux, Goldman, Hasert, Kraemer, Scanlan, Suomi, & Linnoila, 1993).

Although the above research suggests at least partial genetic regulation of stress response systems, studies of nonhuman primates have also revealed a capacity for early, stress-engendering disruptions of social experience to produce long-term changes in neurobiological reactivity (Sanchez et al., 2001).

Research on early deprivation, beginning with the seminal work of Harlow and colleagues at the University of Wisconsin Primate Laboratory (Harlow, Harlow, & Suomi, 1971), has demonstrated the centrality of maternal–infant relationships in the emergence of species normative behavior, and the capacity for isolate- or peer-rearing conditions to disrupt behavioral and psychobiological regulatory functions in several Old and New World primate species. Accompanied by behavioral changes such as protest vocalizations, autistic-like stereotypies, nonnutritive sucking, and self-mutilation, maternal separations produce predictable changes in peripheral and central neural circuitry. Among these are alterations in functional immune competence (Lubach, Coe, & Ershler, 1995), upregulation of autonomic responses to physical stressors (Martin, Sackett, Gunderson, & Goodlin–Jones, 1988), increased CRH expression in CSF (Coplan, Andrews, Rosenblum, Owens, Friedman, Gorman, & Nemeroff, 1996), and dysregulatory changes in HPA axis reactivity (Shannon, Champoux, & Suomi, 1998). As comprehensively reviewed by Sanchez et al. (2001), dysregulatory changes in the CRH system have reliably been found following exposures of infant monkeys to isolated or deprived early rearing conditions, but the character and direction of such changes remain indeterminate in the existing literature. Although some groups have shown consistent elevations in cortisol expression under conditions designed to undermine maternal attentiveness (Champoux, Hwang, Lang, & Levine, 2001; Lyons, Wang, Lindley, Levine, Kalin, & Schatzberg, 1999), others have detected no differences in HPA responses to stressors among isolation reared rhesus infants (Meyer & Bowman, 1972). In one study by Boyce, Champoux, Suomi, and Gunnar (1995), peer rearing of infant macaques appeared associated with blunted, *down*regulatory changes in the circadian periodicity of cortisol secretion. Further, the work of Sapolsky (1990; Sapolsky & Share, 1994) among wild olive baboons has revealed associations between dominance status and adrenocortical activation, suggesting either that experiences related to social adeptness and dominant hierarchical status tended to lower cortisol levels or that constitutionally less reactive individuals occupied higher status positions. Comparable to observations within rodent models of stress reactivity, studies of nonhuman primates offer further evidence for both genetic and contextual influences on the calibration of stress response systems.

Finally, a growing number of studies in *human children and adults* have similarly revealed both genomic and environmental origins for the individual differences observed in biological reactivity (Heim & Nemeroff, 1999). In parallel to genetic evidence from nonhuman primates, studies of children and their parents (Matthews, Manuck, Stoney, Rakaczky, McCann, Saab, Woodall, Block, Visintainer, & Engebretson, 1988), adults in preidentified genetic pedigrees (Cheng, Carmelli, Hunt, & Williams, 1997), as well as mono- and dizygotic twins (Bartels, de Geus, Kirschbaum, Sluyter, & Boomsma, 2003; Busjahn, Faulhaber, Viken, Rose, & Luft, 1996; Turner & Hewitt, 1992) have all affirmed a moderate heritability of reactivity phenotypes. A parental history of hypertension has frequently been shown to be predictive of autonomically mediated blood pressure reactivity in both children (e.g., Lemne, 1998) and young adults (e.g., Adler & Ditto, 1998). Elevated cortisol levels have also been identified in the nondepressed, first-degree relatives of patients with major depression, suggesting that hypercortisolism might be appropriately viewed as a trait measure of a heritable diathesis to affective disorders (Holsboer, Lauer, Schreiber, & Krieg, 1995).

In addition, the research programs of a number of investigators have produced findings supporting experiential, contextual contributions to the emergence of high reactivity. A number of studies in human children suggest, for example, that disruptions in early attachment relationships are associated with regulatory influences on and disturbances in stress–responsive biological systems (Hertsgaard, Gunnar, Erickson, & Nachmias, 1995; Meyer, Chrousos, & Gold, 2001; Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996; Willemsen–Swinkels, Bakermans–Kranenburg, Buitelaar, van, & van Engeland, 2000). Further, a prospective, longitudinal study by Es-

sex, Klein, Cho, and Kalin (2002) demonstrated that, parallel to the findings of animal research, early exposures to stressors such as maternal depression can sensitize children's CRH systems to subsequent adversities, resulting in the development of mental health symptoms. In a singular study of healthy women by Heim, Newport, Heit, Graham, Wilcox, Bonsall, Miller, and Nemeroff (2000), participants with a history of abusive experiences in childhood had dramatically increased levels of pituitary (ACTH) and autonomic reactivity to a standardized laboratory stress protocol. Abused women with current major depression exhibited sixfold greater ACTH responses than age-matched controls and were the only group to show significantly elevated cortisol responses, as well. De Bellis, Baum, Birmaher, Keshavan, Eccard, Boring, Jenkins, and Ryan (1999) similarly found increased 24-hr urinary excretion of cortisol and norepinephrine among children with abuse-related posttraumatic stress disorder (PTSD) symptoms, in comparison to healthy controls, and Perry (1994) reported diminished adrenergic receptors on platelets and increased heart rates in a group of severely abused children. A series of studies by Yehuda and coworkers (Yehuda, 2002; Yehuda, Halligan, & Bierer, 2001; Yehuda, Halligan, & Grossman, 2001) has further documented the psychobiological sequelae of early abusive experiences. Sexual abuse was associated with elevated 24-hr urinary cortisol excretion (Yehuda et al., 2001), increased density of lymphocyte glucocorticoid receptors, and enhanced suppression of plasma cortisol responses to dexamethasone (Stein, Yehuda, Koverola, & Hanna, 1997), each reflecting disturbances in the regulation of the HPA axis. In an interesting parallel to the observations in nonhuman primates mentioned above, Yehuda et al. (2001) also reported that emotional abuse and PTSD were associated with *diminished*, rather than elevated, 24-hr urinary cortisol levels. In studies of broader societal influences on the development of stress responses, Lupien, King, Meaney, and McEwen (2000) found that lower socioeconomic status was associated with higher salivary cortisol levels in children as young as 6 years of age, and Fernald and Grantham–McGregor (1998) observed higher salivary cortisol levels and greater cardiovascular reactivity among growth-stunted children growing up in impoverished neighborhoods in Jamaica.

Taken together, rodent, nonhuman primate, and human research all point to a common conclusion: that both genetic and environmental factors contribute to the calibration of biological stress response systems over the course of early development. These studies further suggest that, while stable individual differences in stress reactivity emerge with maturation, there is pronounced early plasticity in the neurobiological systems that subserve such reactivity (Davidson, Jackson, & Kalin, 2000). Stress reactivity, like many developmentally acquired phenotypic features, appears to become "canalized" over time, revealing progressively greater resistance to change and diminishing plasticity (Turkheimer & Gottesman, 1991; Waddington, 1966). Within the setting of human development, however, little is understood of the developmental time course over which such canalization occurs. Some of the papers reviewed provide evidence for a central developmental role of primary attachment relationships and maternal behavior in shaping, constraining, and regulating psychobiological responses to experiences of future challenge and adversity. Such findings suggest, although with considerable imprecision, that social contextual effects over the first 3–5 years of life may have particular potency in the calibration of stress responsive biological systems.

Stress Reactivity and Health

The CRH and LC-NE systems together constitute a primary integrative pathway by which psychologically and emotionally relevant environmental signals are transmuted into the behavioral, autonomic, and immunologic manifestations of human pathology (Cacioppo et al., 1998; Heilig et al., 1994; McEwen & Stellar, 1993). Dysregulated activation has been implicated, as well, in the genesis and presentation of the major neuropsychiatric disorders (Bloom & Kupfer, 1995), and the experimental administration of neurohormonal products from the CRH and LC-NE systems produces many of the physiological and behavioral symp-

toms that characterize affective and anxiety disorders (Dunn & Berridge, 1990; Heilig et al., 1994). In epidemiologic and observational studies of humans, individual differences in adrenocortical and autonomic reactivity have been associated with a variety of mental and physical disorders, including internalizing and externalizing psychopathology (Boyce, Quas, Alkon, Smider, Essex, & Kupfer, 2001; Kagan, 1994; Raine, Venables, & Mednick, 1997), psychological and physical symptoms (Boyce, Chesney, Alkon–Leonard, Tschann, Adams, Chesterman, Cohen, Kaiser, Folkman, & Wara, 1995; Gannon, Banks, Shelton, & Luchetta, 1989), risk for atherosclerotic heart disease (Lynch, Everson, Kaplan, Salonen, & Salonen, 1998), injuries (Boyce, 1996), and risk-taking behavior (Liang, Jemerin, Tschann, Irwin, Wara, & Boyce, 1995). As reviewed by Cacioppo, Berntson, Sheridan, and McClintock (2000), assessments of stress reactivity to challenge, in a variety of physiological modalities, enhances significantly the clinical predictions of health outcomes that are possible on the basis of resting or static measures alone.

The conventional understanding of stress reactivity is that it represents a pathogenic biobehavioral atavism: a vestige of physiological responsivity to prehistoric environments that is no longer adaptive within the intensity and challenges of modern life and, consequently, increases risk for the development of various morbidities. This view has been convincingly articulated by a generation of investigators, including René Dubos, the pioneering 20th century human biologist, and Randolph Nesse, an eminent evolutionary psychiatrist at the University of Michigan's Institute for Social Research:

> Another illustration of the fact that modern man retains essential traits of his evolutionary past is the persistence in him of hormonal and metabolic responses which were developed to meet threatening situations during his animal ancestry, but which no longer fit the needs of life in civilized societies . . . what was once an advantage is increasingly becoming a handicap under the conditions of modern human life. (Dubos, 1965, p. 29)

> Despite the amount of stress we experience, however, our ancestors almost certainly experienced more. With no police, no food reserves, no medicine, no laws, rampant infections, and prevalent predators, danger could come at any time . . . Perhaps in that environment, where stressors were more often physical, the stress response was more useful than it is now. Today, we mainly face social and mental threats, so the actions of the HPA system may yield net costs. This is plausible and supports the many efforts to reduce stress and to find drugs that block the stress response. (Nesse & Young, 2000, p. 83)

A full rendition of this widely endorsed account comprises the following twin premises: (a) because stress responses evolved in ancestral environments characterized by frequent, severe threats to survival, a unitary system of physiological arousal emerged, which readies the organism for confrontation or retreat, often in a manner disproportionate to the actual hazards encountered; and (b) prolonged activation or acute overactivation of such pathways ultimately undermines the health of organisms by impairing, rather than activating, the function of target organs. Although the essential, protective aspects of these neurobiological responses to adversity are broadly acknowledged, it has become an article of faith that overreactivity promotes the genesis of disease. Compelling and intuitive as the two premises have appeared, both are now challenged by evidence suggesting that stress reactivity is not a unitary physiological process, because adversity often results in down- rather than upregulatory changes in component neural circuits, and that high reactivity exerts bidirectional, rather than univalent, influences on health. It is to this evidence and these findings that the discussion now turns.

Anomalous findings on stress reactivity and health

The *first premise*, that stress reactivity constitutes a unitary, unidirectional set of biological responses to threat, has been contested by a collection of observations and new hypotheses suggesting that the components of the stress response system often act in opposition to, rather than in alliance with, each other. More than 40 years ago, Lacey (1959) criti-

cized the concept of uniform arousal, arguing that, even within the ANS, different neural components pursue different profiles of response. His observations were followed by those of other investigators such as Ekman, Levenson, and Friesen (1983), who maintained that no general state of "arousal" exists, and that specific emotional experiences are linked to specific constellations of ANS and central neural activity. In 1984, Munck, Guyre, and Holbrook advanced a new and then counterintuitive proposal. They hypothesized that glucocorticoids and the HPA axis, rather than constituting the hormonal sine qua non of stress-induced arousal, as had been widely believed since the inception of stress research, function instead as a buffering or counterregulatory influence, essentially "braking" the duration or intensity of an otherwise overly exuberant physiological response. It may thus be the more slowly activated HPA axis that is responsible for the termination or tempering of the immediate, autonomically mediated fight or flight state, by the glucocorticoids' physiological opposition to the effects of adrenergic arousal. Such a view would resolve, they argued, the paradoxical observation that diseases with known associations to psychological stressors, such as rheumatoid arthritis or inflammatory bowel disease, can actually be treated with glucocorticoids. Such sequences of paired, counterregulatory processes are common in biological systems, such as the neuronal action potential and the clotting cascade, in which the same stimuli that activate initial responses (e.g., the opening of sodium channel gates or the cleaving of fibrinogen into fibrin) also activate a delayed suppressor (e.g., the closing of sodium channels or the activation of plasminogen to plasmin to lyse clots), which is required to restore a homeostatic state.

Munck's proposal is, in fact, consistent with a number of empirical observations that have been reported since. Most recently, for example, Bauer (2002) found, in a cross-sectional study, that the absolute levels of activation in either the sympathetic or adrenocortical system were less predictive of serious behavior problems in 4- to 8-year-old children than was the lack of concordance between the systems. Children with symmetrical activation or no activation at all had the fewest behavior problems, whereas children with activation asymmetries had the most, suggesting that dissociations between the sympathetic and adrenocortical arousal under conditions of challenge put children at risk for early psychopathology, a conclusion commensurate with Munck's hypothesis. Similarly, a review paper by Yehuda, McFarlane, and Shalev (1998) concluded that, among adult patients following an acutely stressful event, the combination of low cortisol levels and high heart rates, indicating a disjunction between adrenocortical and sympathetic responses, was most predictive of later PTSD symptoms. The findings of both groups are also consistent with more recent observations that catecholamines and glucocorticoids interact in a variety of complex ways, involving conjoint (but sometimes counterregulatory) effects on such functions as lymphoproliferative responses, appetite, and memory (e.g., Sapolsky, Romero, & Munck, 2000).

Another collection of findings, recently summarized by Gunnar and Vazquez (2001) and Heim, Ehlert, and Hellhammer (2000), also challenges the first premise with evidence of paradoxical *suppression* of HPA activation under conditions of stress. Such "hypocortisolism," that is, lower basal cortisol levels, less HPA reactivity, or a flattening of the circadian cortisol cycle among higher risk samples, has been noted in both animal and human research, by multiple investigators, and in a variety of research settings. In the previously noted study by Boyce, Champoux, et al. (1995), for example, peer rearing of infant macaques was associated with blunted, downregulatory changes in the circadian periodicity of cortisol secretion. Similarly, although Gunnar, Morison, Chisholm, and Schuder (2001) found persistent elevations in salivary cortisol levels among children adopted *out* of Romanian orphanages, Carlson and Earls (1997) found low morning cortisol levels and an absence of the normal circadian decline in cortisol among children continuing to live *inside of* Romanian institutions. Infants with colic (White, Gunnar, Larson, Donzella, & Barr, 2000), children with psychosocial dwarfism (Vazquez, Watson, & Lopez, 2000), and chil-

dren living near the epicenter of a major earthquake (Goenjian, Yehuda, Pynoos, Steinberg, Tashjian, Yang, Najarian, & Fairbanks, 1996) have all shown lower morning cortisol levels and a flattening of the normal circadian cycle, relative to control children without such conditions or experiences. Children characterized as shy or introverted similarly showed diminished cortisol reactivity to normative stressors such as the beginning of a new school year (Davis, Donzella, Krueger, & Gunnar, 1999; de Haan, Gunnar, Tout, Hart, & Stansbury, 1998). These findings with regard to the HPA system are notably similar to those of two other studies in which stressful life events were found inversely related to cardiovascular (rather than adrenocortical) reactivity in children or youth (Chesterman, Boyce, & Winkleby, 1989; Musante, Treiber, Kapuku, Moore, Davis, & Strong, 2000). In an interesting parallel to such observations in children, Heim, Ehrlert, et al. (2000) reviewed evidence for associations between hypocortisolism and stress-related disorders in adults and similarly concluded that low cortisol patterns are sometimes associated with experiences of stress or adversity or with stress-related disorders. Yehuda et al. (2001) reported, for example, that among adult children of Holocaust survivors those with a self-reported history of childhood trauma showed diminished, rather than elevated, 24-hr urinary cortisol levels, relative to a comparison group with no history of trauma.

In summary, the often tacit, but largely conventional, impression that stress reactivity represents a singular, unitary biological response to adversity is weakened by two categories of new evidence. First, recent findings suggest that components of the stress response system may act in a coordinated but counterregulatory manner, as proposed by Munck and colleagues (1989), some operating to dampen, rather than magnify, the physiological effects of others. Second, traumatic events and severe stressors may be associated, in both children and adults, with a downregulated HPA axis, a diminution in circadian cortisol secretion, and a reduction in cardiovascular reactivity.

The *second premise*, that exaggerated or persistent reactivity is univalently associated with stress-related morbidities, has also been questioned in a growing number of studies revealing that high reactivity phenotypes under specific environmental conditions may be associated with protective, rather than harmful, effects and generate normative or improved health outcomes. Such bivalent effects of stress reactivity on human and primate morbidities have thematically characterized a series of studies reported by Boyce and colleagues over the past decade. Examining cardiovascular and immunologic reactivity in two cohorts of 3- to 5-year-old children, for example, significant interactions (see, e.g., Figure 1A) were detected with environmental stressors in the prediction of respiratory illness incidence over the ensuing several month periods (Boyce, Chesney, et al., 1995). Specifically, the noted interactions suggested bidirectional effects of reactivity on illness incidence: highly reactive children in high-stress families or childcare centers sustained significantly higher rates of respiratory illness than their low reactive peers, but equally reactive children in low-stress settings were the healthiest of all children in the samples. By contrast, the respiratory illness incidence of low reactivity children was unresponsive to environmental stress levels, showing approximately the same, midlevel illness rates in both low- and high-stress conditions. Similarly significant interactions were found for injury incidence (Boyce, 1996).

Although prospective in design, both of these studies were observational in nature and lacked experimental data on the incidence of illnesses or injuries among the same group of highly reactive children in both low- and high-stress conditions. In a subsequent study of semifree-ranging rhesus macaques, however, such quasiexperimental conditions were satisfied (Boyce, O'Neill–Wagner, Price, Haines, & Suomi, 1998). The troop of macaques, which had been previously assessed for their degree of biobehavioral reactivity to novel or challenging stimuli, lived in a 5-acre wooded habitat in rural Maryland, on the grounds of the National Institutes of Health Primate Center. In 1993, the troop encountered a 6-month period of protective confinement to a small, 1000-square foot building, during a construction

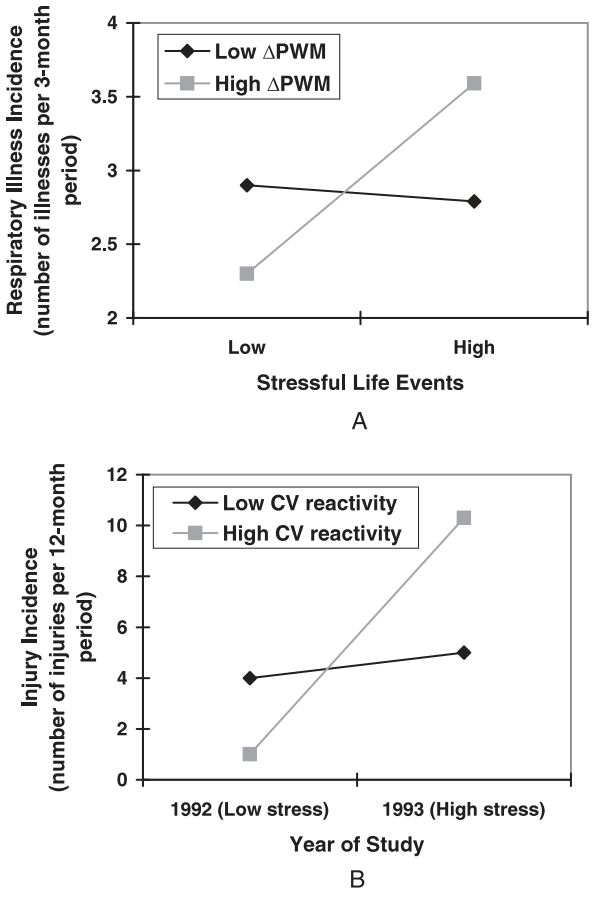

Figure 1. The interactions among laboratory-based stress reactivity and environmental stressors in predicting health outcomes. (A) Immune reactivity (changes in pokeweed nitrogen response) × family stressful events and respiratory illness incidence in kindergartners (N = 99; adapted from Boyce, Chesney, et al., 1995). (B) Biobehavioral reactivity × confinement stress and injury incidence in a troop of semi-free-ranging rhesus monkeys (N = 36; adapted from Boyce et al., 1998).

project on the habitat grounds. The confinement proved highly stressful, however, and the incidence of violent injuries increased fivefold during the 6-month period. Blinded ascertainment of medically attended injury rates from veterinary records produced evidence for a significant interaction between reactivity status and confinement stress, which is plotted in Figure 1B. As with the prior studies of children, low reactivity individuals showed little effect of the confinement, while those with high reactivity showed dramatically higher rates of violent injuries in the high-stress situation but lower rates in the preceding, low-stress condition.

These findings documenting Reactivity × Context interactions in the prediction of biomedical outcomes have been supplemented by recent observations from the same group of investigators and several others on associations among stressors, reactivity and *psychological* symptoms in children and young adults. There is reason to believe that the influence of biological reactivity on mental health outcomes may be even more profound than those observed for biomedical disorders. Worthman, Angold, and Costello (1998), utilizing data from the Great Smoky Mountains Study, found associations in Appalachian children between high adrenocortical reactivity and future diagnoses of anxiety disorders and between low reactivity and diagnoses of conduct disorder. In other work examining cross-sectional data from the Wisconsin Study of Families

and Work, main effects of autonomic reactivity on risk for both internalizing and externalizing spectrum psychopathology in middle childhood have been reported (Boyce et al., 2001). Children with high levels of parasympathetic reactivity to laboratory stressors were significantly more likely to fall into the top 20% on mother and teacher reports of internalizing symptoms, while those with low reactivity in both sympathetic and parasympathetic branches of the ANS were significantly more likely to display high rates of externalizing behavior problems (see also Scarpa & Raine, 1997). A third paper by Gannon et al. (1989), reporting on a cross-sectional study of college students, found that participants with laboratory evidence of exaggerated autonomic reactivity showed higher rates of physical symptoms and depression under stressful circumstances, but lower than average rates under low or minimally stressful conditions. Finally, a recent randomized experimental study by Quas, Bauer, and Boyce (2004) again showed an interaction effect between autonomic reactivity and social context, with highly reactive children showing significantly better memory for a previous, standardized stressful event in a supportive social environment and poorer memory under conditions of low support, relative to a low reactivity comparison group. Although not all research examining Reactivity $\times$ Context interactions have replicated these findings (see, e.g., Musante et al., 2000), a sufficiently substantial number of studies have produced homologous results to suggest a robust phenomenon worthy of further and more explicit analysis.

Recent findings from a variety of investigators and settings thus call into question the second of the premises that circumscribe the prevailing conceptualization of stress reactivity. Rather than acting as a unidirectional risk factor for poor health outcomes, as the second premise would assert, high-stress reactivity has been shown repeatedly to operate in a *bivalent* manner, most often escalating the risk of maladaptive outcomes in high-stress contexts, but diminishing such risk and acting protectively under supportive, low-stress conditions. Such evidence signifies the need for a reconceptualization of high reactivity phenotypes and suggests that highly reactive individuals may be more accurately described as biologically sensitive to the health-eroding or health-sustaining effects of particular social and physical contexts. *BSC* is therefore advanced here as a means of denoting and characterizing this bidirectional influence of high-stress reactivity on psychiatric and biomedical health endpoints. As argued above, stress reactivity is a nonunitary, multifaceted complex of central and peripheral neural responses. The present thesis, however, is that heightened reactivity in each stress response system is reflective of a more elemental biological predisposition to context responsiveness. In the remainder of this paper, we therefore refer to BSC as a phenotypic property of individuals, which has both constitutional and experiential origins, and is indexed by heightened reactivity in one or more of the stress response systems.

BSC: The Dandelion and the Orchid

Such evidence for bivalent, context-dependent health effects of highly reactive phenotypes suggests that reactivity may reflect not simply overarousal of neurobiological pathways, but rather *sensitivity to both harmful and protective contextual effects*. Highly reactive children appear to experience either the best or the worst of psychiatric and biomedical outcomes, within the populations from which they are drawn. Under conditions of adversity, such children sustain higher rates of disease, disorder, and injuries than their more normatively reactive peers from the same environments. On the other hand, equally reactive children in low-stress, protective social environments experience substantially lower rates of health problems than their low reactive peers. These results suggest that the highly reactive biological profiles found in this subset of children reveal a unique sensitivity or "permeability" to the influence of environmental conditions (Boyce, Chesney, et al., 1995).

A Swedish idiomatic expression, *maskrosbarn* (dandelion child), refers to the capacity of some children, not unlike those with *low* reactive phenotypes, to survive and even thrive in whatever circumstances they encounter, in much the same way that dandelions seem to

prosper irrespective of soil, sun, drought, or rain. Observations of such children have generated, for example, an extensive developmental literature on the phenomenon of resilience, the capacity for positive adaptation despite experiences of significant adversity (Luthar, Doernberger, & Zigler, 1993; Masten, 2001). A contrasting Swedish neologism, *orkidebarn* (orchid child), might better describe the context-sensitive individual, whose survival and flourishing is intimately tied, like that of the orchid, to the nurturant or neglectful character of the ambient environment. In conditions of neglect, the orchid promptly declines, while in conditions of support and nurture, it is a flower of unusual delicacy and beauty.

This metaphorical invocation of children with contrasting environmental sensitivities is reminiscent of Belsky's (1997, 2000) theory of individual differences in susceptibility to rearing influence. Belsky has proposed that some individuals have traits and developmental trajectories that are more fixed by their genetic endowment, while others are more plastic and susceptible to rearing influence. Employing an evolutionary framework, he suggests that parents have been selected to "hedge their bets" against an uncertain future by producing both types of offspring: more fixed types capable of achieving higher reproductive success in the particular ecological niche that matches their genotype, and more plastic types capable of fitting and thriving in a wider range of niches, depending upon rearing conditions encountered during ontogeny. The latter, more malleable individuals are hypothesized to monitor features of early childhood environments and to adjust biobehavioral development accordingly. As summarized by Belsky (2000, 2005), it is infants who are high in negative emotional reactivity who appear most susceptible to early rearing influences.

Although Belsky and the theoretical framework presented here both equate heightened reactivity with susceptibility to environmental influence, and both theories posit that reactivity moderates relations between the quality of early family environments and salient developmental outcomes, the theories differ in their definitions of reactivity and their conceptualizations of its origins and consequences. First, the current theory defines BSC as heightened reactivity in one or more of the neurobiological stress response systems, whereas Belsky operationalizes reactivity at the behavioral level. Comparisons between biological and behavioral reactivity have yielded inconsistent results (Kagan, 1994; Quas, Hong, Alkon, & Boyce, 2000), and are in need of further clarification and study. Second, the current theory specifies a conditional adaptation model of the developmental origins of BSC, emphasizing gene–environment interactions. Belsky, by contrast, focuses on heritable variation in susceptibility to rearing influence and does not specify environmental antecedents of this variation. Third, the current theory conceptualizes highly reactive phenotypes as *orchidebarnen*, which are pointedly not adaptable to a broad range of rearing milieus, but tend to do especially well in conditions of high social resources and support. Belsky, by contrast, conceptualizes emotionally reactive infants as more developmentally malleable and capable of entraining biobehavioral development to fit a relatively wide range of niches.

A substantial body of other work provides a broad but reasonably consistent picture of behavioral predispositions found among context-sensitive, biologically reactive orchidebarnen. The research of Kagan and colleagues, for example, has documented the tendency for behaviorally inhibited, shy children to share particular psychobiological features reflecting exaggerated activation of both peripheral and central stress response circuitries (Kagan, 1994, 1997; Snidman, Kagan, Riordan, & Shannon, 1995). In cohorts of infants and children followed by Kagan and others, those displaying shy or fearful behaviors in social or novel situations were significantly more likely to have low heart periods (and thus high heart rates), diminished heart period variability, and greater pupillary dilatation, reflecting sympathetic activation and parasympathetic withdrawal, as well as higher baseline and reactive salivary cortisol levels, indicating heightened adrenocortical activation (Kagan, Reznick, & Snidman, 1987, 1988; Reznick, Kagan, Snidman, Gersten, Baak, & Rosenberg, 1986). Other studies (Calkins, Fox, & Marshall, 1996; Fox, Rubin, Calkins, Marshall,

Coplan, Porges, Long, & Stewart, 1995; Schmidt, Fox, Schulkin, & Gold, 1999) have similarly revealed associations between behavioral inhibition and high, stable heart rates, elevated measures of cortisol secretion, and increased acoustic startle responses. In addition, in a series of studies by Fox and colleagues (Fox, 1991; Fox et al., 1995; Fox, Henderson, Rubin, Calkins, & Schmidt, 2001), a pattern of asymmetrical, right frontal EEG activation was described among shy children, possibly signaling individual differences in prefrontal regulation of amygdalar and limbic fear circuitry (Davidson & Irwin, 1999).

Further, associations noted between behavioral inhibition and biological reactivity have not been limited to human children; the research of Suomi and colleagues at the NIH Laboratory of Comparative Ethology has produced systematic evidence of upregulated adrenocortical and autonomic responses to challenge among the fearful, inhibited subsets of several primate species (Byrne & Suomi, 2002; Suomi, 1997). Although associations between temperamental differences in inhibition and aspects of central and peripheral stress reactivity have been frequently documented, negative results have also been reported (Asendorpf & Meier, 1993; Calkins & Fox, 1992), and there have been suggestions that other developmental factors, such as mother–child attachment, could play moderating roles in such relations. Stevenson–Hinde and Marshall (1999), for example, found the predicted association between low inhibition and high vagal (or parasympathetic) tone only among securely attached children, and Nachmias et al. (1996) reported adrenocortical reactivity in novel coping conditions only for toddlers with both behavioral inhibition and insecure attachment. As reviewed by Fox and Card (1999) and Carter (1998), studies examining attachment, mother–child behavior, and psychophysiological processes have met with varying and sometimes contradictory results. Given the known neuroendocrine correlates of social bonding behavior in lower mammals, however, such relations remain a promising and understudied area of investigation. More generally, the findings noted above indicate associations of moderate magnitude between BSC and behavioral inhibition and suggest the possibility that the character of parent–child relationships could constrain or modify the emergence of high reactivity–high inhibition phenotypes.

Another literature closely related to the construct of context sensitivity, whether by analogy or, more directly, by its status as a potential neurobiological substrate, is a body of work addressing neurosensory "gating." Operationalized as attenuations in either the component amplitudes within auditory event-related potentials or in the capacity for prepulse inhibition,[1] sensory gating is a complex, multifaceted neural function thought to protect higher cortical centers from being flooded with incoming sensory stimuli (Boutros, Torello, Barker, Tueting, Wu, & Nasrallah, 1995), and is thus one candidate modality by which "BSC" might be instantiated in the brain circuitry that could plausibly subserve such sensitivity. Deficits in gating the P50 wave component following paired auditory signals have been found among patients with PTSD (Neylan et al., 1999) and schizophrenia (Braff & Geyer, 1990), and in their first-degree relatives (Waldo, Myles–Worsley, Madison, Byerley, & Freedman, 1995), and similar deficits in prepulse inhibition have been identified among boys with comorbid Tourette syndrome and attention-deficit/hyperactivity disorder (Castellanos, Fine, Kaysen, Marsh, Rapoport, & Hallett, 1996). A variety of brain regions have been implicated in the filtering of incoming sensory information, including the temporal cortex (Boutros et al., 1995), prefrontal cortex (Shimamura, 2000) and thalamus (McCormick & Bal, 1994). Potential linkages between sensory gating deficits and dysregulation in stress response systems are found in observations that glucocorticoids (Stevens, Bullock, & Collins, 2001) and catecholamines (Adler, Pang, Gerhardt, & Rose, 1988) are capable of disrupting gating functions, that impairments in gating are found among clinical populations with stress-related disorders (Neylan et al., 1999), and that laboratory protocols used for the induction of car-

1. Prepulse inhibition is the inhibition of an acoustic startle response by a weaker stimulus presented just before the auditory probe.

diovascular reactivity, such as the cold pressor test, also diminish auditory gating (Johnson & Adler, 1993). BSC, evoked and measured as autonomic or adrenocortical reactivity to challenges in laboratory paradigms, thus appears to have analogous expressions in neural pathways involved in sensory gating.

Personality and BSC

Individual differences relevant to BSC have also received considerable attention from personality researchers interested in the neurobiological bases of personality. Of particular relevance is the personality construct of *reactivity* (Strelau, 1983), which indexes relatively stable individual differences in the intensity (or magnitude) of response to stimulation. Higher reactivity indicates less gating of internal information stemming from external events, and more reactive individuals are therefore susceptible to relatively weak environmental signals, have comparatively low optimal levels of arousal, and are less able than others to endure strong stimulation for prolonged periods of time (Kohn, 1991; Strelau, 1983). Variation in reactivity is, moreover, a common, underlying element of many biologically oriented personality traits. Specifically, high "reactivity" individuals, "introverts," "augmenters," and "sensation avoiders" all tend to avoid situations and activities that involve strong stimulation and arousal, whereas low "reactivity" individuals, "extraverts," "reducers," and "sensation seekers" are all predisposed to pursue such situations and activities (Strelau & Eysenck, 1987). For example, individuals on the reactive end of these personality traits tend to seek out quieter study rooms in libraries (Campbell & Hawley, 1982) and reside in less stimulating suburban neighborhoods (Strelau, 1983); set volume levels lower when listening to music (Davis, Cowles, & Kohn, 1984; Kohn, Hunt, Cowles, & Davis, 1986) or performing a learning task (Geen, 1984); choose hobbies and professions that involve relatively low levels of stimulation (Strelau, 1983; Zuckerman, 1984); and display lower tolerance for pain and discomfort (Kohn, 1991).

Paralleling BSC, a central feature of Strelau's concept of reactivity is susceptibility to environmental influence. Compared with lower reactivity individuals, those with higher reactivity profiles are more adversely affected by environmental stressors and distractions when performing learning and decision-making tasks (Eliasz, 1987; Klonowicz, 1987; Strelau, 1983) and appear more susceptible to social pressure in conformity experiments (Eliasz, 1987). Moreover, unless intense or prolonged stimuli are used, highly reactive people tend to develop conditioned responses more easily and quickly than do their peers with less reactive profiles (Strelau, 1983).

Aron and Aron (1997, p. 362) provide an important further elucidation of the reactivity construct in their discussion of sensory-processing sensitivity and suggest that "there is an underlying differentiating characteristic regarding how some individuals process stimuli, involving a greater sensory-processing sensitivity, reflectivity, and arousability." Anticipated by early investigators noting exceptional sensitivities in young children (Bergman & Escalona, 1949), Aron and Aron posit that individuals high in sensory-processing sensitivity tend to be more attentive, discriminating, and reflective, especially as the complexity of incoming stimuli increases. Linked to both conscientiousness and low impulsivity, sensory-processing sensitivity may function as a "pause and check" system that results in temporary inhibition of activity (Aron & Aron, 1997) and increases susceptibility to environmental influence. This increased susceptibility has been suggested by a series of retrospective studies showing that, for at least men, sensory-processing sensitivity moderates the relation between the quality of early family environments and childhood adjustment (Aron & Aron, 1997).

Complementing these retrospective studies, a number of prospective studies have now documented a similar moderating role for context sensitivity (as indexed by negative emotional reactivity in infants) in associations between family environment and child behavioral outcomes (reviewed in Belsky, 2000, 2005). Specifically, links between quality of parenting and indices of child adjustment have been found to be reliably stronger among emotionally reactive infants. For example, Kochan-

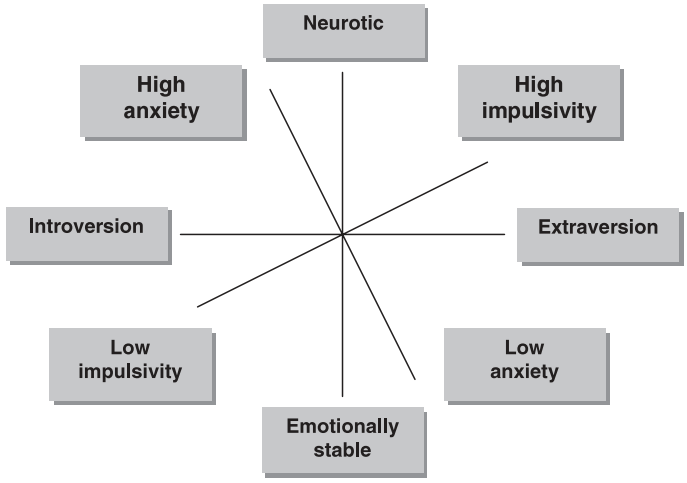

Figure 2. The relation between Eysenck's dimensions of extraversion and neuroticism and Gray's dimensions of impulsivity and anxiety.

ska (1993, 1997) has found much larger effects of maternal discipline (e.g., reliance on gentle guidance vs. forceful control) on development of self-control among infants and toddlers who are high in fearfulness and negative emotionality than among those who are low on these traits (see also Feldman, Greenbaum, & Yirmiya, 1999). Similar moderating effects of infant negative emotionality have also been documented in the relations between quality of parenting and the development of both externalizing and internalizing behavior problems (Belsky, Hsieh, & Crnic, 1998; Blair, 2002; Deater–Decker & Dodge, 1997). Belsky (1997, 2005) has argued convincingly that these interactions provide evidence of greater susceptibility to rearing influence among temperamentally reactive children.

High susceptibility to environmental influence implies that the personality development of reactive children will be especially context dependent, and the multidimensional systems of personality advanced by Eysenck (e.g., 1967; Eysenck & Eysenck, 1985) and Gray (e.g., 1982, 1990) provide a framework for conceptualizing this context dependency (see Figure 2). Eysenck specifies introversion versus extraversion and neuroticism versus emotional stability as major dimensions of personality and locates them at right angles to each other in two-dimensional space. Gray, by contrast, specifies anxiety and impulsivity as major dimensions of personality. Although Gray also locates these dimensions at right angles from each other, he rotates them approximately 30 (conceptual) degrees away from Eysenck's introversion and neuroticism dimensions (see Figure 2). Thus, in Gray's system, individuals who are both neurotic and somewhat introverted are regarded as most anxious, whereas individuals who are both introverted and fairly emotionally stable are viewed as least impulsive. Given that introversion is associated with greater biological reactivity to moderate levels of stimulation (Bullock & Gilliland, 1993; Taub, 1998), and should thus reflect sensitivity to context, the process through which temperamental differences in introversion in children (often labeled shyness or inhibition) develop into stable individual differences in personality should be especially sensitive to the quality of family environments. Under conditions of family stress, characterized by harsh, insensitive, and/or inconsistent parenting, the position of relatively shy, inhibited children (high introversion in Figure 2) may be developmentally rotated to the right toward high anxiety; conversely, under conditions of family support characterized by sensitive and responsive parenting, the position of such children may be developmentally rotated to the left toward low impulsivity (see Figure 2).

This theoretical position is supported by cluster analyses conducted by Aron and Aron (1997) on individuals high in sensory-processing sensitivity. In analysis of three diverse samples, two distinct clusters of highly sensitive people consistently emerged. One group had more troubled childhoods and scored relatively high on measures of social introversion and fearfulness/anxiety. The other group was more similar in childhood adjustment and personality to those who were not highly sensitive. Aron and Aron, who conceptualize sensory-processing sensitivity as a basic dimension of temperament, suggest that the developmental implications of this dimension depend on environmental factors. Based on their interviews with prototypically high sensitivity people, Aron and Aron (1997, p. 363) conclude: "Sensitive individuals from home environments that support their temperament seem quite successful in their lives and adept at making their sensitivity an asset while avoiding shyness or over-self-consciousness."

An Evolutionary–Developmental Theory of BSC

The framework constructed above provides the theoretical groundwork for an alternative explanatory account of the ontogeny and functions of BSC, within an evolutionary model. As reviewed above, there are enduring individual differences in BSC; such differences emerge from the coaction of genetic and environmental influences; different BSC phenotypes yield different costs and benefits in different childhood environments; and individual differences in BSC are hypothesized to constitute variations in susceptibility to environmental influence.

What are the evolutionary origins of such individual differences in BSC? One possibility is that this variation is simply random (i.e., evolutionary noise), much as differences between people in the length of their toes is random, owing to selection-irrelevant genetic variation, the random effects of sexual recombination, and nonadaptive phenotypic plasticity in response to experience. Such variation could still be heritable, and somewhat predictable in response to environmental factors, but would not be the product of natural selection and would have had little bearing on fitness in ancestral environments.

Another possibility is that variation in BSC is adaptively patterned. If this were the case, then different levels of BSC should produce mean differences in survival and reproductive outcomes when all individuals are constrained to a single environment, but similar survival and reproductive outcomes when different BSC phenotypes are allowed to covary with salient features of the environment (i.e., when individuals with different reactivity profiles can employ strategies and inhabit niches that are matched to those profiles; see Mealey, 2001). As cited above, a quasiexperimental study of the effects of stress reactivity under varying environmental conditions in rhesus monkeys suggested that individual differences in stress reactivity may meet these criteria. Specifically, during a 6-month confinement period, when behavioral strategies available to troop members were severely curtailed, highly reactive monkeys suffered dramatically higher rates of violent injuries than did their less reactive peers (Figure 1B). In the free-ranging wooded habitat, however, where a wide range of behavioral strategies could be employed, including escape from conflict, highly reactive monkeys suffered comparatively low rates of violent injury.

The claim advanced here is that variation in stress reactivity has been produced and maintained by natural selection, because differences in BSC reliably produced different fitness outcomes in different childhood environments encountered over evolutionary history. This functional model, which conceptualizes individual differences in BSC as underlying variation in susceptibility to features of the social environment, both positive and negative, can be described by the following three interrelated propositions:

1. As suggested by both the human and animal literature, individuals who experience traumatic, *high-stress environments* in early childhood tend to develop exaggerated stress reactivity profiles. High-stress reactivity in this context may function to increase the overall capacity and readiness of individuals to deal with the very real

dangers in their environment, even when such a strategy results in chronic overarousal and associated sequelae. Gunnar (1994) has suggested, for example, that higher levels of both sympathetic and parasympathetic reactivity in inhibited children cause lower thresholds for anticipating threat in new, unfamiliar situations and support greater vigilance and wariness.

2. In addition to increasing awareness of and sensitivity to threat, the stress response systems may also enable children to experience and absorb more fully the beneficial, protective features of supportive, predictable environments. Reactive, sensitive children have been found, for example, to be more reflective and perhaps more conscious of self and environment (Aron & Aron, 1997; Kagan, Snidman, Zentner, & Peterson, 1999; Lewis & Ramsay, 1997; Patterson & Newman, 1993). Biologically reactive phenotypes should thus also enable children to flourish under stable and nurturant *low-stress environments* and ecological conditions, where they may particularly benefit from high levels of parental investment.
3. The third, complementary assertion is that *low* reactive phenotypes may have also been favored by natural selection, because they enabled children to cope more effectively within the highly prevalent, *moderate stress environments* encompassing the broad, normative range of familial and ecological stressors. Low biological stress reactivity may have served a protective function for children in these environments, by way of increased gating of the emotional signals from chronic stressors, which allowed greater resilience under difficult conditions. These benefits of low reactivity, however, should be specific to conditions of chronic, moderate level stress and threat, and could pose liabilities under developmental conditions characterized by either very high or very low stress (where heavy gating could interfere with responsiveness to the environment).

Thus, phenotypic variation in biological stress reactivity may be adaptively patterned; that is, it may increase the capacity and tendency of different individuals to respond adaptively to specific types of early childhood environments.

This post hoc explanation of observed phenotypic variation in stress reactivity, however, does not meet minimum standards of evolutionary epistemology (Ketelaar & Ellis, 2000). As Lakatos (1970, 1978) has shown, it is relatively easy to construct new explanations or to tinker with old ones to accommodate what is already known. Indeed, there are few empirical findings in psychology and medicine that, after the fact, could not be claimed by multiple theories as falling within their explanatory purview. A good evolutionary explanation must therefore not only account for known facts, but also "stick its neck out" by predicting experimental results that are not known in advance (Ellis & Ketelaar, 2000; Ketelaar & Ellis, 2000). In the final sections of this paper, we present an evolutionary theory of the developmental origins of BSC in children, which is based on the concept of "conditional adaptation," and which generates a novel hypothesis about environmental sources of variation in stress reactivity. This hypothesis is then explored empirically in the second paper of this sequence.

The concept of conditional adaptation

Over the last 2 decades, theory and research in evolutionary biology has begun to acknowledge that, in most species, single "best" strategies for survival and reproduction are unlikely to evolve (Gangestad & Simpson, 2000; Gross, 1996). This is because the best strategy varies as a function of the physical, economic, and social parameters of one's environment (Crawford & Anderson, 1989), and thus a strategy that promotes success in some environmental contexts may lead to failure in others. Selection pressures therefore tend to favor adaptive *phenotypic plasticity*, the capacity of a single genotype to produce a range of phenotypes (manifested in morphology, physiology, and/or behavior) in response to particular ecological conditions that recurrently influenced fitness during a species' evolutionary history (Belsky, Steinberg, & Draper, 1991; Chisholm, 1999; Hrdy, 1999).

Importantly, the development of alternative phenotypes is a nonrandom process; that is, it is the outcome of a structured transaction between genes and environment that was shaped by natural selection to increase the capacity and tendency of individuals to track their developmental environments and adjust their phenotypes accordingly.

Phenotypic plasticity is necessarily a constrained process. Although it would seem advantageous for individuals to respond to environmental changes quickly, appropriately, and flexibly throughout their lives, high levels of responsiveness are not always either possible or desirable. Instead, for many phenotypic characteristics, individuals have been selected to register particular features of their childhood environments as a basis for entraining relevant developmental pathways early in life. There are several reasons to expect early entrainment. First, many complex adaptations are "built" during development and cannot be easily rebuilt when environments fluctuate. For example, the neural and hormonal pathways underlying the stress response systems are set down and calibrated during the early years of development, when the plasticity of neural circuits and structures is at its zenith (Davidson et al., 2000). Second, prolonged practice and attention is required for the successful execution of many behavioral strategies (Draper & Harpending, 1982). For example, individuals who pursue an early and stable life strategy of predatory social interactions (i.e., primary sociopathy) are generally better at executing social deception than individuals who contingently adopt this life strategy at a later age (i.e., secondary sociopathy; see Mealey, 1995). Third, extreme malleability of personality in response to environmental circumstances is not plausible, because different personality systems compete and interfere with each other. For example, high levels of the Big Five Factor Agreeableness, which underlies variation in the extent to which individuals seek out and enjoy intimate, committed relationships (MacDonald, 1995), would surely interfere with sociopathy. Indeed, the social emotions characteristic of individuals high in Agreeableness (e.g., love, compassion, empathy) are essentially absent in sociopaths (Mealey, 1995).

Although the developmental pathways underlying many phenotypic characteristics are likely to be entrained early in life, a capacity for responding to immediate contingencies is also important. As Richard Alexander notes: "It would be the worst of all strategies to enter the competition and cooperativeness of social life, in which others are prepared to alter their responses, with only pre-programmed behaviors" (cited in Mealey, 2000, p. 59). Selection should therefore favor a hierarchy of mechanisms for tracking and responding to environmental information (Slobodkin & Rapoport, 1974; but see also Chisholm, 1999). At the top of this hierarchy are psychological mechanisms underlying general and social intelligence. These mechanisms enable quick and flexible responses to changing opportunities and threats in the immediate environment. Lower in the hierarchy are anatomical, physiological, endocrine, and developmental mechanisms, which track slower and more pervasive changes in the environment. These mechanisms often take the form of *conditional adaptations*: that is, evolved mechanisms that detect and respond to specific features of childhood environments, features that have proven reliable over evolutionary time in predicting the nature of the social and physical world into which children will mature, and entrain developmental pathways that reliably matched those features during a species' natural selective history. Conditional adaptations, which reflect systematic gene–environment interactions, underpin development of contingent survival and reproductive strategies and thus enable individuals to function competently in a variety of different environments.

Illustrations of conditional adaptation

There are myriad examples of conditional adaptation found in the natural world. Although the best examples are found in plants, insects, and fish, there are good mammalian examples, as well, and emerging evidence in humans.

Environmentally triggered polymorphism in caterpillars. The caterpillar *Nemoria arizonaria* develops almost completely different morphologies depending upon its diet in the first 3 days of life (Greene, 1989, 1996). These

caterpillars inhabit oak woodlands in the American Southwest and produce both spring and summer broods. Although the two broods have the same appearance when they first hatch, the spring brood feeds on oak catkins and develops the appearance of the oak's drooping flowers, whereas the summer brood feeds on oak leaves and develops the appearance of twigs. The flower morphology enables the spring brood to blend into the environment while feeding on the ubiquitous spring catkins. Likewise, the twig morph provides camouflage for the leaf-eating summer brood. If either the spring or summer broods are experimentally fed out-of-season food, they develop the corresponding out-of-season morphology and become highly vulnerable to predation. These caterpillars have therefore evolved physiological mechanisms that register features of diet in the first 3 days of life and activate alternative developmental pathways, which function to match the organism's morphology to its feeding ecology (see Greene, 1989, 1996).

Weaning effects on play behavior in kittens. Nursing cats on restricted diets wean their offspring early. Kittens respond to early weaning by engaging in significantly more *object* play, but not more *social* play, compared to normal controls (Bateson, Mendl, & Feaver, 1990). Although early weaning does not affect overall levels of play, it does change the quality of play toward a more object-oriented form. During the cat's natural selective history, early weaning would have been reliably associated with environments where food was scarce, maternal nurturance was limited, and young cats were required to hunt for themselves at a relatively early age. Object play is especially important in the development of hunting skills in cats, and high rates of object play prepare kittens to hunt at an earlier age. Bateson et al. (1990, p. 524) conclude "It seems likely that, by responding to cues from the mother, the individual animal is able to move along a developmental route that is appropriate to the conditions it will encounter in later life." Thus, kittens appear to have evolved mechanisms for registering information about maternal feeding as a basis for upregulating the development of motivational systems involved in object play.

Paternal investment and development of female reproductive strategies. Draper and Harpending (1982, 1988) together with Belsky et al. (1991) have proposed a conditional adaptation theory of adolescent sexual development. Drawing on the concept of sensitive-period learning, the theory posits that the physiological and motivational systems underlying variation in timing of girls' sexual development are especially sensitive to the father's role in the family in approximately the first 5 years of life. Specifically, experiences associated with early father absence and father–daughter distance are hypothesized to entrain the development of reproductive strategies that are matched to the social niche into which the daughter was born, a niche in which male parental investment is relatively unreliable and unimportant. Girls in this context are predicted to develop in a manner that speeds rates of pubertal maturation, accelerates onset of sexual activity, and orients the individual toward relatively unstable pair bonds. Conversely, experiences associated with early father presence and father–daughter closeness are hypothesized to entrain the opposite pattern of sexual development. Either way, the girl "chooses" a developmental trajectory that, in the adult social environment into which she was born, was likely to have promoted reproductive success during human evolutionary history.

There is now a substantial body of empirical data that are consistent with this theory. Specifically, studies in the United States (Doughty & Rodgers, 2000; Ellis & Garber, 2000; Ellis, McFadyen–Ketchum, Dodge, Pettit, & Bates, 1999), Canada (Surbey), New Zealand (Moffitt, Caspi, Belsky, & Silva, 1992), and Australia (Jones, Leeton, McLeod, & Wood, 1972) have all found that girls from homes where the fathers are absent tend to experience earlier pubertal development than girls from homes where the fathers are present. In addition, Ellis et al. (1999) presented longitudinal data showing that girls who had more *distant* relationships with their fathers during the first 5 years of life experienced earlier

pubertal development, and in studies in the United States and New Zealand, early onset of father absence has been found to have a dramatic effect on rates of early sexual activity and teenage pregnancy (Ellis, Bates, Dodge, Fergusson, Horwood, Pettit, & Woodward, 2003). It is important to acknowledge that the apparent effects of father absence and other experiences within families on developmental endpoints, such as pubertal timing, could also be a consequence of genetic influences on both. Comings, Muhleman, Johnson, and MacMurray (2002), for example, have shown that a variant of the X-linked androgen receptor gene is associated both with paternal divorce and father absence in males and with early menarche and early sexual activity in females. Taken together, the cited findings support the plausibility of both experiential and genetic accounts for associations between father absence and the development of precocious reproductive strategies (reviewed in Ellis, 2004).

A conditional adaptation theory of BSC development

The present theory posits that natural selection has favored developmental mechanisms (conditional adaptations) that function to adjust levels of BSC to match familial and ecological conditions encountered early in life. Just as the timing of girls' sexual development may be sensitive to paternal investment, individual differences in BSC may track specific features of childhood environments. Specifically, humans may have evolved developmental mechanisms that detect and internally encode information about levels of supportiveness versus stressfulness in early childhood environments, as a basis for calibrating the activation thresholds and response magnitudes within stress response systems to match those environments.

Based on the claim that individual differences in stress reactivity constitute variation in susceptibility to features of the social environment, both positive and negative, the current theory postulates a U-shaped, curvilinear relationship between levels of supportiveness versus stressfulness in early childhood environments and the development of BSC (see Figure 3). The right side of Figure 3 depicts expected reactivity levels for individuals who experience very high levels of stress in early childhood. Consistent with the experimental animal and epidemiologic human research summarized above, these individuals are hypothesized to develop heightened reactivity profiles. We do not, however, expect reactivity to decline monotonically with decreasing childhood stress. The left side of Figure 3 shows predicted reactivity levels for individuals whose early childhoods are characterized by intensive, stable caregiving and family support. These individuals are also hypothesized to develop exaggerated reactivity profiles, which function in this context to garner the health and survival benefits of highly supportive rearing environments. Finally, the middle of Figure 3 reflects the anticipated, relatively muted reactivity profiles of individuals whose early childhood experiences are characterized by moderate levels of ongoing stress and threat. These individuals, occupying the broad, normative range of species-typical contextual stressors, are hypothesized to develop comparatively low reactivity profiles as a way of gating or filtering highly prevalent, moderate level stressors.[2,3] For the present purposes of sim-

2. Note that we are *not* claiming that moderate level stressors result in extremely low reactivity or biological *insensitivity* to context. Rather, "low reactivity" here refers to low to moderate levels of biological response, relative to the high reactivity individuals we predict would be disproportionately represented in very low and very high stress environments. Our evolutionary–developmental theory of BSC is thus agnostic with regard to the contextual origins of the extremely low reactivity individuals examined, for example, in the work of Raine et al. (1997).
3. Of possible but uncertain interest is the commonality between the hypothesized quadratic association between early adversities and BSC and the historical observations of an inverted U-shaped relation between arousal and performance (Yerkes & Dodson, 1908). Although the former hypothesis addresses the experiential *origins* of biological reactivity, the latter observation describes its *consequences* within cognitive functioning. The Yerkes–Dodson association has been contested in recent years (Neiss, 1988), and the construct of "arousal" may be only distantly allied to reactivity or BSC. Nonetheless, future theoretical and/or empirical work might benefit from a deeper examination of how the two phenomena may be linked or related.

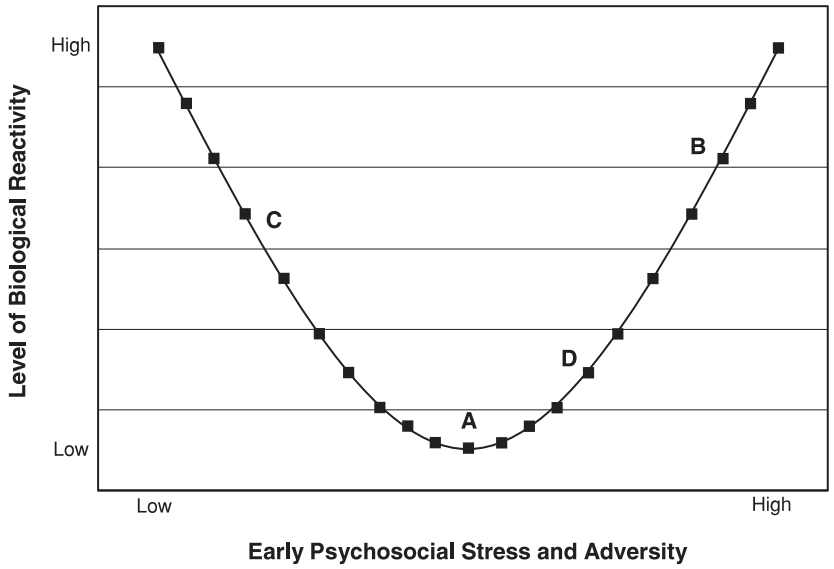

Figure 3. The hyopthesized curvilinear relation of biologic reactivity to early stress and adversity. Comparisons of subjects at points A and B would result in a conclusion that early adversity is associated with greater stress reactivity. Conversely, comparisons at points C and D would generate the inference that early adversity produces diminished reactivity.

plicity and tractability, we further suggest that this curvilinear, quadratic association with early adversity will hold for reactivity in *both* the LC-NE and CRH systems, acknowledging that far greater complexity in the interplay and balancing of the two stress response systems will likely surface as these relations are explored.

Although the U-shaped curve depicted in Figure 3 specifies environmental sources of variation in BSC, genetic sources of variation and gene–environment interactions are also important and need to be addressed in a comprehensive theory of BSC. Children who are genetically disposed toward high reactivity, for example, may alter levels of stress and support in their home environments in ways that further increase BSC. Children may also differ in the location of reaction norms underlying the spectrum of variation in BSC. A reaction norm is a genetically inherited constraint that specifies the range of phenotypes that will be produced by a genotype in different environmental contexts (Schlichting & Pigliucci, 1998). Given equivalent life experiences, children with reaction norms that are located on the upper end of the BSC spectrum should be more likely to develop highly reactive phenotypes than children with reaction norms on the lower end of the spectrum, and vice versa. In addition, children may differ in the breadth of reaction norms. That is, genotypes can be expected to differ in the extent to which they are capable of producing a range of different BSC phenotypes (cf. Belsky, 2005), even given comparable ontogenetic histories. The current evolutionary–development model should be more successful in accounting for the development of individual differences in BSC among children with wider reaction norms. Finally, consistent with the principle of "equifinality" (von Bertalanffy, 1968), we expect that the effects of different BSC phenotypes on child health and adjustment will be equivalent, regardless of whether a child develops a given phenotype primarily as a result of a narrow reaction norm or as a result of particular childhood experiences operating within a relatively broad reaction norm.

Developmental mechanisms for adjusting BSC in response to early childhood experiences may have resulted from a long and recurrent evolutionary history in which (a)

different children confronted substantially different rearing environments; (b) highly reactive children experienced better survival and reproductive outcomes, on average, in both intensely stressful and highly supportive rearing environments; and (c) less reactive children experienced generally better survival and reproductive outcomes in developmental settings characterized by moderate levels of stress and threat. Such an account would reconcile important contradictions, reviewed above, in the existing literature on the origins and consequences of stress reactivity in children. Investigators comparing individuals from points A and B in Figure 3, for example, would conclude, as have Yehuda (2002), De Bellis et al. (1999), and many others, that experiences of family and environmental stress are associated with upregulatory calibrations in biological reactivity systems. On the other hand, studies comparing individuals from points C and D would find, as have those reviewed by Gunnar and Vazquez (2001) and Heim, Ehrlert, et al. (2000), that early stressors are rather associated with downregulatory changes in salient biological responses. The current theory, which posits two oppositionally distinctive ontogenies for BSC, explains both of these up- and downregulatory effects.

Conclusion

Our principal aim in the present paper has been to articulate the precepts and rationale for a new claim about the nature of relations between early life experience and stress reactivity, a claim that we further explore empirically in the companion paper, which follows. The logic of the argument we have sought to present can be summarized in the following way. Biological reactivity to psychological stressors consists of an elaborated, highly coordinated, but phylogenetically primitive set of neural and peripheral neuroendocrine responses, designed to ready the organism for external challenges and threats to survival. Standard explanations of such responses' role in the pathogenesis of human disorders suggest that prolonged or exaggerated reactivity, such as that seen in highly reactive biobehavioral phenotypes, exerts deleterious and impairing effects on a broad range of target organs, including structures within the brain, leading to decrements in health, cognition, and functional capacities. Often overlooked in such accounts is a body of anomalous observations, revealing oppositional, counterregulatory processes within the stress response circuitry itself and, even more compellingly, bivalent effects of reactivity on biomedical and psychiatric outcomes. Highly reactive children sustain disproportionate rates of morbidity when raised in adverse environments but unusually low rates when raised in low-stress, highly supportive settings.

Such bidirectional, environment-dependent health effects suggest that *BSC* is the core, defining feature of highly reactive phenotypes. These observations call into question the presumably unitary pathogenic effects of high reactivity and suggest that its protective effects within specific developmental ecologies might explain the conservation of such phenotypic variation within evolutionary history. Furthermore, conditional adaptations, in which a single genotype supports a range of environmentally contingent phenotypic expressions, enable entrainment of biological and behavioral development to adaptively match early (and predicted future) social environments. Given past evidence that early trauma can evoke upregulatory changes in stress reactivity and new evidence that high reactivity can be protective in highly supportive settings, we postulate a curvilinear, U-shaped relation, shown in Figure 3, between levels of early adversity and the magnitude of biological response dispositions. Specifically, we hypothesize that (a) exposure to acutely stressful childhood environments upregulates BSC, increasing the capacity and tendency of individuals to detect and respond to environmental dangers and threats; (b) exposure to exceptionally supportive childhood environments also upregulates BSC, increasing susceptibility to the social and developmental benefits of such environments; and (c) typical of the large majority of children, exposure to childhood environments that are extreme in neither direction downregulates BSC, buffering individuals against the chronic stressors encountered in a

world that is neither highly threatening nor universally safe.

Because evolutionary "stories" of the kind advanced here are especially vulnerable to the perils of post hoc explanation, it is essential that the current evolutionary–developmental theory of the origins and functions of stress reactivity be put eventually to rigorous tests of its predictive strength. In the paper that follows, we offer a provisional, "promissory note" on such a requirement, by presenting exploratory analyses from two studies, in geographically and culturally distinctive settings, that produce empirical derivations of the same hypothesis first advanced here on conceptual grounds. Such convergence of theoretical and empirical reasoning, we would argue, portends well for the validity of the evolutionary–developmental hypothesis. In the presentation of its conceptual and analytical origins, it is our hope that new knowledge concerning the causes and consequences of children's responsivity to stress will be uncovered, and that richer, more protective environments will be fostered in which highly sensitive children can develop and thrive.

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Policy on Use of Fluoride

Review Council
Council on Clinical Affairs
Latest Revision
2018

Purpose

The American Academy of Pediatric Dentistry (**AAPD**) affirms that the use of fluoride as an adjunct in the prevention of caries is a safe and effective. The AAPD encourages dentist and other health care providers, public health officials, and parents/caregivers to optimize fluoride exposures to reduce the risk for caries and to enhance the remineralization of affected tooth structures.

Methods

This document was originally developed by the Liaison with Other Groups Committee and adopted in 1967. This is an update from the last revision in 2014. An electronic database search using the terms: fluoride, fluoridation, acidulated phosphate fluoride, fluoride varnish, fluoride therapy, and topical fluoride previously was conducted to develop and update this policy. The current update relied upon systematic reviews, expert opinions, and best current practices. The use of silver diamine fluoride is addressed in a separate AAPD policy.[1]

Background

The adjustment of the fluoride level in community water supplies to optimal concentration is the most beneficial and inexpensive method of reducing the occurrence of caries.[2] Long-term use of fluorides has reduced the cost of oral health care for children by as much as 50 percent.[3] When public water is fluoridated to an optimal level, there is a 35 percent reduction in decayed, missing, filled primary teeth and 26 percent fewer decayed, missing, and filled permanent teeth.[4] The occurrence of fluorosis, causing esthetic concerns, has been reported to be 12 percent when public water contains 0.7 ppm F.[4] When combined with other dietary, oral hygiene and preventive measures[5], the use of fluorides can further reduce the incidence of caries.

Professional fluoride products should only be applied by or under the direction of a dentist or physician who is familiar with the child's oral health and has completed a caries risk assessment. When fluoridation of drinking water is impossible, effective fluoride supplementation can be achieved through the intake of daily fluoride supplements according to established guidelines.[2,6-8] Before supplements are prescribed, it is essential to review dietary sources of fluoride (e.g., all drinking water sources, consumed beverages, prepared food, toothpaste) to determine the patient's true exposure to fluoride,[2,9,10] and to take into consideration the caries risk of the child. The mean fluoride concentration of ready-to-feed infant formulas in the U.S. is 0.15 ppm for milk-based formulas and 0.21 ppm for soy-based formulas[11]. The more important issue, however, is the fluoride content of concentrated or powdered formula when reconstituted with fluoridated water. The range of fluoride in ppm for reconstituted powdered or liquid concentrate, when reconstituted with water containing 1ppm fluoride, is 0.64 – 1.07.[11] As the Environmental Protection Agency/Department of Health and Human Services' recommendation[12] for optimizing community water supplies to 0.7 ppm F is instituted, fluorosis due to reconstituting infant formula with fluoridated water is less of an issue.

Significant cariostatic benefits can be achieved by the use of over-the-counter fluoride-containing preparations such as toothpastes, gels, and rinses, especially in areas without water fluoridation.[2] The brushing of teeth with appropriate amounts of fluoride toothpaste twice daily for all children is encouraged.[13] Monitoring children's use of topical fluoride-containing products, including toothpaste, may prevent ingestion of excessive amounts of fluoride.[13,14] Numerous clinical trials have confirmed the anti-caries effect of professional topical fluoride treatments, including 1.23 percent acidulated phosphate fluoride [(APF); 1.23 percent F], five percent sodium fluoride varnish [(NaFV); 2.26 percent F], 0.09 percent fluoride mouth rinse, and 0.5 percent fluoride gel/paste.[15] For children under the age of 6 years, 5 percent sodium fluoride varnish (2.26 percent F) in unit doses, which reduce the potential for harm, is the recommended professionally-applied topical fluoride agent.[15]

A significant number of parents and caregivers are concerned about their child receiving fluoride and may refuse fluoride treatment even though fluoride is safe and effective.[16] This is similar to opposition to community water fluoridation.[17] Topical fluoride refusal and resistance may be a growing problem and mirror trends seen with vaccination refusal in medicine.

Policy statement

The AAPD:

- Endorses and encourages the adjustment of fluoride content of public drinking water supplies to optimal levels where feasible.
- Endorses the supplementation of a child's diet with fluoride according to established guidelines when fluoride levels in public

• Encourages the application of professional fluoride treatments for all individuals at risk for dental caries.
• Encourages dental professionals to inform medical peers of the potential of enamel fluorosis when excess fluoride is ingested prior to enamel maturation.
• Encourages the continued research on safe and effective fluoride products.
• Supports the delegation of fluoride application to auxiliary dental personnel or other trained allied health professionals by prescription or order of a dentist after a comprehensive oral examination or by a physician after a dental screening has been performed.
• Encourages all beverage and infant formula manufacturers to include fluoride concentration with the nutritional content on food labels.
• Recognizes that drinking fluoridated water and brushing with fluoridated toothpaste twice daily are the most effective method in reducing dental caries prevalence in children.
• Encourages dental providers to talk to parents and caregivers about the benefits of fluoride and to proactively address fluoride hesitance through chairside and community education.

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Silver Diamine Fluoride:
Changing the Caries Management Paradigm and Potential Societal Impact

John Timothy Wright, Alex White

Silver diamine fluoride is a topically-applied agent for managing dental caries. It stops caries lesion progression, turning them black and hard in a high percentage of cases. Populations including pediatric, geriatric, special health care needs, and those with limited access to oral health care can all benefit from silver diamine fluoride. This commentary addresses some of the many questions that have arisen with the availability of SDF and marked gaps in our knowledge.

Silver diamine fluoride (SDF) has been used for decades to help manage dental caries in several countries and became available in the United States in March 2015. The FDA approved SDF as a desensitizing agent. Its use as a caries management therapy is an off-label use similar to fluoride varnish [1]. SDF provides a new and effective chemotherapeutic agent that can stop the progression of caries lesions and aid in the management of dental caries. Over the past 100 years, cavitated lesions caused by dental caries have been primarily managed surgically to remove the diseased tissues and replace the lost tooth structure with a variety of dental materials [2]. There have been multiple clinical trials and several systematic reviews indicating application of SDF will arrest or stop progression of caries lesions in a high percentage of cases (30%–70%) [3-6]. Repeated applications increase the caries lesion arrest rate [6]. Treated caries lesions become hard and black when the treatment is successful. In some cases, arrested lesions will become caries active with renewed loss of mineral and tooth structure.

As marketed in the United States, SDF is a solution of 25% silver, 8% amine, 5% fluoride, and 62% water ($AgNH_2F$) and is the most concentrated fluoride product commercially available for caries management (see Table 1). It is a clear liquid (new product is tinted blue to aid in clinical visualization) that is applied to caries lesions with a microbrush [7]. The relative newness of SDF in the United States has led to a variety of questions related to what populations might benefit most from SDF, patient/parent acceptance, practitioner acceptance, if SDF will result in a shift from a traditional surgical to a non-surgical caries management approach, who can and should be able to apply SDF, and cost implications.

TABLE 1.
Fluoride Content of Caries Management Therapeutics

Product	Fluoride %	Fluoride PPM	F Ion mg/ml
SDF	5	44,800	44.8
Varnish 5%NaF	~2.2	22,600	22.6
Toothpaste	~0.11	1100	1.1

Source. Modified from Crystal YO, Niederman R [4].

Potential SDF Benefits to Specific Populations and Patient/Parental Acceptance

Dental caries remains the most common chronic disease in the United States, affecting almost 35% of children (aged 2–5 years) and most adults by the end of adolescence [8, 9]. Populations with lower socioeconomic status and those with special health care needs have disproportionately high disease rates compared with the rest of the general population [10]. Managing dental caries in the pediatric population, especially children under the age of 3 years, often requires pharmacological behavior management approaches, including sedation and/or general anesthesia. These approaches are expensive and carry the potential risk of death. For children under the age of 3, there are concerns about neurological development with prolonged or repeated general anesthesia [11]. The use of SDF to prevent or delay surgical intervention until after the age of 3 years makes it a potentially attractive adjunctive therapy for managing caries in the very young pediatric population.

The use of SDF in the geriatric population has been shown effective in arresting root caries [5, 6]. Surgical approaches for managing root caries remain a challenge in this population, and having a chemotherapeutic option that can be delivered in a non-clinical setting has additional advantages. Both geriatric and patients with special health care needs can benefit from non-surgical SDF treatment that has few contraindications (eg, silver allergy) and is clinically less complicated to deliver than restorative treatment [12].

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Parental acceptance for the pediatric population varies based on the patient's age, patient's sex, and whether the lesion to be treated is in a front or back tooth. Parents are more accepting of SDF treatment and the resulting black lesions for posterior teeth than anterior teeth, and more accepting for boys vs girls. In a recent study, over 60% of parents were accepting of SDF treatment and tooth discoloration of posterior teeth, but only 29% were accepting for anterior teeth [13]. The esthetic consequences of SDF treatment make gaining informed consent critical. Showing parents clinical photographs of treated lesions is recommended (see Figure 1). There are no studies related to acceptance of SDF treatment in adolescents, adults, or patients with special health care needs.

State Dental Boards have different regulations regarding who can apply SDF to patients. In Oregon and Washington, SDF can be applied by hygienists, while in North Carolina the Dental Board has taken the position that SDF should be applied by dentists. The North Carolina State Dental Board feels that arresting a carious lesion and turning it black is an irreversible procedure and should therefore be done by a dentist; the Board will have a hearing in November to discuss whether or not dental providers other than dentists (eg, dental hygienists) can place SDF. Restriction of SDF placement to only dentists has implications related to the availability of SDF to specific segments of the population (eg, nursing home occupants). Physicians and nurses can apply SDF in accordance with their state's practice acts.

Practitioner Acceptance and Adoption (Surgical vs Non-Surgical Management)

Traditionally, dental practitioners have been educated and trained to manage cavitated caries lesions through a surgical approach. Studies show that removing bacteria-laden, demineralized dental tissues does not, in and of itself, change the individual's disease trajectory for developing new dental caries [14]. Adoption of new approaches for managing caries lesions by different providers of the oral health care delivery system varies, but adoption of non-surgical approaches, such as pit and fissure sealants, has been slow [15]. There are barriers to adoption of a new therapeutic approach such as SDF. For example, the use of SDF has not been taught in most dental schools and has only recently become a topic in pediatric dentistry residencies [16]. Common barriers to adopting new therapies and treatments include concerns regarding safety, efficacy, the variation in clinical trial methodologies, lack of clear treatment guidelines, regulation, patient attitudes, and reimbursement, to name a few.

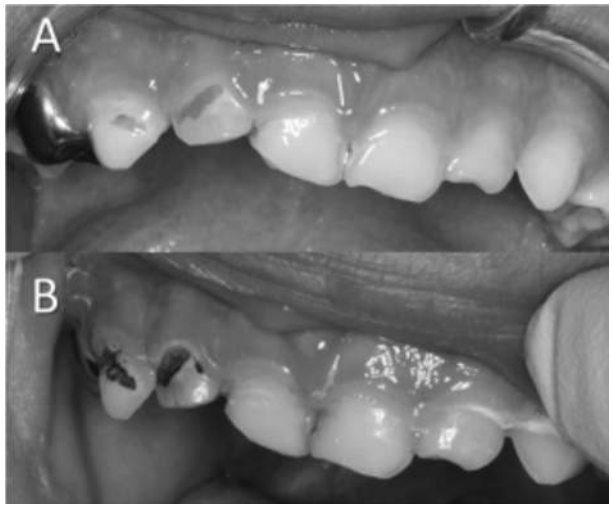
Access to Care and Cost

The American Dental Association (ADA) adopted a billing code (D1354) that can be used for chemotherapeutic caries management that encompasses the use of SDF. There have been different interpretations of this code and whether it should be reimbursed on a per visit bases or per tooth basis. North Carolina Medicaid set a reimbursement rate of $24.18 per visit for application of SDF regardless of the number of teeth or dental surfaces treated. Reimbursement for SDF treatment has only been adopted by a few state Medicaid Programs and insurance carriers at this time, but others are examining inclusion of SDF as a reimbursable caries management therapy. As of August 2017, there were 14 state Medicaid programs that either had adopted (North Carolina has adopted coverage) or were considering reimbursement for SDF treatment. Without coverage by insurance programs, SDF has largely been paid for out-of-pocket, creating a significant barrier to its use. Millions of older and disabled individuals in the United States have to pay for their dental care as these services are not covered by Medicare [17].

There has been no actual cost analysis published regarding the use of SDF and its impact on oral health care expense. Evaluation of caries management approaches and costs for silver nitrate and fluoride varnish (SN/FV) in an Oregon-based study showed the SN/FV group received more preventive services, fewer restorative services, had fewer extractions, and had reduced billing for sedation [18]. However, the overall costs for care were 55% higher in the SN/FV group compared with those patients receiving conventional treatment. It is not known if there would be cost savings realized beyond the 2 years evaluated in the Oregon cohort study.

A fiscal impact study by North Carolina State Dental Medicaid found that $35 million was spent for dental services provided with the aid of general anesthesia to chil-

FIGURE 1.
Carious lesions in these primary teeth prior to SDF show a yellow brownish discoloration (A). These same lesions seen one month after SDF treatment show the typically black discoloration (B) and had hardened or re-mineralized based on probing with a dental explorer.



Source. Modified from Crystal YO, Niederman R [4].

dren 0–8 years of age. If only 10% of dental treatment with general anesthesia were prevented by SDF applications for 4–5-year-olds, the savings would be about $746,000 (North Carolina Division of Medical Assistance, unpublished data, 2016). Other models included allowing SDF-treated teeth to exfoliate without needing restorative care. Treatment protocols for SDF do not require as many applications as are recommended for caries management with SN/FV. This difference could make SDF caries management more cost-effective than SN/FV, but studies need to be completed to better understand the real costs of SDF use in different populations.

States differ significantly in their regulations as to who and what is covered. For example, in Michigan this benefit is covered for all ages, while in North Carolina only children 5 years of age and younger are included. Programs also are limiting the service to 2 treatments per year and a lifetime cap of 6 total treatments. Some Medicaid programs state this is a temporary measure for caries management and is only to be applied when traditional restorative measures are not available (eg, Michigan). This approach has significant cost implications regarding the use of SDF, making it potentially an added cost as opposed to a cost-saving therapy.

Discussion

Dental caries continues to be highly prevalent in the United States and around the world, causing significant morbidity, including pain, suffering, loss of work and school time, loss of income, and the spending of billions of health care dollars [19]. The availability of SDF as a caries management therapeutic provides a potentially valuable new approach that could help stem the tide of the dental caries epidemic. There are diverse implications to broad use of SDF for managing dental caries that range from fiscal issues to oral health-related quality of life. There are numerous perceived and real barriers to the acceptance and application of new treatment approaches in health care, and there is little doubt that adoption of SDF by the dental community will face challenges [20]. Clinical trial methodologies have been variable, although the American Academy of Pediatric Dentistry has developed a recently published clinical care guideline for SDF [21].

The surgical paradigm for caries management is pervasive in dental education and practice, and significant shifts in philosophies for treatment and reimbursement are needed to manage dental caries as a chronic disease. Dental fear is a strong predictor of oral health-related quality of life. Similarly, pain as the reason for seeking recent dental care also is associated with a decreased oral health-related quality of life in children [22]. Caries management with SDF is a relatively simple and painless procedure (moisten caries lesion with the solution). It is not known whether broader use of this chemotherapeutic, non-surgical approach will over time decrease the pervasive fear of dentistry that is often associated with fear of injections and fear of pain. Broader use of SDF could affect the use of protective stabilization or restraint to deliver conventional restorative treatment in young, pre-cooperative children.

It remains to be seen whether SDF will provide an alternative to conventional restorative treatment in significant numbers of children under the age of 3 years that might otherwise require treatment with the aid of sedation or general anesthesia. If SDF treatment is used to successfully manage early childhood caries and reduces the need for sedation and general anesthesia, the cost implications for delivery of care could be significantly reduced. Alternatively, if SDF is primarily used only as a temporary measure to stabilize the disease until conventional restorative treatment can be implemented, then the oral health care costs may actually increase. There is evidence that even if health care costs increase, as was observed with SN/FV treatment, the need for extractions, endodontics, and restorative treatment decreased [18]. Thus, while the cost implications of broadened use of SDF are not clear, there is evidence that a chemotherapeutic approach can decrease the need for surgical care.

In summary, it is likely that SDF will usher in a new caries management approach, moving dentistry toward more frequent non-surgical management of dental caries. There are many potential benefits of broader adoption of SDF that could include lower rates of surgical care, tooth loss, reduction in the prevalence of dental-related infections, and improved oral health-related quality of life. It is not clear what the cost implications will be, and reimbursement remains a barrier to broader adoption of this new therapy by the dental community. NCMJ

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Full Length Research Paper

Comparative inhibitory effect of xylitol and erythritol on the growth and biofilm formation of oral *Streptococci*

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Our aims were to examine the effects of xylitol and a novel polyol sweetener, erythritol, on growth of oral *Streptococci* and compare their effects. The inhibitory effects of xylitol and erythritol on *Streptococcus* strains, as well as on streptococcal biofilm formation were examined. *Streptococcus mutans*, *Streptococcus sobrinus*, and *Streptococcus sanguinis* were used as representatives of oral *Streptococci*. The effects of these polyols on biofilm formation were determined by microtiter plate assay. The growth was compared at each experiment using analysis of variance of repeated measures (SPSS 16.0 for Windows). Our results indicated that in the presence of 4% xylitol and 4% erythritol the growth of *S. mutans* was decreased by 68 and 71%, respectively. Biofilm formation by *S. mutans* was reduced to 31.32% in the presence of 4% erythritol. Regardless of concentration, in general, erythritol was found more effective than xylitol in inhibiting the growth and biofilm formation of *Streptococci* strains used in this study. Xylitol and especially erythritol both inhibited microplate surface adherence of oral *Streptococci*, which are known to contribute to plaque accumulation.

Key words: Xylitol, erythritol, oral *Streptococci*, biofilm, microtiter plate assay.

INTRODUCTION

Dental caries form through a complex interaction over time between acid-producing or acid-tolerating bacteria and fermentable carbohydrates. Acid production by oral bacteria by hydrolysis of the food debris accumulated on the tooth surface, cause demineralization and destruction of the tooth. Thus, it has been an obvious idea to utilize sugar substitutes in order to prevent the disease (Wennerholm et al., 1994; Selwitz et al., 2007). Sugar alcohols are not utilized by the oral bacteria, and so the absence of fermentation and acid production reduces risk of dental caries (Mattos-Graner et al., 2000; Tanzer, 1995). Xylitol and most of other polyols used as bulk sweeteners may have laxative effects and, therefore, they are only suitable for small size products like chewing gums (Soderling, 2009; Soderling et al., 2010). In contrast to other poly alcohols, erythritol is not a laxative and its food applications could be much broader.

The first study to report that xylitol inhibits growth of *S. mutans* was published in 1975 (Knuuttila and Makinen, 1975). Clinical studies have shown xylitol to decrease the number of *S. mutans*, the amount of plaque, and the incidence of caries in children (Ly et al., 2006; Bradshaw and Marsh, 1994; Soderling et al., 1989). A number of studies have also suggested that some *S. sanguinis* and *S. salivarius* strains may be inhibited by xylitol (Isotupa et al., 1995; Kontiokari et al., 1995). Substitution of xylitol for sucrose in the human diet, totally as well as partially, resulted in more than 85% reduction in the incidence of dental caries (Scheinin, 1976). Most of the *S. mutans* strains transport xylitol into the cell via the phosphotransferase system, and xylitol is then phosphorylated to xylitol-5-phosphate and expelled from the cell (Makinen et al., 2001; Soderling, 2009). This futile energy-consuming pathway is thought to be responsible for the growth inhibition of *S. mutans* (Burt, 2006;

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Takahashi and Nyvad, 2008; Trahan, 1995). None of the predominant bacteria found in dental plaques produce acid from xylitol.

Erythritol has been suggested to be caries preventive, but few studies have so far been published on its effects on dental caries (Makinen et al., 2002, 2005; Burt, 2006). The present study shows the effects of xylitol and erythritol on the growth of oral *Streptococci* and compares their potential advantages against adhesion and biofilm development of these bacteria. Since *S. mutans* are considered the most acidogenic microorganisms in dental biofilm, an important aspect of this study is to find high efficiency of erythritol on inhibition of growth and biofilm formation of this bacterium.

MATERIALS AND METHODS

Microorganisms

The following types of *Streptococci* were used: *S. mutans* ATCC35668, *S. sobrinus* ATCC27607, and a clinical isolate of *S. sanguinis* provided by Pasteur Institute of Iran.

Cultivation of the microorganisms

Streptococci species were initially cultured in 5 ml of Brain Heart Infusion (BHI) broth (Merck, Germany) to produce log phase cells. Cultures were incubated at 37 ◦C for approximately 16 h and then transferred (2%) to fresh BHI supplemented with 1% sucrose. The growth media contained 2% (130 mM) or 4% (260 mM) xylitol (Sigma, St. Louis, MO, USA), or 2% (160 mM) or 4% (330 mM) erythritol (Sigma, St. Louis, MO, USA). The polyol concentrations (w/v %) were chosen based on similar study (Soderling et al., 2010). Stock solutions of xylitol and erythritol were prepared in distilled water and sterilized by filtration through a 0.2 Millipore filter (Axiva, India) and afterwards were added aseptically to the medium at the appropriate concentration. The corresponding control medium was free of polyols. The test cultures were incubated in a 5% CO_2 incubator with slowly shaking at 37°C for 24 h. Growth was monitored by measuring the absorbance at a wavelength of 660 nm. Each test was carried out in three independent experiments. The inhibitory effects of xylitol and erythritol were calculated from the growth curves at late log phase.

Microtiter plate assay for efficacy of Streptococcal biofilm removal

Biofilm production by *Streptococci* strains grown in BHI was measured using a semi-quantitative adherence assay on 96-well tissue culture plates. Each *Streptococcus* strain was grown in 10 ml of BHI supplemented with 1% sucrose at 37°C. Overnight cultures in BHI were transferred (0.1 ml) into polystyrene microtiter plates (Greiner Bio-One, Germany) which previously added 0.1 ml of erythritol and xylitol separately. Final concentration of erythritol and xylitol reached to 2 and 4%. Each plate included eight wells of *Streptococci* as control and eight empty wells as blank (Van Loveren, 2004). The plates were covered and incubated aerobically at 37°C for 24 h. At the end of incubation, the liquid in the wells was poured out and each well was washed three times with 0.25 ml of sterile phosphate buffered saline (PBS). Plates then were stained for 5 min with 0.2 ml of 2% crystal violet per well. Excess stain was rinsed off by washing the plate with PBS. Plates were air-dried and the dye was solubilized from the adherent cells by treatment with 0.2 ml of 33% (v/v) glacial acetic acid per well. The cell turbidity was monitored using a microtiter plate reader at 630 nm optical density. The average OD of the blank wells was subtracted from the OD of experimental samples. Inhibitory effect of these polyols on biofilm production and adhesion of *Streptococcus* strains to polystyrene microplate was assayed and determined using the following formula:

Percentage reduction: [(C-B)-(T-B)/(C-B)] × 100%

Where B denotes the average absorbance for blank wells (no biofilm, no treatment); C denotes the average absorbance for control wells (biofilm, no treatment) and T denotes the average absorbance for treated wells (biofilm and treatment) (Djordjevic et al., 2002; Shakeri et al., 2007).

Disc diffusion test

The antimicrobial assay of these polyols was performed by agar disc diffusion method (Baur et al., 1966). The tested bacterial strains were cultured until the cultures attained a turbidity of 0.5 McFarland units. A 0.1 ml volume of the standard suspension of each test bacterial strain was spread evenly on Mueller Hinton (MH) agar (Merck, Germany) using a sterile glass rod spreader and the plates were allowed to dry at room temperature. Blank sterile discs (0.5mm diameter) (Oxoid, Australia) were saturated with 0.2 ml of each polyol solution and allowed to dry before being placed on the top of the agar plate. The controls included distilled water and the commercial antibiotic tetracycline. After holding the plates at room temperature for 2 h, they were incubated for 48 h at 37C in a carbon dioxide environment and the diameter of growth inhibition zone was determined.

Statistical analyses

The growth was compared at each experiment using analysis of variance (ANOVA) repeated measures (SPSS 16.0 for Windows). The level of statistical significance was set at $P< 0.01$.

RESULTS

Cultivation of the microorganisms

Both xylitol and erythritol inhibited the growth of all oral *Streptococci* studied in this study. The presence of 4% xylitol and erythritol resulted in 68 and 71% inhibition of *S. mutans* growth, respectively. The same concentrations of xylitol and erythritol also decreased the growth of *S. sanguinis* by 65 and 77%, respectively (Table 1). As shown in Figure 1, after 24 h of incubation, the optical density of the culture reached the lowest in the presence of erythritol than xylitol, indicating erythritol to be more efficient than xylitol in growth inhibitory of *Streptococcus* strains. In addition our results indicated that the degree of inhibition, as well as the inhibitory pattern differs for most *Streptococcus* strains.

Microtiter plate assay

Inhibitory effect of these polyols on biofilm production and

Table 1. Growth inhibition of xylitol and erythritol on oral *streptococci*.

Polyol	Concentration (%, w/v)	Percentage reduction (%) of [a*]		
		S. mutans	***S. sobrinus***	***S. sanguinis***
Xylitol	2	66	71	57
	4	68	72	65
Erythritol	2	69	71	76
	4	71	76	77

[a]Data points: mean values from three independent experiments, [*]significancy level set at P<0.01.

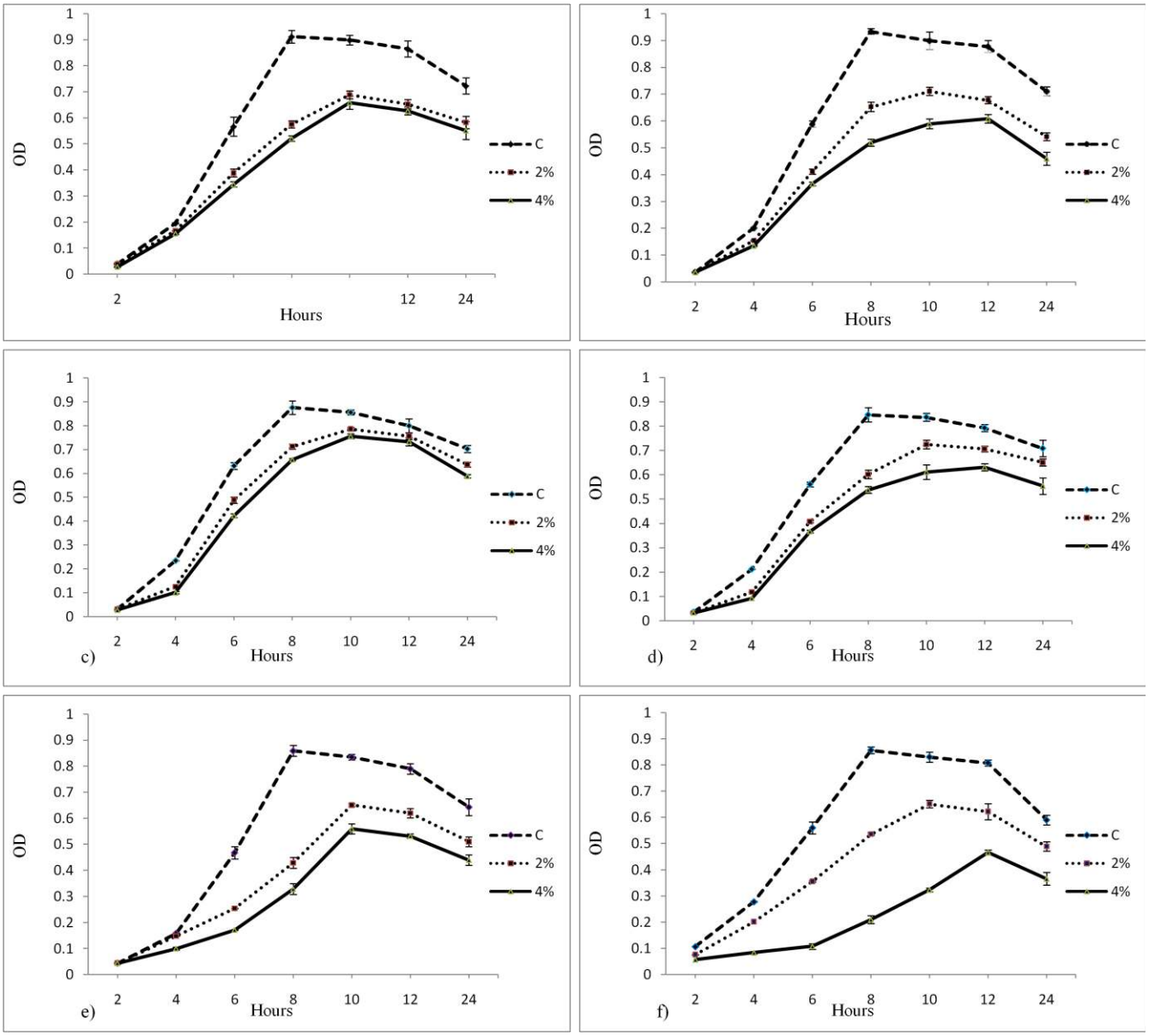

Figure 1. Growth (660 nm) of *S. mutans*, *S. sobrinus* and *S. sanguinis* in the presence of xylitol and erythritol at different concentrations. a) *S. mutans*, xylitol, b) *S. mutans*, erythritol, c) *S. sobrinus*, xylitol, d) S. *sobrinus*, erythritol, e) *S. sanguinis*, xylitol, f) *S. sanguinis*, erythritol (C: Control). Data points: mean values from three independent experiments. Bars represent the standard deviations of means. P < 0.01.

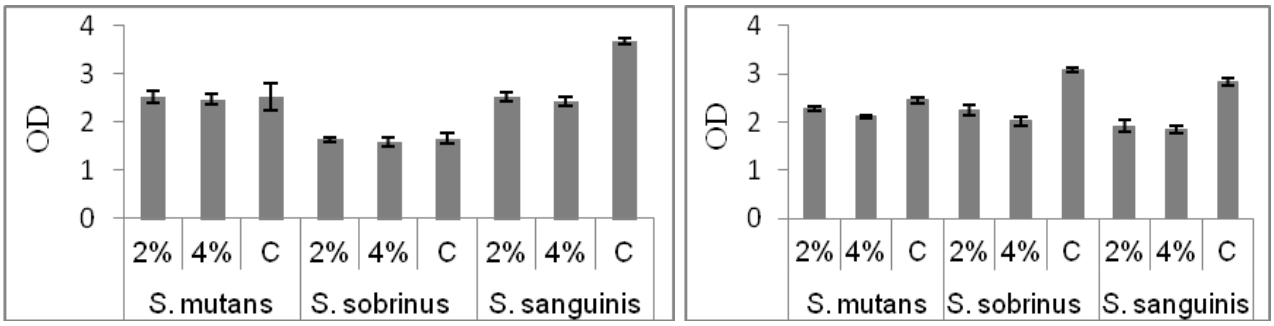

Figure 2. The adhesion (630nm) of three species of *Streptococcus* to polystyrene microplate. (a) The adhesion in presence of xylitol (2%, 4%) b) The adhesion in presence of erythritol (2%, 4%) (C: Control). Data points: mean values from three independent experiments. Bars represent the standard deviations of means.

Table 2. Percent inhibition of biofilm development by xylitol and erythritol on oral *streptococci*.

Polyol	Concentration (%, w/v)	Percentage inhibition of biofilm development (%) of [a]		
		S. mutans	***S. sobrinus***	***S. sanguinis***
Xylitol	2	0.33	3.19	43.80**
	4	3.55	9.9*	47.26**
Erythritol	2	11.65*	38.7**	47.76**
	4	31.32**	50.92**	50.88**

[a]Data points: mean values from three independent experiments, * Significancy level set at P<0.05.** Significancy level set at P<0.01.

adhesion of *Streptococci* strains to polystyrene microplate are shown in Figure 2. The results showed that the inhibitory effect of erythritol is stronger than that of xylitol. Biofilm formation by *S. mutans* was reduced to 3.55% and 31.32% in the presence of 4% xylitol and erythritol, respectively (Table 2).

Disc diffusion test

Solutions of xylitol (2%, 4% w/v) and erythritol (2%, 4% w/v) in distilled water did not cause zones of inhibition in any of the three bacteria plated. The tetracycline that served as control caused observed zones of inhibition.

DISCUSSION

It is considered that high sugar intake and low pH are the most primary mechanisms for disrupting homeostasis, leading to the development of dental caries. Strategies that aim to prevent the disease should include the inhibition of acid production, decrease in sugar consumption and use of non-fermentable sugars. Substitution of sugar by xylitol or erythritol is not only non-acidogenic but also may be considered as anti-cariogenic (Fraga et al., 2010). This study demonstrates higher performance of erythritol than xylitol in inhibiting the growth and biofilm formation of *Streptococci* strains and suggests that erythritol could be a good replacement for xylitol for the future of food industry.

Effect of xylitol and erythritol on growth and biofilm formation of oral *Streptococci*

The objective of this study was to compare the inhibitory effect of erythritol and xylitol on *Streptococcus* strains and a polystyrene microtiter plate assay to compare the inhibitory effect of these polyols on biofilm formation. Our results show that both xylitol and erythritol reduce the growth of oral *Streptococci*, but erythritol has a stronger inhibitory effect on biofilm formation than xylitol. An in vivo study by Makinen et al. (2001) also showed which xylitol, and especially erythritol, inhibited the growth of several strains of *S. mutans*. In addition, this study showed that erythritol significantly inhibited the microplate surface adherence of *S. mutans*, as well as other strains of oral *Streptococci*.

Similar to xylitol, erythritol is not catabolized by

Streptococci species. Xylitol entered to oral *Streptococci* by PEP-PTS system and changed to xylitol 5-phosphate, and then dephosphorylated and expelled as xylitol. The xylitol-5-phosphate inhibits glycolytic enzymes, resultingin the inhibition of both growth and acid production. This futile energy consuming xylitol cycle is thought to be responsible for the growth inhibition of these bacteria when the bacteria are exposed to xylitol. But no theories on the mechanism of growth inhibition by erythritol have so far been published (Soderling et al., 2010; Ly et al., 2008; Fraga et al., 2009) and more studies are clearly needed on this topic. Although ‘‘resistance’’ phenomenon demonstrated for xylitol in many oral *Streptococci*, but this phenomenon did not report for erythritol. Since erythritol is a new polyol and doesn’t have laxative effect therefore; use of erythritol and other same biological sweeteners products are the best way for control of dental caries in future.

Antibacterial effect of xylitol and erythritol on growth of oral *Streptococci*

The purpose of disc diffusion test study was to determine if xylitol or erythritol inhibit the *in vitro* growth of oral *Streptococci*. The results indicated that xylitol and erythritol did not inhibit the growth of bacteria in a carbon dioxide environment on MHA. Furthermore, this study revealed that inhibition is not due to anti-microbial activity but the mechanism of growth inhibition of these polyols is futile energy-consuming in metabolism pathway. Therefore, the main mode of inhibition by xylitol, and probably erythritol, appears to be the replacement of the carbohydrate source.

Some studies established that xylitol decreases the synthesis of insoluble polysaccharides by *S. mutans* (Soderling et al., 1989). This study also demonstrated that not only xylitol but also erythritol decrease the polysaccharide production and consequently decrease the microplate surface adherence of oral *Streptococci*. Hereby, xylitol and especially erythritol are considered as important inhibitors of microplate surface adherence of oral *Streptococci* which are known to contribute to plaque accumulation.

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Original Article

Association Between Breastfeeding and Dental Caries in Japanese Children

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ABSTRACT

Background: Studies investigating the impact of breastfeeding on dental caries have produced contradictory results. This cross-sectional study investigated the relationship between breastfeeding and the prevalence of dental caries in young Japanese children.

Methods: The study subjects were 2056 Japanese children aged 3 years. Information on breastfeeding was obtained by means of a questionnaire. Children were classified as having caries if 1 or more deciduous teeth were decayed, missing, or had been filled at the time of examination.

Results: The prevalence of dental caries was 20.7%. As compared with breastfeeding for less than 6 months, breastfeeding for 18 months or longer was associated with a significantly higher prevalence of dental caries. The relation was J-shaped: the adjusted prevalence ratios for less than 6 months, 6 to 11 months, 12 to 17 months, and 18 months or longer were 1.0, 0.79 (95% confidence interval [CI]: 0.60–1.05), 0.86 (95% CI: 0.66–1.13), and 1.66 (95% CI: 1.33–2.06), respectively (P for linear trend <0.0001, P for quadratic trend <0.0001).

Conclusions: Breastfeeding for 18 months or longer was positively associated with the prevalence of dental caries, while breastfeeding for 6 to 17 months was nonsignificantly inversely associated with the prevalence of dental caries.

Key words: breastfeeding; cross-sectional studies; dental caries; Japan

INTRODUCTION

Breastfeeding is promoted as the preferred method of infant feeding and provides a number of advantages, including health, nutritional, immunological, developmental, psychological, social, economic, and environmental benefits.[1]

However, the evidence has been mixed regarding the association between breastfeeding and dental caries.[2–18] Several studies have reported an inverse association between breastfeeding and dental caries,[2–4] while other studies have failed to show any highly beneficial association.[5–13] Moreover, some studies have demonstrated that breastfeeding was associated with a higher prevalence of dental caries.[2,10,13–18] A US cross-sectional study in children aged 2 to 5 years showed that breastfeeding and its duration were not associated with an increased prevalence of early childhood caries.[5] In a retrospective cohort study of early childhood caries among infants aged 25 to 30 months in Myanmar, the prevalence of early childhood caries was greater in children who were breastfed more than twice at night, whereas there was no association between daytime breastfeeding habits and prevalence.[10]

These inconsistent and even contradictory results among studies are likely mostly due to methodological differences, such as the use of different cut-off points for breastfeeding, lack of adjustment for confounding factors, different definitions of dental caries, and the ages at which outcomes were assessed. Additional data are required to reach a conclusion concerning the association between breastfeeding and dental caries. This cross-sectional study investigated the association between breastfeeding and the prevalence of dental caries among young Japanese children.

METHODS

Study population

Data for the present study came from the Fukuoka Child Health Study (FCHS), a cross-sectional study of the association between various selected factors and child health problems, such as dental caries and allergic disorders.[19–22] In Japan, when children reach age 3 years, the municipality in

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which the family currently resides sponsors a physical examination that includes a dental examination, measurement of height and weight, and an interview with parents or guardians regarding the child's health. Eligible subjects for the present study were children aged 3 years who received this physical examination at any of the 7 public health centers offering it in Fukuoka City, a metropolitan area on Kyushu Island in southern Japan with a total population of approximately 1 414 000. During the period from June 2006 to January 2007, we were granted permission by the Fukuoka City government to provide our questionnaires directly to parents or guardians of children receiving the physical examination at age 3 years. Out of the 8269 eligible children, the parents or guardians of 8064 were provided with a structured self-administered questionnaire, a brief self-administered diet history questionnaire (4 pages), and a postage-paid, addressed, return envelope. The structured self-administered questionnaire consisted of 16 pages: 13 pages for the 68 questions and 3 pages for data transcription from the Maternal and Child Health Handbook. Ultimately, the parents or guardians of 2109 children answered the questionnaires and mailed these materials to the data management center (participation rate = 25.5%). Our research technicians conducted telephone interviews with individual participants when it was necessary to obtain missing data or clarify implausible responses. The current study was restricted to subjects who provided complete information concerning the variables under study; this left 2056 children available for analysis (24.9% of all eligible children). Permission to perform this study was obtained from the ethics committee of the Faculty of Medicine at Fukuoka University.

Outcome variable

During the physical examination, the presence of dental caries was assessed by visual examination without the use of radiographs. Dental examination data were recorded by a dentist in the Maternal and Child Health Handbook provided by the municipality during pregnancy, in which data pertaining to prenatal checkups, postnatal health conditions of both mother and baby, and growth of the child are recorded.[23] The first half of the handbook provides space for recording data on health condition and vaccination records, and the latter half provides information for mothers on pregnancy, delivery, and parenting. In our study, the parents or guardians of the children had to transcribe dental examination data from the Maternal and Child Health Handbook to our self-administered questionnaire. To facilitate the transcription of the oral examination data to the self-administered questionnaire, we used exactly the same format as that used for the records in the Maternal and Child Health Handbook, and parents or guardians transcribed all of the information, including symbols. Children were classified as having dental caries if 1 or more primary teeth had decayed, were missing, or had been filled.

In a sensitivity analysis, we also used data on dental caries gathered at 18 months of age, which were recorded by a dentist in the Maternal and Child Health Handbook when our subjects received their municipal physical examinations at age 18 months. These data were transcribed by parents or guardians from the Maternal and Child Health Handbook to our questionnaire.

Exposure variables and covariates

Information on breastfeeding duration in months was obtained from the structured self-administered questionnaire described above. Breastfeeding duration was the period during which the infants received breast milk, regardless of exclusivity. The questionnaire also included questions on the sex of the child, dental health practice (such as toothbrushing frequency, use of fluoride, and pattern of dental care), between-meal snack habits, maternal smoking during pregnancy, environmental tobacco smoke (ETS) exposure at home, and paternal and maternal educational levels. Use of fluoride was defined as positive if children reported using fluoride toothpaste or receiving topical application of fluoride gel at a health center or a dental clinic.

Data from the brief self-administered diet history questionnaire were not used in the current study.

Statistical analyses

Breastfeeding duration was classified into 4 categories (<6 months, 6–11 months, 12–17 months, and ≥18 months). The following variables were controlled for in the multivariable model: the sex of the child, toothbrushing frequency (<2 or ≥2 times/day), use of fluoride (yes or no), regular dental check-ups (yes or no), between-meal snack frequency (<1, 1, or ≥2 times/day), maternal smoking during pregnancy (yes or no), ETS exposure at home (yes or no), and paternal and maternal educational level (<13 years, 13–14 years, and ≥15 years). In the sensitivity analysis using data on dental caries at age 18 months, adjustment was made for child sex and parental educational levels only. Prevalence ratios (PRs) and 95% confidence intervals (CIs) were estimated using binomial regression with the log link function.[24] Linear trend of the association between duration of breastfeeding and dental caries was assessed using a log-binomial regression model, treating the categories of breastfeeding duration as consecutive integers. For tests of quadratic trend, we included linear and quadratic terms in the model. Two-sided P values less than 0.05 were considered to indicate statistical significance. All statistical analyses were performed using the SAS software package version 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

Of the 2056 children, 425 (20.7%) had dental caries. The mean number of dental caries was 0.70. Toothbrushing 2 or

Table 1. Prevalence ratios (PRs) and 95% confidence intervals for dental caries in relation to study variables (unadjusted analysis)

	Prevalence	Crude PR (95% CI)
Sex		
Female	184/969 (19.0%)	1.00
Male	241/1087 (22.2%)	1.17 (0.98–1.39)
Toothbrushing frequency (times/day)		
<2	252/1244 (20.3%)	1.00
≥2	173/812 (21.3%)	1.05 (0.89–1.25)
Use of fluoride		
No	54/318 (17.0%)	1.00
Yes	371/1738 (21.4%)	1.26 (0.97–1.63)
Regular dental check-ups		
No	301/1166 (25.8%)	1.00
Yes	124/890 (13.9%)	0.54 (0.45–0.65)
Between-meal snack frequency (times/day)		
<1	78/444 (17.6%)	1.00
1	128/754 (17.0%)	0.97 (0.75–1.25)
≥2	219/858 (25.5%)	1.45 (1.15–1.83)
Maternal smoking during pregnancy		
No	346/1787 (19.4%)	1.00
Yes	79/269 (29.4%)	1.52 (1.23–1.87)
ETS exposure at home		
No	205/1156 (17.7%)	1.00
Yes	220/900 (24.4%)	1.38 (1.16–1.63)
Paternal educational level (years)		
<13	145/565 (25.7%)	1.00
13–14	63/307 (20.5%)	0.80 (0.62–1.04)
≥15	217/1184 (18.3%)	0.71 (0.59–0.86)
Maternal educational level (years)		
<13	166/581 (28.6%)	1.00
13–14	151/823 (18.4%)	0.64 (0.53–0.78)
≥15	108/652 (16.6%)	0.58 (0.47–0.72)

ETS, environmental tobacco smoke.

more times per day was reported in about 40% of the subjects. Approximately 85% of children reported fluoride use, either as fluoride toothpaste or topical application of fluoride gel at a health center or a dental clinic. Approximately 44% of children received regular dental check-ups. More than 41% of subjects had between-meal snacks 2 or more times per day. In utero exposure to maternal smoking occurred in 13.1% of children, and 43.8% were exposed to ETS at home at least once. About 20% of children were breastfed for less than 6 months, whereas 27.2% were breastfed for 18 months or longer.

Table 1 shows the results of bivariate analyses for selected covariates. Regular dental check-ups were associated with a lower prevalence of dental caries. In contrast, 2 or more between-meal snacks per day was positively associated with dental caries. With regard to smoking, both maternal smoking during pregnancy and postnatal ETS exposure were associated with an increased prevalence of dental caries. Children with fathers who had more than 15 years of education and those with mothers who had 13 to 14 years, or more than 15 years, of education were less likely to have caries than children with fathers or mothers who had less than 13 years of education.

Table 2. Prevalence ratios (PRs) and 95% confidence intervals for dental caries according to duration of breastfeeding in 2056 children (FCHS, Japan)

	Prevalence	Crude PR (95% CI)	Adjusted PR (95% CI)[a]
Breastfeeding duration (months)			
<6	85/416 (20.4%)	1.00	1.00
6–11	74/498 (14.9%)	0.73 (0.55–0.97)	0.79 (0.60–1.05)
12–17	90/583 (15.4%)	0.76 (0.58–0.99)	0.86 (0.66–1.13)
≥18	176/559 (31.5%)	1.54 (1.23–1.93)	1.66 (1.33–2.06)
P for linear trend		<0.0001	<0.0001
P for quadratic trend		<0.0001	<0.0001

FCHS, Fukuoka Child Health Study.
[a]Adjusted for sex, toothbrushing frequency, use of fluoride, regular dental check-ups, between-meal snack frequency, maternal smoking during pregnancy, exposure to environmental tobacco smoke at home, and paternal and maternal educational levels.

Table 2 shows the PRs and their 95% CIs for the relationship between breastfeeding duration and the prevalence of dental caries. As compared with less than 6 months of breastfeeding, breastfeeding for 18 months or longer was significantly associated with a higher prevalence of dental caries. After adjustment for sex, toothbrushing frequency, use of fluoride, dental check-up history, between-meal snack frequency, maternal smoking during pregnancy, ETS exposure at home, and paternal and maternal educational levels, the positive association remained statistically significant (adjusted PR = 1.66, 95% CI: 1.33–2.06). A J-shaped relationship was observed between breastfeeding duration and dental caries: the lowest PR was among children breastfed for 6 to 11 months (adjusted PR = 0.79, 95% CI: 0.60–1.05) and the highest PR was for children breastfed for 18 months or longer (adjusted PR = 1.66, 95% CI: 1.33–2.06, *P* for linear trend <0.0001, *P* for quadratic trend <0.0001).

We also conducted a sensitivity analysis using dental caries at 18 months of age as the outcome variable. The prevalence of dental caries at age 18 months was 2.9% ($n = 59$). After adjustment for sex and paternal and maternal educational levels, breastfeeding duration was significantly and positively associated with dental caries in children at age 18 months. As compared with children breastfed for less than 6 months, the adjusted PRs for children breastfed for 6 to 11, 12 to 17, and 18 months or longer were 1.52 (95% CI: 0.45–5.17), 2.57 (95% CI: 0.85–7.82), and 6.45 (95% CI: 2.30–18.11), respectively (*P* for linear trend <0.0001).

DISCUSSION

The present study found that breastfeeding for 18 months or longer was significantly associated with a higher prevalence of dental caries. Our results partially agree with those of other studies showing an adverse effect of breastfeeding on dental caries[2,10,13–18] but are at variance with previous findings indicating a null or inverse association between breastfeeding and dental caries.[2–13] We observed a J-shaped relationship

between duration of breastfeeding and the prevalence of dental caries, ie, a nonsignificant inverse association was found between breastfeeding for 6 to 17 months and the prevalence of dental caries. Nevertheless, in a sensitivity analysis using data on dental caries at age 18 months, a nonsignificant positive association was observed between breastfeeding for 6 to 17 months and the prevalence of dental caries, while breastfeeding for 18 months or longer was significantly positively associated with the prevalence of dental caries. With regard to dental caries that had developed before age 18 months, the potential beneficial effects of breastfeeding for 6 to 17 months might not be detected. Alternatively, the results of the sensitivity analysis might have arisen by chance.

A systematic review suggested that breastfeeding for longer than 1 year, as well as nighttime breastfeeding after the eruption of teeth, is associated with some forms of early childhood caries, although the lack of methodological consistency and the inconsistent definitions of caries and breastfeeding used in previous studies make it difficult to draw definitive conclusions.[25] For example, some studies compared breastfeeding for 13 months or longer with a period shorter than 13 months.[7,16] Some studies investigated only the association between breastfeeding at night and dental caries.[6,10,18] One study compared outcomes according to whether breastfeeding was ever performed or never performed.[12] Inconsistency in the definition of dental caries, such as whether it comprises filled or missing teeth[16]; decayed, missing, or filled teeth[4–6,8,17]; 2 or more decayed, missing, or filled labial or palatal surfaces of primary incisors[11]; or cavitated, filled, or missing smooth surfaces in primary maxillary anterior teeth,[18] also reduces the comparability of the available studies. Moreover, many studies were unable to control for important confounding factors such as oral hygiene practices,[4,6–8,10,11,16,18] exposure to fluoride,[4–8,10,11,14,16,18] and socioeconomic status.[4,8,10–12,16,18]

We do not have a definitive explanation regarding the mechanisms that underlie our observations. Several minerals in breast milk, such as phosphate and calcium, help protect tooth enamel. The mineral composition of breast milk changes with advancing lactation, which may affect its cariogenic properties. In a longitudinal investigation, a significant decline was observed in the levels of phosphate and calcium in breast milk over time, with concentrations of phosphate in breast milk at 3, 6, and 26 weeks of lactation of 14.7, 12.7, and 10.7 mg/100 ml, respectively.[26] Corresponding values for calcium concentrations in breast milk during those time frames were 25.9, 27.7, and 24.8 mg/100 ml, respectively.[26] Another possible explanation is that the prophylactic effects of breast milk against dental caries (through the transfer of maternal protective elements such as immunoglobulins, lactoferrin, and casein[27] from mother to infant) decline due to the gradual depletion of these elements after prolonged lactation.[9] Alternatively, some unknown factors related to longer breastfeeding may have confounded the observed association. Mothers who breastfeed longer may be more likely to sleep with their child and breastfeed freely during the night.[17] A cross-sectional study of Brazilian preschool children showed that the prevalence of early childhood caries was higher in children who were breastfed at night after age 12 months than in children who stopped breastfeeding before age 12 months.[18]

Our study has several strengths. The study subjects were similar in age and geographic background, which likely reduced potential confounding produced by unmeasured factors related to age and geographic background. Data on dental caries were obtained by means of oral examinations by dentists, and we were able to control for a variety of potential confounding factors.

This study also has some limitations that should be considered. Of the 8269 eligible subjects in Fukuoka City, only 2057 (24.9%) were included in this analysis, so selection bias may be a factor. We were unable to assess differences between participants and nonparticipants because, except for age, no information on the personal characteristics of nonparticipants was available. Thus, our subjects cannot be considered representative of Japanese children in the general population, and the present findings should not be generalized. In fact, the educational levels of parents in the present study were higher than those of the general population. According to the 2000 population census of Japan, the proportions of men aged 35 to 39 years in Fukuoka City with less than 13 years, 13 to 14 years, 15 years or more, and unknown years of education were 39.6%, 8.0%, 43.3%, and 9.1%, respectively.[28] The corresponding figures among fathers in the present study were 27.5%, 14.9%, 57.6%, and 0.0%, respectively. The proportions of women 30 to 34 years of age in Fukuoka City with less than 13 years, 13 to 14 years, 15 years or more, and unknown years of education were 41.3%, 34.4%, 16.1%, and 8.3%, respectively.[28] The corresponding figures among mothers in the present study were 28.3%, 40.1%, 31.7%, and 0.0%, respectively. In addition, the prevalence of dental caries in the study population (20.7%) was lower than that in a sample of 3-year-old Japanese children assessed in a 2005 survey of dental diseases (24.4%).[29]

The data on dental caries used in the present study were gathered during routine examinations by a number of dentists at public health centers. The dentists were given detailed criteria for performing the examination but were not specifically trained so as to ensure standardization of their examinations. The unstandardized nature of the examinations could lead to nondifferential misclassification of caries and thus bias the results toward the null, that is, toward a lack of association between breastfeeding and early childhood caries. Moreover, because parents or guardians of the children transcribed the data gathered at the dental examinations from their Maternal and Child Health Handbook to our self-administered questionnaire, we cannot exclude the possibility

that transcription errors occurred. Nevertheless, misclassification of outcome is unlikely to differ across categories of breastfeeding status. If this had occurred, the consequence would have been an underestimation of values in our results. Breastfeeding duration was assessed 3 years after the birth of the children, and this delay could have led to recall bias. Most questionnaires, however, were completed by the child's mother, and it is unlikely that a mother would not remember the breastfeeding history of her child. In a validation study, breastfeeding duration reported by the mother has been shown to be accurate for 20 years or even longer after the birth of a child.[30] Even if errors in measurement of breastfeeding duration occurred, misclassification is unlikely to differ across exposure categories. If this had occurred, the results would be biased toward the null.

Although we have adjusted our analyses for numerous potential confounders, such as toothbrushing frequency, use of fluoride, and parental educational levels, residual confounding effects cannot be ruled out. Additionally, it is possible that our results remain confounded by other potentially important factors such as frequency of bedtime breastfeeding, age at introduction of foods and fluids other than breast milk, and dietary intake (eg, sugar intake). Our study design was cross-sectional, so the results of this analysis should not be interpreted as providing evidence of a cause-effect relationship between breastfeeding and dental caries.

In conclusion, we found a J-shaped association between breastfeeding duration and the prevalence of dental caries among young Japanese children. Our findings indicate that breastfeeding for 18 months or longer is associated with a higher prevalence of dental caries. From the perspective of avoiding any harmful impacts of breastfeeding on infant dental health, the ideal duration of breastfeeding might be shorter than 18 months, given that a nonsignificant inverse association was observed between breastfeeding for 6 to 17 months and the prevalence of dental caries. Further, prospective, studies with clearly defined breastfeeding variables (eg, exclusive breastfeeding duration and breastfeeding frequency) that also account for confounding factors need to be undertaken before a definitive conclusion can be reached.

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Association of Long-Duration Breastfeeding and Dental Caries Estimated with Marginal Structural Models

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Abstract

Purpose—Estimate the association between breastfeeding ≥24 months and severe early childhood caries (ECC).

Methods—Within a birth cohort (n=715) from low-income families in Porto Alegre, Brazil, the age 38-month prevalence of severe-ECC (≥4 affected tooth surfaces or ≥1 affected maxillary anterior teeth) was compared over breastfeeding duration categories using marginal structural models to account for time-dependent confounding by other feeding habits and child growth. Additional analyses assessed whether daily breastfeeding frequency modified the association of breastfeeding duration and severe-ECC. Multiple imputation and censoring weights were used to address incomplete covariate information and missing outcomes, respectively. Confidence intervals (CI) were estimated using bootstrap re-sampling.

Results—Breastfeeding ≥24 months was associated with the highest adjusted population-average severe-ECC prevalence (0.45, 95% CI: 0.36, 0.54) compared with breastfeeding <6 months (0.22, 95% CI: 0.15, 0.28), 6–11 months (0.38, 95% CI: 0.25, 0.53), or 12–23 months (0.39, 95% CI: 0.20, 0.56). High frequency breastfeeding enhanced the association between long-duration breastfeeding and caries (excess prevalence due to interaction: 0.13, 80% CI: −0.03, 0.30).

Conclusions—In this population, breastfeeding ≥24 months, particularly if frequent, was associated with severe-ECC. Dental health should be one consideration, among many, in evaluating health outcomes associated with breastfeeding ≥24 months.

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Keywords

breastfeeding; dental caries; epidemiologic methods; feeding behavior; marginal structural models; prospective studies

Introduction

The World Health Organization (WHO) recommends continued breastfeeding up to age 2 years or beyond [1], and failure to breastfeed is associated with poor health consequences for both mother and child [2,3]. However, the nature of the relationship between dental caries and the age to which children are breastfed remains uncertain. Caries is among the most common diseases worldwide and often goes untreated, particularly in low-resource settings [4–6], with negative quality of life implications [7]. Some laboratory models suggest that human milk can cause caries [8,9], particularly in combination with added sugars [10], while some report no demineralization of tooth material by human milk alone [11]. The epidemiological literature [12] includes studies that support a positive association between long-duration breastfeeding and early childhood caries (ECC) [13–16] and others that do not [17,18].

Breastfeeding timing relative to other feeding habits complicates study of breastfeeding duration and ECC. Early breastfeeding cessation might accelerate the introduction of particular foods [19,20], and the foods consumed early in life likely influence caries development [21–23]. In turn, early-life food experiences might also influence the duration to which a breastfeeding child continues nursing [19]. Regression modeling is problematic in the presence of such time-dependent confounding, in which a variable (e.g. early-life food experiences) can be part of a causal pathway between an earlier aspect of exposure (e.g. early breastfeeding) and the outcome, while simultaneously operating as confounder with respect to a later aspect of exposure (e.g. continued breastfeeding). Marginal structural models (MSMs), in contrast, have been used to make causal inference from observational data in the presence of time-varying covariates [24–28]. Such techniques are particularly relevant for exposures, such as breastfeeding, that cannot be easily assigned as a randomized intervention.

We aimed to estimate the association between long-duration breastfeeding (≥24 months) and severe-ECC (S-ECC) in a birth cohort of urban, low-income Brazilian children. We hypothesized that long-duration breastfeeding is associated with greater caries occurrence. We secondarily hypothesized that the association between long-duration breastfeeding and S-ECC is stronger if daily breastfeeding episodes are more frequent.

Methods

Participants

We followed a birth cohort nested in a cluster-randomized trial in Porto Alegre, Brazil. The community water supply is optimally fluoridated [29], and 52 public healthcare centers provide primary medical services predominantly to low-income residents. A stratified

random sample (n=20 health centers) was selected from 31 eligible clinics for participation in the original trial of healthcare worker training [30,31].

In 2008, 715 of 736 eligible pregnant women with appointments at participating clinics agreed to enroll their children in a cohort to track health outcomes (Figure 1). The trial had provided intervention clinics with healthcare worker training that promoted healthful infant complementary feeding for incorporation into maternal counseling. After 3 years, the intervention did not extend the total duration of breastfeeding (hazard ratio for breastfeeding cessation: 0.94, 95% confidence interval: 0.79, 1.11), although the mean duration of exclusive breastfeeding was increased [30]. S-ECC was not lowered significantly among children born to intervention group clinic attendees [31].

Baseline variables

Trained fieldworkers collected baseline (during pregnancy) socio-demographic information via structured questionnaires. Data included maternal age, household size, maternal education (≤8 years), maternal smoking (current vs. never/former smoker), indoor bathroom (yes/no), city region (indicators for eight geo-administrative districts), parity (first child yes/no), maternal partner status (married or partnered vs. single, separated, or widowed), household income (≤1500 Brazilian reais monthly; approximately 900 US dollars in 2008), outside income source (e.g. government support), social class (Brazilian Association of Economic Research Institutes classification ≤C), low body mass index (BMI) (≤18, based on measured height and self-reported pre-pregnancy weight). Child sex and birth date were collected at age 5–9 months.

Time-varying behaviors and anthropometry

Infant growth and feeding habits were recorded at each of three home visits, corresponding to mean ages of 6 months (range: 5–9), 12 months (range: 11–15), and 38 months (range: 31–46). Infant length and child height were collected following standard protocol and converted to height/length-for-age Z-scores using WHO standards [32]. At each visit, mothers were asked whether they had ever breastfed and whether they were currently breastfeeding. Breastfeeding duration represented the age to which any breastfeeding continued, regardless of complementary feeding. Breastfeeding mothers were asked how frequently they nursed daily (0, 1, 2–3, or "many times," separately for day and night). Mothers no longer breastfeeding were asked at what age (in months) breastfeeding ceased.

At the 6-month assessment, the number of feeding bottles consumed in the preceding day was recorded (later categorized 0, 1–3, ≥4). Sugar in the bottle corresponded to consuming ≥1 bottle containing any sweet additive: table sugar, powdered or liquid artificial chocolate, soft drinks, or powdered artificial juice. Questionnaires addressed use of commercially prepared infant formula and the age of introduction of 32 specific foods (e.g. fruits, beans, soft drinks, candies). At the 12-month assessment, the questionnaire posed whether 29 specific items were consumed in the previous month and the weekly consumption frequency of 5 complementary foods (fruits, vegetables, beans, meats, organ meats). Two feeding indices measured dietary patterns to account for foods consumed in combination and to increase the efficiency of the analysis [33]. The indices were created specifically for this

analysis due to a lack of existing diet indices specific to cariogenic feeding behaviors in comparable populations. The first, referred to here as the food introduction index, was the count of low nutrient-density and/or presumably cariogenic foods introduced before age 6 months: added sugar, candy, chips, chocolate, chocolate milk, cookies, fruit-flavored drink, gelatin, honey, ice cream, soft drinks, and sweet biscuits. The second, termed here as the first-year feeding index, summed the food introduction index with the count of the following foods recorded at the 12-month assessment: added sugar in a drink, candy, cake, chips, chocolate, chocolate milk, cookies, creamed caramel, fruit-flavored drink, gelatin, honey, ice cream, other confection, soft drinks, and sweet biscuits.

At the 38-month assessment, data were collected regarding bottle use, height-for-age Z-scores, and tooth brushing with fluoride dentifrice. While these variables are likely associated with S-ECC, we did not consider them confounders, because our cut-point for defining the exposure (breastfeeding ≥24 months) temporally preceded these measures. However, we estimated separate models that included these variables as a sensitivity check.

Dental caries

Dental status was evaluated at 38 months following WHO protocol [34], with non-cavitated (white-spot) lesions also recorded. Assessments took place in participants' homes, aided by a lighted intraoral mirror. Teeth were brushed and dried with gauze. Severe-ECC was defined as ≥1 affected maxillary anterior teeth or ≥4 decayed, missing due to caries, or filled tooth surfaces (dmfs ≥4) [35]. Two dentist-examiners completed the evaluations following identical protocol (inter-rater unweighted kappa=0.75; intra-rater unweighted kappa=0.83 for both examiners).

Statistical methods

The proportion of children with S-ECC was compared across four breastfeeding duration categories: <6 months, 6–11 months, 12–23 months, and ≥24 months. Three marginal structural models were fit. The weights for estimating unadjusted models incorporated only clinic allocation status to account for the nested study design. Adjusted models additionally accounted for baseline socio-demographic variables: maternal age, education, parity, pre-pregnancy BMI, smoking status, social class, and child age and sex. Fully-adjusted models included those variables, as well as time-varying bottle use, feeding habits, and length-for-age Z-scores (see below).

In estimating MSMs, inverse probability weighting was used to generate a “pseudo-population” representative of a hypothetical population in which breastfeeding duration (A) had been allocated independently of confounding variables. Weights were assigned inversely to the predicted probability of observed exposure, given baseline characteristics (W) and longitudinally recorded variables (L_t), giving the greatest weight to observations with exposure and confounder combinations least represented in the sample, relative to what would have been observed under random exposure allocation. Figure 2 depicts the assumed relationships among variables as a directed acyclic graph. Exposure probabilities were estimated using Super Learner, a data-adaptive machine-learning tool [36].

To account for time-dependent confounding, weights were based on treatment models for three probabilities: the probability of breastfeeding at 6 months (Pr[A_6=1]); the probability of breastfeeding at 12 months, given breastfeeding at 6 months (Pr[A_{12}=1 | A_6=1]); and the probability of breastfeeding at 24 months, given breastfeeding at 12 months (Pr[A_{24}=1 | A_{12}=1]). Each treatment model was estimated while incorporating temporally appropriate putative confounders: in fully-adjusted models, the 6-month treatment model included clinic allocation status and baseline socio-demographic variables only; the 12-month treatment model included these variables and added the 6-month bottle use variables, formula use, food introduction index, and 6-month length-for-age Z-scores; the 24-month treatment model replaced the food introduction index and 6-month Z-scores with the first-year feeding index and 12-month Z-scores, respectively, and added complementary food frequency. To stabilize the weights, we multiplied by the marginal probability of the observed exposure category [25].

Equation 1 gives the stabilized treatment weights, where indicators (I) take a value of 1 when the exposure category was observed and 0 otherwise.

$$
\begin{aligned}
SW^{T} - & \frac{\Pr[A<6months]I[A<6months]}{\Pr[A_6-1|W]} \\
& + \frac{\Pr[A-6-11months]I[A-6-11months]}{\Pr[A_6-1|W]\Pr[A_{12}-1|A_6-1,W,L_6]} \\
& + \frac{\Pr[A-12-23months]I[A-12-23months]}{\Pr[A_6-1|W]\Pr[A_{12}-1|A_6-1,W,L_6]\Pr[A_{24}-0|A_{12}-1,W,L_6,L_{12}]} \\
& + \frac{\Pr[A\geq 24months]I[A\geq 24months]}{\Pr[A_6-1|W]\Pr[A_{12}-1|A_6-0,W,L_6]\Pr[A_{24}-1|A_{12}-1,W,L_6,L_{12}]}
\end{aligned}
\tag{1}
$$

For each model, missing variables and missing or incomplete breastfeeding histories were multiply imputed from probabilities estimated using Super Learner, corresponding to 2.6% of data among children with observed outcomes. Censoring weights, equal to the inverse probability of having an observed outcome, given exposure and covariates, up-weighted observations most resembling those with missing outcomes. The probability of an observed outcome (Pr[C=0]) was estimated via Super Learner using predictor variables clinic allocation status, maternal age, education, partner status, parity, smoking in pregnancy, and pre-pregnancy BMI, household income, indoor bathroom, number of inhabitants, outside income source, city region, and social class, and child breastfeeding duration, first-year feeding index, height-for-age Z-score, and sex. Stabilized censoring weight numerators were the product of the probability of having outcome data, given breastfeeding duration category, and a 1/0 indicator for having outcome data (equation 2).

$$
SW^{C} = \frac{\Pr[C=0|A]I[C=0]}{\Pr[C=0|A,W,L_t]} \tag{2}
$$

Final MSM weights were the product of stabilized treatment weights and stabilized censoring weights: $SW = SW^{T} \times SW^{C}$. In the fully-adjusted model, non-zero stabilized weights had a mean of 0.997 (minimum: 0.28, maximum: 8.90).

For each model, point estimates (prevalence ratio, PR; prevalence difference, PD) were averaged over 200 multiple imputations. Percentile-based 95% confidence intervals (CI) were estimated as the 2.5 and 97.5 quantiles from 5000 bootstrap iterations to account for variance from sampling, imputation, and weighting. For comparison, an analogous complete-case regression analysis was completed using log-linear models and robust variance. Analyses were completed in R version 3.0.1 (http://www.r-project.org).

Secondarily, we examined whether frequent daytime breastfeeding (≥4 daily episodes) intensified the association of breastfeeding duration and S-ECC, restricting the analysis to children breastfed ≥6 months, the earliest age at which frequency data were collected. High frequency daytime breastfeeding and a long duration-high frequency interaction term were included as MSM covariates, and frequent breastfeeding was added to the treatment models. We defined the excess prevalence due to interaction (EPI) as a departure from additivity, following an example proposed for the relative risk [37]. If D and F represent the presence of long-duration and high frequency breastfeeding, respectively, and $\bar{D}$ and $\bar{F}$ the absence of these factors, then the EPI = PD(DF) – PD($D\bar{F}$) – PD($\bar{D}F$). As a sensitivity check, we also estimated models in which frequency strata were defined by high frequency breastfeeding in either the day or night, versus high frequency breastfeeding in neither. Nighttime breastfeeding was not used alone to define strata, because high frequency daytime breastfeeding was common (>50%) when nighttime high frequency breastfeeding was absent. Because tests for statistical interaction may have low power [38], 80% confidence intervals were provided.

Ethics

This study proposal received ethical approval from committees at the Federal University of Health Sciences of Porto Alegre (UFCSPA) and the University of California Berkeley and is in accordance with the Declaration of Helsinki. Informed consent was reached with mothers on behalf of their children. Children with caries or suspected anemia, under-nutrition, or overweight status were referred to their local health center.

Results

Table 1 demonstrates selected characteristics of the study population. The fraction of mothers interviewed at the 6-month assessment to report initiating any breastfeeding was 0.99 (627/633); the fraction who breastfed to 12 months was 0.47 (282/598). Exclusive breastfeeding continued to mean age 2.1 months. Nearly half the children were introduced to commercially prepared infant formula by 6 months (0.49, 309/632), but few children used formula at 12 months (0.03, 18/539). Bottle use and soft drink consumption were common, while inadequate length-for-age was rare (Table 1). S-ECC prevalence at 38 months was 0.34 (157/458); the prevalence of at least one affected tooth was 0.55 (250/458). Caries was most common among children breastfed for ≥24 months (Figure 3).

The highest S-ECC prevalence was associated with breastfeeding ≥24 months in all marginal structural models (Table 2). As a sensitivity check, including 38-month variables (bottle use, fluoride toothpaste, height-for-age) in the fully adjusted model did not

appreciably alter estimates; for example, the prevalence ratio comparing breastfeeding ≥24 months to <6mo changed to 2.11 (95% CI: 1.50, 3.30) from 2.10 (95% CI: 1.50, 3.25).

Compared to breastfeeding 6–23 months, breastfeeding ≥24 months was associated with elevated S-ECC prevalence, although not statistically significant (unadjusted PR: 1.31, 95% CI: 0.97, 1.79; adjusted PR: 1.22, 95% CI: 0.89, 1.66; fully-adjusted PR: 1.17, 95% CI: 0.85, 1.78). However, breastfeeding ≥24 months was more strongly associated with S-ECC when daytime breastfeeding was frequent (fully-adjusted PR: 1.38, 95% CI: 0.97, 2.16) (Table 3). The EPI was 0.13 (80% CI: −0.03, 0.30), suggesting positive interaction between frequent daytime nursing and breastfeeding ≥24 months. Results were similar defining high frequency based on frequent nursing in either the day or night versus neither day or night (fully-adjusted PR with frequent breastfeeding: 1.43, 95% CI: 1.01, 2.18); the EPI increased to 0.23 (80% CI: 0.03, 0.41).

Complete-case regression analysis yielded modest differences in estimates (Table 4). However, findings were qualitatively consistent with MSM results, supporting a positive association between S-ECC and breastfeeding ≥24 months.

Discussion

In this population of low-income Brazilian families, we estimated an increase in severe early childhood caries prevalence with breastfeeding 24 months or beyond. While the overall health benefits of breastfeeding are considerable, this work adds evidence that, in some contexts, very extended and frequent breastfeeding might increase caries risk. In addition to exposing teeth to bacterially fermentable milk sugars, prolonged breastfeeding might enhance the fidelity with which caries-causing oral bacteria are transmitted from mothers [39].

Several investigations have reported positive associations between breastfeeding duration and caries when using cut-points exceeding 18 months to define the uppermost duration category [13–15,40,41]. Studies that reported no association between caries and breastfeeding generally used earlier cut-points to define long-duration breastfeeding (i.e. ≥8 or ≥13 months) and have featured populations in which breastfeeding to age 2 years is uncommon, such as in Germany [42], Italy [17], and the United States [18]. A large hospital-based breastfeeding promotion intervention in Belarus did not affect caries prevalence at age 6 years [43]; however, the study did not directly compare caries outcomes among children who were or were not breastfed for extended durations (e.g. ≥24 months), which was an uncommon behavior in that trial population.

Daily breastfeeding frequency was associated with S-ECC in a previous study of Brazilian preschoolers, in which breastfeeding frequency, but not duration ≥12 months, maintained statistical significance in multi-variable models [21]. A combined measure of breastfeeding duration and frequency was strongly associated with ECC in Mayanmar [44], and a measure of nighttime breastfeeding burden, which was based on frequency, was positively associated with ECC in Iran [45], although not statistically significant.

An important strength of this study was the longitudinal, prospective collection of feeding information. We use weighting estimators to respect the temporal sequence of exposure and covariate information, accounting for time-dependent confounding by early-life feeding habits [46]. These methods have not been broadly used in oral health epidemiology.

Causal interpretation of marginal structural model results depends on unverifiable assumptions, specifically, positivity, exchangeability, and correct specification of the treatment models used to generate the weights [25,28]. In this analysis, the distribution of the weights was not extreme, important socioeconomic and nutritional confounders were prospectively collected, and Super Learner estimation reduced the reliance on parametric model-building assumptions [36]. These strengths give credence to causal interpretations, however, as with any observational study, residual confounding cannot be ruled out. For instance, our main estimates do not adjust for earlier oral hygiene habits. However, adjustment for 38-month toothbrushing habits did not affect estimates, and we have no evidence that oral hygiene habits would be associated with breastfeeding duration. Also, while we adjust for the age of introduction and weekly frequency of selected foods, not every aspect of the diet was recorded. Conservatively, we consider our estimates to represent meaningful associations, for which definitive causal claims await confirmation from future studies.

Losses to follow-up were relatively high but not unusual among cohort studies in low-resource settings, where participants frequently change address and contact information. Inverse probability censoring weights can account for losses, and, in this study, weighted estimates were similar to those from complete-case regression. However, losses remain a limitation, as strategies to account for missing data introduce additional assumptions [47]. EPI estimates were imprecise, because a relatively small number of children breastfed both infrequently and to long durations limited the statistical power to confirm interactions. Finally, this study population, featuring a relatively high prevalence of breastfeeding and of caries, might not be representative of the breastfeeding-caries relationship in all historical, geographical, and socioeconomic contexts.

A critical question raised by our findings is why breastfeeding, a normative and otherwise health-promoting human behavior, would be associated with deleterious dental outcomes. One possibility is that the mechanism through which repeated, prolonged exposure to human milk could enhance caries progression might operate differently under the near universal availability of highly refined sugars in the modern diet versus historically. Laboratory studies demonstrating greater cariogenic potential of human milk with the addition of outside sugars support this hypothesis [10,11]. Future research is recommended to better define the relationship between particular breastfeeding practices and dental caries in the context of a high-sugar food supply. Our results in no way suggest that breastfeeding itself be discouraged but are congruent with guidelines from professional dental organizations, which recommend supporting a mother's decision to breastfeed but avoiding *ad libitum* breastfeeding after tooth eruption [48].

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Abbreviations

BMI	body mass index
CI	confidence interval
ECC	early childhood caries
EPI	excess prevalence due to interaction
MSM	marginal structural model
PD	prevalence difference
PR	prevalence ratio
S-ECC	severe early childhood caries

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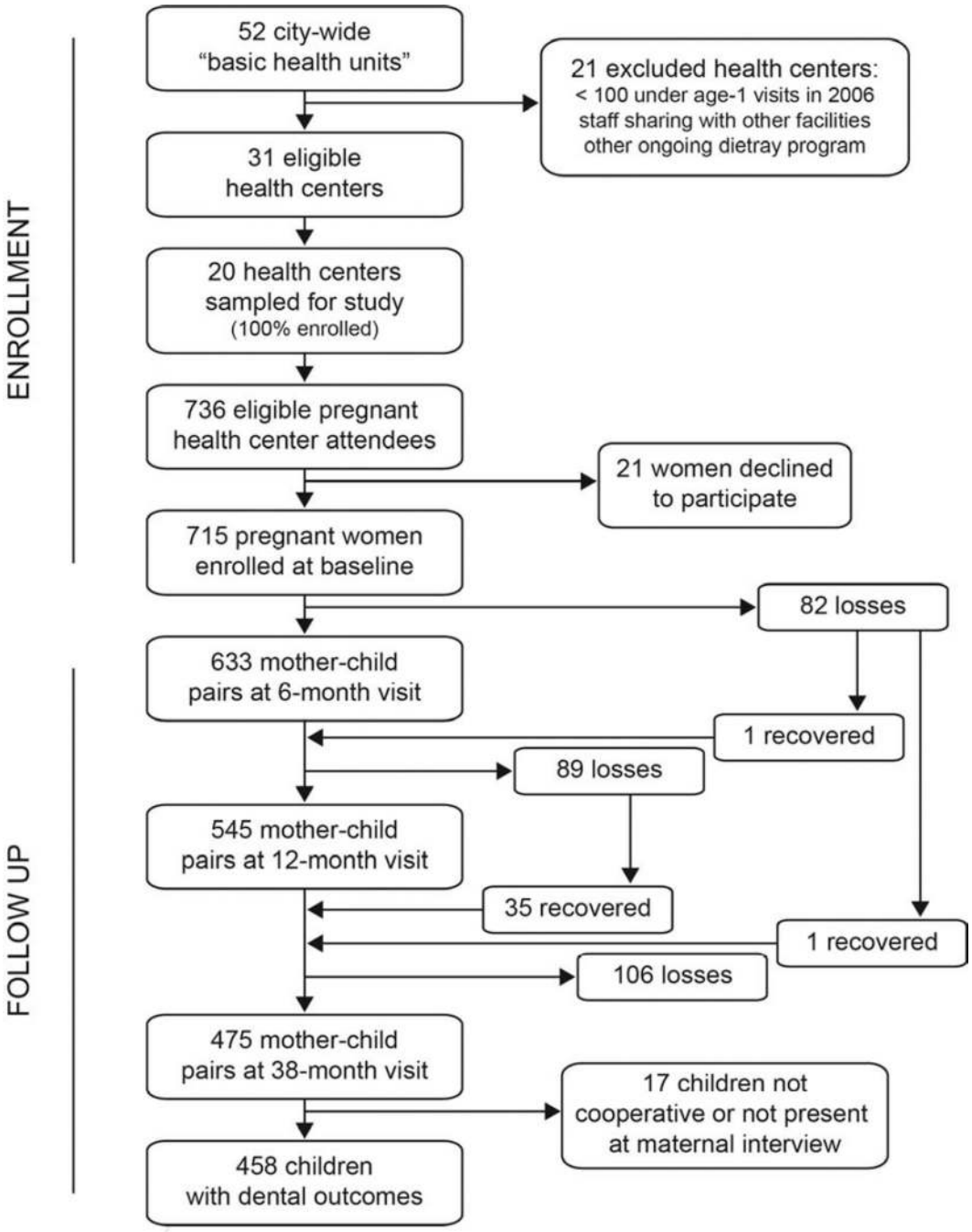

Figure 1. Flow of Participants

Pregnant women were recruited from 20 municipal health centers in the city of Porto Alegre, Brazil and followed to a mean child age of 38 months.

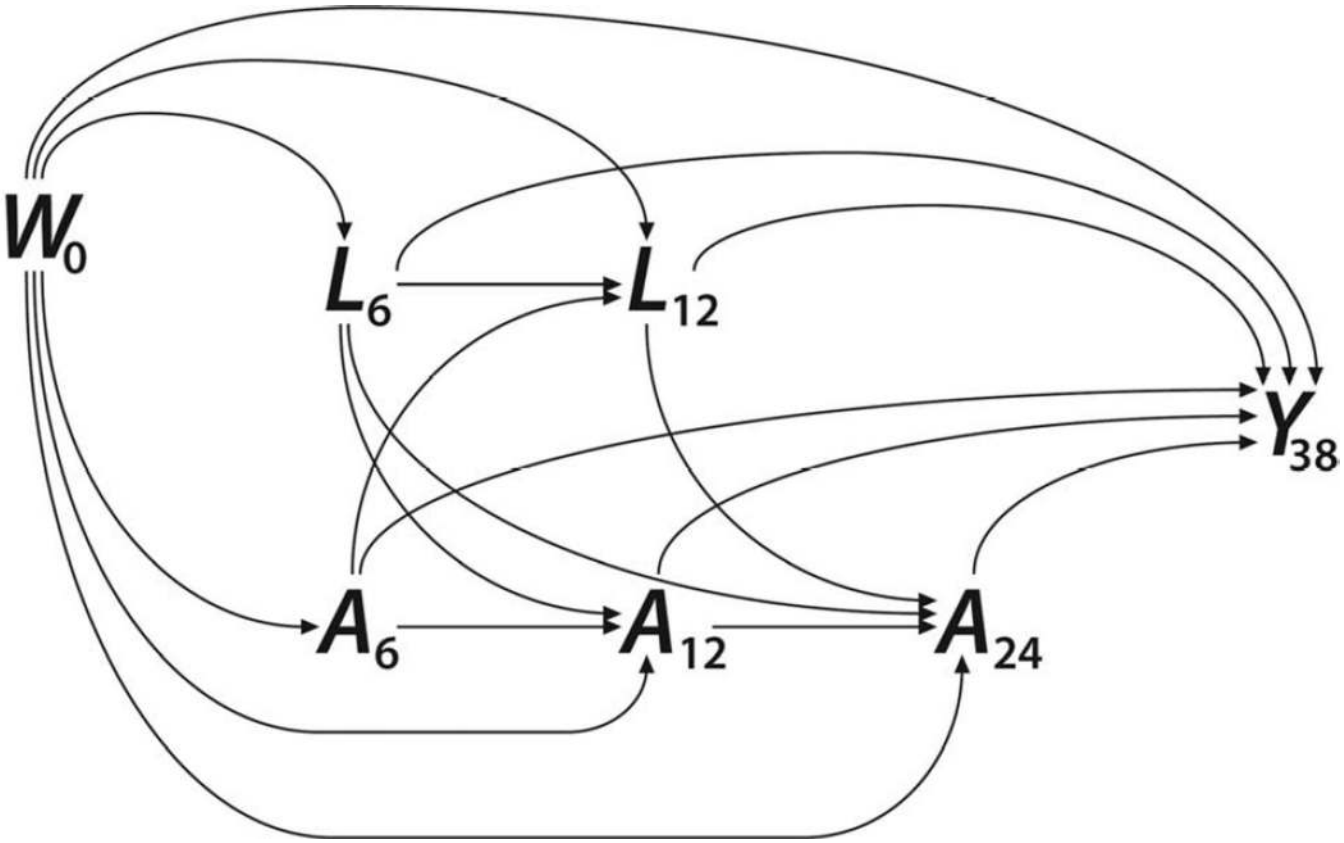

Figure 2. Directed acyclic graph

The graph depicts the assumed relationships between study variables. W_0 = baseline socio-demographic characteristics; A_t = breastfeeding at time t months; L_t = time-varying feeding behaviors and anthropometry at time t months; Y_{38} = severe early childhood caries at 38-months. Time-dependent confounding occurs through L_{12} variables, which are part of a directed path from A_6 to Y_{38} but a back-door path from A_{24} to Y_{38}.

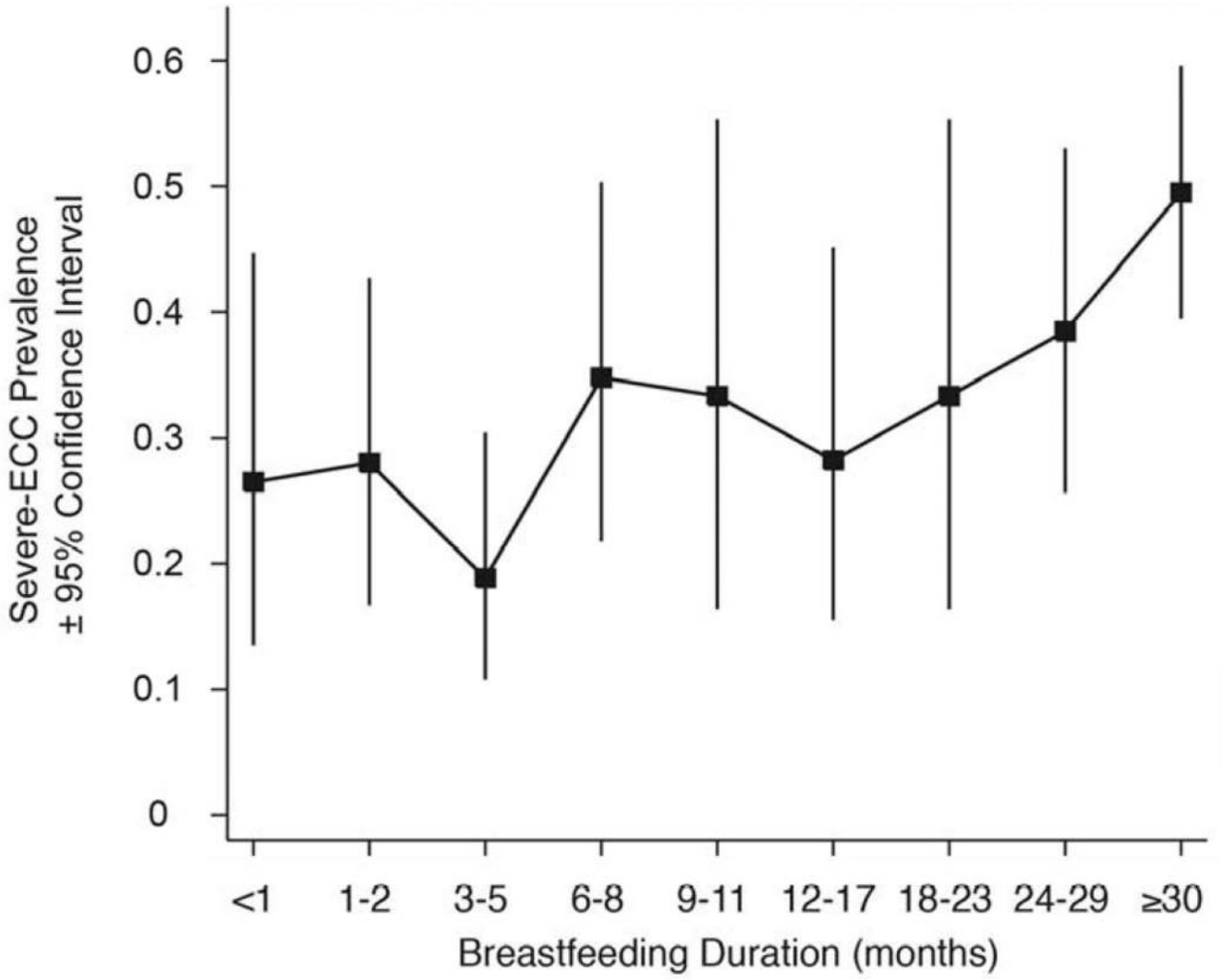

Figure 3. Observed Prevalence of Severe Early Childhood Caries at Age 38 Months by Categories of Breastfeeding Duration

The unadjusted (crude) prevalence of severe early childhood caries by categories of breastfeeding duration is shown for the 439 children with complete observed data for both breastfeeding duration and dental health.

Table 1

Characteristics of Participants

Characteristic		Number of observations[1]
Socio-demographic characteristics		
Maternal age at expected delivery date, mean (SD), years	26.0 (6.7)	715
Mother has ≤8 y of formal education, n (%)	340 (47.6)	715
Household income ≤3 times minimum salary[2], n (%)	565 (81.9)	690
Social class C or lower by ABIPEME index[3], n (%)	569 (79.8)	713
Self-identified maternal race white, n (%)	395 (55.2)	715
Self-identified maternal race black, mixed, or other, n (%)	320 (44.8)	715
Male child, n (%)	333 (52.4)	635
Anthropometry		
Length-for-age Z-score at age 5–9 months, mean (SD)	−0.13 (1.2)	631
Length-for-age Z-score <−2 at age 5–9 months, n (%)	31 (4.9)	631
Length-for-age Z-score at age 11–15 months, mean (SD)	−0.03 (0.9)	527
Length-for-age Z-score <−2 at age 11–15 months, n (%)	4 (0.8)	527
Feeding Habits		
Introduced to soft drinks before age 6 months, n (%)	192 (30.3)	633
Introduced to any sweets before age 6 months, n (%)	557 (90.8)	613
Consumed soft drinks in prior month at age 11–15 months, n (%)	413 (76.6)	539
Consumed vegetables ≥4 days per week at age 11–15 months, n (%)	341 (63.5)	537
Ever initiated breastfeeding	627 (98.9)	633
Duration exclusive breastfeeding, mean (SD), months	2.1 (1.6)	633
Exclusive breastfeeding to age ≥4 months, n (%)	152 (24.0)	633
Breastfeeding duration to age <6 months, n (%)	216 (34.1)	633
Breastfeeding duration to age 6–11 months, n (%)	100 (16.7)	598
Breastfeeding duration to age 12–23 months, n (%)	65 (12.1)	537
Breastfeeding duration to age ≥24 months, n (%)	156 (29.1)	537
Consuming sweet substances in bottle at age 5–9 months, n (%)	198 (32.3)	614
Consuming sweet substances in bottle at age 2–3 years, n (%)	312 (68.4)	456
Dental Caries Experience at age 2–3 years		
Any affected tooth, n (%)	250 (54.6)	458
S-ECC, n (%)	157 (34.3)	458
dmfs (any decay), mean (SD)	3.2 (6.1)	458
dmfs (cavitated decay only), mean (SD)	2.6 (5.9)	458

Abbreviations: SD, standard deviation; ABIPEME, Brazilian Association of Economic Research Institutes; S-ECC, severe early childhood caries; dmfs, decayed missing filled surfaces index

[1] Number of observations differ for some variables due to missing data and/or losses to follow-up

[2] Monthly income of ≤1500 Brazilian reais; approximately 900 US dollars in 2008

[3] Socioeconomic classification scale based on material possessions and education, A = highest status, E = lowest status

Table 2

Unadjusted and Adjusted Associations of Breastfeeding Duration and Severe Early Childhood Caries in Preschoolers

	Marginal Prevalence[1]	95% CI	Prevalence Ratio	95% CI	Prevalence Difference	95% CI
Breastfeeding Duration						
Unadjusted[2] Model						
<6 months (reference)	0.23	0.16, 0.30	1		0	
6–11 months	0.38	0.27, 0.50	1.66	1.06, 2.57	0.15	0.02, 0.29
12–23 months	0.31	0.20, 0.43	1.35	0.81, 2.16	0.08	−0.05, 0.22
≥24 months	0.45	0.38, 0.53	1.98	1.44, 2.87	0.22	0.12, 0.33
Adjusted[3] Model						
<6 months (reference)	0.22	0.15, 0.28	1		0	
6–11 months	0.39	0.27, 0.53	1.79	1.13, 2.80	0.17	0.03, 0.32
12–23 months	0.35	0.22, 0.49	1.59	0.97, 2.65	0.12	−0.01, 0.29
≥24 months	0.45	0.38, 0.53	2.06	1.51, 3.00	0.23	0.14, 0.34
Fully-Adjusted[4] Model						
<6 months (reference)	0.22	0.15, 0.28	1		0	
6–11 months	0.38	0.25, 0.53	1.77	1.12, 2.85	0.17	0.03, 0.32
12–23 months	0.39	0.20, 0.56	1.82	0.85, 3.20	0.18	−0.03, 0.42
≥24 months	0.45	0.36, 0.54	2.10	1.50, 3.25	0.24	0.13, 0.36

Abbreviation: CI, confidence interval

[1] Population-average prevalence of severe early childhood caries at given categories of breastfeeding duration, as estimated from marginal structural models

[2] Includes allocation status from nesting intervention study only

[3] Includes allocation status from nesting intervention study and maternal age, education, parity, pre-pregnancy BMI, smoking status, social class, and child age and sex

[4] Includes all adjusted model variables and time-varying bottle use variables, feeding habits, and height-for-age Z-scores

Table 3

Unadjusted and Adjusted Associations of Breastfeeding ≥24 Months and Severe Early Childhood Caries, Stratified by Frequency of Daytime Breastfeeding

	Marginal Prevalence[1]	95% CI	Prevalence Ratio	95% CI	EPI	80% CI
Breastfeeding Duration and Frequency						
Unadjusted[2] Model						
Duration 6–23 months and low frequency	0.38	0.25, 0.51	1			
Duration ≥24 months and low frequency	0.37	0.22, 0.52	0.97	0.53, 1.68		
Duration 6–23 months and high frequency	0.31	0.22, 0.42	1			
Duration ≥24 months and high frequency	0.48	0.39, 0.57	1.53	1.06, 2.30	0.18	–0.07, 0.43
Adjusted[3] Model						
Duration 6–23 months and low frequency	0.38	0.25, 0.51	1			
Duration ≥24 months and low frequency	0.36	0.20, 0.53	0.94	0.51, 1.67		
Duration 6–23 months and high frequency	0.33	0.23, 0.44	1			
Duration ≥24 months and high frequency	0.47	0.38, 0.56	1.42	0.99, 2.12	0.16	–0.10, 0.41
Fully-Adjusted[4] Model						
Duration 6–23 months and low frequency	0.36	0.24, 0.49	1			
Duration ≥24 months and low frequency	0.37	0.20, 0.55	1.01	0.52, 1.81		
Duration 6–23 months and high frequency	0.35	0.23, 0.45	1			
Duration ≥24 months and high frequency	0.48	0.38, 0.58	1.38	0.97, 2.16	0.13	–0.03, 0.30

Abbreviations: CI, confidence interval; EPI, excess prevalence due to interaction

[1] Population-average prevalence of severe early childhood caries at given categories of breastfeeding duration, as estimated from marginal structural models

[2] Includes allocation status from nesting intervention study only

[3] Includes allocation status from nesting intervention study and maternal age, education, parity, pre-pregnancy BMI, smoking status, social class, and child age and sex

[4] Includes all adjusted model variables and time-varying bottle use variables, feeding habits, and height-for-age Z-scores

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Table 4

Unadjusted and Adjusted Associations from Regression Models of Breastfeeding Duration and Severe Early Childhood Caries

	Unadjusted Model n = 439		Adjusted Model n = 422		Fully Adjusted Model n = 338	
Model Variables	**Prevalence Ratio**	**95% CI**	**Prevalence Ratio**	**95% CI**	**Prevalence Ratio**	**95% CI**
Breastfeeding <6 months (reference)	1		1		1	
Breastfeeding 6–11 months	1.45	0.93, 2.23	1.52	0.99, 2.33	1.45	0.83, 2.53
Breastfeeding 12–23 months	1.28	0.80, 2.05	1.44	0.89, 2.32	1.39	0.73, 2.64
Breastfeeding ≥24 months	1.96	1.40, 2.73	2.04	1.45, 2.85	1.85	1.11, 3.08
Clinic allocation (intervention)	0.85	0.65, 1.09	0.89	0.69, 1.15	0.95	0.71, 1.27
Maternal age (years)			0.98	0.96, 1.01	0.98	0.96, 1.01
Maternal education (≤8 years)			1.24	0.93, 1.65	1.35	0.97, 1.89
Maternal smoking (current)			1.49	1.13, 1.95	1.12	0.81, 1.55
Parity (has previous child)			1.18	0.85, 1.63	1.22	0.87, 1.71
Social class (C or lower)			1.09	0.77, 1.54	1.06	0.74, 1.52
Pre-pregnancy BMI ≤18			1.43	1.06, 1.93	1.44	1.03, 2.01
Child age at dental assessment (years)			1.25	0.64, 2.42	0.99	0.46, 2.12
Child sex (male)			1.18	0.91, 1.53	1.34	1.01, 1.77
Length-for-age Zscore at 11–15 months (per SD)					1.05	0.90, 1.23
First-year feeding index (per unit)					1.05	1.01, 1.09
Daily bottles at 5–9 months (1–3)					0.62	0.38, 1.02
Daily bottles at 5–9 months (≥4)					0.84	0.47, 1.52
Added sugar in bottle at 5–9 months					1.46	0.94, 2.26
Ever formula fed					0.82	0.59, 1.14
Frequency of fruits at 11–15 months					0.95	0.90, 1.01
Frequency of vegetables at 11–15 months					1.05	0.98, 1.12
Frequency of beans at 11–15 months					1.02	0.94, 1.10
Frequency of meat at 11–15 months					1.04	0.97, 1.12
Frequency of organ meat at 11–15 months					1.17	0.98, 1.39

Abbreviation: CI, confidence interval; BMI, body mass index; SD, standard deviation