Mechanisms of sympathetic regulation in orthostatic intolerance

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The mechanisms of sympathetic regulation in orthostatic intolerance (OI) are key to the rapid cardiovascular adjustments that occur within seconds of standing upright (orthostasis) and which are required for bipedal stance. Indeed, patients with ineffective sympathetic adrenergic vasoconstriction rapidly develop orthostatic hypotension, prohibiting effective upright activities. One speaks of orthostatic intolerance (OI) when signs, such as hypotension, and symptoms, such as light-headedness, occur when upright and are relieved by recumbence. The experience of transient mild OI is part of daily life. However, many people experience episodic acute OI as postural faint or chronic OI in the form of orthostatic tachycardia and orthostatic hypotension that significantly reduce the quality of life. Potential mechanisms for OI are discussed including forms of sympathetic hypofunction, forms of sympathetic hyperfunction, and OI that results from regional blood volume redistribution attributable to regional adrenergic hypofunction.

Normal Stressors and the Autonomic Regulatory Framework

According to Rowell (74) there are two quotidian physical stressors: upright posture and dynamic exercise that “demand the full capabilities of the reflexes that govern cardiovascular function.” Optimum orthostasis and exercise performance depend on intact intrinsic vascular structure and function, intact control of vasomotor function, adequate central blood volume and oxygen carrying capacity, and intact physical compensatory mechanisms including the integrity of skeletal and respiratory muscle pumps (59, 102). Compensatory mechanisms are often multiply redundant to offset inadequacy of any one system. Thus, for example, a small to moderate change in blood volume is well tolerated.

Short-time adjustments in hemodynamics depend most on the autonomic nervous system, although the kinetics of the myogenic response (54) and flow mediated dilation (81) are comparable. It may be fair to state that the autonomic nervous system comprises the framework in which rapid adjustments of the circulation produced by heart rate changes, arterial vasoconstriction, reflex venoconstriction, adrenal secretion, renovascular adjustments, and cardiac contractility maintain blood pressure. Apart from parasympathetic contributions to heart rate changes, these are efferent actions of the sympathetic nervous system, although recent work indicates strong vagal influences on sympathoexcitation (5). These rapid autonomic adjustments also depend on a “tonic milieu” produced by slower endocrine, paracrine, and autocrine regulatory mechanisms that may exert both direct effects on the circulation and also modulate autonomic function. Notable examples include the effects of nitric oxide and angiotensin II acting at both central (52) and peripheral (56) levels. Although parasympathetic mechanisms can play an important complementary role in the beat to beat maintenance of blood pressure, the sympathetic nervous system and its primary vascular neurotransmitter norepinephrine (101), and cotransmitters neuropeptides Y and ATP (56) are of paramount importance. Sympathetic control is
NORMAL ORTHOSTATIC RESPONSE

Standing up reduces venous return by translocating a large fraction of central blood volume, in excess of 500 ml in the adult human, to the dependent body parts. There is an initial transient dynamic state during which mechanical equilibrium must be re-established causing a decrease in blood pressure dependent on initial vascular tone (85); a further delay, on the order of 10–15 s, occurs in the onset of active compensatory responses. The delay coincides with the gravitationally driven redistribution of blood from the central circulation to the periphery, predominantly into the venous vasculature of the lower limbs and splanchnic circulation (79). The initial response denoted “initial orthostatic hypotension” (103) is complete within 30–60 s and blood pressure is restored. Tonically active adrenergic sympathetic activity contributes to resting vasoconstriction (3) and can alter the time to recovery. However, major interindividual variability in sympathetic activity exists (9) and could alter both baseline vascular resistance and the extent of vasoconstriction during standing. That being said, it appears that either differences in adrenergic transduction at the smooth muscle neurovascular synapse or alterations in blood volume ensure a measure of blood pressure uniformity across subjects (40). Thus peripheral resistance varies much less than muscle sympathetic nerve activity (8).

Even after mechanical equilibrium is re-established during continued standing, microvascular filtration from plasma to interstitium continues to reduce blood volume (49). Partial restitution of blood volume depends on lymphatic activity and reabsorption of interstitial fluid into the blood volume (35). Nevertheless, there is a net reduction in blood volume and venous return and thus a net reduction in cardiac output, cerebral blood flow, central blood volume, and stroke volume during quiet standing. Total peripheral resistance (TPR), sympathetic nervous activity, and blood pressure are increased (Fig. 1). Diastolic BP increases more than systolic blood pressure, and the resultant decrease in pulse pressure coincides with the reduction in stroke volume when upright.

Common wisdom dictates that the restoration of blood pressure and venous return during standing is attributable in large part to the reduced stretch and inactivation (unloading) of the inhibitory arterial baroreflexes. These cause adrenergic vasoconstriction, active vasoconstriction within the splanchnic circulation (33), and passive elastic recoil of pooled blood within the lower extremities and splanchnic vasculature, which partially counteract the loss of central blood volume (16). The cardiopulmonary baroreflexes are simultaneously unloaded when upright and markedly potentiate the actions of the arterial reflexes (100). A reduction in BP typically occurs only during the transient mechanical dysequilibrium of initial hypotension. Afterward both systolic and diastolic blood pressures are usually slightly increased compared with the supine position. Despite unchanged or even increased BP, increased sympathetic activity continues (Fig. 1), which again speaks to the importance of cardiopulmonary reflexes. Because diastolic blood pressure correlates best with muscle sympathetic nerve activity, it is reasonable to infer that the sympathetic nervous system is involved in the regulation of orthostatic hypotension.
activity (MSNA) in humans (94) and is increased at the level of the carotid sinus, a reduction of diastolic arterial baroreflex stretch does not occur while upright. Studies using lower body negative pressure (LBNP) as an orthostatic stress emphasize this apparent paradox in the absence of any hemostatic pressure difference between heart and carotid sinus. Both cardiovascual and sympathetic vasomotor baroreflex reflex curves are reset when upright, as occurs during exercise (20), presumably through the influence of cardiopulmonary receptors. This resetting enables a sustained increase in heart rate through vagal withdrawal and sympathoexcitation and increase in sympathetic nerve activity and vasoconstrictive characteristic of the normal compensatory response to orthostasis (13).

DEFINITION OF ORTHOSTATIC INTOLERANCE

Orthostasis means standing up. Orthostatic intolerance (OI) can be defined by the inability to tolerate upright posture relieved by recumbency (72). Typical signs and symptoms include loss of consciousness or lesser cognitive deficits, visual difficulties, lightheadedness-dizziness, headache, fatigue, orthostatic hypotension and sometimes hypertension, weakness, nausea and abdominal pain, sweating, tremulousness, and exercise intolerance. Of these, loss of consciousness or severe lightheadedness and neurocognitive loss, “CNS symptoms,” are most likely to directly provoke recumbence whereas the other findings are more directly related to increased adrenergic activity. The CNS symptoms are related to reduced perfusion of the brain (67, 68) as illustrated in Fig. 2 during orthostatic stress for two common forms of OI, vasovagal syncope (simple faint), and postural tachycardia syndrome (POTS). Cerebral blood flow (CBF) is autoregulated and thus CBF should remain nearly constant within a range of perfusion pressure. Reductions of CBF indicate impaired cerebral autoregulation; thus CBF is no longer independent of perfusion pressure (67, 68). However, a well-defined quantitative relationship between lightheadedness and CBF has not been established. Most people experience some degree of episodic OI during their lives, if only transiently during infectious diseases or during dehydration (36). Abnormally reduced CBF is not explained by postural hydrostatic decreases in cerebral perfusion pressure because CBF is independent of changes in mean arterial blood pressure (MAP) within a range of ~60 to 150 mmHg (48). Rather, cerebral blood flow is reduced by hypocapnia, which can accompany OI (45, 88), and is also dependent on parasympathetic (nitrergic) withdrawal (96) primarily at the level of pial resistance vessels. CBF is relatively independent of sympathetic influences except during very rapid changes and extremes of blood pressure (30).

ORTHOSTATIC STRESS TEST AND TOOLS TO STUDY OI

As exercise stress tests are designed to test aerobic exercise capacity, so orthostatic stress tests test orthostatic capability. Approaches to standardize orthostatic testing vary. The most physiological approach is simply to have subjects stand without restriction, although exercising in place is avoided. However, OI patients can ameliorate symptoms by means of increased skeletal muscle pump activity (11). Thus many investigators use devices such as the motorized tilt table (42), which passively places the patient upright and reduces movement. More dramatic results can be obtained by upright suspension (70). Also, LBNP or suction has been used to duplicate some findings of orthostasis even while remaining supine, but it more closely simulates hemorrhage. Indeed, large negative pressures or combination of LBNP with upright tilt can evoke a fainting response in everyone.

Physiological measurements made supine and during orthostasis employ a variety of instrumentation that measure BP, heart rate and cardiac rhythm, cardiac output (e.g., indicator dilution, inert gas rebreathing), regional blood flow (e.g., ultrasound, venous occlusion plethysmography, impedance plethysmography), blood volume, and blood chemistry, protein and genetic analyses. However, human in vivo studies of sympathetic adrenergic activity began in earnest with the advent of specific methods to measure sympathetic nerve activity with microneurography (94), to measure the resultant spillover of norepinephrine from the adrenergic synapse (19),

Why can’t we tolerate the upright position?

Vasovagal Syncope

Fig. 2. Top: arterial pressure (AP); bottom: cerebral blood flow (CBF). Left: data from a representative vasovagal syncope patient; Right: data from a postural tachycardia syndrome (POTS) patient. AP and CBF are at first stable (Stage 1), fall slowly (Stage 2), and then abruptly decrease by >50% in the syncope patient at which time consciousness is lost. This compares with the POTS patient who has no decrease in AP but has a >20% reduction in CBF throughout tilt.
to measure the effect of adrenergic vasoconstriction on local blood flow (15), and most recently to directly assess the integrity of norepinephrine synthesis and metabolic products by vascular biopsy (18, 46) (Fig. 3).

**INEFFECTIVE SYMPATHETIC VASOCONSTRICTION PRODUCES NEUROGENIC ORTHOSTATIC HYPOTENSION**

Orthostatic hypotension (OH) is defined as a sustained reduction of systolic BP >20 mmHg or diastolic BP >10 mmHg within 3 min of standing or head-up tilt to ≥60° (22). Nonneurogenic OH can be caused by drugs, age, and illnesses that secondarily cause acute or chronic hypovolemia. Neurogenic OH is identified with autonomic failure attributable to inadequate release of norepinephrine from sympathetic vasomotor neurons leading to vasoconstrictor failure (22). Autonomic failure can be primary with preganglionic, postganglionic, or both (e.g., Parkinson disease) forms of sympathetic failure (80); it can be genetic as in dopamine-beta-hydroxylase deficiency (73); it can be autoimmune (43); and it can be acquired as a secondary aspect of systemic disease such as diabetes (63). Sympathetic cardiac denervation is a central aspect of Parkinson’s disease (38) and may be found in other forms of autonomic failure. Cardiac parasympathetic innervation also is often defective, resulting in a steady fall in BP with little reflex tachycardia during orthostatic challenge.

Treatment of the underlying illness is essential. General therapy focuses on decreasing symptomatic orthostatic hypotension and syncope. Such therapy would include physical countermeasures including compression garments, dietary changes (increased salt, rapid water drinking), as well as pharmacotherapy. Pharmacotherapy is aimed at increasing blood volume by promoting salt and water retention (fludrocortisone) or by increasing red blood cell mass (recombinant erythropoietin). Short-acting pressor drugs such as midodrine or Droxidopa or drugs that enhance autonomic activity (atomoxetine, yohimbine, pyridostigmine) are also used (80).

POTS can be defined by day-to-day symptoms of OI coincident with excessive upright tachycardia but not hypotension that is improved by recumbence (25, 76). Excessive tachycardia is defined in adults by an increase exceeding 30 beats/min or to a heart rate exceeding 120 beats/min when upright. Higher heart rate changes are expected in the young with POTS (82). Tachycardia and concurrent symptoms are observed during orthostatic testing. POTS has often loosely been partitioned into patients with neuropathic POTS, in which often selective or partial dysautonomic de facto sympathetic adrenergic vasoconstriction occurs, and hyperadrenergic POTS, in which upright sympathetic overactivity dominates the picture.

As originally described, neuropathic POTS is caused by decreased sympathetic adrenergic vasoconstriction in the lower limbs, associated with reduced leg norepinephrine spillover (37) and lower extremity vasodilation (84). This results in increased blood flow (high flow) in the lower extremities even while supine. A recently described neuropathic variant has normal lower extremity hemodynamics (normal flow) but decreased splanchnic resistance when upright caused by impaired regional sympathetic vasoconstriction (89). Autonomic autoimmune neuropathy (43), when presenting as POTS, may have a similar mechanism of action. When neuropathic POTS patients are upright, a redistributive central hypovolemia causes baroreflex-mediated tachycardia; indeed, baroreflex inhibition with intravenous phenylephrine eliminates the POTS response (90). This is complicated by known defects in the cardiovasal and sympathetic baroreflex in similar POTS patients (21), by the central effects of unexplained hyperpnea and hypocapnia in 50% of patients (88), and by observations of increased circulating catecholamines during orthostasis (37) even in these neuropathic patients.
The tachycardia of hyperadrenergic POTS is presumably driven by increased presynaptic or postsynaptic adrenergic potentiation. This might include central sympathoexcitation causing an increase in sympathetic nerve activity at the adrenergic synapse. Although increased sympathetic supine activity has been reported by some (25), it has not been reported by others (4). To date my laboratory has only observed increased muscle sympathetic activity in POTS when upright. Alternatively, supine NE may be increased: as epitomized by the norepinephrine transporter deficiency heterozygote (77), an autosomal mutation, found so far in only one pedigree with variable penetrance. Non-Mendelian NET deficiency with a smaller reduction in the transporter has been recently described and has wider prevalence (46).

Sympathetic nerve activity, and norepinephrine synthesis, release, and binding are also modulated by endocrine, paracrine, and autocrine mediators perhaps epitomized by the reciprocal actions of nitric oxide (NO) and angiotensin II. Data support a role for NO as an inhibitory neurotransmitter (105). Nitric NO, in particular, can act at prejunctional and postjunctional sites to reduce sympathetic transduction (93). This includes reduction of the release and binding of norepinephrine from the neurovascular junction (44) and postjunctional interference with neurotransmission (31). Downregulation of adrenergic receptors (34) and chemically denaturing of norepinephrine (55) have also been reported. Such mechanisms may contribute to the reduction of norepinephrine spillover in neuropathic POTS. Conversely, studies of sympathoexcited states show that ANG II acts via AT1R and reactive oxygen and nitrogen species (ROS) as an excitatory neurotransmitter within the brain at presynaptic sympathetic neurons (32) and in the periphery, where it exerts pre- and postjunctional modulation of sympathetic transduction, upregulation of adrenergic receptors (34), the release and binding of norepinephrine from the neuromuscular junction (44), and facilitation of the effects of norepinephrine. As in the CNS, this depends critically on the formation of ROS (7), which decrease NO (104), often uncoupling NOS (47), thus further enhancing superoxide production. This mechanism occurs in an important variant of hyperadrenergic POTS associated with a phenotype of pallor, supine tachycardia and vasovasostion (low flow), and absolute hypovolemia (71). Bioavailable NO, plasma renin, and serum aldosterone are decreased (58), while plasma ANG II (86) is increased by a defect in ACE 2 (91).

Therapy for POTS to date is much like the treatment for neurogenic orthostatic hypotension in the use of physical countermeasures, salt and water intake, and even pharmacotherapy. Innovative treatment with ARBs and Droxidopa are under investigation. Exercise has always been a mainstay of rehabilitation in these patients. Recent work indicates that gravitational deconditioning (e.g., bedrest) is a frequent concomitant of the illness and that a graded exercise program can be very effective in improving overall patient well being (24).

**Postural Syncope (Vasovagal Syncope, Acute OI, Simple Faint)**

Syncope (fainting) may be defined as “complete loss of consciousness (and postural tone) attributable to transient global cerebral hypoperfusion characterized by rapid onset, short duration, and spontaneous complete recovery” (61). During a lifetime, ~40% of people will faint, half of these presenting during adolescence with a maximum incidence at 15 yr old (26). Most syncope is caused by systemic hypotension. Syncope may be attributable to sympathetic adrenergic failure and orthostatic hypotension, which we have already discussed and which is easily ruled out by a 3-min standing test. Otherwise syncope is partitioned among cardiovascular syncope, frequently attributable to arrhythmic or structural heart disease and reflex or neurally mediated syncope. Cardiovascular syncope has a poor prognosis unless successful steps are taken to treat specific cardiac pathophysiology. Reflex syncope has a good prognosis (83). Orthostatic stress syncope and emotional stress syncope together comprise vasovagal syncope (VVS) (27), which is the largest subgroup within reflex syncope group. Regional or system-wide loss of sympathetic adrenergic vasoconstriction is an element in all vasovagal syncope, at least as a terminal event, and will be discussed in greater detail below. Orthostatic or postural syncope may be thought of as acute OI. Indeed, loss of consciousness is most often preceded by a prodrome of OI symptoms, particularly lightheadedness, nausea, sweating, weakness, and visual disturbance (e.g., black-out). Until recently postural syncope was thought to be caused by reflexes from a hypercontractile underfilled heart analogous to the Bezold-Jarisch reflex (1). This mechanism was favored despite evidence to the contrary: thus any such stimulus could only be short lived because baroreceptors would immediately be unloaded (28); few afferent nerves were excited in the original Oberg and Thoren (66) hemorrhaged cat model; VVS can occur in a ventricular denervated transplant recipient given the sodium nitroprusside (75); and the heart before syncope is neither empty nor hypercontractile (51). Thus to date, the pathophysiology of simple faint remains elusive (60) and findings are largely descriptive without necessarily informing on specific molecular mechanisms.

In the most common variant of postural faint that occurs in young patients, postural faint often comprises three stages (Fig. 4) that closely emulate the circulatory changes that occur during hemorrhage (2). Following initial orthostatic hypotension, mechanical and neurovascular equilibrium are reestablished, and BP stabilizes while HR increases in Stage 1. This stability distinguishes postural faint from true OH, in which BP falls early and remains low. BP is often highly oscillatory during this stage. These oscillations are sometimes referred to as Mayer waves (41) and correspond to approximately sinusoidal fluctuations in BP with an approximate 10-s period (0.1 Hz). Similar periodicity is shared by fluctuations in MSNA. The oscillations represent the time it takes for the closed loop sympathetic baroreflex to sense and compensate for a change in BP (29). Similar oscillations can be observed to a lesser extent in HR transduced by the cardiac baroreflex. Oscillations are accentuated during baroreflex unloading as occurs with orthostasis, in part attributable to resetting of the sympathetic baroreflex with orthostasis (23) and in part the result of thoracic hypovolemia.

During Stage 2 BP slowly declines as HR reflexively increases further. This decrease in BP is often related to a reduction in cardiac output (99) despite sustained and even increased MSNA (12), although peripheral arterial resistance (95) and Mayer wave activity (65) are sustained. Both resistance and pressure oscillations subsequently diminish despite...
sustained sympatheoexcitation. Hyperpnea and hypocapnia is observed at this point (45). In some patients Stage 2 is abbreviated. This is especially true for patients with convulsive syncope in whom episodes occur abruptly in association with asystole. Some explanations offered for the early phases of postural faint include reduced tyrosine hydroxylase and NE synthesis in patients with supine low BP, excess NET (98), or selective deficit of splanchnic adrenergic vasoconstriction/venoconstriction (89). Prodromal OI symptoms often begin during this second stage; combined with tachycardia that may lead one to diagnose POTS in the laboratory setting. However, a history of episodic faints interspersed with long periods free of signs and symptoms of OI distinguishes postural syncope from POTS, in which symptoms are chronically present. Medical history is paramount. Admittedly, the prodrome of simple faint and the signs and symptoms of neuropathic POTS are similar because they can have similar pathophysiology, namely reflex tachycardia from excessive reduction in central blood volume (84, 87, 89). On the other hand, postural fainters corresponding to the pale and vasoconstricted hyperadrenergic POTS patients are rarae aves. In our experience, POTS patients typically have day-to-day symptoms but do not faint, whereas fainters do not have daily symptoms; however, this distinction has blurred and there are some POTS patients who faint and a few fainters with daily or nearly daily symptoms of OI. Nevertheless, fainting in POTS is relatively uncommon outside the laboratory where POTS patients can be made to faint.

In the final Stage 3, CBF, BP, and HR fall precipitously in that order, seemingly defying the expected causal relationship between BP and CBF (14). Recent data suggest loss of cardiovagal and sympathetic baroreflex integrity and loss of cerebral autoregulation with entrainment of CBF, BP, and HR by an extrinsic oscillator that may be hyperpneic hyperventilation (67, 69). Why baroreflex integrity is lost is unknown. Thus, instead of the usual reciprocal BP-HR and BP-MSNA functional relationships (BP decreases, HR and MSNA increase), HR, BP, and MSNA decrease synchronously. This may result in asystole and sympathetic silence (39). Typically the faint is associated with marked systemic vasodilation while CBF becomes strictly dependent on declining BP. The requirement of sympathetic nerve withdrawal as the precipitant of final hypotension has recently been challenged (97). Although vasodilation always occurs, the sympathetic baroreflex can fail with or without MSNA silence. Similar findings occur in patients with vasodepressor syncope where vasodilation without bradycardia occurs along with loss of the sympathetic efferent baroreflex causing progressive loss of compensatory vasoconstriction. The vagal baroreflex remains intact.

Therapy for vasovagal syncope associated with a lengthy prodrome is largely avoidance and physical countermeasures; the most efficacious of these is to lie down or squat. Other countermeasures include those that enhance the skeletal muscle pump (e.g., leg crossing) or activate the exercise pressor reflex (isometric hand grip). Enhanced salt and water intake is often encouraged and has shown some efficacy in small studies employing large amounts of salt loading (10). In older patients, confounding use of antihypertensives or diuretics need to be considered. Pharmacotherapy has not been shown to be particularly effective in large multicenter studies (78). Asystolic faints can be improved by pacemaker insertion (6).

**Respiration and Postural Hyperpnea**

Both POTS and postural faint are associated with hyperventilation, more specifically hypocapnia (45, 64, 88). Hyperpnea and hypocapnia precede loss of consciousness in virtually every vasovagal syncope patient. Hypotension and bradycardia might be explained by the pulmonary stretch reflex unfettered by compensatory baroreflex effects (53, 69). The cause of hyperpnea is unclear. However, a ventilatory efferent arm of the arterial baroreflex has recently been found in humans that is independent of respiratory chemoreflexes (92). Thus unloaded baroreflexes in Stage 2 of fainting cause an increase in tidal volume but not in respiratory rate, resulting in markedly hyperpneic respirations. Similar findings of hyperpnea are found in POTS patients with central hypovolemia who do not faint. Measurements indicate that while cardiovagal baroreflex gain is reduced in POTS (21), the sympathetic nerve response is augmented (62).

**POSTURAL HYPERPNEA AS A SEPARATE VARIANT OF OI**

The final figure (Fig. 5) shows the results of a representative patient with involuntary hyperpnea in the upright position. Similar hemodynamic findings can be induced in healthy volunteers.
during upright voluntary hyperpnea. Findings include marked increase in MSNA and peripheral resistance, decreased cardiac output (CO), and decreased cerebral blood flow as a result of hypocapnia. A large initial reduction in central blood volume, an extraordinary hyperpneic breath, and rapid reduction of cerebral blood flow start a self-perpetuated process. Respiratory chemoreflex assessment is normal. During subsequent upright experiments infusion of phenylephrine or inhalation of supplemental CO₂ to correct end-tidal carbon dioxide from 24 to 38 Torr decreased upright HR from 130 to 100, reduced MSNA, and normalized cerebral blood flow. There were no findings consistent with anxiety including low resting MSNA. Once upright, sympathoexcitation occurred and preceded obvious anxiety. Withdrawal of supplemental CO₂ increased MSNA followed thereafter by hyperpnea, suggesting a causal relation. Similar findings were reported previously (17). Postural hyperventilation has been observed for years and often attributed to panic disorder (57). A more complex pathophysiology involves sympathetic stimulation of ventilation and cerebral alkalosis (92).

Perspective

Once true neurogenic orthostatic hypotension is ruled out, orthostatic intolerance comprises non-life threatening phenomena that occur in large numbers of people and relate to inappropriate sympathetic adrenergic function. Although most of us have at least experienced mild OI as the transient initial orthostatic hypotension of rapid standing and its associated light-headedness, other forms of OI can have a serious impact on quality of life. Postural vasovagal faint and postural tachycardia syndrome are two well described common forms of OI. Other forms of OI, such as postural hyperpnea, remain to be investigated.

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AUTHOR CONTRIBUTIONS

Author contributions: J.M.S. conception and design of research; J.M.S. performed experiments; J.M.S. analyzed data; J.M.S. interpreted results of experiments; J.M.S. prepared figures; J.M.S. drafted manuscript; J.M.S. edited and revised manuscript; J.M.S. approved final version of manuscript.
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