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Potential Applications of Hydrogen Sulfide–induced Suspended Animation

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Abstract

A suspended animation–like state has been induced in rodents with the use of hydrogen sulfide, resulting in hypothermia with a concomitant reduction in metabolic rate. Also oxygen demand was reduced, thereby protecting against hypoxia. Several therapeutic applications of induction of a hibernation–like state have been suggested, including ischemia–reperfusion injury. More recently, hydrogen sulfide has been found to be protective in states of exaggerated inflammatory responses, such as acute lung injury. Possible mechanisms of this protective effect may include reduction of metabolism, as well as reduction of inflammation. In this manuscript, the methods of inducing a suspended animation–like state in experimental models using hydrogen sulfide are described. We discuss the effects of hydrogen sulfide–induced hypo–metabolism on hemodynamic, metabolic and inflammatory changes in animal models of various hypoxic and inflammatory diseases. In addition, potential therapeutic possibilities of hydrogen sulfide–induced hibernation are outlined.

Introduction

In October 2006, the first known case of a human going into “hibernation” was described. After slipping and breaking his pelvis, a hiker survived 24 days in a mountain forest without food or water. He was found unconscious, with a body temperature close to 22°C, a weak pulse and suffering from blood loss (1). After referral to a hospital, he made a full recovery. His treating physicians believed that his survival was a result of going into a state akin to hibernation, as mountain temperature dropped as low as 10°C. This report does not stand alone. Other cases of survival of accidental deep hypothermia have been mentioned, including patients after a prolonged cardiac arrest and resuscitation (2–6).

Organ damage occurs after more than five minutes of circulatory arrest as a reaction to low oxygen levels (hypoxia) or no oxygen (anoxia). Hypoxia leads to the generation of free radicals, release of excitatory amino acids and calcium shifts lead to mitochondrial damage and apoptosis, causing cerebral injury (7–9). In nature, tolerance to hypoxia is seen in hibernating mammals (10). These animals can transiently arrest cellular processes as a results of unfavorable environmental changes by changing their metabolism, substrate selection, oxygen consumption, heart rate and body temperature (10–14). During hibernation, body temperature can drop to ambient temperature even below freezing points. Under these circumstances, hibernating animals are resistance to otherwise lethal hypoxia.

Several animals adapt to hypoxic or anoxic atmospheres (15–19). Some invertebrates exhibit an arrest of development and a reduction in protein synthesis in the absence of oxygen (17). A clinically relevant example of adaptation to anoxia was provided in a dog model of prolonged exsanguination–induced cardiac arrest, followed by flushing 4°C saline in the aortic arch, resulting in profound hypothermia. After resuscitation, a complete recovery without neurological damage was observed (20), suggestive of a mechanism to adapt to a lack of oxygen when energy supply and energy demand is limited.

From a philosophical point of view, these adaptive mechanisms may be a rudiment from early development of life on earth, when the atmosphere lacked oxygen, but was full of sulfur–containing molecules, such as hydrogen sulfide (21;22). Primordial organisms may have generated their energy supply by using hydrogen sulfide, resembling the way that modern life–forms use oxygen (23). In some bacteria, sulphur based respiration is still present (24;25). Indeed, hydrogen sulfide is produced endogenously in humans (26). By binding to cytochrome c oxidase, hydrogen sulfide can competitively inhibit the oxidative phosphorylation pathway in the inner mitochondrial membrane in the presence of oxygen (27). The ability of hydrogen sulfide to compete with oxygen may have become part of an intrinsic cellular program to naturally slow or stop oxidative phosphorylation under anoxic conditions (27;28).

Recently, it was shown that a hibernation-like state can be induced in animals that do not naturally hibernate (29). Mice exposed to hydrogen sulfide gas experienced a drop in core body temperature to 15°C, along with the ambient temperature, together with a drop in metabolic rate. This was termed a *suspended animation like state*. As described above, comparable physiological changes have been seen in naturally hibernating mammals (11).

The observations in animal models of anoxia and hypoxia suggest that inhibiting metabolism on demand is feasible. Although humans are considered to be homeothermic mammals, the anecdotal reports on otherwise healthy people surviving accidental deep hypothermia and circulatory arrest with no or minimal cerebral impairment, suggest that the ability to hibernate may have persisted throughout mammalian evolution (23).

If mammalian hibernation proves to be possible, induction of a hypo-metabolic state may be beneficial in a number of situations when supply of oxygen is jeopardized. An example of the protective effect of a treatment that reduces metabolic processes is induced hypothermia. Hypothermia increases the tolerance of the brain to ischemia. Induction of deep hypothermia has been applied in the clinical setting successfully in the last decades as a means of protecting the brain from ischemic injury when circulatory arrest is required, e.g. during cardiothoracic surgery (30–33). Moderate hypothermia is now an accepted clinical treatment in patients after cardiac arrest and is associated with better neurological outcomes in these patients (34). Although the mechanisms of the protective effect of induced hypothermia are complex, reduction of cerebral oxygen consumption is likely to contribute (35–38).

In this review, we will discuss the methods of induction of suspended animation with H₂S in non-hibernating animals, as well as the metabolic, hemodynamic and inflammatory effects of H₂S in different animal models. Furthermore, we speculate on potential applications of hydrogen sulfide-induced suspended animation.

Methods – systematic search of the literature

The following keywords were used to obtain papers published in the Medline database; *hypothermia*, *hydrogen sulfide*, *suspended animation*, *metabolism*, and *hypoxia*. The relevance of each paper was assessed using the online abstracts. In addition, the reference list of the retrieved papers was screened for potentially important papers.

Induction of a suspended animation-like state using H₂S

Hydrogen sulfide (H₂S) is a flammable gas, which has long been considered an environmental hazard because of its cytotoxic effects (39). However, H₂S is also endogenously produced in small amounts and plays a significant role as a second messenger in cellular signaling.

H₂S is synthesized in the cysteine biosynthesis pathway under the enzymatic regulation of cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) (26). The substrate of CBS and CSE is L-cysteine, which is derived from nutritional sources or synthesized from L-methionine. The activity of CBS and CSE depends upon pyridoxal 5'-phosphate as a cofactor. Production of H₂S generating enzymes is organ-specific. CBS is expressed in tissue of the nervous system, whereas CSE is expressed in vascular muscle tissue. The major metabolic detoxification of sulfide is hepatic oxidation to sulfate (SO₄²⁻) and the subsequent elimination of sulfate in the urine (40;41). H₂S may also be methylated (CH₃SCH₃) by the liver.

Biological effects of H₂S.

Like nitric oxide (NO) and carbon monoxide (CO), H₂S is a gas transmitter, which is highly soluble in aqueous and lipid environments, readily passes between cells and is rapidly oxidized. H₂S induces a variety of posttranslational protein modifications, ranging from cytotoxic to cytoprotective actions. Biological activities of H₂S include dilation of blood vessels and smooth muscle cells (42) and modulation of neurotransmission (43;44). Furthermore, it displays both pro- and anti-inflammatory activities.

Similar to NO and CO, H₂S can inhibit mitochondrial respiration. Mitochondria utilize most of total body O₂ to produce adenosine triphosphate (ATP). Oxidative phosphorylation is the last step in the generation of ATP in the mitochondrial membrane, a process which involves the reduction of O₂ and which is catalyzed by cytochrome c oxidase. At high doses, H₂S competes with O₂ in binding to cytochrome c oxidase, thereby reducing cellular oxygen consumption and resulting in profound hypo-metabolism, with metabolic and cardiovascular responses consistent with the physiology of hibernating.

Induction of a suspended animation–like state using H₂S gas.

The first experiments showing that a suspended animation–like state can be induced by H₂S in non-hibernating mammals were done in conscious mice (29). In this study, mice were exposed to H₂S gas in concentrations ranging from 20 to 80 ppm and 17.5% O₂ in a customized sealed glass cage. The metabolic rate, measured by CO₂- production and O₂-consumption, were analyzed using portable gas analyzers. A dose-dependent reduction of metabolic rate by 50% was observed. Body temperature dropped as low as 15°C, which was 2°C higher than the ambient temperature. Animals ceased all movement and respiratory rate declined to 10 breaths per minute. After cessation of H₂S exposure, the mice awoke, without displaying any neurologic or behavioural deficits. Further experiments showed that pre-treatment with H₂S increased the survival rate of mice exposed to hypoxia, whereas hypoxia led to death in all the control mice (45). The authors speculated that hypoxic damage stems from the consequences of inefficient mitochondrial activity in the presence of low oxygen tension, including limited energy supplies and free radical formation. In

suspended animation, high levels of hydrogen sulfide may directly interfere with oxygen sensing through competitive inhibition of cytochrome c oxidase, thereby reducing damaging free radical formation. Studies performed in *Caenorhabditis elegans* embryos, which enter into suspended animation in response to anoxia (17), may support this notion. Exposure to CO protected *C. elegans* from hypoxic damage (46).

After these experiments, suspended animation in non-hibernating mammals was also achieved by others, using the same experimental set up with awake mice, exposed to 80 ppm of H₂S in a chamber (47).

Induction of a suspended animation-like state using H₂S-donors.

Parenteral solutions have a number of practical advantages: ease of administration, no need for an inhalation delivery system, no risk of exposure of gas to personnel and no issues related to the characteristic odor of H₂S gas. In aqueous solutions, H₂S readily dissociates to give HS⁻ which then exists in equilibrium with H₂S and S²⁻ according to the equations (48;49): NaHS → Na⁺ + HS⁻ (1st reaction), 2HS⁻ ↔ H₂S + S²⁻ (2nd reaction), HS⁻ + H⁺ ↔ H₂S (3rd reaction).

Parental solutions have been used to inhibit metabolism in animal models. In anesthetized pigs, Na₂S was used, of which a bolus of 0,2 mg/kg was given, followed by an infusion of 2 mg/kg/hour. This dose resulted in reduction of energy expenditure, exemplified by reduced O₂-uptake and CO₂-production (50). In preliminary experiments performed in our laboratory, we used NaHS to induce hypo-metabolism in an anesthetized and mechanically ventilated rat model. Continuous infusion at a dose of 2 mg/kg/hour resulted in a reduction in body temperature, heart rate and exhaled CO₂ (unpublished data).

Whether parenteral solutions of H₂S donors in doses that induce hypo-metabolism are less toxic than gaseous H₂S, remains to be explored. It has been shown that bolus injections of very high doses of NaHS (10 mg/kg) cause acute lung injury (51).

Induction of suspended animation in larger animals.

It should be pointed out that small animal models of metabolism have important limitations. The metabolic rate of mammals increases with decreasing body mass, resulting in a faster exhaustion of energy reserves. Due to a large surface/mass ratio, small animals can reduce core body temperature rapidly when challenged with hypoxic conditions or other noxious insults (52). Thermal inertia of large mammals and humans may resist reductions in body temperature. However, in the experiments discussed above, H₂S depressed metabolic rate within minutes, whereas a decrease in body temperature to ambient temperature occurred gradually, over the course of hours. Therefore, a large body mass may not impede H₂S-induced suppression of metabolism. H₂S-induced suspended animation has been studied in several larger animal models, yielding contrasting results so far.

In anesthetized pigs, a parenteral formula of an H₂S–donor in a dose of 2 mg/kg/hour, resulted in a reduction of heart rate and body temperature, as well as a reduction in O₂–uptake and CO₂–production, indicating a reduction of metabolism (50). Others have not succeeded in inducing suspended animation–like state in pigs. In anesthetized and paralyzed animals, H₂S gas was delivered via mechanical ventilation (53). Ambient temperature was kept at 23°C. Ventilation with 20 to 80 ppm of H₂S resulted in a drop of O₂ consumption and CO₂ production, as well as a decrease in body temperature, in both H₂S exposed animals and controls exposed to air. However, as all metabolic effects were related to a decrease in body temperature, the authors concluded that H₂S did not influence metabolic processes. In sheep, H₂S gas was supplied through a silicone, plastic respiratory mask, which was adapted to each animal (54). The sheep were sedated with ketamine, were breathing spontaneously and lying calm on their side. Airflow was measured by a pneumotachograph. Gas from the mask was drawn for CO₂ measurements. Also in this study, H₂S 80 ppm did not reduce O₂–consumption or exhaled CO₂.

Effects of H₂S on circulation

H₂S is endogenously generated in blood vessels, under control of the enzyme CSE. H₂S induces relaxation of arterial blood vessels and cardiomyocytes, thereby regulating vascular tone and myocardial contractility (26;42;55). Similar to the gas transmitter NO, H₂S predominantly exerts vasodilatation, an effect that is mediated by opening of K_{ATP} channels (56;57). Indeed, an intravenous bolus injection of H₂S in rats transiently decreased blood pressure (41). The vasodilatory effect of H₂S depends on oxygen tension. At high concentrations of oxygen, H₂S has been found to contract arterial vessels *in vitro* (58).

The cardiovascular effects of H₂S depend on its concentration. The cardioprotective effect of sulfide (59–61), which may be mediated – at least in part – by ERK and phosphatidylinositol 3 – kinase (PI3K)/AKT pathways (49;62), was dose–dependent, ranging from 0,1 μM to 1 μM of NaHS. Increasing the dose to 10 μM of NaHS did not further reduce infarct size, but rather aggravated myocardial injury. The cardioprotective effect appeared to be bell–shaped, with the optimal effect at hibernation inducing concentrations. A similar biphasic response in the infarct limiting effects of NO and NO donors has been reported (63). Cardioprotection probably involved the ability of H₂S to activate myocardial K_{ATP} channels, as evidenced by an abolishment of the beneficial effect by K_{ATP} channels blockers (49;59). Other pathways of cardioprotection have also been reported. High doses of sodium sulfide administered to mice after myocardial ischemia and reperfusion injury, resulted in a reduction in infarct size, an effect which was associated with a reduction in apoptosis and concomitant preservation of mitochondrial function (60). An anti–apoptotic effect was also observed in a pig model of myocardial infarction (61).

High doses of H₂S that inhibit cytochrome c oxidase, induce distinct cardiovascular responses, as seen during hibernation (47). Mice exposed to high doses of H₂S gas showed a decrease in heart rate by 50%, returning to baseline value after cessation of H₂S exposure. H₂S induced a sinus bradycardia with sinus arrest, which recovered to a slow sinus rhythm within 5 minutes after cessation of exposure. The reduction in heart rate did not result in a change of mean arterial pressure. Echocardiographic analysis showed a decrease in cardiac output by 60%, while systolic volume remained unaffected, suggestive of systemic vasoconstriction. However, this is not in line with the vasorelaxant properties of H₂S. Alternatively, a low body temperature could have resulted in systemic vasoconstriction. However, all hemodynamic effects occurred independently of body temperature, in hypothermic as well as normothermic mice. Therefore, it seems unlikely that hypothermia alone accounted for the observed cardiovascular effects. Alternatively, vasoconstriction may have been triggered by baroreceptors to compensate for a low cardiac output.

The sulfide-induced hemodynamic effects were confirmed in an anesthetized and mechanically ventilated pig model of ischemia-reperfusion injury (50). Infusion of an H₂S donor resulted in a parallel fall in both heart rate and cardiac output, whereas stroke volume did not change. The animals showed a drop in body temperature, concomitant with lower O₂ uptake and CO₂ production. High doses of a H₂S donor have also been reported to lower arterial blood pressure during ischemia-reperfusion injury (61). Also in this experiment, H₂S preserved left ventricular function.

Contrasting hemodynamic effects were found in anesthetized pigs exposed to high doses of H₂S gas (53). In this study, heart rate and cardiac output did not change, whereas arterial blood pressure increased after exposure to H₂S, suggestive of a stimulating effect. However, body temperature and oxygen consumption did not differ from controls, suggesting that hibernation was not induced in these animals. Overall, in doses that influence metabolism, a reduction in heart rate and cardiac output is most consistently noted, while myocardial function seems to be preserved. No conclusion can be made about oxygen demand from these experiments. Therefore, whether high doses of H₂S result in a better balance between oxygen supply and demand remains to be determined.

Effects of H₂S on inflammation

Hydrogen sulfide exerts a wide range of biological effects, which, like NO, may be due to the absence of a specific target receptor. Both pro- and anti-inflammatory activities of hydrogen sulfide have been reported. In several models of septic shock as well as in septic patients, levels of H₂S were elevated (64;65), together with an increase in the expression of the H₂S generating enzyme CSE (66), pointing to an increased endogenous production of H₂S during inflammation. In accordance, an inhibitor of CSE was found to abolish the increase in H₂S (48;67). H₂S has been implicated as a mediator of inflammation, as inhibiting endogenous

H₂S production with the use of a CSE inhibitor was found to reduce organ damage, as well as myeloperoxidase activity (67). Blocking CSE also reduced the production of pro-inflammatory cytokines and chemokines in a model of sepsis (51), an effect which was mediated by the activation of NF- κ B (68). Inflammatory effects of H₂S are thought to occur at the leukocyte–endothelium interface, as inhibition of endogenous H₂S synthesis reduced leukocyte rolling and adherence to blood vessels (7;69). In addition, H₂S has been found to regulate the production of pro-inflammatory neuropeptides (substance P and calcitonin gene related peptide) during sepsis, thereby contributing to the inflammatory response (8;9). Therefore, during inflammation, endogenous H₂S seems to act as an important endogenous regulator of leukocyte activation and trafficking during an inflammatory response.

However, endogenous hydrogen sulfide has also been found to have anti-inflammatory effects. In a model of carrageen–induced inflammation in the rat, a CSE–inhibitor enhanced edema formation as well as the infiltration of inflammatory cells (7;69). Also, endogenous H₂S seems to play a role in healing of gastric ulcers, as inhibition aggravated gastric injury (70).

Administration of exogenous H₂S resulted in anti-inflammatory activities in multiple experimental designs. In vitro, neutrophils pretreated with 2 mM sulfide showed suppressed calcium dependent cytoskeleton activities such as chemotaxis and granule release (71). Furthermore, a cytoprotective effect has been demonstrated during myocardial ischemia reperfusion injury (60), which related to peroxynitrite (ONOO⁻) scavenging capabilities of H₂S (72), thereby decreasing intracellular tyrosine nitration and oxidative stress. Similar to the protective effect of inhibiting endogenous H₂S, the addition of exogenous H₂S improved healing of gastric ulcers. Rats exposed to 160 ppm H₂S in a water–immersion and restraint stress model, were protected from the occurrence of gastric lesions (73). In addition, H₂S diminished local inflammation and neutrophil activation, as measured by a decrease in myeloperoxidase activity. In a mouse model of trauma–induced lung injury, treatment with NaHS (2 mg/kg) reduced the level of pro-inflammatory cytokine IL–1 in the lung compared to control animals, while increasing IL–10 concentration (74). Furthermore, burn and smoke inhalation injury increased the presence of protein carbonyl formation in the lung, while administration of hydrogen sulfide reversed this effect and reduced protein carbonyl formation, indicative of an overall anti–oxidant effect. In contrast, in mechanically ventilated pigs in suspended animation, a H₂S donor did not affect pro-inflammatory cytokine levels or markers of oxidative stress (50).

Therefore, H₂S can exert both anti-inflammatory and cytotoxic effects, which appear to be dose dependent. The considerable differences in design of the models hamper definite conclusions on the inflammatory effects of H₂S.

Potential applications of H₂S induced suspended animation

Organs for transplantation.

The success of organ transplantation is critically dependent on the quality of the donor organ. Donor organ quality, in turn, is determined by a variety of factors including donor age and pre-existing disease, the mechanism of brain death, donor management prior to organ procurement, the duration of hypothermic storage, and the circumstances of reperfusion (75). Measures to prolong viability of donor tissue comprise infusion of a preservative solution and placement on ice during transportation. Cold ischemic times which are accepted for transplantation in a recipient vary from 4 hours (heart) to 30 hours (kidney) (76). After this time window, ischemic injury causes early non-function or delayed graft function, which plays an important role in the development of chronic graft failure and late graft loss. A more detailed mechanism of ischemic donor organ injury includes the development of tissue edema, cellular and mitochondrial swelling and loss of cell membrane integrity. Cellular swelling is initiated by inhibition of Na⁺ K⁺ ATP-ase, followed by suppression of oxidative phosphorylation, ATP depletion, and an increase in the cytosolic calcium concentration. Mitochondrial calcium overload follows this increase in cytosolic calcium and is associated with the opening of the permeability transition pore, and mitochondrial swelling (77;78), a process which correlates with apoptosis (79;80).

In the last decades, different preservation solutions and techniques have been developed. Some experiments have used H₂S in their solution. Hu *et al* (81) reported an improvement in preservation of isolated rat hearts by adding 1 μmol/L NaHS. The solution showed comparable preservation effects with St. Thomas solution, but had a better ATP production and a reduction in apoptosis, as showed by TUNNEL staining. It can be speculated that organs placed in a suspended state by adding H₂S, may preserve their viability for days, which may reduce the number of unused organs due to mismatching or too long transportation time. Of interest, H₂S has been shown to reduce metabolic demand during excessive blood loss, thus in a state of low oxygen supply (82). Indeed, exposure of mice to H₂S resulted in survival during hypoxic conditions that otherwise would have been lethal (45).

Patients undergoing coronary artery bypass surgery.

Deep hypothermia (to 20°C) is already applied during cardiothoracic surgery to protect organs from ischemia-induced organ damage. Interestingly, patients with coronary arterial disease can develop transient cardiac failure, which recovers after perfusion is restored. This phenomenon is termed myocardial hibernation, which has been postulated as an adaptive response to an oxygen deficit (83;84). To identify molecular adaptations responsible for myocardial hibernation, swines were instrumented with a chronic left anterior descending coronary artery stenosis (85). After 5 months, myocardial analysis showed an intrinsic down regulation of many of the mitochondrial enzymes responsible for oxidative metabolism and

electron transport. Also, a chronic up–regulation of stress proteins coupled with a transient increase in cytoskeleton proteins was seen. These myocardial adaptations support the notion that the molecular mechanisms operative in hibernating myocardium are dynamic, protecting myocytes from irreversible injury, which does not always lead to fibrosis. Inducing a suspended animation–like state with H₂S in patients undergoing coronary artery bypass surgery may not only be beneficial due to hypothermic effects, but could also influence mitochondrial enzymes responsible for energy production, thereby protecting myocytes from injury. In addition, H₂S in non–hibernating doses has shown cardioprotective effects in various models of myocardial ischemia–reperfusion injury, which may involve a vasorelaxant effect of coronary arteries via opening of K_{ATP}–channels and a reduction of apoptotic cell death (41;49).

Patients suffering a cardiac arrest.

Mild hypothermia (to 32–34°C) improves neurological outcome when applied in patients after a cardiac arrest. Ischemic brain injury results from a loss of ATP production and dysfunction of membrane ATP–dependent pumps, resulting in cell swelling and global cerebral dysfunction, as well as overproduction of free oxygen radicals and induction of apoptosis (86;87). The ability of hypothermia to affect multiple points of the injury cascade may contribute significantly to its success as an intervention. It should be noted that induced hypothermia and reduction in body temperature due to H₂S exposure are different mechanisms. Hypothermia can be induced by external cooling of the body with the use of ice–cold infusions and with cooling devices, such as a cooling mattress. H₂S associated suspended animation in animal models inhibits metabolism, followed by a drop in body temperature. However, although a distinct condition, hypothermia is a significant characteristic of suspended animation and may be a consequence of competitively inhibiting oxidative phosphorylation in the presence of oxygen. Remarkably, a 17 month old patient with cytochrome c oxidase deficiency presented with several severe episodes of hypothermia (31°C – 33°C) during daytime when the child was normal active (88). Also, hypothermia has been observed in children with other mitochondrial respiratory chain dysfunctions.

Possibly, H₂S–induced suspended animation could also improve neurological outcome after a circulatory arrest, which might not only be due to a hypothermic effect, but also to protection against hypoxia as described earlier. Several observations may support this notion. The H₂S–regulating enzyme CBS is highly expressed in the brain. H₂S enhances sensitivity of aspartate receptor to glutamate and plays a central role in long–term potentiation of the neuronal circuitry. H₂S also regulates synaptic activity by modulating the activity of both neurons and glia cells. A neuroprotective effect of exogenous H₂S has been reported. H₂S decreased oxidative stress in neurons *in vitro*, by increasing levels of the antioxidant glutathione in

neurons (43;44;89). Research into mitigating brain damage after cardiac arrest has shown outcome benefits in clinically relevant dog models of a prolonged exsanguination–induced cardiac arrest as described earlier (20;90). In these dogs, a suspended state was achieved with the use of cold fluids within the first 5 minutes of no flow, resulting in profound hypothermia. Resuscitation after 90–120 minutes resulted in complete recovery of function and normal histology. We speculate that the addition of H₂S may increase the effectivity of induced suspended animation in these models, or prolong the time after arrest in which inducing suspended animation is possible.

Critically ill patients with multiple organ failure.

In critical illness, a hyper–metabolic response to insults is a common clinical entity. Sepsis is characterized by an exaggerated systemic inflammatory response to infection. In the absence of infection, a systemic inflammatory response syndrome (SIRS) can occur as a reaction to a variety of non–infectious insults, including trauma, cardiothoracic surgery and ischemia–reperfusion injury. The dysregulated host inflammatory response in sepsis and SIRS results in multiple organ failure (91;92). A deficiency in tissue oxygen delivery cannot solely account for the development of organ failure. In the presence of an adequate tissue oxygen tension, metabolic dysfunction persists, suggestive of a disturbance in cellular metabolic pathways (93;94). Although disparate results of mitochondrial function have been reported in short–term models of sepsis, a decrease in mitochondrial activity has been a consistent finding in longer term sepsis models (95;96) including a decrease in oxidative phosphorylation capability with subsequent decreased oxygen consumption and ATP synthesis. In agreement, a depletion of skeletal muscle ATP concentrations, a marker of mitochondrial oxidative phosphorylation, was found in septic patients, which was associated with worse outcome (96;97).

Preservation or optimization of residual mitochondrial function may prevent ATP levels from dropping below threshold levels that induce apoptosis and cell death. Treatment of multiple organ failure traditionally consists of supportive care, ensuring that high metabolic demands are met. Also, regulating mitochondrial substrate may provide a protective measure in the critically ill. Intensive insulin therapy aimed at maintaining strict normoglycemia has been found to preserve mitochondrial ultra structure and function (98) and improve outcome (99;100) in critically ill patients. It can be hypothesized that inducing a hypo–metabolic state during critical illness may limit organ injury by protection from prolonged energetic failure, enabling a faster recovery when the inflammatory insult has resolved.

Patients with acute lung injury.

Acute lung injury (ALI) is a common finding in the critically ill patients. ALI is characterized by hypoxia, for which intubation and mechanical ventilation is often indicated (101;102).

However, mechanical ventilation can induce or aggravate ALI. The use of lower tidal volumes has been shown to be protective in patients with ALI (103). In addition, lowering the number of breaths/minute reduced pulmonary inflammation in an animal model of ALI (104). Protective mechanical ventilation using low tidal volumes and respiratory rate is limited by low minute ventilation, resulting in elevated CO₂ and severe hypercapnia. H₂S–induced suspended animation may be a promising treatment strategy to reduce damage inflicted by mechanical ventilation, by decreasing CO₂ production and O₂ demand, with a concomitant lower minute ventilation needed for adequate gas exchange, thereby allowing for lower tidal volumes and less breaths per minute. Experiments with H₂S in ALI are limited. In a model of combined burn and smoke inhalation, high doses of H₂S attenuated lung injury, by inhibition of inflammatory mediators and oxidative stress (74). The effect on gas exchange was not studied.

Discussion and recommendations for future research

Reducing the metabolic rate on demand using H₂S seems to be feasible in naturally non–hibernating mammals. The anecdotal reports on people surviving deep accidental hypothermia and anoxic conditions suggest that the ability to hibernate might be latently present. Before clinical application of inducing hypo–metabolism can be considered, a number of issues need to be resolved in pre–clinical experiments.

The conflicting results in larger animals need to be clarified. It is feasible that the use of sedation and/or paralysis may have blunted the metabolic effect related to reduction in activity during H₂S–exposure, an effect which was observed in mice. Alternatively, the differences in response to H₂S may rely on the relation between body weight and basal metabolic rate. Small animals have a higher basal metabolic rate, a large portion of which is used for heat production, not for ATP generation. H₂S–induced lowering of metabolism in small animals may therefore not affect ATP–production. In mammals with a higher body mass, higher doses of the gas may be needed to induce hypo–metabolism. Indeed, inhalation of doses of H₂S that already induced hypo–metabolism in mice, were reported to have no apparent side effects in exercising men (105–107). Obviously, increasing the dose carries the risk of toxicity. However, also doses of 50 ppm of H₂S have been shown to affect cell metabolism by reducing cytochrome c oxidase activity (108). In addition, using doses that induce hibernation did not result in considerable toxicity in animal models, and even tended to decrease the inflammatory response. Dose–finding studies that induce hibernation–like states but do not increase toxicity are mandated in appropriately–sized animal models.

Even when inducing hypo–metabolism proves to be feasible, potential adverse effects of inhibiting metabolism should be elucidated, such as the effect on host defense. It can

be hypothesized that suspended animation hampers adequate immune response during bacterial infections, possibly leading to diminished clearance of bacteria. Indeed, the production of heat shock proteins has been suggested to enhance host defense against bacteria (109). This issue needs to be studied in animal models of bacterial infections. Another potential adverse effect may include the rebound effect of nitric oxide. After cessation of suspended animation, it is possible that excessive rebound NO production may result in reactive oxygen species, when recovery of mitochondria has not yet been established.

We have suggested that hypo-metabolism can be considered to restore the imbalance between oxygen demand and consumption in critically ill patients. In these patients, additional issues need to be studied, such as whether beneficial results of hypo-metabolism also apply to anesthetized patients. Indeed, most of the clinical applications we mentioned require intubation and respiratory support, and a number of these patients will be sedated. In addition, critically ill patients most often require different types of pharmacological support, including vasoactive drugs and antibiotics. Future experiments are needed to study possible interaction of drugs with H₂S.

Lastly, there are a number of practical hurdles. H₂S has the smell of rotten eggs. In a closed system of mechanical ventilation, exposure to the odor may be limited, but accidental disconnections from the ventilation may pose a risk to medical personnel. Also, corrosion of metal parts and tubes may result in shortening of the durability of the mechanical ventilator.

Conclusions

H₂S-induced suspended animation has resulted in a reduction of CO₂-production and O₂-consumption, and thus resistance to hypoxia in various experimental models. Inducing a hypo-metabolic state may be a beneficial therapy in various conditions of hypoxia-induced organ damage, in particular in critically ill patients, in whom bio-energetic failure is a hallmark of multiple organ damage. Restoring the imbalance between oxygen demand and consumption may provide a novel therapeutic approach towards critically ill patients. Future studies are required to address feasibility as well as issues of toxicity before clinical application can be considered.

References

- (1) McCurry J, Jha A. Injured hiker survived 24 days on mountain by ‘hibernating’. *Guardian* 2006 Dec 21.
- (2) http://www.cbc.ca/canada/story/2001/02/28/baby_erika010228.html.
- (3) Oberhammer R, Beikircher W, Hormann C, Lorenz I, Pycha R, dler–Kastner L, Brugger H. Full recovery of an avalanche victim with profound hypothermia and prolonged cardiac arrest treated by extracorporeal re–warming. *Resuscitation* 2008 March;76(3):474–80.
- (4) Walpoth BH, Walpoth–Aslan BN, Mattle HP, Radanov BP, Schroth G, Schaeffler L, Fischer AP, von SL, Althaus U. Outcome of survivors of accidental deep hypothermia and circulatory arrest treated with extracorporeal blood warming. *N Engl J Med* 1997 November 20;337(21):1500–5.
- (5) Walpoth BH, Locher T, Leupi F, Schupbach P, Muhlemann W, Althaus U. Accidental deep hypothermia with cardiopulmonary arrest: extracorporeal blood rewarming in 11 patients. *Eur J Cardiothorac Surg* 1990;4(7):390–3.
- (6) Hughes A, Riou P, Day C. Full neurological recovery from profound (18.0 degrees C) acute accidental hypothermia: successful resuscitation using active invasive rewarming techniques. *Emerg Med J* 2007 July;24(7):511–2.
- (7) Zhang H, Zhi L, Moochhala SM, Moore PK, Bhatia M. Endogenous hydrogen sulfide regulates leukocyte trafficking in cecal ligation and puncture–induced sepsis. *J Leukoc Biol* 2007 October;82(4):894–905.
- (8) Lowicka E, Beltowski J. Hydrogen sulfide (H₂S) – the third gas of interest for pharmacologists. *Pharmacol Rep* 2007 January;59(1):4–24.
- (9) Zhang H, Hegde A, Ng SW, Adhikari S, Moochhala SM, Bhatia M. Hydrogen sulfide up–regulates substance P in polymicrobial sepsis–associated lung injury. *J Immunol* 2007 September 15;179(6):4153–60.
- (10) Carey HV, Andrews MT, Martin SL. Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. *Physiol Rev* 2003 October;83(4):1153–81.
- (11) Heldmaier G, Klingenspor M, Werneyer M, Lampi BJ, Brooks SP, Storey KB. Metabolic adjustments during daily torpor in the Djungarian hamster. *Am J Physiol* 1999 May;276(5 Pt 1):E896–E906.
- (12) Wang SQ, Lakatta EG, Cheng H, Zhou ZQ. Adaptive mechanisms of intracellular calcium homeostasis in mammalian hibernators. *J Exp Biol* 2002 October;205(Pt 19):2957–62.
- (13) Yoshimura T, Yasuo S, Watanabe M, Iigo M, Yamamura T, Hirunagi K, Ebihara S. Light–induced hormone conversion of T₄ to T₃ regulates photoperiodic response of gonads in birds. *Nature* 2003 November 13;426(6963):178–81.
- (14) Andrews MT. Advances in molecular biology of hibernation in mammals. *Bioessays* 2007 May;29(5):431–40.
- (15) Gorr TA, Gassmann M, Wappner P. Sensing and responding to hypoxia via HIF in model invertebrates. *J Insect Physiol* 2006 April;52(4):349–64.
- (16) Jonz MG, Nurse CA. Ontogenesis of oxygen chemoreception in aquatic vertebrates. *Respir Physiol Neurobiol* 2006 November;154(1–2):139–52.
- (17) Padilla PA, Roth MB. Oxygen deprivation causes suspended animation in the zebrafish embryo. *Proc Natl Acad Sci U S A* 2001 June 19;98(13):7331–5.
- (18) Clegg J. Embryos of *Artemia franciscana* survive four years of continuous anoxia: the case for complete metabolic rate depression. *J Exp Biol* 1997;200(Pt 3):467–75.
- (19) MacRae TH. Molecular chaperones, stress resistance and development in *Artemia franciscana*. *Semin Cell Dev Biol* 2003 October;14(5):251–8.
- (20) Woods RJ, Prueckner S, Safar P, Radovsky A, Takasu A, Stezoski SW, Stezoski J, Tisherman SA. Hypothermic aortic arch flush for preservation during exsanguination cardiac arrest of 15 minutes in dogs. *J Trauma* 1999 December;47(6):1028–36.
- (21) Anbar AD. Oceans. Elements and evolution. *Science* 2008 December 5;322(5907):1481–3.
- (22) Farquhar J, Peters M, Johnston DT, Strauss H, Masterson A, Wiechert U, Kaufman AJ. Isotopic evidence for Mesoarchaean anoxia and changing atmospheric sulphur chemistry. *Nature* 2007 October 11;449(7163):706–9.
- (23) Roth MB, Nystul T. Buying time in suspended animation. *Sci Am* 2005 June;292(6):48–55.

- (24) Kelly DP, Shergill JK, Lu WP, Wood AP. Oxidative metabolism of inorganic sulfur compounds by bacteria. *Antonie Van Leeuwenhoek* 1997 February;71(1–2):95–107.
- (25) Tripp HJ, Kitner JB, Schwalbach MS, Dacey JW, Wilhelm LJ, Giovannoni SJ. SAR11 marine bacteria require exogenous reduced sulphur for growth. *Nature* 2008 April 10;452(7188):741–4.
- (26) Moore PK, Bhatia M, Moochhala S. Hydrogen sulfide: from the smell of the past to the mediator of the future? *Trends Pharmacol Sci* 2003 December;24(12):609–11.
- (27) Kakkur P, Singh BK. Mitochondria: a hub of redox activities and cellular distress control. *Mol Cell Biochem* 2007 November;305(1–2):235–53.
- (28) Kemp M, Go YM, Jones DP. Nonequilibrium thermodynamics of thiol/disulfide redox systems: a perspective on redox systems biology. *Free Radic Biol Med* 2008 March 15;44(6):921–37.
- (29) Blackstone E, Morrison M, Roth MB. H₂S induces a suspended animation–like state in mice. *Science* 2005 April 22;308(5721):518.
- (30) Bigelow WG. Hypothermia. *Surgery* 1958 April;43(4):683–7.
- (31) Bernard SA, Jones BM, Horne MK. Clinical trial of induced hypothermia in comatose survivors of out–of–hospital cardiac arrest. *Ann Emerg Med* 1997 August;30(2):146–53.
- (32) Grimm M, Czerny M, Baumer H, Kilo J, Madl C, Kramer L, Rajek A, Wolner E. Normothermic cardiopulmonary bypass is beneficial for cognitive brain function after coronary artery bypass grafting—a prospective randomized trial. *Eur J Cardiothorac Surg* 2000 September;18(3):270–5.
- (33) Boldt J, Osmer C, Linke LC, Goriach G, Hempelmann G. Hypothermic versus normothermic cardiopulmonary bypass: influence on circulating adhesion molecules. *J Cardiothorac Vasc Anesth* 1996 April;10(3):342–7.
- (34) Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. *N Engl J Med* 2002 February 21;346(8):549–56.
- (35) Bigelow WG, LINDSAY WK. . Oxygen transport and utilization in dogs at low body temperatures. *Am J Physiol* 1950 January;160(1):125–37.
- (36) Rosomoff HL. PATHOPHYSIOLOGY OF THE CENTRAL NERVOUS SYSTEM DURING HYPOTHERMIA. *Acta Neurochir Suppl* 1964;14:SUPPL–22.
- (37) Chopp M, Knight R, Tidwell CD, Helpert JA, Brown E, Welch KM. The metabolic effects of mild hypothermia on global cerebral ischemia and recirculation in the cat: comparison to normothermia and hyperthermia. *J Cereb Blood Flow Metab* 1989 April;9(2):141–8.
- (38) Kramer RS, Sanders AP, Lesage AM, Woodhall B, Sealy WC. The effect profound hypothermia on preservation of cerebral ATP content during circulatory arrest. *J Thorac Cardiovasc Surg* 1968 November;56(5):699–709.
- (39) Beauchamp RO, Jr., Bus JS, Popp JA, Boreiko CJ, Andjelkovich DA. A critical review of the literature on hydrogen sulfide toxicity. *Crit Rev Toxicol* 1984;13(1):25–97.
- (40) Szabo C. Hydrogen sulphide and its therapeutic potential. *Nat Rev Drug Discov* 2007 November;6(11):917–35.
- (41) Zhao W, Zhang J, Lu Y, Wang R. The vasorelaxant effect of H(2)S as a novel endogenous gaseous K(ATP) channel opener. *EMBO J* 2001 November 1;20(21):6008–16.
- (42) Bhatia M. Hydrogen sulfide as a vasodilator. *IUBMB Life* 2005 September;57(9):603–6.
- (43) Kimura Y, Kimura H. Hydrogen sulfide protects neurons from oxidative stress. *FASEB J* 2004 July;18(10):1165–7.
- (44) Kimura H, Nagai Y, Umemura K, Kimura Y. Physiological roles of hydrogen sulfide: synaptic modulation, neuroprotection, and smooth muscle relaxation. *Antioxid Redox Signal* 2005 May;7(5–6):795–803.
- (45) Blackstone E, Roth MB. Suspended animation–like state protects mice from lethal hypoxia. *Shock* 2007 April;27(4):370–2.
- (46) Nystul TG, Roth MB. Carbon monoxide–induced suspended animation protects against hypoxic damage in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* 2004 June 15;101(24):9133–6.
- (47) Volpato GP, Searles R, Yu B, Scherrer–Crosbie M, Bloch KD, Ichinose F, Zapol WM. Inhaled hydrogen sulfide: a rapidly reversible inhibitor of cardiac and metabolic function in the mouse. *Anesthesiology* 2008 April;108(4):659–68.
- (48) Li L, Moore PK. Putative biological roles of hydrogen sulfide in health and disease: a breath of not so fresh air? *Trends Pharmacol Sci* 2008 February;29(2):84–90.

- (49) Johansen D, Ytrehus K, Baxter GF. Exogenous hydrogen sulfide (H₂S) protects against regional myocardial ischemia–reperfusion injury—Evidence for a role of K ATP channels. *Basic Res Cardiol* 2006 January;101(1):53–60.
- (50) Simon F, Giudici R, Duy CN, Schelzig H, Oter S, Groger M, Wachter U, Vogt J, Speit G, Szabo C, Radermacher P, Calzia E. Hemodynamic and metabolic effects of hydrogen sulfide during porcine ischemia/reperfusion injury. *Shock* 2008 October;30(4):359–64.
- (51) Zhang H, Zhi L, Moore PK, Bhatia M. Role of hydrogen sulfide in cecal ligation and puncture–induced sepsis in the mouse. *Am J Physiol Lung Cell Mol Physiol* 2006 June;290(6):L1193–L1201.
- (52) Singer D. Metabolic adaptation to hypoxia: cost and benefit of being small. *Respir Physiol Neurobiol* 2004 August 12;141(3):215–28.
- (53) Li J, Zhang G, Cai S, Redington AN. Effect of inhaled hydrogen sulfide on metabolic responses in anesthetized, paralyzed, and mechanically ventilated piglets. *Pediatr Crit Care Med* 2008 January;9(1):110–2.
- (54) Haouzi P, Notet V, Chenuel B, Chalon B, Sponne I, Ogier V, Bihain B. H₂S induced hypometabolism in mice is missing in sedated sheep. *Respir Physiol Neurobiol* 2008 January 1;160(1):109–15.
- (55) Wang R. Two's company, three's a crowd: can H₂S be the third endogenous gaseous transmitter? *FASEB J* 2002 November;16(13):1792–8.
- (56) Hosoki R, Matsuki N, Kimura H. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. *Biochem Biophys Res Commun* 1997 August 28;237(3):527–31.
- (57) Zhao W, Ndisang JF, Wang R. Modulation of endogenous production of H₂S in rat tissues. *Can J Physiol Pharmacol* 2003 September;81(9):848–53.
- (58) Koenitzer JR, Isbell TS, Patel HD, Benavides GA, Dickinson DA, Patel RP, rley–Usmar VM, Lancaster JR, Jr., Doeller JE, Kraus DW. Hydrogen sulfide mediates vasoactivity in an O₂–dependent manner. *Am J Physiol Heart Circ Physiol* 2007 April;292(4):H1953–H1960.
- (59) Sivarajah A, McDonald MC, Thiemermann C. The production of hydrogen sulfide limits myocardial ischemia and reperfusion injury and contributes to the cardioprotective effects of preconditioning with endotoxin, but not ischemia in the rat. *Shock* 2006 August;26(2):154–61.
- (60) Elrod JW, Calvert JW, Morrison J, Doeller JE, Kraus DW, Tao L, Jiao X, Scalia R, Kiss L, Szabo C, Kimura H, Chow CW, Lefer DJ. Hydrogen sulfide attenuates myocardial ischemia–reperfusion injury by preservation of mitochondrial function. *Proc Natl Acad Sci U S A* 2007 September 25;104(39):15560–5.
- (61) Sodha NR, Clements RT, Feng J, Liu Y, Bianchi C, Horvath EM, Szabo C, Sellke FW. The effects of therapeutic sulfide on myocardial apoptosis in response to ischemia–reperfusion injury. *Eur J Cardiothorac Surg* 2008 May;33(5):906–13.
- (62) Hu Y, Chen X, Pan TT, Neo KL, Lee SW, Khin ES, Moore PK, Bian JS. Cardioprotection induced by hydrogen sulfide preconditioning involves activation of ERK and PI3K/Akt pathways. *Pflugers Arch* 2008 January;455(4):607–16.
- (63) Bell RM, Maddock HL, Yellon DM. The cardioprotective and mitochondrial depolarising properties of exogenous nitric oxide in mouse heart. *Cardiovasc Res* 2003 February;57(2):405–15.
- (64) Li L, Bhatia M, Zhu YZ, Zhu YC, Ramnath RD, Wang ZJ, Anuar FB, Whiteman M, Salto–Tellez M, Moore PK. Hydrogen sulfide is a novel mediator of lipopolysaccharide–induced inflammation in the mouse. *FASEB J* 2005 July;19(9):1196–8.
- (65) Hui Y, Du J, Tang C, Bin G, Jiang H. Changes in arterial hydrogen sulfide (H(2)S) content during septic shock and endotoxin shock in rats. *J Infect* 2003 August;47(2):155–60.
- (66) Chunyu Z, Junbao D, Dingfang B, Hui Y, Xiuying T, Chaoshu T. The regulatory effect of hydrogen sulfide on hypoxic pulmonary hypertension in rats. *Biochem Biophys Res Commun* 2003 March 21;302(4):810–6.
- (67) Collin M, Anuar FB, Murch O, Bhatia M, Moore PK, Thiemermann C. Inhibition of endogenous hydrogen sulfide formation reduces the organ injury caused by endotoxemia. *Br J Pharmacol* 2005 October;146(4):498–505.
- (68) Zhang H, Zhi L, Moochhala S, Moore PK, Bhatia M. Hydrogen sulfide acts as an inflammatory mediator in cecal ligation and puncture–induced sepsis in mice by upregulating the production of cytokines and chemokines via NF–kappaB. *Am J Physiol Lung Cell Mol Physiol* 2007 April;292(4):L960–L971.

- (69) Zanardo RC, Brancaleone V, Distrutti E, Fiorucci S, Cirino G, Wallace JL. Hydrogen sulfide is an endogenous modulator of leukocyte-mediated inflammation. *FASEBJ* 2006 October;20(12):2118–20.
- (70) Fiorucci S, Antonelli E, Distrutti E, Rizzo G, Mencarelli A, Orlandi S, Zanardo R, Renga B, Di Sante M, Morelli A, Cirino G, Wallace JL. Inhibition of hydrogen sulfide generation contributes to gastric injury caused by anti-inflammatory nonsteroidal drugs. *Gastroenterology* 2005 October;129(4):1210–24.
- (71) Persson S, Claesson R, Carlsson J. Chemotaxis and degranulation of polymorphonuclear leukocytes in the presence of sulfide. *Oral Microbiol Immunol* 1993 February;8(1):46–9.
- (72) Whiteman M, Armstrong JS, Chu SH, Jia-Ling S, Wong BS, Cheung NS, Halliwell B, Moore PK. The novel neuromodulator hydrogen sulfide: an endogenous peroxynitrite ‘scavenger’? *J Neurochem* 2004 August;90(3):765–8.
- (73) Lou LX, Geng B, Du JB, Tang CS. Hydrogen sulphide-induced hypothermia attenuates stress-related ulceration in rats. *Clin Exp Pharmacol Physiol* 2008 February;35(2):223–8.
- (74) Esehie A, Kiss L, Olah G, Horvath EM, Hawkins H, Szabo C, Traber DL. Protective effect of hydrogen sulfide in a murine model of acute lung injury induced by combined burn and smoke inhalation. *Clin Sci (Lond)* 2008 August;115(3):91–7.
- (75) naya-Prado R, gado-Vazquez JA. Scientific basis of organ preservation. *Curr Opin Organ Transplant* 2008 April;13(2):129–34.
- (76) Eurotransplant International Foundation online site, <http://www.eurotransplant.nl/?id=organs>.
- (77) Salahudeen AK. Cold ischemic injury of transplanted kidneys: new insights from experimental studies. *Am J Physiol Renal Physiol* 2004 August;287(2):F181–F187.
- (78) Bopassa JC, Michel P, Gateau-Roesch O, Ovize M, Ferrera R. Low-pressure reperfusion alters mitochondrial permeability transition. *Am J Physiol Heart Circ Physiol* 2005 June;288(6):H2750–H2755.
- (79) Gunter KK, Gunter TE. Transport of calcium by mitochondria. *J Bioenerg Biomembr* 1994 October;26(5):471–85.
- (80) Hausenloy DJ, Duchon MR, Yellon DM. Inhibiting mitochondrial permeability transition pore opening at reperfusion protects against ischaemia-reperfusion injury. *Cardiovasc Res* 2003 December 1;60(3):617–25.
- (81) Hu X, Li T, Bi S, Jin Z, Zhou G, Bai C, Li L, Cui Q, Liu W. Possible role of hydrogen sulfide on the preservation of donor rat hearts. *Transplant Proc* 2007 December;39(10):3024–9.
- (82) Morrison ML, Blackwood JE, Lockett SL, Iwata A, Winn RK, Roth MB. Surviving blood loss using hydrogen sulfide. *J Trauma* 2008 July;65(1):183–8.
- (83) Vanoverschelde JL, Wijns W, Depre C, Essamri B, Heyndrickx GR, Borgers M, Bol A, Melin JA. Mechanisms of chronic regional postischemic dysfunction in humans. New insights from the study of noninfarcted collateral-dependent myocardium. *Circulation* 1993 May;87(5):1513–23.
- (84) Fallavollita JA, Perry BJ, Canty JM, Jr. 18F–2–deoxyglucose deposition and regional flow in pigs with chronically dysfunctional myocardium. Evidence for transmural variations in chronic hibernating myocardium. *Circulation* 1997 April 1;95(7):1900–9.
- (85) Page B, Young R, Iyer V, Suzuki G, Lis M, Korotchkina L, Patel MS, Blumenthal KM, Fallavollita JA, Canty JM, Jr. Persistent regional downregulation in mitochondrial enzymes and upregulation of stress proteins in swine with chronic hibernating myocardium. *Circ Res* 2008 January 4;102(1):103–12.
- (86) Greer DM. Mechanisms of injury in hypoxic-ischemic encephalopathy: implications to therapy. *Semin Neurol* 2006 September;26(4):373–9.
- (87) Harukuni I, Bhardwaj A. Mechanisms of brain injury after global cerebral ischemia. *Neurol Clin* 2006 February;24(1):1–21.
- (88) Cholley F, Edery P, Ricquier D, Peudener S, Slama A, Tardieu M. Mitochondrial respiratory chain deficiency revealed by hypothermia. *Neuropediatrics* 2001 April;32(2):104–6.
- (89) Boehning D, Snyder SH. Novel neural modulators. *Annu Rev Neurosci* 2003;26:105–31.
- (90) Kentner R, Rollwagen FM, Prueckner S, Behringer W, Wu X, Stezoski J, Safar P, Tisherman SA. Effects of mild hypothermia on survival and serum cytokines in uncontrolled hemorrhagic shock in rats. *Shock* 2002 June;17(6):521–6.

- (91) Bell RC, Coalson JJ, Smith JD, Johanson WG, Jr. Multiple organ system failure and infection in adult respiratory distress syndrome. *Ann Intern Med* 1983 September;99(3):293–8.
- (92) American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992 June;20(6):864–74.
- (93) Boekstegers P, Weidenhofer S, Pilz G, Werdan K. Peripheral oxygen availability within skeletal muscle in sepsis and septic shock: comparison to limited infection and cardiogenic shock. *Infection* 1991 September;19(5):317–23.
- (94) Vandermeer TJ, Wang H, Fink MP. Endotoxemia causes ileal mucosal acidosis in the absence of mucosal hypoxia in a normodynamic porcine model of septic shock. *Crit Care Med* 1995 July;23(7):1217–26.
- (95) Brealey D, Singer M. Mitochondrial Dysfunction in Sepsis. *Curr Infect Dis Rep* 2003 October;5(5):365–71.
- (96) Singer M. Mitochondrial function in sepsis: acute phase versus multiple organ failure. *Crit Care Med* 2007 September;35(9 Suppl):S441–S448.
- (97) Brealey D, Brand M, Hargreaves I, Heales S, Land J, Smolenski R, Davies NA, Cooper CE, Singer M. Association between mitochondrial dysfunction and severity and outcome of septic shock. *Lancet* 2002 July 20;360(9328):219–23.
- (98) Vanhorebeek I, De Vos R, Mesotten D, Wouters PJ, Wolf–Peeters C, Van den BG. Protection of hepatocyte mitochondrial ultrastructure and function by strict blood glucose control with insulin in critically ill patients. *Lancet* 2005 January 1;365(9453):53–9.
- (99) Van den BG, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P, Bouillon R. Intensive insulin therapy in the critically ill patients. *N Engl J Med* 2001 November 8;345(19):1359–67.
- (100) Van den BG, Wilmer A, Hermans G, Meersseman W, Wouters PJ, Milants I, Van Wijngaerden E, Bobbaers H, Bouillon R. Intensive insulin therapy in the medical ICU. *N Engl J Med* 2006 February 2;354(5):449–61.
- (101) Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, Stern EJ, Hudson LD. Incidence and outcomes of acute lung injury. *N Engl J Med* 2005 October 20;353(16):1685–93.
- (102) Ware LB, Matthay MA. The acute respiratory distress syndrome. *N Engl J Med* 2000 May 4;342(18):1334–49.
- (103) Amato MB, Barbas CS, Medeiros DM, Magaldi RB, Schettino GP, Lorenzi–Filho G, Kairalla RA, Deheinzelin D, Munoz C, Oliveira R, Takagaki TY, Carvalho CR. Effect of a protective–ventilation strategy on mortality in the acute respiratory distress syndrome. *N Engl J Med* 1998 February 5;338(6):347–54.
- (104) Hotchkiss JR, Jr., Blanch L, Murias G, Adams AB, Olson DA, Wangenstein OD, Leo PH, Marini JJ. Effects of decreased respiratory frequency on ventilator–induced lung injury. *Am J Respir Crit Care Med* 2000 February;161(2 Pt 1):463–8.
- (105) Bhambhani Y, Singh M. Physiological effects of hydrogen sulfide inhalation during exercise in healthy men. *J Appl Physiol* 1991 November;71(5):1872–7.
- (106) Bhambhani Y, Burnham R, Snydmiller G, MacLean I, Lovlin R. Effects of 10–ppm hydrogen sulfide inhalation on pulmonary function in healthy men and women. *J Occup Environ Med* 1996 October;38(10):1012–7.
- (107) Bhambhani Y, Burnham R, Snydmiller G, MacLean I. Effects of 10–ppm hydrogen sulfide inhalation in exercising men and women. Cardiovascular, metabolic, and biochemical responses. *J Occup Environ Med* 1997 February;39(2):122–9.
- (108) Khan AA, Schuler MM, Prior MG, Yong S, Coppock RW, Florence LZ, Lillie LE. Effects of hydrogen sulfide exposure on lung mitochondrial respiratory chain enzymes in rats. *Toxicol Appl Pharmacol* 1990 May;103(3):482–90.
- (109) Schroeder S, Bischoff J, Lehmann LE, Hering R, von ST, Putensen C, Hoefl A, Stuber F. Endotoxin inhibits heat shock protein 70 (HSP70) expression in peripheral blood mononuclear cells of patients with severe sepsis. *Intensive Care Med* 1999 January;25(1):52–7.